Virginia Department of Environmental Quality

Draft 2014 Water Quality Integrated Report

Public Comment-Response Document

Comments received

December 15, 2014 to January 30, 2015

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION III
1650 Arch Street
Philadelphia, Pennsylvania 19103-2029

Mr. John Kennedy, Director
Office of Ecology
Virginia Department of Environmental Quality
629 E. Main Street
P.O. Box 1105
Richmond, Virginia 23218

JAN 29 2015

Dear Mr. Kennedy:

Thank you for the opportunity to review the Virginia Department of Environmental Quality's (VADEQ) Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report (Integrated Report). The U.S. Environmental Protection Agency (EPA) is providing comments related to specific impaired waterbody segments that EPA could not track from 2012 to 2014 in the enclosure. EPA has also outlined additional comments related to the Shenandoah River below. These comments are in addition to the delisting comments submitted to VADEQ electronically on December 22, 2014. EPA is in the process of the reviewing the responses to our comments and may request additional clarification.

Virginia has proposed for delisting 35 waterbody segments from category 5 to 4C due to natural swamp water conditions. VADEQ did not provide delisting rationales for each of these waters, and therefore, there was insufficient information for EPA to review or comment on these 35 delistings. Please provide rationales for each water proposed for delisting due to natural conditions. Once EPA has had a chance to fully evaluate the delisting rationales for these waters, we will submit more detailed comments on the proposed delistings and Virginia's natural condition assessment methodology. EPA is currently developing a draft national "*Framework for Defining and Documenting Natural Conditions for Development of Site-Specific Natural Background Water Quality Criteria for Temperature, Dissolved Oxygen, and pH to Protect Aquatic Life Uses: Interim Document.*" When this document becomes available, it may be useful in informing our future conversations regarding Virginia's natural condition assessment methodology.

As you are aware, over the past few years VADEQ and EPA have received numerous complaints from the Shenandoah Riverkeeper related to excess algal growth on the North Fork Shenandoah River, South Fork Shenandoah River and mainstem Shenandoah River. EPA believes the Riverkeeper's information warrants serious consideration and further investigation. EPA outlined expectations related to algal issues in the Shenandoah River in our September 23, 2014 approval of Virginia's 2012 Section 303(d) List. Specifically, EPA expects VADEQ to make significant progress, commit to concrete steps toward, and provide a timeline for developing an assessment methodology and impairment threshold to evaluate algal impacts to

recreation uses in at least the Shenandoah Basin and preferably for all of Virginia's rivers and streams. We note the draft 2014 IR has not outlined any additional information related to development of an assessment methodology.

The draft 2014 Virginia Integrated Report represents the third listing cycle the Shenandoah River is listed in category 2B (having an observed effect, but no water quality standard exists for a particular pollutant). VADEQ details not having an assessment methodology or sufficient data available to assess algal impacts to recreation uses in the Shenandoah Basin. EPA anticipates that the Shenandoah Riverkeeper will provide information regarding algal growth in the Shenandoah Basin in connection with the public comment period. EPA reminds VADEQ that states are required to assess approved water quality standards for Clean Water Act Section 303(d)/305(b) purposes and that, where sufficient information for a specific water is provided, waters can be assessed on a case-by-case basis using information provided without first developing a generalized assessment methodology. EPA's 2014 Integrated Report Guidance outlines examples of how States have assessed their narrative water quality standard utilizing public feedback, complaints and photo documentation of excess algal growth. Impairments based upon a case-by-case assessment can be refined as future methodologies are developed and implemented or as additional data are collected.

In October 2012, the Shenandoah Riverkeeper submitted directly to EPA over 900 photos of various stream segments along the mainstem Shenandoah River, North Fork Shenandoah River and South Fork Shenandoah River. In addition to the photos, the Riverkeeper supplied copies of the 80 citizen complaints of excess algal growth within the Shenandoah Basin that were submitted to VADEQ during the 2012 Integrated Report public comment period. EPA's Decision Rational for the 2012 Section 303(d) list encouraged the public to provide information to VADEQ consistent with the established public process and discussed the need for additional data to confirm the presence of algal blooms and to facilitate analysis of the spatial and temporal extent of any impairment. EPA requests VADEQ to assess the information provided by the Shenandoah Riverkeeper to determine if sufficient evidence has been provided to document a violation of VADEQ's narrative water quality standard (9VAC25-260-20) due to excess algal growth and an impairment of the recreation use on any segments of the Shenandoah River. In connection with VADEQ's final submission, EPA will consider information provided to VADEQ by the public during the 2014 Integrated Report public comment period.

With funding assistance from EPA, the Interstate Commission on the Potomac River Basin (ICPRB) worked on a 2014 pilot project in the Shenandoah Basin to study algal issues raised by the Riverkeeper. ICPRB worked with volunteer/citizen monitoring groups to collect algal cover information and develop an assessment methodology that can be used to assess Virginia's free-flowing rivers and streams. A final report from ICPRB is expected to be submitted to both VADEQ and EPA by February 6, 2015. We request that VADEQ evaluate the ICPRB report to seriously consider how the methodologies developed by ICPRB could be incorporated into VADEQ's future assessment methodology.

Virginia may not indefinitely postpone evaluation of information in the form of visual observations and statements by users of the River due to lack of an assessment methodology and impairment threshold. We anticipate that VADEQ will provide a full response to any

information regarding algal growth received from the public. We anticipate that such a response could include the following:

1) A discussion that outlines the steps to date taken by Virginia to evaluate and address the algal issues raised by the Riverkeeper;
2) A timeline in your 2014 IR that includes your plans to develop an assessment methodology that will evaluate the algal impacts to recreation uses; and
3) Virginia's plans to use that methodology for evaluating use attainment in the Shenandoah River.

EPA appreciates the continued cooperation from VADEQ staff in evaluating the water quality issues in the Commonwealth. Once VADEQ staff have had time to evaluate public comments related to the Shenandoah River, EPA would like to continue our discussions on the Shenandoah River prior to VADEQ submitting a final Integrated Report. If you have any questions, please feel free to contact me, or have your staff call Mr. William Richardson at (215) 814-5675.

Sincerely,

Evelyn S. MacKnight

Evelyn S. MacKnight, Associate Director
Office of Standards, Assessment and TMDLs
Water Protection Division

Enclosure

EPA comments on draft 2014 IR - Category 5 - 303(d) list
KEY:
ILD: Initial List Date
IR: Integrated Report

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed
A26R-02-BAC	VAN-A26R_POW02A02	Powells Creek	Listed on 2012 IR for E. coli	VAN-A26R_POW02A02 identified as nested; please provide nesting rationale
A28R-01-BAC	VAN-A28R_AUS02A06	Austin Run	Listed on 2012 IR for E. coli	VAN-A28R_AUS02A06 identified as nested; please provide nesting rationale
A29E-02-BAC	VAN-A29E_POT01A06	Fairview Beach (Potomac River)	Listed on 2012 IR for 0.012 square miles; 0.005 square miles on 2014 IR	Please clarify mileage change.
A29R-02-BAC	VAN-A29R_POM02A06	Potomac Creek	Listed on 2012 IR for E. coli	VAN-A29R_POM02A06 identified as nested; please provide nesting rationale
A31E-01-SF2	VAP-A31E_ROS01A08	Rosier Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A31E-04-PH	VAP-A31E_MON01A00	Monroe Creek	Proposed delisting to 4C for 0.176 Sq. Miles	Please provide delisting rationale for this area.
A31R-03-DO	VAP-A31R_POP01A00	Popes Creek watershed	Proposed delisting to 4C for 29.88 miles	Please provide delisting rationale for this segment.
A31R-03-PH	VAP-A31R_POP01A00	Popes Creek watershed	Proposed delisting to 4C for 29.88 miles	Please provide delisting rationale for this segment.
A31R-04-DO	VAP-A31R_MRC01A98	Monroe Creek Watershed	Proposed delisting to 4C for 12.27 Miles	Please provide delisting rationale for this segment.
A31R-04-PH	VAP-A31R_MRC01A98	Monroe Creek Watershed	Proposed delisting to 4C for 12.27 Miles	Please provide delisting rationale for this segment.
A31R-05-DO	VAP-A31R_CAS01A00	Canal Swamp Watershed	Proposed delisting to 4C for 12.57 Miles	Please provide delisting rationale for this segment.
A31R-05-PH	VAP-A31R_CAS01A00	Canal Swamp Watershed	Proposed delisting to 4C for 12.57 Miles	Please provide delisting rationale for this segment.
A32E-04-SF2	VAP-A32E_NOM01A04	Nomini Creek, Pierce Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A32E-13-SF	VAP-A32E_CUR01B08	Currioman Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A32E-14-SF	VAP-A32E_POO01A08	Poor Jack Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A32E-15-SF	VAP-A32E_DAV01A08	Davis Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A32E-16-SF	VAP-A32E_JUL01A08	Jules Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A32E-17-SF	VAP-A32E_MAT01A08	Matthews Cove	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A32E-21-SF	VAP-A32E_NOP02A08	North Prong Buckner Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed
A32R-01-DO	VAP-A32R_THP01A06	Thompson Branch	Proposed delisting to 4C for 1.60 Miles	Please provide delisting rationale for this segment.
A32R-01-PH	VAP-A32R_THP01A06	Thompson Branch	Proposed delisting to 4C for 1.60 Miles	Please provide delisting rationale for this segment.
A33E-12-SF	VAP-A33E_SHA01A98	Shannon Branch	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A33E-16-SF	VAP-A33E_WES01A06	West Yeocomico River	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A33R-01-BAC	VAP-A33R_MIA01A00	Mill Creek	Listed on 2012 IR for E. coli	Please provide nesting rationale.
A33R-02-DO	VAP-A33R_LOG01A04	Lodge Creek	Proposed delisting to 4C for 3.44 Miles	Please provide delisting rationale for this segment.
A34E-01-SF2	VAP-A34E_GLE01A04	The Glebe	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A34E-17-SF	VAP-A34E_BRI01C98	Bridge Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A34E-21-SF	VAP-A34E_KIN04A06	Kingscote Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A34E-25-SF	VAP-A34E_BOT01A04	Boathouse Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A34E-28-SF	VAP-A34E_SLO05A98	Slough Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A34E-31-BAC	VAP-A34E_LIS01A02, VAP-A34E_LIS01A14, VAP-A34E_LIS01A98, VAP-A34E_LIS01C14	Little Wicomico River	Listed on 2012 IR for Enterococcus	Please provide nesting rationale.
A34E-32-SF	VAP-A34E_COA01A02	Coan River	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A34E-33-SF	VAP-A34E_BBC01A08	Bridgemans Back Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A34E-34-SF	VAP-A34E_BAC01A12	Back Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
A34E-35-SF	VAP-A34E_LIS04B12	Little Wicomico River, UT	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C01E-01-SF2	VAP-C01E_GWR01B08,	Great Wicomoico River, Blackwells Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C01E-02-SF2	VAP-C01E_BLS02A08	Balls Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C01E-03-SF2	VAP-C01E_TIP02A08	Tipers Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C01E-06-SF2	VAP-C01E_WCO02A08	Warehouse Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C01E-07-SF	VAP-C01E_HHB01A98	Horn Harbor	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C01E-09-SF2	VAP-C01E_PNT02B10	Prentice Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C01E-12-SF2	VAP-C01E_MIL02A08	Mill Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C01E-14-SF	VAP-C01E_GSK01A10	Gaskin Pond	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C01E-17-PCB		Chesapeake Bay and Tidal Tributaries	Three listings on 2012 IR for PCB in Fish Tissue	Where is this listing?
C01E-31-SF	VAP-C01E_JAR01A02	Jarvis Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C01E-33-SF	VAP-C01E_BMC01A04	Betts Mill Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C01E-37-SF	VAP-C01E_HAV01A08	Harveys Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C01E-38-SF	VAP-C01E_CRN01A06	Cranes Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C01E-40-SF	VAP-C01E_COL01A08	Coles Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C01E-56-SF	VAP-C01E_PEN01A12	Head River Branch	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed
C01E-58-SF	VAP-C01E_XEV01A12	XEV - Mill Creek, UT	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C01E-60-SF	VAP-C01E_LEE02A12	Lees Cove	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C03E-03-SF	VAP-C03E_FRE01A02	Frenchs Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C03E-09-SF		Piankatank River, UT	Listed on 2012 IR for Fecal Coliform	Where is this listing?
C03E-14-EBEN		Piankatank River	Listed on 2012 IR for Estuarine Bioassessments under Cause Group Code "C03E-14-EBEN"; listed under "C03E-10-EBEB" in 2014 IR	Please identify correct cause group code.
C03E-15-SF	VAP-C03E_DAN01A08	Dancing Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C03E-19-SF	VAP-C03E_PNK04D08	Porpoise Cove	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-01-SF2	VAP-C04E_QUE02A12	Queens Creek, UT	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-05-SF2	VAP-C04E_STT01B10	Stutts Creek and Morris Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-07-SF2	VAP-C04E_BLP01B10	Billups Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-08-SF2	VAP-C04E_NOR01B08	North River	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-12-SF	VAP-C04E_DAV01A98	Davis Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-25-SF	VAP-C04E_MID01A02	Winder Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-28-SF	VAP-C04E_BRN01A04	Barn Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-30-SF	VAP-C04E_LAN01B08	Lanes Creek, UT	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-31-SF	VAP-C04E_STT01B06	Stutts Creet, (Hole in the Wall)	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-32-SF	VAP-C04E_HUD01A08	Hudgins Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-42-SF	VAP-C04E_WON01A08	Weston Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-47-SF	VAP-C04E_BEV01A08	Belleville Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-48-SF	VAP-C04E_WOO01A10	Woodas Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-49-SF	VAP-C04E_EST01B10	East River	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-52-SF	VAP-C04E_LAN01A02	Lanes Creek, UT	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-53-BAC		Whites Creek	Listed on 2012 IR as "Whites Creek", "Whites Creek - Festival Branch" on 2014 IR	Please identify correct water name.
C04E-54-SF	VAP-C04E_DVS03A12	Davis Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04E-55-SF	VAP-C04E_RAY01A12	Raymond Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C04R-01-DO	VAP-C04R_EST01A12	East River	Listed in 2012 IR for 1.79 miles; 0.59 miles in 2014 IR	Please clarify mileage changes.
C04R-01-PH	VAP-C04R_EST01A12	East River	Listed in 2012 IR for 1.79 miles; 0.59 miles in 2014 IR	Please clarify mileage changes.
C05E-01-SF2	VAP-C05E_WAR01B08	Ware River	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C05E-02-SF2	VAP-C05E_WIL01B08	Wilson Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
C06R-01-DO	VAP-C06R_SEN01A00	Northwest Branch of Severn River	Proposed delisting to 4C for 2.16 Miles	Please provide delisting rationale for this segment.

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed
C07E-01-PCB		Chesapeake Bay and Tidal Tributaries VDH Fish Consumption Advisory for PCBs	Listed in 2012 IR for PCB in Fish Tissue	Partial 4a (VAT-C07E_BRK01A06 TMDL ID 31233), where is this listing?
C08E-01-EBEN		Lynnhaven River - Mainstem	Identified on 2012 IR as "Lynnhaven River - Mainstem", "London Bridge Creek" on 2014 IR	Please identify correct water name.
C08L-01-CHLA		Lake Whitehurst	Listed in 2012 IR for 494.93 acres, 481.42 acres in 2014 IR (-13.52 acres)	Please clarify acreage change.
C08L-01-DO		Lake Whitehurst	Listed in 2012 IR for 494.93 acres, 481.42 acres in 2014 IR (-13.52 acres)	Please clarify acreage change.
C08L-01-HG		Lake Whitehurst	Listed in 2012 IR for 494.93 acres, 481.42 acres in 2014 IR (-13.52 acres)	Please clarify acreage change.
C08L-01-PCB		Lake Whitehurst VDH Fish Consumption Advisory	Listed in 2012 IR for 494.93 acres, 481.42 acres in 2014 IR (-13.52 acres)	Please clarify acreage change.
C08L-01-TP		Lake Whitehurst	Listed in 2012 IR for 494.93 acres, 481.42 acres in 2014 IR (-13.52 acres)	Please clarify acreage change.
C10E-12-SF2	VAT-C10E_GLF01B08	Guilford Creek - Upper (No TMDL)	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
C10R-01-BAC2	VAT-C10R_XCX01A08	Unnamed tributary from Tyson Foods	Listed in 2012 IR for E. coli	Please provide nesting rationale.
C13E-02-BAC		Nassawadox Creek - Lower	Listed in 2012 IR for Enterococcus	Where is this listing?
C13E-11-SF2		Church Creek - Middle, UT North Cove [No TMDL]	Listed in 2012 IR for Fecal Coliform	Where is this listing?
C13E-22-SF	VAT-C13E_OCH03A08	Shields Cove	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
C13E-23-SF		Fisher Cove	Listed in 2012 IR for Fecal Coliform	Where is this listing?
C15E-13-SF	VAT-C15E_KNS02A00	Kings Creek - Upper Middle [No TMDL]	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
C16E-10-SF2	VAT-C16E_OPC01A06	Old Plantation Creek - Upper	Listed in 2012 IR for Fecal Coliform	Is this nested? If so, please provide nesting rationale
CB7PH-EBEN-BAY		Chesapeake Bay segment CB7PH	Listed in 2012 IR for Estuarine bioassessments	Where is this listing?
D06R-03-BAC	VAT-D06R_TOM01A08	Tommy's Ditch	Listed in 2012 IR for 2.29 miles; 1.44 miles in 2014 IR	Please clarify mileage change.
D06R-03-DO	VAT-D06R_TOM01A08	Tommy's Ditch	Listed in 2012 IR for 2.29 miles; 1.44 miles in 2014 IR	Please clarify mileage change.
E06R-02-BEN		Battle Run	Listed in 2012 IR for Benthic-macroinvert.	Where is this listing?
E22R-05-DO		Baylors Creek	Listed in 2012 IR for DO	Where is this listing?
E22R-07-DO	VAP-E22R_XGI01A10	Occupacia Creek UT-XGI	Proposed delisting to 4C for 1.96 Miles	Please provide delisting rationale for this segment.

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed
E22R-07-PH	VAP-E22R_XGI01A10	Occupacia Creek UT-XGI	Proposed delisting to 4C for 1.96 Miles	Please provide delisting rationale for this segment.
E23L-01-HG	VAP-E23L_CMR01A08	Chandlers Millpond	Listed in 2012 IR for 51.87 acres; 47.99 acres in 2014 IR	Please clarify acreage change.
E25E-10-SF2	VAP-E25E_DEE01B08	Deep Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E25E-11-SF2	VAP-E25E_LAN01B08	Lancaster Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E25E-12-SF	VAP-E25E_MTT01A00	Morattico Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E25E-22-SF	VAP-E25E_ROS02A04	Robinson Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E25E-25-SF	VAP-E25E_MUB03A08	Mulberry Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E25E-27-SF	VAP-E25E_PRR02A08	Parrotts Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E25E-28-SF	VAP-E25E_PAY01A02	Paynes Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E25E-29-SF	VAP-E25E_GEE02A06	Goldenvale Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E25R-01-PH	VAP-E25E_MUC01A04	Mud Creek	Proposed delisting to 4C for .204 Square Miles	Please provide delisting rationale for this segment.
E25R-17-DO	VAP-E25E_MUC01A04	Masons Mill Swamp	Proposed delisting to 4C for 3.37 Miles	Please provide delisting rationale for this segment.
E26E-18-SF	VAP-E26E_MYE01C04	Myer Creek	Listed in 2012 IR for Fecal Coliform	Where is this listing?
E26E-22-SF		Windmill Point Creek	Listed in 2012 IR for Fecal Coliform	Where is this listing?
E26E-31-SF	VAP-E26E_MYE01B02	Myer Creek, UT	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E26E-33-SF	VAP-E26E_WHR01A00	Whitehouse Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E26E-35-SF	VAP-E26E_DAS01A02	Davis Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E26E-36-SF	VAP-E26E_MYE01C04	Myer Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E26E-41-SF		Woods Creek	Listed in 2012 IR for Fecal Coliform	Where is this listing?
E26E-42-SF	VAP-E26E_HNU01A08	Hunting Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E26E-46-SF	VAP-E26E_CEB01B08	Eastern Branch Carter Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E26E-49-SF	VAP-E26E_MOR01A08	Moran Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E26E-50-SF	VAP-E26E_EWE02A08	Ewells Prong	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E26E-51-SF	VAP-E26E_CTM02A08	Eastern Branch Corrotoman River, UT	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E26E-53-SF	VAP-E26E_JON01A08	John Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
E26E-54-SF	VAP-E26E_LOW01A08	Lowrey Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
F12R-03-DO	VAP-F12R_HQT01A00	Hornquarter Creek	Proposed delisting to 4C for 6.77 Miles	Please provide delisting rationale for this segment.
F12R-03-PH	VAP-F12R_HQT01A00	Hornquarter Creek	Proposed delisting to 4C for 6.77 Miles	Please provide delisting rationale for this segment.
F13R-02-PH	VAP-F13R_TPT01A98	Totopotomy Creek	Proposed delisting to 4C for 10.26 Miles	Please provide delisting rationale for this segment.

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed
F13R-03-DO	VAP-F13R_ACQ01A14, VAP-F13R_JKC01A98, VAP-F13R_MLY01A12	Jacks Creek and major tributaries	Proposed delisting to 4C for 21.18 Miles	Please provide delisting rationale for this segment.
F13R-04-PH	VAP-F13R_MNQ01A98	Moncuin Creek, Webb Creek	Proposed delisting to 4C for 12.12 Miles	Please provide delisting rationale for this segment.
F13R-05-DO	VAP-F13R_GOV01A10	Governor Swamp	Proposed delisting to 4C for 3.32 Miles	Please provide delisting rationale for this segment.
F13R-05-PH	VAP-F13R_GOV01A10	Governor Swamp	Proposed delisting to 4C for 3.32 Miles	Please provide delisting rationale for this segment.
F13R-06-DO	VAP-F13R_SLN01A00	Sullens Creek	Proposed delisting to 4C for 4.60 Miles	Please provide delisting rationale for this segment.
F13R-06-PH	VAP-F13R_SLN01A00	Sullens Creek	Proposed delisting to 4C for 4.60 Miles	Please provide delisting rationale for this segment.
F13R-10-PH	VAP-F13R_MLY01A12	Mallory Creek	Proposed delisting to 4C for 4.02 Miles	Please provide delisting rationale for this segment.
F13R-14-PH	VAP-F13R_MHX01A12	Mehixen Creek and tributary XIV	Proposed delisting to 4C for 6.44 Miles	Please provide delisting rationale for this segment.
F23R-02-DO	VAP-F23R_DKW01A00, VAP-F23R_DKW01B00, VAP-F23R_DKW01C98, VAP-F23R_DWD01A00, VAP-F23R_FTS01A10, VAP-F23R_GNT01A00, VAP-F23R_MKT01A00, VAP-F23R_MKT01B00,	Dickeys Swamp, Garnetts Creek, and Tributaries	Proposed delisting to 4C for 24.27 Miles	Please provide delisting rationale for this segment.
F23R-07-PH		Walkerton Branch	Listed in 2012 IR for pH	Where is this listing?
F26E-01-PCB		York River and Tribs	Two listings in 2012 IR for PCB in Fish Tissue	Where is this listing?
F26E-02-SF		Aberdeen Creek	Listed in 2012 IR for Fecal Coliform	Where is this listing?
F27E-20-SF	VAT-F27E_CDB02A00	Cedarbrush Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.
G01E-03-PCB		James River and Various Tribs	2008 ILD Listed in 2012 IR for 0.012 square miles; 0.002 square miles in 2014 IR	Please clarify mileage change.
G01R-02-CU		XVP Almond Creek, UT	Listed in 2012 IR as G01R-02-CU; listed in 2014IR as G01R-22-CU	Identify correct cause group code.
G01R-02-ZN		XVP Almond Creek, UT	Listed in 2012 IR as G01R-02-ZN; listed in 2014 IR as G01R-22-ZN	Identify correct cause group code.
G01R-05-PH	VAP-G01R_KSL01A04	Kingsland Creek	Proposed delisting to 4C for 8.54 Miles	Please provide delisting rationale for this segment.

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed
G05R-03-BAC	VAP-G05R_CHK01B10, VAP-G05R_CHK01C12, VAP-G05R_CHK02A04	Chickahominy River	Listed on 2012 IR for E. coli	Please provide nesting rationale.
G05R-12-BAC	VAP-G05R_SNF02A12	Upper Stony Run and Tribs	Listed on 2012 IR for E. coli	Please provide nesting rationale.
G05R-12-DO	VAP-G05R_SNF02A12	Upper Stony Run and Tribs	Proposed delisting to 4C for 39.87 Miles	Please provide delisting rationale for this segment.
G05R-12-PH	VAP-G05R_SNF02A12	Upper Stony Run and Tribs	Proposed delisting to 4C for 39.87 Miles	Please provide delisting rationale for this segment.
G07R-03-DO	VAP-G07R_PEL01B10	Pelham Swamp	Proposed delisting to 4C for 1.57 Miles	Please provide delisting rationale for this segment.
G07R-04-BAC	VAP-G07R_SMN01A00	Collins Run	Listed on 2012 IR for E. coli	Please provide nesting rationale.
G07R-05-PH	VAP-G07R_XAA01A10	XAA-Rumley Marsh, UT	Proposed delisting to 4C for 1.76 Miles	Please provide delisting rationale for this segment.
G10E-06-BAC		College Creek	Listed in 2012 IR for Enterococcus	Where is this listing?
G11E-16-SF2	VAT-G11E_PGN01A08	Pagan River, Upstream of Chalmers Point	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.
G15E-06-01-BAC	VAT-G15E_HAI01A06	Hampton River	Listed in 2012 IR for Enterococcus as "Hampton River" and listed in 2014IR as "James River-King/Lincoln Park Beach Area"	Please identify correct water name.
H03R-04-PCB (2006)		James River	2006 ILD listed in 2012 IR for 164.27 miles; 158.32 miles in 2014 IR	Please clarify mileage change.
H03R-04-PCB (2006)		James River	2006 ILD listed in 2012 IR for 164.27 miles; 158.32 miles in 2014 IR	Please clarify mileage change.
H11R-01-BAC	VAC-H11R_BUF04A08	Buffalo River	Two Listings on 2012 IR for E. Coli	VAC-H11R_BUF04A08 identified as nested; please provide nesting rationale
H14R-01-HG	VAC-H14R_JMS01A00	James River	Listed in 2012 IR as 21.44 Miles, 18.57 Miles on 2014 IR (-2.87 miles)	Please clarify mileage change.
H15R-03-BAC	VAV-H15R_TLR01A08	Taylor Creek	Listed on 2012 IR for E. coli	Please provide nesting rationale.
H17R-02-BAC		James River	Listed in 2012 IR for 20.40 miles (2008 ILD) and 16.32 miles (2012 ILD); 20.40 miles (2008 ILD) and 16.32 miles (2012 ILD) in 2014 IR	Please clarify mileage change.
I19R-01-BAC	VAW-I19R_CRG02A02	Craig Creek	Listed in 2012 IR for 7.79 miles; 6.55 miles in 2014 IR	Please clarify mileage change.
I37R-03-BAC	VAV-I37R_PGH01A00	Maury River	Listed in 2012 IR for E. coli, Listed on 2014 IR as "Poague River" with an initial list date of 2014.	Please clarify correct water name and initial list date.

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed
J11R-03-PH		Bland Creek	Listed on 2012 IR for pH	Where is this listing?
J11R-04-PH		Cellar Creek	Listed on 2012 IR for pH	Where is this listing?
J15R-05-BEN	VAP-J15R_RHC01A06	Rohoic Creek	Listed in 2012 IR for 6.78 miles; 4.61 miles in 2014 IR	Please clarify mileage change.
K05R-05-DO	VAP-K05R_HAY01A10	Hayes Creek	Listed in 2012 IR as Hayes; listed in 2014 IR as Hays	Please clarify correct water name.
K05R-05-PH	VAP-K05R_HAY01A10	Hayes Creek	Listed in 2012 IR as Hayes; listed in 2014 IR as Hays	Please clarify correct water name.
K11R-03-DO	VAP-K11R_CTT01A02, VAP-K11R_CTT01B10	Cattail Creek	Proposed delisting to 4C for 7.38 Miles	Please provide delisting rationale for this segment.
K11R-03-pH	VAP-K11R_CTT01A02	Cattail Creek	Proposed delisting to 4C for 5.33 Miles	Please provide delisting rationale for this segment.
K13R-03-BAC		Meherrin River (Lower)	Listed in 2012 IR for E. coli	Where is this listing?
K26R-04-BAC	VAP-K26R_MCC01A00	Maclins Creek	Listed in 2012 IR for E. coli	Please provide nesting rationale.
K35R-03-BAC	VAT-K35R_XDY01A04	UT Seacock Swamp	Listed on 2012 IR for 1.03 miles; 0.68 miles on 2014 IR	Please clarify mileage change.
K35R-03-DO	VAT-K35R_XDY01A04	UT Seacock Swamp	Listed on 2012 IR for 1.03 miles; 0.68 miles on 2014 IR	Please clarify mileage change.
K39R-01-HG	VAT-K39R_XCK01A00	Dismal Swamp Canal & Feeder Ditch to Lake Drummond	Listed on 2012IR for 17.58 Miles, 13.21 Miles on 2014 IR (-4.37)	Please clarify mileage change.
K39R-02-HG	VAT-K39R_ZZZ01B08	Unsegmented Rivers in k39R	Listed in 2012 IR for 15.80 miles; 15.28 miles in 2014 IR	Please clarify mileage change.
L04R-02-BEN	VAW-L04R_MDL01A06	Mud Lick Creek	Listed in 2012 IR for Benthic-macroinvert.	Please provide nesting rationale.
L04R-05-BEN	VAW-L04R_MSN01A00	Mason Creek	Listed in 2012 IR for Benthic-macroinvert.	Please provide nesting rationale.
L04R-06-BEN	VAW-L04R_BHT01A10	Barnhardt Creek	Listed in 2012 IR for Benthic-macroinvert.	Please provide nesting rationale.
L04R-07-BEN	VAW-L04R_MUR01A00	Murray Run	Listed in 2012 IR for Benthic-macroinvert.	Please provide nesting rationale.
L09R-01-TEMP	VAW-L09R_MEE05A00	Maggodee Creek	2012 IR identifies ILD as 2008; 2014 IR identifies ILD as 2012	Which initial list date is correct?
L79L-02-HG	VAC-L79L_MES01L00	Lake Gordon	Listed in 2012 IR for 114.58 acres; 105.96 acres in 2014 IR	Please clarify acreage change.
L80L-01-PCB	VAC-L78R_ROA06A98, VAC-L79L_ROA07A98, VAC-L80L_ROA08A04	Lake Gaston	Listed in 2012 IR for 4478.72 acres and 5.72 miles; 4439.72 acres and 5.69 miles in 2014 IR	Please clarify acreage and mileage change.

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed
L81R-02-BAC	VAC-L81R_LIZ01A10	Lizard Creek	Listed in 2012 IR for 4.26 miles; 2.73 miles in 2014 IR	Please clarify mileage change.
M02R-01-BAC (2010)	VAS-M02R_STE01A02	Lovills Creek	Listed in 2012 IR as Lovills Creek, Appendix 3 - 42 identifies M02R-01-BAC (2010) as "Stewarts Creek"	Please clarify correct water name.
N10R-02-BAC	VAS-N10R_HOL01A12, VAS-N10R_MCE01A02, VAS-N10R_RSF01A00, VAS-N10R_RSF01A02	South Fork Reed Creek and Mill Creek	Two listings in 2012 IR for E. coli	VAS-N10R_HOL01A12 6, VAS-N10R_RSF01A02 identified as nested; please provide nesting rationale
N11R-01-BAC	VAS-N10R_GLN01A04, VAS-N10R_PRN01A12, VAS-N10R_RDC01A00, VAS-N10R_RDC01A02, VAS-N10R_RDC01B00, VAS-N10R_RDC01C02, VAS-N11R_RDC01B00, VAS-N11R_RDC01C02	Reed Creek	Five listings in 2012 IR for Fecal Coliform and E. coli	VAS-N10R_GLN01A04, VAS-N10R_PRN01A12, VAS-N11R_RDC01C02 ,VAS-N10R_RDC01C02 identified as nested; please provide nesting rationale.
N11R-02-BAC	VAS-N11R_MER02A10	Miller Creek	Two listings on 2012 IR for E. coli	VAS-N11R_MER02A10 identified as nested; please provide nesting rationale
N30R-01-BAC		Wolf Creek	Listed on 2012 IR for E. coli, 2006 ILD at 23.24 miles, 14.36 miles for 2006 ILD on 2014 IR (-8.88 miles)	Please clarify mileage change.
N32R-01-BAC (2012)		Wolf Creek	Listed on 2012 IR for E. coli	Where is this listing? Specifically, the listing identified with 2012 ILD.
N36R-01-PCB (2012)		Bluestone River	Listed in 2012 IR for 3.03 miles (2012 ILD); 2.99 miles in 2014 IR	Please clarify mileage change.
O01R-02-PH	VAS-O01R_XEE01A08	Hurricane Creek Trib	Listed on 2012 IR for pH	Where is this listing?
O09R-03-BAC	VAS-O09R_NFH01B02	North Fork Holston River	Three listings in 2012 IR for Fecal Coliform and E. coli	VAS-O09R_NFH01B02 identified as nested; please provide nesting rationale
O10R-01-BAC	VAS-O10R_NFH01A94, VAS-O10R_NFH02A00, VAS-O11R_NFH03A94	North For Holston River	Three listings in 2012 IR for Fecal Coliform and E. coli	VAS-O13R_NFH01A94 VAS-O14R_BMC04A00

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed
O10R-05-BAC	VAS-O10R_BVR01A02, VAS-O10R_BVR01B04, VAS-O10R_LAE01A02, VAS-O10R_LAE02A02, VAS-O10R_LOC01A02, VAS-O10R_RRB01A02, VAS-O10R_TUR01A10	North Fork Holston River Tribs	Six Listings in 2012 IR for Fecal Coliform and E. Coli	VAS-O10R_BVR01B04, VAS-O10R_LAE02A02 identified as nested; please provide nesting rationale
O10R-08-BAC		Beaver Creek	Listed in 2012 IR for E. coli	Where is this listing?
O11R-04-BAC	VAS-O11R_LOG01A02	Logan Creek	Listed in 2012 IR for E. coli	Please provide nesting rationale.
O11R-09-BAC	VAS-O11R_EWF01A12	East Fork Wolf Creek	Listed in 2012 IR for E. coli	Please provide nesting rationale.
O11R-11-BAC	VAS-O11R_FIN01A12	Finley Creek	Listed in 2012 IR for E. coli	Please provide nesting rationale.
O11R-12-BAC	VAS-O11R_WOC01A12	West Fork Wolf Creek	Listed in 2012 IR for E. coli	Please provide nesting rationale.
O12R-05-BAC		Nordyke Creek	Listed in 2012 IR for E. coli	Where is this listing?
O14R-01-BAC	VAS-O14R_BMC01A98, VAS-O14R_BMC04A00, VAS-O14R_BMC05A02, VAS-O14R_BMC06A02, VAS-O14R_BMC07A02	Big Moccasin Creek	Three listings in 2012 IR for E. coli	VAS-O14R_BMC04A00 identified as nested; please provide nesting rationale.
P01R-02-BAC	VAS-P01R_NCL01A04, VAS-P01R_PLU01A04	Plum Creek and North Fork Clinch River	Three listings in 2012 IR for Fecal Coliform and E. coli	VAS-P01R_NCL01A04 identified as nested; please provide nesting rationale.
P07R-01-BEN		Thompson Creek	Listed in 2012 IR as "Thompson Creek", 2014 IR as "Clinch River Tributaries"	Please identify correct water name.
P10R-05-BAC		Gravel Lick Creek	Listed in 2012 IR for E. coli	Where is this listing?
P17R-08-BEN		Powell River	Listed in 2012 IR for Benthic-macroinvertebrate impairment.	Where is this listing?
P22R-01-TEMP	VAS-P22R_WAL02A02, VAS-P22R_WAL02B02	Wallen Creek	Listed on 2012 IR for 27.92 Miles, 21.06 Miles on 2014 IR (-6.86 miles)	Please clarify mileage change.
P22R-02-TEMP		Wallen Creek	Listed on 2012 IR for Water Temp.	Where is this listing?
Q03R-03-BAC	VAS-Q01R_JBF01A10, VAS-Q03R_PPW01A94	Guess Fork, Pawpaw Creek, and Jacobs Fork	Listed on 2012 IR for Fecal Coliform (2004 ILD), and E. coli (2010 ILD, 8.43 Miles)	Where are these two listings?
Q12R-01-BAC	VAS-Q12R_RPC01A96	Russell Prater Creek	Listed on 2012 IR for 17.02 Miles, 11.72 Miles on 2014 IR (-5.3 miles)	Partial delist Q12R-01-BAC under "Barts Lick Creek" and waterbody ID VAS-Q12R_BAI01A08. Please clarify relation to Russell Prater Creek VAS-Q12R_RPC01A96

DEQ's Response to EPA Region III

During the public comment period for the 2012 Integrated Report (March 26 to April 24, 2012), DEQ received over 80 comments from citizens regarding the presence of algae in the Shenandoah River. Commenters expressed concern that algae in the river have impaired the recreation designated use. However, DEQ did not list the Shenandoah as impaired for the recreation use for the following principal reasons:

- Waters that do not meet Water Quality Standards due to a pollutant(s) may be listed as impaired. "Pollutant" is defined in Federal law and either narrative or numeric water quality standards may be used to list waters as impaired. However, an "impaired" designation can only be made based on specific and objective monitoring data, in terms of location, extent, and duration, as well as an accepted, scientifically valid assessment method that compares monitoring data to water quality standards or criteria.
- Virginia's General Water Quality Criteria (narrative) provides that state waters shall be free from substances that interfere with designated uses, including those which *nourish* undesirable or nuisance aquatic plant life. The terms "undesirable or nuisance" are subjective and require interpretation and no numeric thresholds have been developed for Virginia waters. DEQ currently has no reliable methodology for specifically assessing the level of recreation use attainment in the context of what may or may not be a "nuisance".
- There are no Federal numeric criteria classifying algae production as excessive or in violation of aesthetic criteria. Virginia currently has nutrient criteria for lakes designed to protect aquatic life and recreation. Virginia doesn't currently have adopted water quality standards for nutrient levels in free flowing waters, but DEQ has been working for a number of years on a screening approach for nutrient criteria that appears very promising. Progress on this approach can be viewed at this web link: http://www.deq.virginia.gov/Programs/Water/WaterQualityInformationTMDLs/WaterQualityStandards/NutrientCriteriaDevelopment.aspx.
- Virginia's recreational use assessment for rivers and streams is based on violations of E. coli bacteria, a numeric human health risk criterion. DEQ believes that standard is appropriate to determine recreational use support.
- There is insufficient data to list entire stretches of the North Fork, South Fork, and mainstem Shenandoah Rivers as impaired. The information received from concerned citizens was largely anecdotal. DEQ has maintained a long-standing policy of basing impairment decisions solely on data collected with an agency-approved quality assurance plan (Level III data).
- Local TMDLs have been developed in 17 tributaries to the Shenandoah Basin to address sediment and nutrients as causes for benthic impairments.
- In addition to the local TMDL's, the Chesapeake Bay TMDL with its Virginia Watershed Implementation Plan will result in significant controls for inputs of nitrogen, phosphorus and sediment throughout the Shenandoah Basin.

To date, for several "impaired" designations across the State, DEQ has taken into account well-documented, systematic observations of excessive algae, but only in relation to assessment of the aquatic life use and only when combined with other lines of evidence (e.g., dissolved oxygen, pH, nutrients, and Virginia Stream Condition Index scores). However, public comments and complaints are also important to DEQ. In response to the comments received during the 2012 assessment, DEQ classified the unimpaired portions of the Shenandoah river system as "Category 2B" (having an observed effect, but no water quality standard exists for a particular pollutant). This category is used to identify waters with potential problems so they can be prioritized for additional monitoring.

Starting in the spring of 2014, the Interstate Commission on the Potomac River Basin (ICPRB)—with guidance provided by DEQ—conducted a study focused on scientifically valid field methods for evaluating algae cover in both wadeable and boatable streams. ICPRB's final report was submitted to DEQ in March 2015. DEQ is currently reviewing the recommendations of the report in preparation of the next 305(b)/303(d) assessment. Additionally, because concerns over nuisance algae have been pushed to

the national forefront, DEQ has proposed a collaborative effort among the Mid-Atlantic jurisdictions that comprise EPA Region III (PA, WV, MD, VA, DEQ and DC) to develop a consistent strategy for discerning the causes of nuisance algae blooms and quantifying designated use impacts in a defensible manner. The findings of the ICPRB report will aid in this process and a workplan will be jointly developed over the next year.

The following table contains DEQ's response to your 2014 303(d) List comments.

DEQ's Response to EPA's Comments

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
A26R-02-BAC	VAN-A26R_POW02A02	Powells Creek	Listed on 2012 IR for E. coli	VAN-A26R_POW02A02 identified as nested; please provide nesting rationale	Nested with the *Bacteria TMDL for Tributaries to the Potomac River: Prince William and Stafford Counties* (follows Part VII, Delisting Rule 3 of the WQA Guidance Manual - no rationale needed)	
A28R-01-BAC	VAN-A28R_AUS02A06	Austin Run	Listed on 2012 IR for E. coli	VAN-A28R_AUS02A06 identified as nested; please provide nesting rationale	Nested with the *Bacteria TMDL for Tributaries to the Potomac River: Prince William and Stafford Counties* (follows Part VII, Delisting Rule 3 of the WQA Guidance Manual - no rationale needed)	
A29E-02-BAC	VAN-A29E_POT01A06	Fairview Beach (Potomac River)	Listed on 2012 IR for 0.012 square miles; 0.005 square miles on 2014 IR	Please clarify mileage change.	Nested with the *Bacteria TMDL for Tributaries to the Potomac River: Prince William and Stafford Counties* (follows Part VII, Delisting Rule 3 of the WQA Guidance Manual - no rationale needed)	
A29R-02-BAC	VAN-A29R_POM02A06	Potomac Creek	Listed on 2012 IR for E. coli	VAN-A29R_POM02A06 identified as nested; please provide nesting rationale	Nested with the *Bacteria TMDL for Tributaries to the Potomac River: Prince William and Stafford Counties* (follows Part VII, Delisting Rule 3 of the WQA Guidance Manual - no rationale needed)	
A31E-01-SF2	VAP-A31E_ROS01A08	Rosier Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A31E-04-PH	VAP-A31E_MON01A00	Monroe Creek	Proposed delisting to 4C for 0.176 Sq. Miles	Please provide delisting rationale for this area.	See the "Natural Conditions Assessment for Low Dissolved Oxygen and pH, Monroe Creek and Tributaries in Westmoreland County, Virginia" revised 2/5/2015	
A31R-03-DO	VAP-A31R_POP01A00	Popes Creek watershed	Proposed delisting to 4C for 29.88 miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low Dissolved Oxygen and pH, Popes Creek and Tributaries in Westmoreland County, Virginia"	
A31R-03-PH	VAP-A31R_POP01A00	Popes Creek watershed	Proposed delisting to 4C for 29.88 miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low Dissolved Oxygen and pH, Popes Creek and Tributaries in Westmoreland County, Virginia"	
A31R-04-DO	VAP-A31R_MRC01A98	Monroe Creek Watershed	Proposed delisting to 4C for 12.27 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low Dissolved Oxygen and pH, Monroe Creek and Tributaries in Westmoreland County, Virginia"	
A31R-04-PH	VAP-A31R_MRC01A98	Monroe Creek Watershed	Proposed delisting to 4C for 12.27 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low Dissolved Oxygen and pH, Monroe Creek and Tributaries in Westmoreland County, Virginia"	

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
A31R-05-DO	VAP-A31R_CAS01A00	Canal Swamp Watershed	Proposed delisting to 4C for 12.57 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low Dissolved Oxygen and pH, Popes Creek and Tributaries in Westmoreland County, Virginia"	
A31R-05-PH	VAP-A31R_CAS01A00	Canal Swamp Watershed	Proposed delisting to 4C for 12.57 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low Dissolved Oxygen and pH, Popes Creek and Tributaries in Westmoreland County, Virginia"	
A32E-04-SF2	VAP-A32E_NOM01A04	Nomini Creek, Pierce Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A32E-13-SF	VAP-A32E_CUR01B08	Currioman Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A32E-14-SF	VAP-A32E_POO01A08	Poor Jack Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A32E-15-SF	VAP-A32E_DAV01A08	Davis Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A32E-16-SF	VAP-A32E_JUL01A08	Jules Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A32E-17-SF	VAP-A32E_MAT01A08	Matthews Cove	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A32E-21-SF	VAP-A32E_NOP02A08	North Prong Buckner Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A32R-01-DO	VAP-A32R_THP01A06	Thompson Branch	Proposed delisting to 4C for 1.60 Miles	Please provide delisting rationale for this segment.		The natural conditions report for this water will be rescinded in the final 2014 integrated report and the categories moved back to 4C. We may resubmit this in the future.
A32R-01-PH	VAP-A32R_THP01A06	Thompson Branch	Proposed delisting to 4C for 1.60 Miles	Please provide delisting rationale for this segment.		The natural conditions report for this water will be rescinded in the final 2014 integrated report and the categories moved back to 4C. We may resubmit this in the future.
A33E-12-SF	VAP-A33E_SHA01A98	Shannon Branch	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A33E-16-SF	VAP-A33E_WES01A06	West Yeocomico River	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A33R-01-BAC	VAP-A33R_MIA01A00	Mill Creek	Listed on 2012 IR for E. coli	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
A33R-02-DO	VAP-A33R_LOG01A04	Lodge Creek	Proposed delisting to 4C for 3.44 Miles	Please provide delisting rationale for this segment.		The natural conditions report for this water will be rescinded in the final 2014 integrated report and the categories moved back to 4C. We may resubmit this in the future.
A34E-01-SF2	VAP-A34E_GLE01A04	The Glebe	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A34E-17-SF	VAP-A34E_BRI01C98	Bridge Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A34E-21-SF	VAP-A34E_KIN04A06	Kingscote Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A34E-25-SF	VAP-A34E_BOT01A04	Boathouse Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A34E-28-SF	VAP-A34E_SLO05A98	Slough Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A34E-31-BAC	VAP-A34E_LIS01A02, VAP-A34E_LIS01A14, VAP-A34E_LIS01A98, VAP-A34E_LIS01C14	Little Wicomico River	Listed on 2012 IR for Enterococcus	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A34E-32-SF	VAP-A34E_COA01A02	Coan River	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A34E-33-SF	VAP-A34E_BBC01A08	Bridgemans Back Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A34E-34-SF	VAP-A34E_BAC01A12	Back Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
A34E-35-SF	VAP-A34E_LIS04B12	Little Wicomico River, UT	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-01-SF2	VAP-C01E_GWR01B08,	Great Wicomoico River, Blackwells Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-02-SF2	VAP-C01E_BLS02A08	Balls Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-03-SF2	VAP-C01E_TIP02A08	Tipers Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-06-SF2	VAP-C01E_WCO02A08	Warehouse Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-07-SF	VAP-C01E_HHB01A98	Horn Harbor	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-09-SF2	VAP-C01E_PNT02B10	Prentice Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-12-SF2	VAP-C01E_MIL02A08	Mill Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-14-SF	VAP-C01E_GSK01A10	Gaskin Pond	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-17-PCB		Chesapeake Bay and Tidal Tributaries	Three listings on 2012 IR for PCB in Fish Tissue	Where is this listing?	C01E-17-PCB mistakenly excluded from Appendix 1a	C07E-01-PCB has been re-added to Appendix 1a.

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
C01E-31-SF	VAP-C01E_JAR01A02	Jarvis Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-33-SF	VAP-C01E_BMC01A04	Betts Mill Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-37-SF	VAP-C01E_HAV01A08	Harveys Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-38-SF	VAP-C01E_CRN01A06	Cranes Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-40-SF	VAP-C01E_COL01A08	Coles Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-56-SF	VAP-C01E_PEN01A12	Head River Branch	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-58-SF	VAP-C01E_XEV01A12	XEV - Mill Creek, UT	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C01E-60-SF	VAP-C01E_LEE02A12	Lees Cove	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C03E-03-SF	VAP-C03E_FRE01A02	Frenchs Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C03E-09-SF		Piankatank River, UT	Listed on 2012 IR for Fecal Coliform	Where is this listing?	VAP-C03E_PNK07B08 was submitted for partial delisting and was included in the delisting package	
C03E-14-EBEN		Piankatank River	Listed on 2012 IR for Estuarine Bioassessments under Cause Group Code "C03E-14-EBEN"; listed under "C03E-10-EBEB" in 2014 IR	Please identify correct cause group code.	VAP-C03E_PNK05A02 - 2012's C03E-14-EBEN was incorporated into 2014's C03E-10-EBEN due to a large new mainstem benthic impairment	
C03E-15-SF	VAP-C03E_DAN01A08	Dancing Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C03E-19-SF	VAP-C03E_PNK04D08	Porpoise Cove	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-01-SF2	VAP-C04E_QUE02A12	Queens Creek, UT	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-05-SF2	VAP-C04E_STT01B10	Stutts Creek and Morris Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-07-SF2	VAP-C04E_BLP01B10	Billups Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-08-SF2	VAP-C04E_NOR01B08	North River	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-12-SF	VAP-C04E_DAV01A98	Davis Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-25-SF	VAP-C04E_MID01A02	Winder Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-28-SF	VAP-C04E_BRN01A04	Barn Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-30-SF	VAP-C04E_LAN01B08	Lanes Creek, UT	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-31-SF	VAP-C04E_STT01B06	Stutts Creet, (Hole in the Wall)	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-32-SF	VAP-C04E_HUD01A08	Hudgins Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-42-SF	VAP-C04E_WON01A08	Weston Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-47-SF	VAP-C04E_BEV01A08	Belleville Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
C04E-48-SF	VAP-C04E_WOO01A10	Woodas Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-49-SF	VAP-C04E_EST01B10	East River	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-52-SF	VAP-C04E_LAN01A02	Lanes Creek, UT	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-53-BAC		Whites Creek	Listed on 2012 IR as "Whites Creek", "Whites Creek - Festival Branch" on 2014 IR	Please identify correct water name.		Corrected to "Whites Creek"
C04E-54-SF	VAP-C04E_DVS03A12	Davis Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04E-55-SF	VAP-C04E_RAY01A12	Raymond Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C04R-01-DO	VAP-C04R_EST01A12	East River	Listed in 2012 IR for 1.79 miles; 0.59 miles in 2014 IR	Please clarify mileage changes.	Changed due to NHD conversion from 100k to 24K	
C04R-01-PH	VAP-C04R_EST01A12	East River	Listed in 2012 IR for 1.79 miles; 0.59 miles in 2014 IR	Please clarify mileage changes.	Changed due to NHD conversion from 100k to 24K	
C05E-01-SF2	VAP-C05E_WAR01B08	Ware River	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C05E-02-SF2	VAP-C05E_WIL01B08	Wilson Creek	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
C06R-01-DO	VAP-C06R_SEN01A00	Northwest Branch of Severn River	Proposed delisting to 4C for 2.16 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low Dissolved Oxygen, Northwest Branch Severn River in Gloucester County, Virginia"	
C07E-01-PCB		Chesapeake Bay and Tidal Tributaries VDH Fish Consumption Advisory for PCBs	Listed in 2012 IR for PCB in Fish Tissue	Partial 4a (VAT-C07E_BRK01A06 TMDL ID 31233), where is this listing?	C07E-01-PCB was mistakenly excluded from Appendix 1a. This cause group code is still in need of a TMDL (5A). The Fecal Coliform TMDL (31233) is 4A in the 2014 IR with Cause Group Code C07E-01-SF (C07E-01-SF is in Appendix 1c)	C07E-01-PCB has been re-added to Appendix 1a.
C08E-01-EBEN		Lynnhaven River - Mainstem	Identified on 2012 IR as "Lynnhaven River - Mainstem", "London Bridge Creek" on 2014 IR	Please identify correct water name.		Revised Appendix 1 a to Lynnhaven River System.
C08L-01-CHLA		Lake Whitehurst	Listed in 2012 IR for 494.93 acres, 481.42 acres in 2014 IR (-13.52 acres)	Please clarify acreage change.	The delineation of water quality assessment units (AUs) are based on National Hydrography Dataset (NHD).The size changes in the 2014 are primarily due to the use of more accurate and precise mapping.	
C08L-01-DO		Lake Whitehurst	Listed in 2012 IR for 494.93 acres, 481.42 acres in 2014 IR (-13.52 acres)	Please clarify acreage change.	The delineation of water quality assessment units (AUs) are based on National Hydrography Dataset (NHD).The size changes in the 2014 are primarily due to the use of more accurate and precise mapping.	
C08L-01-HG		Lake Whitehurst	Listed in 2012 IR for 494.93 acres, 481.42 acres in 2014 IR (-13.52 acres)	Please clarify acreage change.	The delineation of water quality assessment units (AUs) are based on National Hydrography Dataset (NHD).The size changes in the 2014 are primarily due to the use of more accurate and precise mapping.	

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
C08L-01-PCB		Lake Whitehurst VDH Fish Consumption Advisory	Listed in 2012 IR for 494.93 acres, 481.42 acres in 2014 IR (-13.52 acres)	Please clarify acreage change.	The delineation of water quality assessment units (AUs) are based on National Hydrography Dataset (NHD).The size changes in the 2014 are primarily due to the use of more accurate and precise mapping.	
C08L-01-TP		Lake Whitehurst	Listed in 2012 IR for 494.93 acres, 481.42 acres in 2014 IR (-13.52 acres)	Please clarify acreage change.	The delineation of water quality assessment units (AUs) are based on National Hydrography Dataset (NHD).The size changes in the 2014 are primarily due to the use of more accurate and precise mapping.	
C10E-12-SF2	VAT-C10E_GLF01B08	Guilford Creek - Upper (No TMDL)	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	Nesting follows Part VII, Delisting Rule 3 of the WQA Guidance Manual: a bacteria impairment that is within the tidal range of an existing bacteria TMDL, land use is comparable, reductions apply to TMDL watershed, and point sources of bacteria are already identified. Nesting statement and description is found in the cause comment of the bacteria nested water.	
C10R-01-BAC2	VAT-C10R_XCX01A08	Unnamed tributary from Tyson Foods	Listed in 2012 IR for E. coli	Please provide nesting rationale.	Nesting follows Part VII, Delisting Rule 3 of the WQA Guidance Manual: a bacteria impairment that is within the tidal range of an existing bacteria TMDL, land use is comparable, reductions apply to TMDL watershed, and point sources of bacteria are already identified. Nesting statement and description is found in the cause comment of the bacteria nested water.	
C13E-02-BAC		Nassawadox Creek - Lower	Listed in 2012 IR for Enterococcus	Where is this listing?	Listed in Appendix 1c.	
C13E-11-SF2		Church Creek - Middle, UT North Cove [No TMDL]	Listed in 2012 IR for Fecal Coliform	Where is this listing?	Listed in Appendix 1c- Nested see below from ADB Cause Comment. NEST 2014 : 33777, 9/20/2007 Category 4A: 2006 00409 / 2008 C13E-11-SF/ 2010 C13E-11-SF2 The Shellfishing Use is impaired due to the DSS shellfish direct harvesting condemnation # 085-185 A (effective 20111007). Not covered under TMDL for "Nassawadox Creek Watershed" (33777) EPA approved 9/20/2007. However will nest since SF impairment is within tidal range of Nassawadox TMDL, newly impaired segments are comparable and all existing sources are accounted for in the TMDL.	

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
C13E-22-SF	VAT-C13E_OCH03A08	Shields Cove	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	Nesting follows Part VII, Delisting Rule 3 of the WQA Guidance Manual: a bacteria impairment that is within the tidal range of an existing bacteria TMDL, land use is comparable, reductions apply to TMDL watershed, and point sources of bacteria are already identified. Nesting statement and description is found in the cause comment of the bacteria nested water.	
C13E-23-SF		Fisher Cove	Listed in 2012 IR for Fecal Coliform	Where is this listing?	Listed in Appendix 1c. Fisher and Shields are now merged together with Cause Group Code C13E-22-SF. Now both included in AU VAT-C13E_OCH03A08.	
C15E-13-SF	VAT-C15E_KNS02A00	Kings Creek - Upper Middle [No TMDL]	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	Nesting follows Part VII, Delisting Rule 3 of the WQA Guidance Manual: a bacteria impairment that is within the tidal range of an existing bacteria TMDL, land use is comparable, reductions apply to TMDL watershed, and point sources of bacteria are already identified. Nesting statement and description is found in the cause comment of the bacteria nested water.	
C16E-10-SF2	VAT-C16E_OPC01A06	Old Plantation Creek - Upper	Listed in 2012 IR for Fecal Coliform	Is this nested? If so, please provide nesting rationale	AU VAT-C16E_OPC01B08 is nested. Nesting follows Part VII, Delisting Rule 3 of the WQA Guidance Manual: a bacteria impairment that is within the tidal range of an existing bacteria TMDL, land use is comparable, reductions apply to TMDL watershed, and point sources of bacteria are already identified. Nesting statement and description is found in the cause comment of the bacteria nested water.	
CB7PH-EBEN-BAY		Chesapeake Bay segment CB7PH	Listed in 2012 IR for Estuarine bioassessments	Where is this listing?	CB7PH-EBEN-BAY is listed in Appendix 3 Delist.	
D06R-03-BAC	VAT-D06R_TOM01A08	Tommy's Ditch	Listed in 2012 IR for 2.29 miles; 1.44 miles in 2014 IR	Please clarify mileage change.	The delineation of water quality assessment units (AUs) are based on National Hydrography Dataset (NHD).The size changes in the 2014 are primarily due to the use of more accurate and precise mapping.	
D06R-03-DO	VAT-D06R_TOM01A08	Tommy's Ditch	Listed in 2012 IR for 2.29 miles; 1.44 miles in 2014 IR	Please clarify mileage change.	The delineation of water quality assessment units (AUs) are based on National Hydrography Dataset (NHD).The size changes in the 2014 are primarily due to the use of more accurate and precise mapping.	

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
E06R-02-BEN		Battle Run	Listed in 2012 IR for Benthic-macroinvert.	Where is this listing?	VAN-E06R_BTL01A02 was submitted for partial delisting; can be found in Appendix 3 pg 38	
E22R-05-DO		Baylors Creek	Listed in 2012 IR for DO	Where is this listing?	VAP-E22R_BAY01A08 - was submitted for partial delisting and was included in the delisting package	
E22R-07-DO	VAP-E22R_XGI01A10	Occupacia Creek UT-XGI	Proposed delisting to 4C for 1.96 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low pH Occupacia Creek Essex County, Virginia"	
E22R-07-PH	VAP-E22R_XGI01A10	Occupacia Creek UT-XGI	Proposed delisting to 4C for 1.96 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low pH Occupacia Creek Essex County, Virginia"	
E23L-01-HG	VAP-E23L_CMR01A08	Chandlers Millpond	Listed in 2012 IR for 51.87 acres; 47.99 acres in 2014 IR	Please clarify acreage change.	Changed due to NHD conversion from 100k to 24K	
E25E-10-SF2	VAP-E25E_DEE01B08	Deep Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E25E-11-SF2	VAP-E25E_LAN01B08	Lancaster Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E25E-12-SF	VAP-E25E_MTT01A00	Morattico Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E25E-22-SF	VAP-E25E_ROS02A04	Robinson Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E25E-25-SF	VAP-E25E_MUB03A08	Mulberry Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E25E-27-SF	VAP-E25E_PRR02A08	Parrotts Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E25E-28-SF	VAP-E25E_PAY01A02	Paynes Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E25E-29-SF	VAP-E25E_GEE02A06	Goldenvale Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E25R-01-PH	VAP-E25E_MUC01A04	Mud Creek	Proposed delisting to 4C for .204 Square Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low Dissolved Oxygen and Low pH, Mud Creek in Middlesex County, Virginia"	
E25R-17-DO	VAP-E25E_MUC01A04	Masons Mill Swamp	Proposed delisting to 4C for 3.37 Miles	Please provide delisting rationale for this segment.	Do you mean VAP-E25R_MAO01A00? If so, see "Natural Conditions Assessment for Low Dissolved Oxygen, Masons Mill Swamp in Middlesex County, Virginia"	
E26E-18-SF	VAP-E26E_MYE01C04	Myer Creek	Listed in 2012 IR for Fecal Coliform	Where is this listing?	Included under E26E-36-SF in 2014 IR	
E26E-22-SF		Windmill Point Creek	Listed in 2012 IR for Fecal Coliform	Where is this listing?	VAP-E26E_WID01A12 - was submitted for delisting because of use removal	
E26E-31-SF	VAP-E26E_MYE01B02	Myer Creek, UT	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E26E-33-SF	VAP-E26E_WHR01A00	Whitehouse Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E26E-35-SF	VAP-E26E_DAS01A02	Davis Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E26E-36-SF	VAP-E26E_MYE01C04	Myer Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E26E-41-SF		Woods Creek	Listed in 2012 IR for Fecal Coliform	Where is this listing?	VAP-E26E_WOO01A08 - was submitted for delisting because of use removal	
E26E-42-SF	VAP-E26E_HNU01A08	Hunting Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
E26E-46-SF	VAP-E26E_CEB01B08	Eastern Branch Carter Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E26E-49-SF	VAP-E26E_MOR01A08	Moran Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E26E-50-SF	VAP-E26E_EWE02A08	Ewells Prong	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E26E-51-SF	VAP-E26E_CTM02A08	Eastern Branch Corrotoman River, UT	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E26E-53-SF	VAP-E26E_JON01A08	John Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
E26E-54-SF	VAP-E26E_LOW01A08	Lowrey Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
F12R-03-DO	VAP-F12R_HQT01A00	Hornquarter Creek	Proposed delisting to 4C for 6.77 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low pH and Low Dissolved Oxygen, Hornquarter Creek in King William County, Virginia"	
F12R-03-PH	VAP-F12R_HQT01A00	Hornquarter Creek	Proposed delisting to 4C for 6.77 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low pH and Low Dissolved Oxygen, Hornquarter Creek in King William County, Virginia"	
F13R-02-PH	VAP-F13R_TPT01A98	Totopotomy Creek	Proposed delisting to 4C for 10.26 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low pH and Dissolved Oxygen in Totopotomoy Creek, Hanover County, Virginia"	
F13R-03-DO	VAP-F13R_ACQ01A14, VAP-F13R_JKC01A98, VAP-F13R_MLY01A12	Jacks Creek and major tributaries	Proposed delisting to 4C for 21.18 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low pH and Low Dissolved Oxygen, Jacks Creek and Tributaries in King William County, Virginia"	
F13R-04-PH	VAP-F13R_MNQ01A98	Moncuin Creek, Webb Creek	Proposed delisting to 4C for 12.12 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low pH and Low Dissolved Oxygen, Monquin Creek and Tributaries in King William County, Virginia"	
F13R-05-DO	VAP-F13R_GOV01A10	Governor Swamp	Proposed delisting to 4C for 3.32 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low pH and Low Dissolved Oxygen, Monquin Creek and Tributaries in King William County, Virginia"	
F13R-05-PH	VAP-F13R_GOV01A10	Governor Swamp	Proposed delisting to 4C for 3.32 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low pH and Low Dissolved Oxygen, Monquin Creek and Tributaries in King William County, Virginia"	
F13R-06-DO	VAP-F13R_SLN01A00	Sullens Creek	Proposed delisting to 4C for 4.60 Miles	Please provide delisting rationale for this segment.		The natural conditions report for this water will be rescinded in the final 2014 integrated report and the categories moved back to 4C. We may resubmit this in the future.

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
F13R-06-PH	VAP-F13R_SLN01A00	Sullens Creek	Proposed delisting to 4C for 4.60 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low Dissolved Oxygen and Low pH, Sullens and Mehixen Creeks and Tributaries in King William County, Virginia"	
F13R-10-PH	VAP-F13R_MLY01A12	Mallory Creek	Proposed delisting to 4C for 4.02 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low pH and Low Dissolved Oxygen, Jacks Creek and Tributaries in King William County, Virginia"	
F13R-14-PH	VAP-F13R_MHX01A12	Mehixen Creek and tributary XIV	Proposed delisting to 4C for 6.44 Miles	Please provide delisting rationale for this segment.		The natural conditions report for this water will be rescinded in the final 2014 integrated report and the categories moved back to 4C. We may resubmit this in the future.
F23R-02-DO	VAP-F23R_DKW01A00, VAP-F23R_DKW01B00, VAP-F23R_DKW01C98, VAP-F23R_DWD01A00, VAP-F23R_FTS01A10, VAP-F23R_GNT01A00, VAP-F23R_MKT01A00, VAP-F23R_MKT01B00,	Dickeys Swamp, Garnetts Creek, and Tributaries	Proposed delisting to 4C for 24.27 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low Dissolved Oxygen, Dickeys Swamp, Garnetts Creek and Tributaries in King and Queen County, Virginia"	
F23R-07-PH		Walkerton Branch	Listed in 2012 IR for pH	Where is this listing?	VAP-F23R_WKN02A12 - delist statement was mistakenly excluded from ADB; was properly included in the delisting package	
F26E-01-PCB		York River and Tribs	Two listings in 2012 IR for PCB in Fish Tissue	Where is this listing?	Listed in Appendix 1a and described in detail in Appendix 5	
F26E-02-SF		Aberdeen Creek	Listed in 2012 IR for Fecal Coliform	Where is this listing?	Changed Cause Group Code to F26E-15-SF and joined to VAT-F26E_ABD01A00 as a 4A impairment.	
F27E-20-SF	VAT-F27E_CDB02A00	Cedarbrush Creek	Listed in 2012 IR for Fecal Coliform	Please provide nesting rationale.		
G01E-03-PCB		James River and Various Tribs	2008 ILD Listed in 2012 IR for 0.012 square miles; 0.002 square miles in 2014 IR	Please clarify mileage change.	VAP-G03E_PTH01A00 - changed due to conversion from NHD 100K to 24K	
G01R-02-CU		XVP Almond Creek, UT	Listed in 2012 IR as G01R-02-CU; listed in 2014IR as G01R-22-CU	Identify correct cause group code.	G01R-22-CU is correct - the impairments were renamed during the 2014 cycle.	
G01R-02-ZN		XVP Almond Creek, UT	Listed in 2012 IR as G01R-02-ZN; listed in 2014 IR as G01R-22-ZN	Identify correct cause group code.	G01R-22-ZN is correct - the impairments were renamed during the 2014 cycle.	
G01R-05-PH	VAP-G01R_KSL01A04	Kingsland Creek	Proposed delisting to 4C for 8.54 Miles	Please provide delisting rationale for this segment.	"Natural Conditions Assessment for Low pH, Kingsland Creek in Chesterfield County, Virginia"	Report was not submitted in time for triennial review. Will return to 5C.

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
G05R-03-BAC	VAP-G05R_CHK01B10, VAP-G05R_CHK01C12, VAP-G05R_CHK02A04	Chickahominy River	Listed on 2012 IR for E. coli	Please provide nesting rationale.	See the approved Nesting Process Guidance	
G05R-12-BAC	VAP-G05R_SNF02A12	Upper Stony Run and Tribs	Listed on 2012 IR for E. coli	Please provide nesting rationale.	See the approved Nesting Process Guidance	
G05R-12-DO	VAP-G05R_SNF02A12	Upper Stony Run and Tribs	Proposed delisting to 4C for 39.87 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low Dissolved Oxygen and pH, Stony Run and Tributaries in Hanover County, Virginia"	
G05R-12-PH	VAP-G05R_SNF02A12	Upper Stony Run and Tribs	Proposed delisting to 4C for 39.87 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low Dissolved Oxygen and pH, Stony Run and Tributaries in Hanover County, Virginia"	
G07R-03-DO	VAP-G07R_PEL01B10	Pelham Swamp	Proposed delisting to 4C for 1.57 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low pH and Low Dissolved Oxygen, Rumley Marsh, Pelham Swamp and Tributaries in New Kent County, Virginia"	
G07R-04-BAC	VAP-G07R_SMN01A00	Collins Run	Listed on 2012 IR for E. coli	Please provide nesting rationale.	Do you mean Schminoe Creek? If so, see the approved nesting guidance	
G07R-05-PH	VAP-G07R_XAA01A10	XAA-Rumley Marsh, UT	Proposed delisting to 4C for 1.76 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low pH and Low Dissolved Oxygen, Rumley Marsh, Pelham Swamp and Tributaries in New Kent County, Virginia"	
G10E-06-BAC		College Creek	Listed in 2012 IR for Enterococcus	Where is this listing?	VAT-G10E_CLG01A06 was submitted for delist; can be found in Appendix 3 pg 28	
G11E-16-SF2	VAT-G11E_PGN01A08	Pagan River, Upstream of Chalmers Point	Listed on 2012 IR for Fecal Coliform	Please provide nesting rationale.	Nesting follows Part VII, Delisting Rule 3 of the WQA Guidance Manual: a bacteria impairment that is within the tidal range of an existing bacteria TMDL, land use is comparable, reductions apply to TMDL watershed, and point sources of bacteria are already identified. Nesting statement and description is found in the cause comment of the bacteria nested water.	
G15E-06-01-BAC	VAT-G15E_HAI01A06	Hampton River	Listed in 2012 IR for Enterococcus as "Hampton River" and listed in 2014IR as "James River-King/Lincoln Park Beach Area"	Please identify correct water name.		Revised the Fact Sheet to include new Cause Group Code : G15E-06-05-BAC for Hampton River.
H03R-04-PCB (2006)		James River	2006 ILD listed in 2012 IR for 164.27 miles; 158.32 miles in 2014 IR	Please clarify mileage change.	During the mapping conversion from 1:100,000 to 1:24,000 in the 2014 cycle, I cleaned up all areas of braiding or divided river paths. The loss of mileage is due to the double counting that was elimiated in this process.	

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
H03R-04-PCB (2006)		James River	2006 ILD listed in 2012 IR for 164.27 miles; 158.32 miles in 2014 IR	Please clarify mileage change.	During the mapping conversion from 1:100,000 to 1:24,000 in the 2014 cycle, I cleaned up all areas of braiding or divided river paths. The loss of mileage is due to the double counting that was elimiated in this process.	
H11R-01-BAC	VAC-H11R_BUF04A08	Buffalo River	Two Listings on 2012 IR for E. Coli	VAC-H11R_BUF04A08 identified as nested; please provide nesting rationale	The 2012 impaired segment was extended to include an additional upstream segment where monitoring was conducted during the TMDL study and showed bacteria impairment.	
H14R-01-HG	VAC-H14R_JMS01A00	James River	Listed in 2012 IR as 21.44 Miles, 18.57 Miles on 2014 IR (-2.87 miles)	Please clarify mileage change.	During the mapping conversion from 1:100,000 to 1:24,000 in the 2014 cycle, I cleaned up all areas of braiding or divided river paths. The loss of mileage is due to the double counting that was elimiated in this process.	
H15R-03-BAC	VAV-H15R_TLR01A08	Taylor Creek	Listed on 2012 IR for E. coli	Please provide nesting rationale.	Refer to Part VII, Delisting Rule 3 of the WQA Guidance Manual. The nesting is entered into ADB according to this guidance.	
H17R-02-BAC		James River	Listed in 2012 IR for 20.40 miles (2008 ILD) and 16.32 miles (2012 ILD); 20.40 miles (2008 ILD) and 16.32 miles (2012 ILD) in 2014 IR	Please clarify mileage change.	During the mapping conversion from 1:100,000 to 1:24,000 in the 2014 cycle, I cleaned up all areas of braiding or divided river paths. The loss of mileage is due to the double counting that was elimiated in this process.	
I19R-01-BAC	VAW-I19R_CRG02A02	Craig Creek	Listed in 2012 IR for 7.79 miles; 6.55 miles in 2014 IR	Please clarify mileage change.	Assessment Units VAW-I19R_CRG02A02 (6.55 mi.) and VAW-I19R_CRG02A14 (1.36 mi.) Increase due to conversion from 100K scale to 24K scale.	Correction made to mileage: 7.91.
I37R-03-BAC	VAV-I37R_PGH01A00	Maury River	Listed in 2012 IR for E. coli, Listed on 2014 IR as "Poague River" with an initial list date of 2014.	Please clarify correct water name and initial list date.	Upon review of ADB, I cannot find any reference to the Maury River with this assessment unit except in its description of the AU being from the headwaters downstream to the Maury River. Poague Run (not River) was not listed for e-coli in the 2012 cycle. The AU did not have data available for assessment and was a 3A water. It is newly listed in the 2014 cycle under the name Poague Run and identified as VAV-I37R_PGH01A00.	
J11R-03-PH		Bland Creek	Listed on 2012 IR for pH	Where is this listing?	pH was delisted in 2014 (by a partial delist)	
J11R-04-PH		Cellar Creek	Listed on 2012 IR for pH	Where is this listing?	pH was delisted in 2014 (by a partial delist)	
J15R-05-BEN	VAP-J15R_RHC01A06	Rohoic Creek	Listed in 2012 IR for 6.78 miles; 4.61 miles in 2014 IR	Please clarify mileage change.		Added tributaries back into segment
K05R-05-DO	VAP-K05R_HAY01A10	Hayes Creek	Listed in 2012 IR as Hayes; listed in 2014 IR as Hays	Please clarify correct water name.	Hays Creek is correct.	

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
K05R-05-PH	VAP-K05R_HAY01A10	Hayes Creek	Listed in 2012 IR as Hayes; listed in 2014 IR as Hays	Please clarify correct water name.	Hays Creek is correct.	
K11R-03-DO	VAP-K11R_CTT01A02, VAP-K11R_CTT01B10	Cattail Creek	Proposed delisting to 4C for 7.38 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low pH and Low Dissolved Oxygen, Cattail Creek in Greensville County, Virginia"	
K11R-03-pH	VAP-K11R_CTT01A02	Cattail Creek	Proposed delisting to 4C for 5.33 Miles	Please provide delisting rationale for this segment.	See "Natural Conditions Assessment for Low pH and Low Dissolved Oxygen, Cattail Creek in Greensville County, Virginia"	
K13R-03-BAC		Meherrin River (Lower)	Listed in 2012 IR for E. coli	Where is this listing?	VAT-K13R_MHN01A00 was submitted for delist; can be found in Appendix 3 pg 49	
K26R-04-BAC	VAP-K26R_MCC01A00	Maclins Creek	Listed in 2012 IR for E. coli	Please provide nesting rationale.	See the approved Nesting Process Guidance	
K35R-03-BAC	VAT-K35R_XDY01A04	UT Seacock Swamp	Listed on 2012 IR for 1.03 miles; 0.68 miles on 2014 IR	Please clarify mileage change.	The delineation of water quality assessment units (AUs) are based on National Hydrography Dataset (NHD).The size changes in the 2014 are primarily due to the use of more accurate and precise mapping.	
K35R-03-DO	VAT-K35R_XDY01A04	UT Seacock Swamp	Listed on 2012 IR for 1.03 miles; 0.68 miles on 2014 IR	Please clarify mileage change.	The delineation of water quality assessment units (AUs) are based on National Hydrography Dataset (NHD).The size changes in the 2014 are primarily due to the use of more accurate and precise mapping.	
K39R-01-HG	VAT-K39R_XCK01A00	Dismal Swamp Canal & Feeder Ditch to Lake Drummond	Listed on 2012IR for 17.58 Miles, 13.21 Miles on 2014 IR (-4.37)	Please clarify mileage change.	The delineation of water quality assessment units (AUs) are based on National Hydrography Dataset (NHD).The size changes in the 2014 are primarily due to the use of more accurate and precise mapping.	
K39R-02-HG	VAT-K39R_ZZZ01B08	Unsegmented Rivers in k39R	Listed in 2012 IR for 15.80 miles; 15.28 miles in 2014 IR	Please clarify mileage change.	The delineation of water quality assessment units (AUs) are based on National Hydrography Dataset (NHD).The size changes in the 2014 are primarily due to the use of more accurate and precise mapping.	
L04R-02-BEN	VAW-L04R_MDL01A06	Mud Lick Creek	Listed in 2012 IR for Benthic-macroinvert.	Please provide nesting rationale.	See attached nesting rationale	
L04R-05-BEN	VAW-L04R_MSN01A00	Mason Creek	Listed in 2012 IR for Benthic-macroinvert.	Please provide nesting rationale.	See attached nesting rationale	
L04R-06-BEN	VAW-L04R_BHT01A10	Barnhardt Creek	Listed in 2012 IR for Benthic-macroinvert.	Please provide nesting rationale.	See attached nesting rationale	
L04R-07-BEN	VAW-L04R_MUR01A00	Murray Run	Listed in 2012 IR for Benthic-macroinvert.	Please provide nesting rationale.	See attached nesting rationale	
L09R-01-TEMP	VAW-L09R_MEE05A00	Maggodee Creek	2012 IR identifies ILD as 2008; 2014 IR identifies ILD as 2012	Which initial list date is correct?		Corrected to 2008
L79L-02-HG	VAC-L79L_MES01L00	Lake Gordon	Listed in 2012 IR for 114.58 acres; 105.96 acres in 2014 IR	Please clarify acreage change.	The 1:24,000 scale provided a different total acreage for this lake.	

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
L80L-01-PCB	VAC-L78R_ROA06A98, VAC-L79L_ROA07A98, VAC-L80L_ROA08A04	Lake Gaston	Listed in 2012 IR for 4478.72 acres and 5.72 miles; 4439.72 acres and 5.69 miles in 2014 IR	Please clarify acreage and mileage change.	During the mapping conversion from 1:100,000 to 1:24,000 in the 2014 cycle, I cleaned up all areas of braiding or divided river paths. The loss of mileage is due to the double counting that was elimiated in this process. These segments were also altered to only include those miles included within the State boundaries.	
L81R-02-BAC	VAC-L81R_LIZ01A10	Lizard Creek	Listed in 2012 IR for 4.26 miles; 2.73 miles in 2014 IR	Please clarify mileage change.	The segment was shortened to only include those miles included within the State boundaries.	
M02R-01-BAC (2010)	VAS-M02R_STE01A02	Lovills Creek	Listed in 2012 IR as Lovills Creek, Appendix 3 - 42 identifies M02R-01-BAC (2010) as "Stewarts Creek"	Please clarify correct water name.	This is Stewarts Creek and is near Lovills Creek, so was included in Cause Group Code due to geographic proximity. It was delisted.	
N10R-02-BAC	VAS-N10R_HOL01A12, VAS-N10R_MCE01A02, VAS-N10R_RSF01A00, VAS-N10R_RSF01A02	South Fork Reed Creek and Mill Creek	Two listings in 2012 IR for E. coli	VAS-N10R_HOL01A12 6, VAS-N10R_RSF01A02 identified as nested; please provide nesting rationale	See the approved Nesting Process Guidance for bacteria	
N11R-01-BAC	VAS-N10R_GLN01A04, VAS-N10R_PRN01A12, VAS-N10R_RDC01A00, VAS-N10R_RDC01A02, VAS-N10R_RDC01B00, VAS-N10R_RDC01C02, VAS-N11R_RDC01B00, VAS-N11R_RDC01C02	Reed Creek	Five listings in 2012 IR for Fecal Coliform and E. coli	VAS-N10R_GLN01A04, VAS-N10R_PRN01A12, VAS-N11R_RDC01C02 ,VAS-N10R_RDC01C02 identified as nested; please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
N11R-02-BAC	VAS-N11R_MER02A10	Miller Creek	Two listings on 2012 IR for E. coli	VAS-N11R_MER02A10 identified as nested; please provide nesting rationale	See the approved Nesting Process Guidance for bacteria	
N30R-01-BAC		Wolf Creek	Listed on 2012 IR for E. coli, 2006 ILD at 23.24 miles, 14.36 miles for 2006 ILD on 2014 IR (-8.88 miles)	Please clarify mileage change.		The total mileage for this cause group mile will be revised to 30.82.
N32R-01-BAC (2012)		Wolf Creek	Listed on 2012 IR for E. coli	Where is this listing? Specifically, the listing identified with 2012 ILD.	This is listed in Appendix 1a and described in detail in Appendix 5.	
N36R-01-PCB (2012)		Bluestone River	Listed in 2012 IR for 3.03 miles (2012 ILD); 2.99 miles in 2014 IR	Please clarify mileage change.	Changed due to NHD conversion from 100k to 24K	
O01R-02-PH	VAS-O01R_XEE01A08	Hurricane Creek Trib	Listed on 2012 IR for pH	Where is this listing?	This is listed in Appendix 1a and described in detail in Appendix 5.	
O09R-03-BAC	VAS-O09R_NFH01B02	North Fork Holston River	Three listings in 2012 IR for Fecal Coliform and E. coli	VAS-O09R_NFH01B02 identified as nested; please provide nesting rationale	See the approved Nesting Process Guidance for bacteria	
O10R-01-BAC	VAS-O10R_NFH01A94, VAS-O10R_NFH02A00, VAS-O11R_NFH03A94	North For Holston River	Three listings in 2012 IR for Fecal Coliform and E. coli	VAS-O13R_NFH01A94 VAS-O14R_BMC04A00	VAS-O13R_NFH01A94 TMDL is complete; VAS-O14R_BMC04A00 was not listed for fecal coliform in 2012 and TMDL is completed for E.coli.	

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
O10R-05-BAC	VAS-O10R_BVR01A02, VAS-O10R_BVR01B04, VAS-O10R_LAE01A02, VAS-O10R_LAE02A02, VAS-O10R_LOC01A02, VAS-O10R_RRB01A02, VAS-O10R_TUR01A10	North Fork Holston River Tribs	Six Listings in 2012 IR for Fecal Coliform and E. Coli	VAS-O10R_BVR01B04, VAS-O10R_LAE02A02 identified as nested; please provide nesting rationale	See the approved Nesting Process Guidance for bacteria	
O10R-08-BAC		Beaver Creek	Listed in 2012 IR for E. coli	Where is this listing?	Changed Cause Group Code to O10R-05-BAC (VAS-O10R_BVR01B04) as a nested 4A impairment.	
O11R-04-BAC	VAS-O11R_LOG01A02	Logan Creek	Listed in 2012 IR for E. coli	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
O11R-09-BAC	VAS-O11R_EWF01A12	East Fork Wolf Creek	Listed in 2012 IR for E. coli	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
O11R-11-BAC	VAS-O11R_FIN01A12	Finley Creek	Listed in 2012 IR for E. coli	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
O11R-12-BAC	VAS-O11R_WOC01A12	West Fork Wolf Creek	Listed in 2012 IR for E. coli	Please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
O12R-05-BAC		Nordyke Creek	Listed in 2012 IR for E. coli	Where is this listing?	VAS-O12R_NOR01A02 was submitted for delist; can be found in Appendix 3 pg 55	
O14R-01-BAC	VAS-O14R_BMC01A98, VAS-O14R_BMC04A00, VAS-O14R_BMC05A02, VAS-O14R_BMC06A02, VAS-O14R_BMC07A02	Big Moccasin Creek	Three listings in 2012 IR for E. coli	VAS-O14R_BMC04A00 identified as nested; please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
P01R-02-BAC	VAS-P01R_NCL01A04, VAS-P01R_PLU01A04	Plum Creek and North Fork Clinch River	Three listings in 2012 IR for Fecal Coliform and E. coli	VAS-P01R_NCL01A04 identified as nested; please provide nesting rationale.	See the approved Nesting Process Guidance for bacteria	
P07R-01-BEN		Thompson Creek	Listed in 2012 IR as "Thompson Creek", 2014 IR as "Clinch River Tributaries"	Please identify correct water name.	Cause Group Code includes Clinch River, Weaver Creek, and Thompson Creek. Thus, "Clinch River Tributaries" is the correct name.	
P10R-05-BAC		Gravel Lick Creek	Listed in 2012 IR for E. coli	Where is this listing?	Changed Cause Group Code to P10R-01-BAC (VAS-P10R_GRV01A10) as a nested 4A impairment.	
P17R-08-BEN		Powell River	Listed in 2012 IR for Benthic-macroinvertebrate impairment.	Where is this listing?	Cause Group Code was corrected back to the original code used in 2010 P17R-02-BEN (VAS-P17R_POW02B06). It is now a 4A impairment.	
P22R-01-TEMP	VAS-P22R_WAL02A02, VAS-P22R_WAL02B02	Wallen Creek	Listed on 2012 IR for 27.92 Miles, 21.06 Miles on 2014 IR (-6.86 miles)	Please clarify mileage change.		The tributary streams in this AU were omitted and now have been added back.
P22R-02-TEMP		Wallen Creek	Listed on 2012 IR for Water Temp.	Where is this listing?	Changed Cause Group Code to P22R-01-TEMP (VAS-P22R_WAL02B02). It remains 5A impaired and will be in the final.	
Q03R-03-BAC	VAS-Q01R_JBF01A10, VAS-Q03R_PPW01A94	Guess Fork, Pawpaw Creek, and Jacobs Fork	Listed on 2012 IR for Fecal Coliform (2004 ILD), and E. coli (2010 ILD, 8.43 Miles)	Where are these two listings?	VAS-Q03R_PPW01A94 and VAS-Q03R_PPW01A94 are listed in the 2014IR	

Cause Group Code	Waterbody ID	Water name	Discrepancy	Clarification/Action Needed	DEQ Response	DEQ Action
Q12R-01-BAC	VAS-Q12R_RPC01A96	Russell Prater Creek	Listed on 2012 IR for 17.02 Miles, 11.72 Miles on 2014 IR (-5.3 miles)	Partial delist Q12R-01-BAC under "Barts Lick Creek" and waterbody ID VAS-Q12R_BAI01A08. Please clarify relation to Russell Prater Creek VAS-Q12R_RPC01A96	Both Barts Lick Creek and Russell Prater Creek are tributaries to Russell Fork, have same land uses, geology, and are in geographic proximity.	

Catoctin Creek Scenic River Advisory Committee
Protect, Preserve and Explore!

Via email to John.Kennedy@deq.virginia.gov
John Kennedy
Virginia Department of Environmental Quality
Office of Ecology
P.O. Box 1105
Richmond, VA 23218-1105

January 30, 2015

Subject: Comments on "**Draft 2014 305(b)/303(d) Water Quality Assessment (WQA) Integrated Report**"

Dear Mr. Kennedy:

On behalf of the Catoctin Creek Scenic River Advisory Committee we offer the following comments. In order to more fully understand the origins of our comments, I have provided a brief background on the committee and the Virginia scenic rivers program, followed by a discussion of monitoring and finally our statements on the WQA and the Catoctin Creek watershed.

Catoctin Scenic River Background
Established in 1970's the Scenic Rivers Program within the Virginia Department of Conservation and Recreation is intended to identify, designate and help protect rivers and streams that possess outstanding scenic, recreational, historic and natural characteristics of statewide significance for future generations. The program does not address water quality issues directly, however, the program does encourage and promotes a greater awareness of the value of rivers through partnership forged between citizens, local government and the state. The Committee's focus is to build and utilize those partnerships in support of the health of the creek and preservation of the scenic corridor.

Duties of the committee include:

- to preserve and protect the river's natural beauty and to assure its use and enjoyment for its scenic, recreational, geologic, fish, wildlife, historic, cultural or other assets, and to encourage the continuance of existing agricultural, horticultural, forestry, and open space land and water uses.

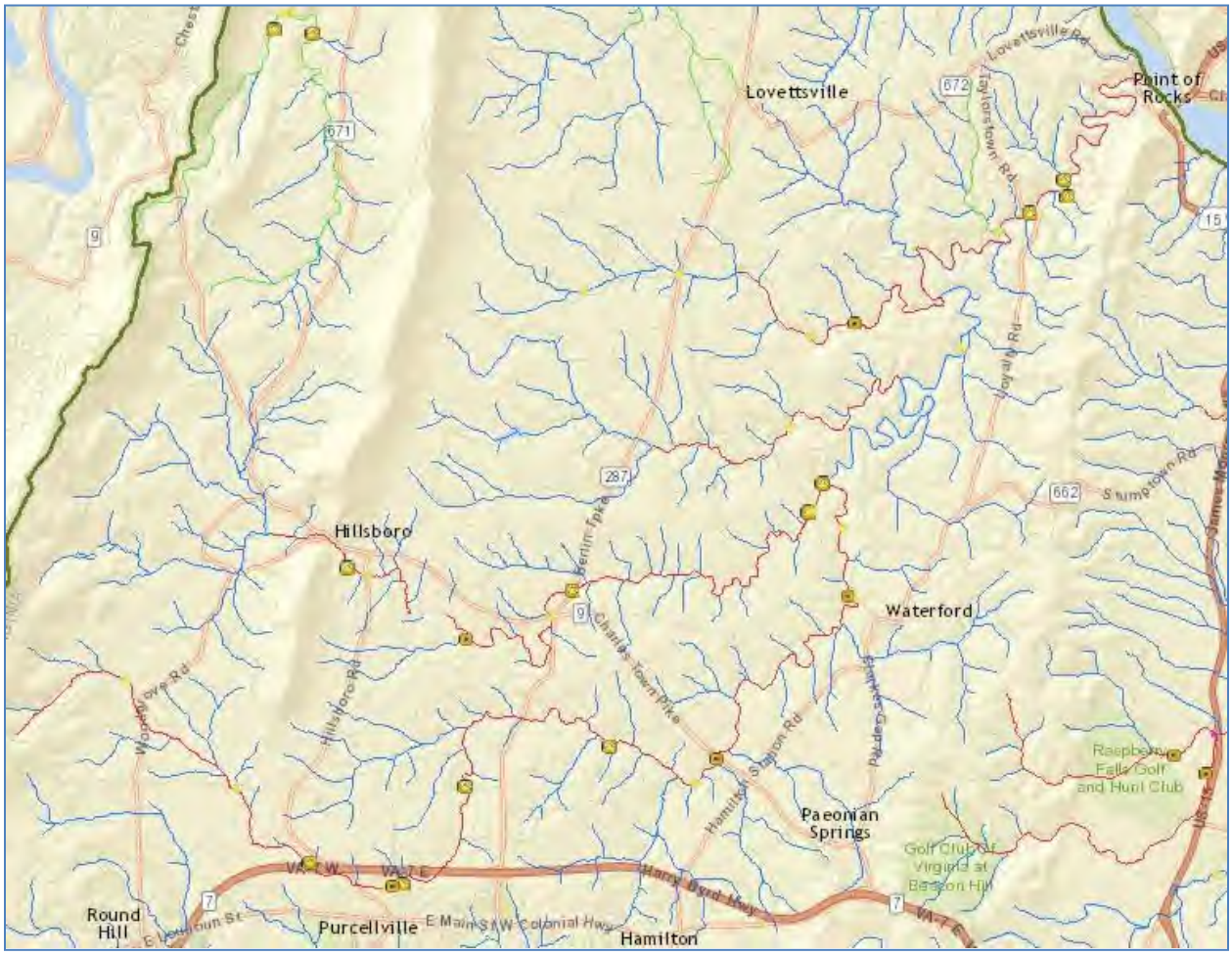
- to periodically survey each scenic river and its immediate environs and monitor of all existing and proposed uses of each scenic river and its related land resources.

The scenic portion of Catoctin Creek originates in Waterford and continues northward approximately 16 miles to the Potomac River. At the request of the Committee, Loudoun County Government added the scenic rivers in their on-line mapping as shown below with metadata.

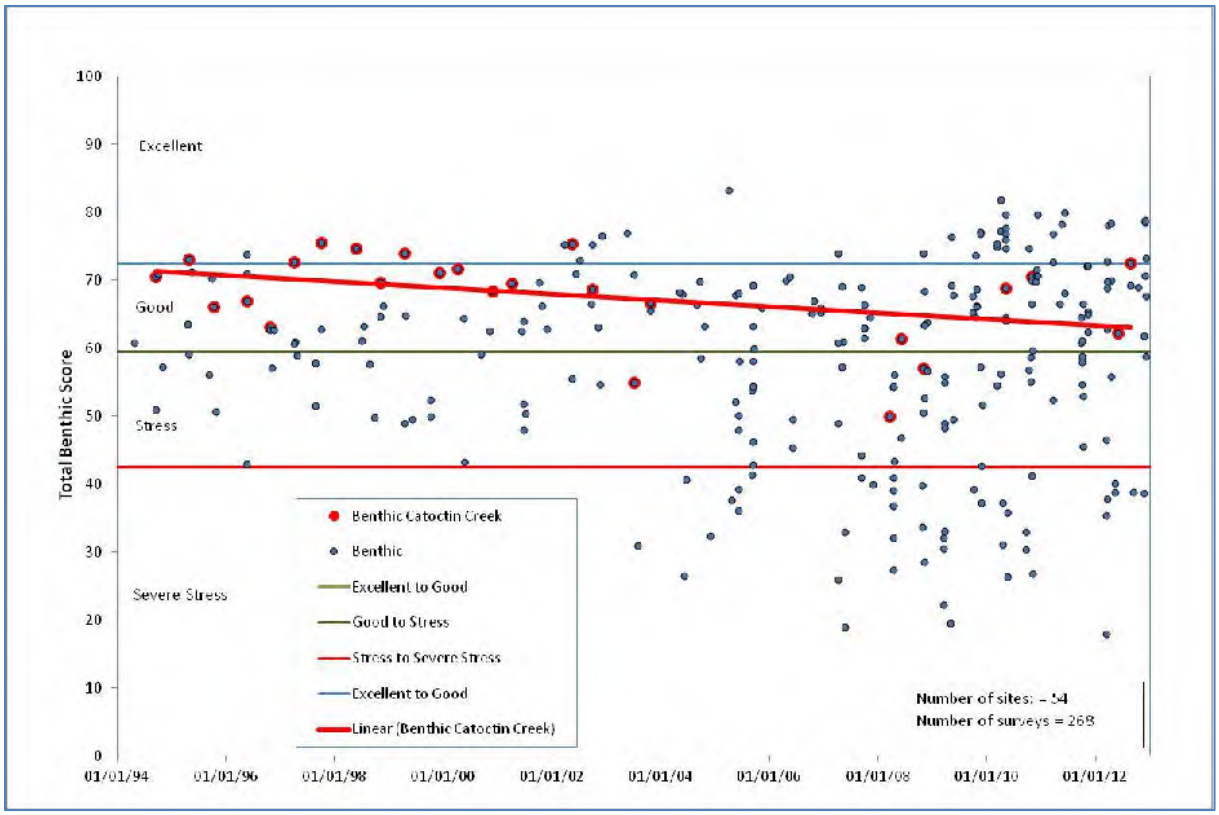
1:64000
0
0.5
1mi
Dutchman Creek
Lower Catoctin Creek
BERKLINE PKE
CHARLESTOWN PKE
287

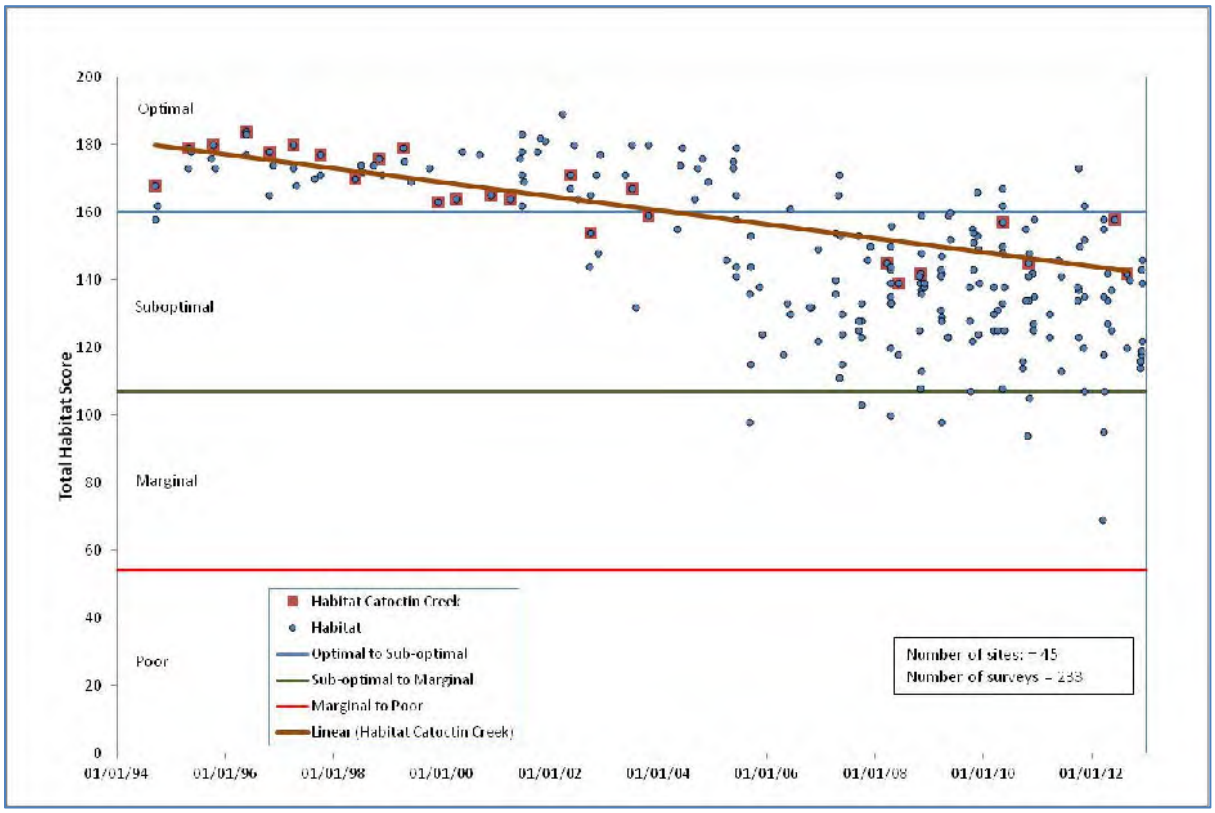
Scenic River and Stream Monitoring
While there is no rigorous scientific relationship between the scenic aesthetics of a river and water quality monitoring, one can clearly understand and appreciate that a reduced benthic community, which is almost always associated with excessive sediments, is often associated with reduced stream habitat and health. It is often found that reduce benthic conditions are associated with increased turbidity in the water and detracts from "scenic beauty". Increase stream flashiness resulting construction and increased imperviousness leads to bank erosion and channel scaring. Recreational use is guided by unsafe bacteria levels in the creek. It is our believe that virtually all of the Catoctin Watershed suffers from excessive bacteria levels, particularly during high flows even though DEQ monitoring is limited. Unsafe rivers means reduced "scenic beauty" and impacts resident from wading and enjoying the creeks.

Stream Monitoring in Catoctin Watershed
The most prominent location where DEQ monitors the creek is the long-term trend station at Taylorstown Rd, Station 1ACAX004.57. DEQ also conducts stream monitoring on the North and South Fork of Catoctin Creek and some tributaries to Catoctin Creek. There are also several citizen stream monitoring locations as shown below from DEQ's website.

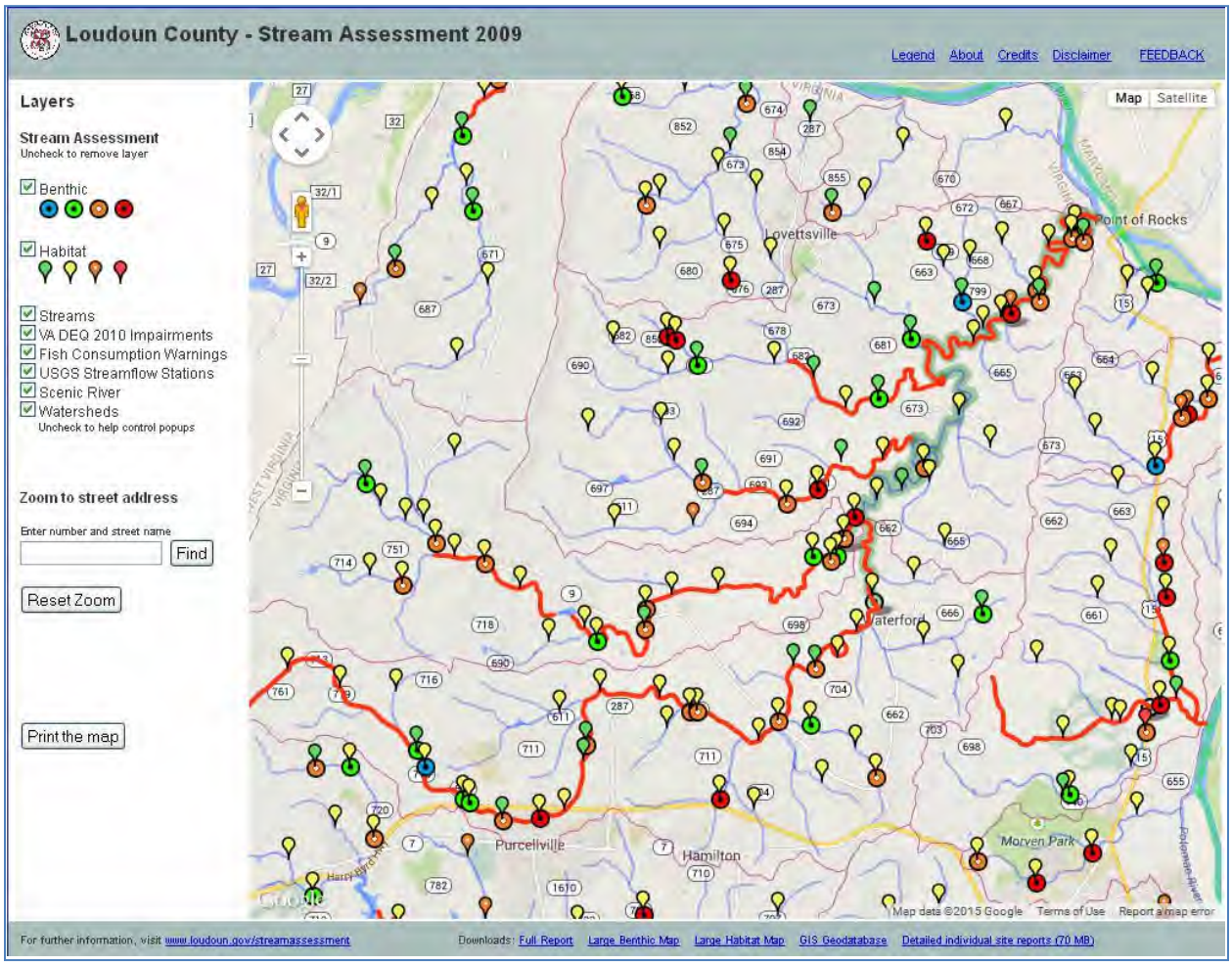


For several decades DEQ has been conducting stream assessment and habitat evaluations. Below are VA DEQ "ProbMon" data for Loudoun County with data along Catoctin Creek highlighted. The purpose of displaying this data is to clearly demonstrate the long-term trend on the decrease in stream health and habitat.





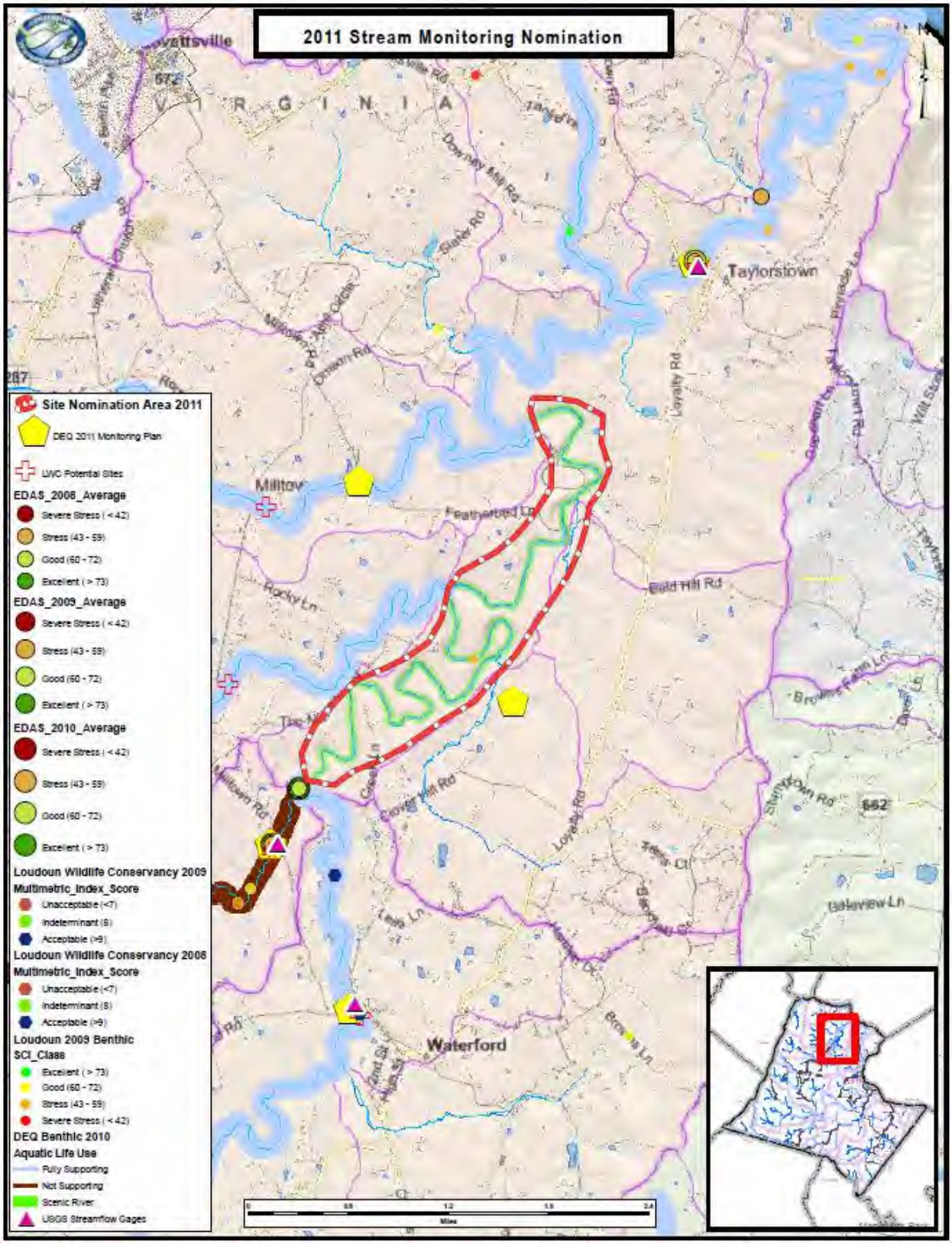
DEQ does not show the comprehensive stream assessment conducted by Loudoun County in 2009 as the County did not "officially" submit the data to DEQ. Below is a display of a map of this data within the in Catoctin Watershed. The EPA protocols for both benthic and habitat used are identical to those of DEQ biologists. The benthic scores of "stress" and "severe stress" shown in orange and red, respectively would lead to listing these reaches as impaired. These poor benthic scores are evident on the tributaries, but also the main channel where DEQ has not completed an assessment.



Catoctin Creek Water Quality Assessments

For over a decade reaches of streams within the Catoctin watershed has been listed, typically for recreational use and occasionally for aquatic life use. The end result has been a bacteria TMDL and TMDL Implementation Plan (and implementation of said plan) for the watershed which effectively ended in 2009 with limited reduction in bacteria levels, thereby impacting the recreational use.

Note however that the density and intensity of monitoring has left many reaches unassessed. While Loudoun Watershed Watch has submitted several letters to DEQ to nominate streams in the watershed, in 2011, a specific request was submitted for the mainstem of Catoctin Creek (along the scenic portion) as shown below.


2011 Stream Monitoring Nomination
Site Nomination Area 2011
DEQ 2011 Monitoring Plan
LWC Potential Sites
EDAS_2008_Average
Severe Stress (< 42)
Stress (43 - 59)
Good (60 - 72)
Excellent (> 73)
EDAS_2009_Average
Severe Stress (< 42)
Stress (43 - 59)
Good (60 - 72)
Excellent (> 73)
EDAS_2010_Average
Severe Stress (< 42)
Stress (43 - 59)
Good (60 - 72)
Excellent (> 73)
Loudoun Wildlife Conservancy 2009
Multimetric_Index_Score
Unacceptable (<7)
Indeterminant (8)
Acceptable (>9)
Loudoun Wildlife Conservancy 2008
Multimetric_Index_Score
Unacceptable (<7)
Indeterminant (8)
Acceptable (>9)
Loudoun 2009 Benthic
SCI_Class
Excellent (> 73)
Good (60 - 72)
Stress (43 - 59)
Severe Stress (< 42)
DEQ Benthic 2010
Aquatic Life Use
Fully Supporting
Not Supporting
Scenic River
USGS Streamflow Gages
Taylorstown
Milltov
Waterford
Bald Hill Rd
Rocky Ln
Clover Hill Rd
Lovely Rd
Downey Mill Rd
Slater Rd
Dream Rd
Milltown Rd
Featherbed Ln
Lewis Ln
Brownsville Ln
Galaxview Ln
Miles

The response from DEQ was that past and present benthic monitoring are meeting this request.

Our Comments on the Draft 2014 Water Quality Assessment
In the 2014 WQA, 2.5 miles of North Fork Catoctin Creek was added for benthic impairment on assessment unit VAN-A02R_NOC03A02 . This has been added to the schedule in 2026. We appreciate the efforts to identify and list an additional reach on the North Fork Catoctin Creek. We believe that symptoms in the headwater could reasonably affect the downstream scenic portion on the mainstem of Catoctin Creek.

We further understand that in 2010 DEQ delisted the benthic impairment on South Fork Catoctin Creek. It is our belief that such a decision, while scientifically valid, may have been premature. It is our opinion that using 6 or 8 data points is severely limited. Basically, we understand that the benthic scores were such that they were just under the threshold for a limited period of time. Based on the fluctuations on benthic scores year-to-year and the additional evidence in the comprehensive 2009 stream assessment, **we seek to have DEQ re-examine this delisting.**

The mainstem of Catoctin Creek, the scenic portion, has yet to be adequately assessed in our opinion. **We suggest that DEQ re-evaluate the monitoring plans for this reach.**

Existing Bacteria TMDL:
Some of the bacteria listings in the Catoctin Watershed date back to 1996. Although a TMDL Implementation Plan was in effect from 2005 to 2009, **we ask DEQ not to designate this as a completed TMDL Implementation**. Citizen monitoring of over 1,000 data points do not support such a position. We ask that DEQ reword maps such as "Water Quality Restoration Progress". We do not believe that it is appropriate to label streams as being restored simply because a five-year cycle of actions was accomplished as show in the map. The table indicates only "some improvement for the Catoctin bacteria TMDL. This has yet to be a "Success Story".

Future Benthic TMDL:
It is our understanding that DEQ plans to develop a benthic TMDL, but only in north Fork Catoctin Creek. **We suggest that in light of the overall conditions of the watershed that DEQ prepare a benthic TMDL for the entire Catoctin Creek Watershed (North Fork, South Fork and mainstem (scenic)).**

Thank you for your efforts on behalf of our Nation's Water Quality. We look forward to your response and positive consideration of the requests made in this letter. The Catoctin Creek Scenic River Advisory Committee can be reached at CatoctinScenicRiver@gmail.com. You may also contact me directly at 540.454.6880 or pattipsaris@rstarmail.com.

Sincerely,

Patti J. Psaris
Catoctin Scenic River Advisory Committee, Chair

Committee Members:
Eleanor Adams
Otto Guttenson
Bruce Johnson
Anne Larson
David Nelson
David Ward
Norma Wilson

cc: Lynn Crump, Environmental Programs Planner;
Virginia Department of Conservation and Recreation,
Planning & Recreation Resources

DEQ's Response to Catoctin Creek Scenic River Advisory Committee

Comment:

We further understand that in 2010 DEQ delisted the benthic impairment on South Fork Catoctin Creek. It is our belief that such a decision, while scientifically valid, may have been premature. It is our opinion that using 6 or 8 data points is severely limited. Basically, we understand that the benthic scores were such that they were just under the threshold for a limited period of time. Based on the fluctuations on benthic scores year-to-year and the additional evidence in the comprehensive 2009 stream assessment, we seek to have DEQ re-examine this delisting….

It is our understanding that DEQ plans to develop a benthic TMDL, but only in north Fork Catoctin Creek. We suggest that in light of the overall conditions of the watershed that DEQ prepare a benthic TMDL for the entire Catoctin Creek Watershed (North Fork, South Fork and mainstem (scenic)).

Response:

EPA approved the 2010 delisting of the benthic impairment on South Fork Catoctin Creek based on four Virginia Stream Condition Index scores (out of a total of six) that were above the impairment threshold. Subsequent site visits since then have corroborated this decision. Moreover, the VSCI and Total Habitat Scores in the current assessment period (2007-2012) indicate use attainment, which is why DEQ has proposed the delisting of two additional segments of SF Catoctin Creek. Thus, the information that DEQ is authorized to use in assessments does not support re-classifying SF Catoctin Creek as impaired at this time.

DEQ has detected increasing total nitrogen, phosphorus, and bacteria concentrations in Catoctin Creek, however. We hope that these trends are arrested when current bacteria and future benthic implementation plans are put into practice. SF Catoctin Creek will continue to be monitored and assessed as resources allow. If at any time new data indicates it is impaired, it will be returned to the Impaired Waters List and scheduled for a TMDL.

DEQ strongly encourages Loudoun County to submit its benthic data to DEQ so it can be used in listing/delisting decisions. These data could also be useful in TMDL and Implementation Plan development.

Comment:

Some of the bacteria listings in the Catoctin Watershed date back to 1996. Although a TMDL Implementation Plan was in effect from 2005 to 2009, we ask DEQ not to designate this as a completed TMDL Implementation. Citizen monitoring of over 1,000 data points do not support such a position. We ask that DEQ reword maps such as "Water Quality Restoration Progress". We do not believe that it is appropriate to label streams as being restored simply because a five-year cycle of actions was accomplished as show in the map. The table indicates only "some improvement for the Catoctin bacteria TMDL. This has yet to be a "Success Story".

Response:

DEQ considers a restored waterbody to be one that previously failed water quality standards, but is now attaining them. SF Catoctin Creek appears in the "Water Quality Restoration Progress" map for this reason. A waterbody with an impairment addressed by a completed TMDL or Implementation Plan is not considered "restored" until the applicable water quality standards for that impairment have been attained. But they are tracked and reported so that citizens and EPA can see where and how Virginia's impairments are being addressed.

CHESAPEAKE BAY FOUNDATION
Saving a National Treasure

January 30, 2015

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Mr. John Kennedy
Director of Ecology
Department of Environmental Quality
P.O. Box 1105
Richmond, 23218

On behalf of the Chesapeake Bay Foundation (CBF), I submit the following comments in reference to the 2014 Virginia Water Quality Assessment 305(b)/303(d) Integrated Report. First, I'd like to express our appreciation for the tremendous effort that Virginia Department of Environmental Quality (DEQ) staff and Virginia's citizen monitoring groups have put into monitoring, analyzing, and reporting upon the health of our state's waters. The 2014 report includes assessments of more than 22,000 miles of Virginia streams, which represents the most ever included in one of Virginia's integrated reports and nearly a 4,000 mile increase from the previous report.

The report indicates there are twice as many miles of streams as previously thought in the Commonwealth. While we also applaud DEQ for using updated resources to collect these data, we think a much more robust discussion, which better illustrates the new techniques and data used, would add significantly to the report. We also suggest that DEQ investigate whether future technological upgrades will lead to additional increases, as it is critical for the department to have an accurate quantification of Virginia's aquatic resources in order to accurately determine restoration efforts and monitoring needs.

CBF applauds DEQ for developing an Academic Advisory Committee and working with EPA to address the need for freshwater nutrient criteria. We hope the next draft of the integrated report can incorporate these freshwater nutrient criteria in order to improve our assessment of designated use impairment in the state of Virginia.

CBF recommends DEQ utilize their probabilistic monitoring data set to estimate the total number of impaired streams for the state of Virginia. In Chapter 4 (Probabilistic Monitoring), the report indicates 43.5% of streams and rivers are impaired based upon bio-monitoring, yet this result is not included in the executive summary. Given that an intent of the report is to help with planning and management, we feel this critical result should be interpreted further with an effort to highlight it. Specifically, it is important to provide the best estimate of the total number of Virginia stream miles that are currently expected to be impaired. While these estimates do have some uncertainty, they are essential for informing localities, decision makers, and the public of the resources needed to address water quality concerns.

Unfortunately, the one overarching message is clear: a significant proportion of Virginia's waters are impaired due to nutrients, sediment, and E. coli that are directly related to agriculture and stormwater runoff. The necessary actions to improve the water quality of these small non-tidal streams is consistent with actions necessary to improve the water quality in Chesapeake Bay. The proportion of impaired estuarine waters in Virginia has declined slightly

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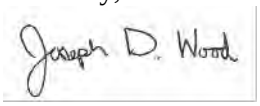
(~5% from the previous integrative report) but is still alarmingly high (75%). This modest improvement may be attributed to significant advancements in wastewater treatment, but they clearly demonstrate the need for nutrient and sediment reductions from all sources. The Chesapeake Bay TMDL and Virginia's WIP call for such reductions from agriculture, stormwater, and wastewater. From the onset of the Bay cleanup, there have been significant reductions in wastewater nutrient loads, but agriculture and stormwater reductions have been modest at best.

Thank you for your consideration of our comments on this important report. If you have any questions regarding these comments, please feel free to contact me at 804-780-1392 ext. 3130 or at jwood@cbf.org.

Sincerely,



Joseph D. Wood, Ph.D.
Virginia Staff Scientist
Chesapeake Bay Foundation

CC: Ann Jennings, Virginia Executive Director, CBF
Peggy Sanner, Virginia Senior Attorney, CBF
Chris Moore, Virginia Senior Scientist, CBF

DEQ's Response to the Chesapeake Bay Foundation

Comment:

The report indicates there are twice as many miles of streams as previously thought in the Commonwealth. While we also applaud DEQ for using updated resources to collect these data, we think a much more robust discussion, which better illustrates the new techniques and data used, would add significantly to the report. We also suggest that DEQ investigate whether future technological upgrades will lead to additional increases, as it is critical for the department to have an accurate quantification of Virginia's aquatic resources in order to accurately determine restoration efforts and monitoring needs.

Response:

The expansion in stream mileage can be attributed to DEQ's adoption of the 1:24,000 scale resolution of the National Hydrography Dataset (NHD) in its water quality assessments. The NHD is maintained by the United States Geological Service (USGS), and it is considered to be the most accurate geographic representation of the country's surface waters. In the early 2000s, EPA launched the Watershed Assessment, Tracking and Environmental Results System (WATERS), which integrates the nation's surface water quality databases into a common geographic platform based on the 1:24K NHD, allowing for more consistent tracking and reporting. The conversion of Virginia's older 1:100K NHD to the higher resolution was accomplished in 2013 through a collaborative effort of federal and state agencies. EPA put together a document illustrating how the move to higher resolution mapping can affect water quality characterization. This document can be found here: http://epadev.induscorp.com/waters/doc/p0340.pdf.

Much of the additional stream mileage is due to the inclusion of previously unmapped waterways—mostly small unnamed tributaries. These waters are counted as "unassessed" until they are monitored and properly characterized by DEQ staff. The non-impaired/impaired waterbody size estimates presented in the report are largely unaffected by the addition of these waters into DEQ's database. The mileage also increased due to the refinement of previously mapped stream courses and shorelines. But this had a relatively small impact on the non-impaired/impaired waterbody sizes compared to changes dictated by monitoring data.

DEQ does not anticipate significant changes to the resolution of its surface water mapping in the foreseeable future, though it is likely statewide stream mileage will decrease slightly as ephemeral streams are better distinguished from perennial waters.

Comment:

CBF applauds DEQ for developing an Academic Advisory Committee and working with EPA to address the need for freshwater nutrient criteria. We hope the next draft of the integrated report can incorporate these freshwater nutrient criteria in order to improve our assessment of designated use impairment in the state of Virginia.

Response:

The progress that DEQ's Academic Advisory Committee has made on freshwater criteria can be found on the Water Quality Standard's webpage: http://www.deq.virginia.gov/Programs/Water/WaterQualityInformationTMDLs/WaterQualityStandards/NutrientCriteriaDevelopment.aspx#Nutrient_Criteria_Streams_Rivers. CBF may find the report "A Screening Approach for Nutrient Criteria in Virginia" of interest, in particular. This work will likely not be completed in time for the 2016 Integrated Report, as proposed criteria will have to go through Virginia's rule-making process and be approved by EPA.

Comment:

CBF recommends DEQ utilize their probabilistic monitoring data set to estimate the total number of impaired streams for the state of Virginia. In Chapter 4 (Probabilistic Monitoring), the report indicates 43.5% of streams and rivers are impaired based upon bio-monitoring, yet this result is not included in the executive summary. Given that an intent of the report is to help with planning and management, we feel this critical result should be interpreted further with an effort to highlight it.

Response:

The executive summary provides an overview of water quality assessment results based on information gathered from all of DEQ's surface water monitoring programs, including both estuarine and freshwater ProbMon. DEQ will take into consideration the suggestion to report ProbMon survey results in addition to this general overview.

Cleaning wastewater every day for a better Bay.

January 30, 2015

By e-mail

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
John.Kennedy@deq.virginia.gov

RE: Comments on Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

The Hampton Roads Sanitation District appreciates the opportunity to comment on the above referenced document. HRSD continues to appreciate the magnitude of the effort involved in assessing the waters of the Commonwealth and in preparing the Integrated Report. The report continues to evolve with each iteration as evidenced in the 2014 report by the addition of two new assessment categories and the updated GIS layers to improve the accuracy of watershed mapping. As DEQ continues to improve the assessment process, HRSD would like to offer comment on issues with VPDES regulatory implications.

PCB Fish Advisory Discussion

The discussion in Chapter 7.4 (page 233) regarding fish consumption advisories due to polychlorinated biphenyls (PCBs) contamination needs to be updated to reflect the *increase* in the Virginia Department of Health's (VDH) fish tissue advisory threshold. It would be beneficial to include in this section information on whether this increase in the threshold resulted in a removal of any fish consumption advisories. This section would further benefit by clarifying the relationship, or lack thereof, between the VDH fish tissue threshold (100 ppb) and the DEQ fish tissue threshold (20 ppb) given that both have been identified to be protective of human health. Due to the large disparity between the agency thresholds, it is possible that some waterbodies deemed safe for fish consumption by VDH may be identified as impaired for this same use based on the DEQ threshold. Since both of these thresholds were developed to protect human health, discussion on this issue is warranted.

Analytical Methodology

The VPDES permit program requires that only 40CFR Part 136 analytical procedures be used to analyze samples when such a method is available. The assessment program does not have a similar requirement. Both the ambient monitoring program and the VPDES program determine the potential for impact in state waters. Though all data used for supporting and listing purposes is collected using an EPA accepted and DEQ approved method, this does not address the concern that the use of varying methodologies between the two programs could

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result in conflict and undermine the credibility of the listings and subsequent TMDLs. It is true that the analytical laboratories are accredited through the Division of Consolidated Laboratory Services. However, this does not address the concern that different analytical methods have varying levels of precision and detection limits. The use of these different analytical methods can result in disparate findings.

Citizen Monitoring Data

Citizen monitoring data can be an invaluable tool in identifying waters that require more in-depth evaluation and, when collected and analyzed in a manner consistent with appropriate quality assurance protocols, can be useful in expanding the network of assessed streams. However, ensuring that this data was collected under the stringent quality assurance measures required of Level III data demands significant resources. It is unclear that DEQ has the appropriate funding and staffing needed for this oversight given the dramatic increase in Level III data. In light of the importance of these listing determinations, resources must be adequately assigned to ensure a thorough review of SOPs and QA/QC plans for each citizen group.

Thank you for the opportunity to comment. The report is an invaluable tool in characterizing the waters of the state and in identifying waters in need of improvement.

Respectfully,

Jamie S. Heisig-Mitchell
Chief of Technical Services
Hampton Roads Sanitation District
1434 Air Rail Avenue
Virginia Beach, VA 23455
757-460-4220
jmitchell@hrsd.com

DEQ's Response to Hampton Roads Sanitation District

Comment:

The discussion in Chapter 7.4 (page 233) regarding fish consumption advisories due to polychlorinated biphenyls (PCBs) contamination needs to be updated to reflect the *increase* in the Virginia Department of Health's (VDH) fish tissue advisory threshold. It would be beneficial to include in this section information on whether this increase in the threshold resulted in a removal of any fish consumption advisories.

Response:

The aforementioned section will be revised as follows (additions in italics): "In 1998, VDH developed its own fish consumption advisory guidelines and set its levels of concern for PCBs in fish to no more than 600 ppb. *This limit was lowered to 50 ppb in 2004*. The latest guidelines announced by VDH in 2012 increased that level to 100 ppb. The change reflects VDH's adoption of the updated exposure factors (such as average body weight) and human behavior-related assumptions recommended by EPA. *This change does not affect previously issued consumption advisories or current impairment listings*. The watersheds covered by fish consumption advisories due to PCB contamination are described below. "

Comment:

This section would further benefit by clarifying the relationship, or lack thereof, between the VDH fish tissue threshold (100 ppb) and the DEQ fish tissue threshold (20 ppb) given that both have been identified to be protective of human health. Due to the large disparity between the agency thresholds, it is possible that some waterbodies deemed safe for fish consumption by VDH may be identified as impaired for this same use based on the DEQ threshold. Since both of these thresholds were developed to protect human health, discussion on this issue is warranted.

Response:

The discrepancies in fish tissue thresholds can be partially explained by the different roles served by VDH and DEQ. With regards to fish consumption, VDH protects public health when contamination has been identified. VDH's thresholds are based on risk assessment guidance designed for sites with known contamination (like Superfund sites)--where the potential for exposure is generally limited to a finite geographical area. In contrast, DEQ is charged with preventing contamination from happening in the first place, and thus the thresholds it uses minimize risks in all waterbodies.

Differences in decision-making processes between the two agencies also account for the discrepancies. VDH has the authority to change its advisory levels when it has reasonable cause to do so. In contrast, DEQ must undertake an extensive process to change its thresholds. This process involves the participation of EPA, environmental groups, the regulated community, the State Water Control Board, and the public at large. EPA has final approval of the water quality standards DEQ employs for designated use assessments.

DEQ and VDH operate under a Memorandum of Understanding which ensures the timely exchange and evaluation of reliable water quality and fish advisory information between the two agencies.

Comment:

The VPDES permit program requires that only 40CFR Part 136 analytical procedures be used to analyze samples when such a method is available. The assessment program does not have a similar requirement. Both the ambient monitoring program and the VPDES program determine the potential for impact in state waters. Though all data used for supporting and listing purposes is collected using an EPA accepted and DEQ approved method, this does not address the concern that the use of varying methodologies between the two programs could result in conflict and undermine the credibility of the listings and subsequent TMDLs. It is true that the analytical laboratories are accredited through the Division of Consolidated Laboratory Services. However, this does not address the concern that different analytical methods have varying levels of precision and detection limits. The use of these different analytical methods can result in disparate findings.

Response:

DEQ will continue to emphasize the importance of coordination amongst all water quality management planning programs--watershed monitoring and assessment, TMDLs, and Virginia Pollution Discharge Elimination System permitting. Not only do stakeholders benefit when these programs work in concert, but the agency also has a vested interest in minimizing conflicts arising from different program requirements.

Per Virginia Regulations 1 VAC 30-46, all analyte data submitted to DEQ for regulatory purposes, including both permit-mandated reporting and 303(d) listing, must be from a NELAC-accredited laboratory. This ensures that only data of high quality are used. Because laboratory certification and auditing are the responsibilities of the Division of Consolidated Laboratory Services (DCLS), concerns about inconsistencies in analytical procedures should be directed to DCLS.

Comment:

Citizen monitoring data can be an invaluable tool in identifying waters that require more in-depth evaluation and, when collected and analyzed in a manner consistent with appropriate quality assurance protocols, can be useful in expanding the network of assessed streams. However, ensuring that this data was collected under the stringent quality assurance measures required of Level III data demands significant resources. It is unclear that DEQ has the appropriate funding and staffing needed for this oversight given the dramatic increase in Level III data. In light of the importance of these listing determinations, resources must be adequately assigned to ensure a thorough review of SOPs and QA/QC plans for each citizen group.

Response:

During each assessment cycle, DEQ receives a large quantity of citizen volunteer and other non-DEQ monitoring data. Based on review of the protocols and procedures used, these data are classified into one of three levels. The highest level, Level III, comprises of submitted data that follows DEQ recognized protocols and uses quality assurance procedures that ensure the data generated is of the highest quality. Level III data are of the most value to the agency since they can be used directly to assess water quality conditions, without the need of additional agency sampling.

To ensure data meet the highest possible standard, groups are required to submit a Quality Assurance Project Plan (QAPP) and may be asked to provide additional relevant documentation such as calibration log sheets. The submitted data are independently reviewed by DEQ quality assurance staff. Questionable data are not included in the assessment report if their accuracy cannot be verified by the data submitter. This is consistent with the procedure DEQ follows for its own data.

Many of the Level III groups monitor basic parameters such as temperature and dissolved oxygen using standard field equipment. DEQ routinely verifies the accuracy and precision of this equipment if a group lacks the ability to do so itself. Verification of laboratory-derived data is done either through a VLEAP certified laboratory or by DEQ on a regular basis following similar VLEAP-auditing protocols. Finally, DEQ staff routinely assists with, reviews, and leads training events with volunteer groups to ensure the sample teams are following established methods. To supplement in-person training, DEQ is developing videos that will help train and refresh volunteers in proper calibration, maintenance, and sampling procedures.

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Reid Engineering
Whitman, Requardt & Associates
Wiley & Wilson
William P. Johnson II, PE, PC
WW Associates

LEGAL COUNSEL
Christopher D. Pomeroy, Esq.

January 30, 2015

By Electronic Mail & First Class U.S. Mail

Mr. John Kennedy
Virginia Department of Environmental Quality
Office of Ecology
P.O. Box 1105
Richmond, VA 23218-1105
John.Kennedy@deq.virginia.gov

Re: Virginia Draft Water Quality Assessment 305(b)/303(d) Integrated Report 2014

Dear Mr. Kennedy:

As you know, the Virginia Association of Municipal Wastewater Agencies, Inc. ("VAMWA") is a statewide association of local governments and authorities that own and operate municipal wastewater treatment plants. On behalf of VAMWA, please accept the enclosed comments on Virginia's Draft Water Quality Assessment 305(b)/303(d) Integrated Report 2014.

VAMWA appreciates the substantial effort by the Department in preparing the Draft Integrated Report. We look forward to continuing to work with the Department in the further development of the Integrated Report, and more generally working toward our shared goal of improving water quality in the Commonwealth.

Sincerely,

Ted Henifin
VAMWA President

cc: VAMWA Board
VAMWA Members
Chris Pomeroy, VAMWA General Counsel

COMMENTS OF THE
VIRGINIA ASSOCIATION OF MUNICIPAL WASTEWATER AGENCIES, INC.
ON THE VIRGINIA WATER QUALITY ASSESSMENT 305(b)/303(d) INTEGRATED REPORT 2014

JANUARY 30, 2015

The Virginia Association of Municipal Wastewater Agencies, Inc. ("VAMWA") appreciates the Virginia Department of Environmental Quality's ("DEQ's") substantial efforts in preparing the Virginia Water Quality Assessment 305(b)/303(d) Integrated Report for 2014 ("Integrated Report"). The Integrated Report identifies several changes to Virginia's assessment process and VAMWA is encouraged to see that these changes are likely to result in an increase in the soundness of DEQ's assessment of the quality of Virginia's waters. Comments regarding particular portions of the Integrated Report follow.

1. VAMWA Supports DEQ's Determination to Not List Waters of the Shenandoah as Impaired for Recreational Use at This Time.

VAMWA strongly supports DEQ's decision to not list the Shenandoah River as impaired for the recreational use designated use. As DEQ correctly points out, there is no data to justify a decision to list the Shenandoah as impaired for recreational use. DEQ's decision is correct for several reasons as follows.

a. Public Data Submitted Did Not Meet Data Quality Requirements.

While some citizens have expressed concerns over algae impeding recreational use in the Shenandoah River, DEQ lacks the kind of specific and objective water quality monitoring data necessary to list this water segment as impaired for the recreational use designated use. Data submitted to DEQ during the 2012 Integrated Report public comment period was largely anecdotal and according to DEQ's own protocol for data quality, fell short of being categorized as Level III data, which is the only level of non-agency submitted data that DEQ will consider for listing purposes. DEQ properly did not rely on this data to evaluate compliance with the recreational use designated use. Additionally, DEQ's QA/QC of the data submitted by citizen monitoring organizations since the 2012 Integrated Report shows that some of the data submitted by these organizations was collected from within DEQ-approved mixing zones. Appropriately, DEQ did not consider such data.

DEQ correctly states that an impaired designation "can only be made based on specific and objective monitoring data, in terms of location, extent, and duration, as well as an accepted, scientifically valid assessment method that compares monitoring data to water quality standards

or criteria." (Draft Integrated Report, pg. 61). As monitoring data quality and quantity increase for the Shenandoah River, DEQ will be in a better position to determine recreational use impairment in future Integrated Reports.

b. Shenandoah River Recreational Use Impairment is Best Addressed via E. Coli for the 2014 Integrated Report.

At this time, DEQ correctly limits its assessment of the recreational use designated use to human health impacts tied to E. Coli bacteria because this is the only objective water quality data currently available to assess this designated use. Contrary to the anecdotal citizen monitoring data submitted, DEQ's E. Coli bacteria data are quantifiable and objective. Therefore, VAMWA supports DEQ's decision to base its recreational use assessment only on this E. Coli data until such time as other sufficient water quality data is available to assess the recreational use designated use.

c. Many Different Programs Are Already Addressing Algal Growth in the Shenandoah River.

While additional water quality data is being collected, the Shenandoah River's algal problem is already being addressed. For instance, EPA correctly notes in its September 23, 2014 letter to DEQ approving DEQ's 2012 303(d) list that "the Chesapeake Bay TMDL and WIP controls should reduce sources of sediment and nutrients to the River and thus decrease causative factors for algal growth." (See Comments at pg. 3). In addition to implementation of the Chesapeake Bay TMDL, DEQ notes that 17 TMDLs have been developed in tributaries to the Shenandoah River basin to address nutrients and sediments for benthic impairments. These TMDLs will lead to positive water quality impacts that will help correct any existing algal overgrowth in the Shenandoah River.

d. DEQ Properly Lists the Shenandoah River In Category 2B for Recreational Use; Listing as Category 3B or 3C Also Appropriate.

DEQ properly determined to list the Shenandoah River as a Category 2B Water - "waters are of concern to the state but no water quality standard exists for a specific pollutant, or the water exceeds a state screening value or toxicity test." Given the anecdotal nature of the data submitted by citizens, Category 3B ("some data exist but are insufficient to determine support of designated uses") or Category 3C ("data collected by citizen monitoring or another organization indicating water quality problems may exist but the methodology and/or data quality has not been approved for a determination of support of designated use(s)") are also appropriate Listing Categories for the Shenandoah River for the recreational use designated use. Listing the Shenandoah River under Categories 3B or 3C would also be appropriate given DEQ's emphasis on obtaining additional objective water quality data. For example, the Integrated Report mentions a cooperative pilot study between DEQ and EPA to evaluate the spatial and temporal extent of algal growth in Virginia's non-tidal flowing waters in a quantitative and repeatable way, which will provide additional data prior to the next Integrated Report. Listing the Shenandoah River in Category 3B or 3C rather than Category 2B may more accurately reflect this focus of DEQ's efforts.

2. VAMWA Is Concerned Over Adequate Staffing to QA/QC Non-Agency Submitted Data, Given Increase in Non-Agency Submitted Data.

VAMWA is concerned about adequate staffing to evaluate and assign appropriate data quality levels to volunteer-generated data, given the unprecedented volume of such data used in the Integrated Report. We want to ensure that DEQ has sufficient funding and staffing to properly assess this increased volume of volunteer data. The Integrated Report indicates that this assessment cycle marks the highest-ever number of Level III stations included in an Integrated Report. Because Level III data is used for 303(d) listings and delistings, it is critical that this non-agency submitted data receive sufficient oversight. Forty out of the 120 citizen monitoring organizations who have submitted water quality data to the agency have been assigned as Level III status, with several such organizations submitting substantial volumes of data.

3. DEQ Should Require Submitted Data to Comply with 40 CFR Part 136 Analytical Methods.

DEQ's assessment program does not require the use of only 40 CFR Part 136 analytical procedures. However, the VPDES permit program does requires the use of these methods. See 40 CFR 136.1. Because data submitted under the requirements of DEQ's assessment program are used to help determine potential impacts to state waters and ultimately to help determine whether designated uses are being met (which may lead to a state water being added to the 303(d) list, and subsequent TMDL development with ultimate impacts on VPDES permittees), all data used to support such determinations should comply with the required methods. By explicitly requiring the use of 40 CFR Part 136 analytical methods, DEQ will bolster the legitimacy of its data collection and its use of such data for regulatory purposes.

4. VAMWA is Concerned about Potential Impacts from Substantial Increase in Total Stream Mileage.

The 2014 Integrated Report briefly notes a substantial increase in total stream miles (a 92 percent increase) relative to previously estimated reports. DEQ indicates that the increase in stream miles is due to improved precision and accuracy of its mapping tools, specifically its Geographic Information System data and a move to high-resolution imagery to conform to 1:24K USGS National Hydrography Dataset. VAMWA applauds DEQ's efforts to more accurately characterize the Commonwealth's waterbodies. However, given DEQ's limited discussion of this substantial increase in total stream miles, VAMWA is concerned about the public perception that may result from this substantial increase. As DEQ states, the increase in total stream miles means even more Virginia stream miles will be classified as unassessed. VAMWA supports increased explanation and publicity of this technical improvement so there is no public misperception that DEQ has assessed fewer state waters than previously reported. Additionally, VAMWA (and all interested parties) would benefit from a greater explanation of how these new stream miles fit within existing waterbody designations. For instance, do newly mapped stream miles substantially impact the boundaries of existing USGS Hydrologic Unit Codes? VAMWA assumes that all newly mapped stream miles will be listed as unassessed. However, for newly mapped stream miles in water segments that have already been assessed as impaired by DEQ, will the newly mapped stream miles undergo an independent assessment for impairment?

5. Minor Reference Issue.

In addition to the above comments, VAMWA also identified an errant reference that should be corrected. In Chapter 4.1, subsection 4 discusses Toxic Pollutant assessment. (pg. 44-45) DEQ references its 2014 Assessment Guidance Manual and Part VI, Section 6.5.3 as providing additional information on the "weight of evidence" approach. Part VI, Section 6.5.3 does not exist in the Assessment Guidance Manual. This reference may be more appropriately drawn to Appendix G of the Guidance Manual (Weight-of-Evidence Aquatic Life Use Assessment in Estuarine Waters).

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DEQ's Response to Virginia Association of Municipal Wastewater Agencies (VAMWA)

Comments:

VAMWA is concerned about adequate staffing to evaluate and assign appropriate data quality levels to volunteer-generated data, given the unprecedented volume of such data used in the Integrated Report. We want to ensure that DEQ has sufficient funding and staffing to properly assess this increased volume of volunteer data. The Integrated Report indicates that this assessment cycle marks the highest-ever number of Level III stations included in an Integrated Report. Because Level III data is used for 303(d) listings and delistings, it is critical that this non-agency submitted data receive sufficient oversight. Forty out of the 120 citizen monitoring organizations who have submitted water quality data to the agency have been assigned as Level III status, with several such organizations submitting substantial volumes of data.

Response:

During each assessment cycle, DEQ receives a large quantity of citizen volunteer and other non-DEQ monitoring data. Based on review of the protocols and procedures used, these data are classified into one of three levels. The highest level, Level III, comprises of submitted data that follows DEQ recognized protocols and uses quality assurance procedures that ensure the data generated is of the highest quality. Level III data are of the most value to the agency since they can be used directly to assess water quality conditions, without the need of additional agency sampling.

To ensure data meet the highest possible standard, groups are required to submit a Quality Assurance Project Plan (QAPP) and may be asked to provide additional relevant documentation such as calibration log sheets. The submitted data are independently reviewed by DEQ quality assurance staff. Questionable data are not included in the assessment report if their accuracy cannot be verified by the data submitter. This is consistent with the procedure DEQ follows for its own data.

Many of the Level III groups monitor basic parameters such as temperature and dissolved oxygen using standard field equipment. DEQ routinely verifies the accuracy and precision of this equipment if a group lacks the ability to do so itself. Verification of laboratory-derived data is done either through a VLEAP certified laboratory or by DEQ on a regular basis following similar VLEAP-auditing protocols. Finally, DEQ staff routinely assists with, reviews, and leads training events with volunteer groups to ensure the sample teams are following established methods. To supplement in-person training, DEQ is developing videos that will help train and refresh volunteers in proper calibration, maintenance, and sampling procedures.

Comment:

DEQ's assessment program does not require the use of only 40 CFR Part 136 analytical procedures. However, the VPDES permit program does requires the use of these methods. See 40 CFR 136.1. Because data submitted under the requirements of DEQ's assessment program are used to help determine potential impacts to state waters and ultimately to help determine whether designated uses are being met (which may lead to a state water being added to the 303(d) list, and subsequent TMDL development with ultimate impacts on VPDES permittees), all data used to support such determinations should comply with the required methods. By explicitly requiring the use of 40 CFR

Part 136 analytical methods, DEQ will bolster the legitimacy of its data collection and its use of such data for regulatory purposes.

Response:

DEQ will continue to emphasize the importance of coordination amongst all water quality management planning programs--watershed monitoring and assessment, TMDLs, and Virginia Pollution Discharge Elimination System permitting. Not only do stakeholders benefit when these programs work in concert, but the agency also has a vested interest in minimizing conflicts arising from different program requirements.

Per Virginia Regulations 1 VAC 30-46, all analyte data submitted to DEQ for regulatory purposes, including both permit-mandated reporting and 303(d) listing, must be from a NELAC-accredited laboratory. This ensures that only data of high quality are used. Because laboratory certification and auditing are the responsibilities of the Division of Consolidated Laboratory Services (DCLS), concerns about inconsistencies in analytical procedures should be directed to DCLS.

Comment:

The 2014 Integrated Report briefly notes a substantial increase in total stream miles (a 92 percent increase) relative to previously estimated reports. DEQ indicates that the increase in stream miles is due to improved precision and accuracy of its mapping tools, specifically its Geographic Information System data and a move to high-resolution imagery to conform to 1:24K USGS National Hydrography Dataset. VAMWA applauds DEQ's efforts to more accurately characterize the Commonwealth's waterbodies. However, given DEQ's limited discussion of this substantial increase in total stream miles, VAMWA is concerned about the public perception that may result from this substantial increase. As DEQ states, the increase in total stream miles means even more Virginia stream miles will be classified as unassessed. VAMWA supports increased explanation and publicity of this technical improvement so there is no public misperception that DEQ has assessed fewer state waters than previously reported. Additionally, VAMWA (and all interested parties) would benefit from a greater explanation of how these new stream miles fit within existing waterbody designations. For instance, do newly mapped stream miles substantially impact the boundaries of existing USGS Hydrologic Unit Codes? VAMWA assumes that all newly mapped stream miles will be listed as unassessed. However, for newly mapped stream miles in water segments that have already been assessed as impaired by DEQ, will the newly mapped stream miles undergo an independent assessment for impairment?

Response:

The expansion in stream mileage can be attributed to DEQ's adoption of the 1:24,000 scale resolution of the National Hydrography Dataset (NHD) in its water quality assessments. The NHD is maintained by the United States Geological Service (USGS), and it is considered to be the most accurate geographic representation of the country's surface waters. In the early 2000s, EPA launched the Watershed Assessment, Tracking and Environmental Results System (WATERS), which integrates the nation's surface water quality databases into a common geographic platform based on the 1:24K NHD, allowing for more consistent tracking and reporting. The conversion of Virginia's older 1:100K NHD to the higher resolution was accomplished in 2013 through a collaborative effort of federal and state agencies. EPA

put together a document illustrating how the move to higher resolution mapping can affect water quality characterization. This document can be found here: http://epadev.induscorp.com/waters/doc/p0340.pdf.

Much of the additional stream mileage is due to the inclusion of previously unmapped waterways—mostly small unnamed tributaries. These waters are counted as "unassessed" until they are monitored and properly characterized by DEQ staff. The non-impaired/impaired waterbody size estimates presented in the report are largely unaffected by the addition of these waters into DEQ's database. The mileage also increased due to the refinement of previously mapped stream courses and shorelines. But this had a relatively small impact on the non-impaired/impaired waterbody sizes compared to changes dictated by monitoring data.

DEQ does not anticipate significant changes to the resolution of its surface water mapping in the foreseeable future, though it is likely statewise stream mileage will decrease slightly as ephemeral streams are better distinguished from perennial waters.

Comment:

In addition to the above comments, VAMWA also identified an errant reference that should be corrected. In Chapter 4.1, subsection 4 discusses Toxic Pollutant assessment. (pg. 44-45) DEQ references its 2014 Assessment Guidance Manual and Part VI, Section 6.5.3 as providing additional information on the "weight of evidence" approach. Part VI, Section 6.5.3 does not exist in the Assessment Guidance Manual. This reference may be more appropriately drawn to Appendix G of the Guidance Manual Weight-of-Evidence Aquatic Life Use Assessment in Estuarine Waters).

Response:

Thanks for bringing this to our attention. The correction will appear in the final report.

Technical Review of Evidence to Determine the Presence, Extent, and Consequences of Excessive Algal Growths in the Shenandoah River and its Tributaries

Submitted to the Virginia Department of Environmental Quality on Behalf of Potomac Riverkeeper and Shenandoah Riverkeeper

David Sligh

January 30, 2015

I. Introduction

Shenandoah Riverkeeper ("SRK" or "Riverkeeper") has collected and analyzed a huge volume of information related to algal growths in the mainstem of the Shenandoah River and in the North and South Forks. This report presents findings and conclusions from this effort. The evidence presented, supported by hundreds of attachments and references, overwhelmingly supports the following conclusions:

A. Excessive algal growths in the Shenandoah River, North Fork Shenandoah River, South Fork Shenandoah River, North River, and South River interfere with and sometimes prevent human uses of these streams, including but not limited to boating, swimming, wading, fishing, and aesthetic enjoyment of the environment.
B. Excessive algal growths in the streams cited in A. damage the biological integrity and cause imbalances in aquatic communities in each stream.
C. Excessive algal growths in the streams cited in A. constitute undesirable and nuisance plant growths in each stream.
D. Excessive algal growths in the streams cited in A. result in the presence of floating mats of algae and decaying plant materials, color, odors, and turbidity in each of the streams.
E. The excessive algal growths and impacts described in A. through D. occur throughout the following sections: on the Shenandoah River, from its beginning near Front Royal to its confluence with the Potomac River; on the North Fork Shenandoah River, from its beginning near Bergton to its confluence with the South Fork; on the South Fork Shenandoah River, from its beginning near Grottoes to its confluence with the North Fork; on North River, from River Mile 4 to the mouth; and on South River, from River 4 to the mouth.
F. The excessive algal growths and impacts described in A. through D. have occurred on a persistent basis, throughout at least the period from 2007 to 2014, with variations from season to season and year to year. Impacts are most frequently observed in summer and early fall periods, when recreational users are most affected.

The succeeding sections of this document are as follows. Appendices A through H are attached
:

II. Citizen Reports
III. Expert Findings and Opinions
IV. Photographs
V. Transect Data
VI. Water Quality Goals
VII. Comparison of Data to Water Quality Goals
VIII. Conclusions
IX. References
X. Expert Testimony - Kelble
XI. Qualifications of David Sligh

II. Citizen Reports

Attached to this report are one hundred and twenty six (126) separate submittals from citizens describing conditions in the Shenandoah watershed and the citizens' responses to those conditions. These letters tell of algae-related problems in the River and the two Forks; many listing specific times and locations when they observed conditions caused by an overabundance of algae. Other citizen statements include observations gathered over wider time periods and larger areas and changes observed through the last several decades.

Most of the submittals are from people who use the rivers for recreational and aesthetic purposes, some of whom have done so for many years. Complaints from less frequent or newer users are also represented among the citizen statements. Those whose properties border one of the waterbodies are also well represented and have obvious economic interests that they believe to be affected by the degradation of the streams by excessive algal growths and die-off. Almost all of the commenters have long and intimate familiarity with one or more of the streams addressed and with the conditions that have been conducive to their enjoyment of activities in, on, and around the waters. Many explain in some detail the problems they have observed, the ways in which these problems interfere with their uses, and the areas and time periods affected.

A spreadsheet summarizing much of the information gleaned from the submittals is contained in Appendix A to this report and electronic versions of all of the submittals are submitted with this report. Some of the general patterns we can observe from the table in Appendix A are:

A. Numbers of comments addressing problems on the mainstem, North Fork, and South Fork are 61, 58, and 70, respectively.

B. Cumulatively, the complaints cite algae problems spanning the entire lengths of each of the three streams.

C. The numbers of comments citing specific uses that were impaired includes: Fishing - 102, Primary Contact Recreation - 44, Boating - 55, Wading - 40, General Aesthetic Enjoyment - 57.

D. The numbers of comments citing specific problems that impaired their uses includes: Periphyton, general - 31, Filamentous Algae - 55, Plankton and/or Floating Masses - 50, Color - 40, Odor - 60, Turbidity - 10, Health Concerns: Toxicity and/or pathogens - 19, Fish Lesions and Diseases - 26. Almost all commenters named more than one of the problems listed.

E. Thirty (30) of the comments specifically compared the conditions in the Shenandoah streams with those they have experienced in other waters and noted that the conditions here were worse than those in any of the other streams they have used.

In addition to the summary of comments described above, quotes from some of the comment letters are provided below, to provide a fuller sense of the facts and opinions included in the comments. The C# notations match those that are used in Appendix A and in the file names for the individual comments. The dates on the written comments are included, next to each person's name. The specific locations discussed in these comments are spread throughout the watershed and on all three of the major streams.

Quotes from Comment Letters

C1 - Alan Lehman (9/3/14)
"In the late afternoons and early evenings on late summer and fall days, floating globs of algae nearly fill the river at my house. This discourages me and my guests from swimming in the river, since it is extremely gross when it gets on our bodies and in our hair."

C2 - Alan Lehman (4/10/12)
"I've seen globs of floating algae on the back eddies and channels on the North Fork Shenandoah River in March and April of this and past years, near Woodstock, New Market, and Toms Brook. In May and June of each of the past few years, I've seen filamentous algae on the bottom of the North Fork near Toms Brook, and Strasburg, and also on the bottom of the South Fork near Island Ford, Elkton, Shenandoah, Luray, and on the Main Stem Shenandoah River near Morgans Ford Landing, Rt. 50 and Rt. 7 in Clarke County. I've seen the smelly floating algae on the North Fork in July, August, and September near New Market, Mt. Jackson, Edinburg, Woodstock, Strasburg, Toms Brook, and Riverton. This smelly floating algae is also persistent on the South Fork in late summer around Port Republic, Island Ford, Elkton, Shenandoah, Newport, Alma, Luray, and near the Andy Guest/Shenandoah River State Park in Warren County."

C3 - Allan Thomson (4/12)
"I have noticed that there is often in the spring and summer a slimy mat of algae covering the rocks and native grasses which makes the river not only unsightly but also hazardous to walk in. This is especially true in the North fork and the main stem north of Front Royal."

C4 - Amy Mrstik (9/16/14)
"As we approached the lower end of our trip, near Front Royal, we stopped so Rick could spend some time in an area he said looked fishy. But I noticed the area was full of dark green algae, and it smelled way worse than fishy. I didn't want to get into the water here because of the smell, so I took pictures of some wild flowers growing along the bank. Rick waded in but soon complained that his lure was getting full of algae and his favorite fishing shirt was getting stained green. I was never able to get that gunk completely out of his shirt."

C6 - Andrew Riccobono (4/13/12)
"I still regularly fish the for smallmouth bass and panfish at the Shenandoah River Andy Guest State park near Bentonville and my experiences from spring through summer have become alarmingly predictable. . . . By July my flies are covered in green muck after every cast – whether I am fishing on the surface or with a sinking lure. When the algae die off, the decomposing clumps smell pretty nasty."

C7 - Andrew Thayer (4/12)
"During that time on the river in April and early May, I witnessed something at the area known as Shenandoah Shores on the main stem below Front Royal. There were large clumps of green and brown stuff that were floating around. As I passed, the clumps had a sewage-like stench that could be smelled."

C14 - Bernard Griswold (4/12/12)
"I have had riverfront property directly on the North Fork outside of Woodstock since 1991. . . . During dry low water spells, planktonic algae also increases dramatically to the point that it covers and clogs grass beds from shore to shore. . . . Fifteen years ago, vegetation in this area consisted primarily of rooted grasses which provided cover and food for a variety of river creatures, especially from mid-June through early fall. Now, beginning in early June, rooted filamentous brown algae begins to coat rocks and rubble in pools and runs and increases by mid-August to provide floating clumps of brown gunk in such quantity as to collect in masses around any object at the surface. This has resulted, in recent years, in much reduced use of the river in late summer and early fall for all our activities and provides a real eyesore from our vantage point on shore. It also provides a severe odor problem during hot dry, low water periods in late summer."

C16 - Bill Millhouser (4/6/12)
"When these algaes are blooming, the fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I just cannot use the River due to the odors and annoyance. I found this problem in the following areas last year from July through August: Strasburg, Bentonville, Front Royal, Luray Dam, 211, Shenandoah, Route 50, and Route 7."

C24 - Charles V. Loudermilk, II (4/18/12)
"I have seen in recent years when on the river that the water has an odd dark greenish color that seems almost like it could glow in the dark to it in the spring. I have witnessed this just last weekend on 4-15-12 when I had floated from Rt. 50 to a takeout 4 miles downstream."

C25 - Charles V. Loudermilk, II (8/14)
"I can recall a float from Alma to Whitehouse on the South Fork in July of 2012 that there were section of the river that the algae was so thick that that my canoe in about 2 foot of water would get stuck. I had to use my oar to push myself off the long strings of algae in these sections. I would wade Edinburg area or should I say tried to wade this area. It was very hard to even get in the water and move around because the algae were so thick."

C31 - Douglas Lees (8/31/14)
" I fished the South Fork and Main Stem of the river this summer in July and August as follows: . . . July 20, . . . on the South Fork near Luray, catching no bass and no sunfish and noticing numerous clumps of foul-smelling algae—this section of the river smelled like an outhouse."

C41 - Elwyn "Chip" Comstock (4/10/12)
"The places I like to wade and fish are mile 13, 16 and up stream from Andy Guest Park. The past two years I've fished these locations less due to the fact that I not only find algae as mentioned above but I find fish that have abnormal growths on them. I typically fish these areas from May through September; however, I've begun seeking out other locations due to the poor conditions of the water."

C63 - Jeff Browne (8/25/14)
"Last week I was paddling up-river from the Hazard Mill landing in Bentonville (Hazard Ford Road) and for the first quarter mile you can see the devastation caused by algae as the river grass has been killed over time. The largest smallmouth bass I've caught on the river have been in that stretch, but no more. Now I just paddle through it in order to get to faster waters that haven't been hit as hard."

C64 - Jeff Little (4/9/12)
"The last time I visited the Shenandoah River to fish was last September near Pugh's Run on the North Fork I took my two sons to wade and fish where I have fond memories of catching feisty smallmouth bass. We spent about t})rte hours wading downstream, catching a few small bass, a far cry from my memories of this previously impressive fishery. The slippery filamentous algae that permeated the river bottom made wading treacherous for my sons. The "snot grass" coated their water sandals and when the decided to jump in further:, the rest of their clothes. It also made for frustrating fishing as each cast yielded a crop of algae that prevented our catching many fish."

C68 John F. Ehrlich (4/6/12)
"Finally in 2008, I decided to visit the low water bridge at Bentonville to see if the river quality had improved since I last fished here. . . . It was during a particularly hot period in July. I was shocked to see the amount of algae both upstream and downstream from the bridge. The slime that clung to the rocks was clearly evident and the noxious odors that l first noticed in the 1990s had become worse . lt is a sad epitaph for a river that so many considered one of the premier rivers in the Mid Atlantic."

C69 - John Holmes (8/27/14)
"I have a cottage on the North Fork near Woodstock Virginia and my sole reason for choosing this location was to have access to the river and the ability to enjoy the river.

. . .

I want to share a specific problem that occurred this year. In early July we had some heavy rain that washed out some of the algae that had been accumulating. I was at the cottage on the weekend of July 12th and was able to wade and fish. With that good experience I invited my partner's family of five ... two adults and three boys ... to come out the following weekend of July 19th and 20th. We and they had planned to canoe, fish, and swim. When we got to the cottage, we found the algae was back with vengeance. The swimming and tube floating were cancelled. We fished a little from the low water bridge but could not wade fish. Clumps of algae in the slow water near the bridge made it a stinky environment. The older boys asked what was wrong with the river, when they smelled the algae and saw the slimy floating clumps during a brief canoe trip."

C80 - Leslie D. Mitchell (4/12/12)
"I am a volunteer water monitor for Friends of the North Fork of the Shenandoah River, so I observe the portions of the North Fork near Strasburg on a bimonthly basis. . . . In recent years, I have noticed or been alerted to numerous and different types of algae blooms and observed thick algae growth on the river's surface and below the surface. The blooms I have observed have been in the summer and early fall of the year. Please see photos below of an algae blooms: 1) June 22,

2010 about a mile downstream of Deer Rapids Bridge; 2) Same location and date; 3) and 4) Bloom that occurred in the North Fork between Deer Rapids south of Strasburg and the Rt. 55 Bridge across the North Fork, northeast of Strasburg in July of 2011. The algae smells bad, is difficult to paddle through and creates an unpleasant recreational experience in general, especially as it causes one to wonder what it is that is causing these imbalances in the water, allowing this unusual algae growth to occur."

C81- Mark J. Frondorf (4/13/12)
"This past summer, I donated my time as a fishing guide to the Shenandoah River Rodeo that took place in Front Royal, VA. I was embarrassed to take major contributors out on the supposed crown jewel of Virginia rivers as rock snot coated every rock and eddy pocket on the river."

C82 - Mark R. Myers (4/5/12)
"When my wife and I were dating in the 1980s we found several locations along the North Fork of the Shenandoah in the Strasburg area where we would picnic, wade and fish. For a number of years we occasionally returned to these spots, such as the VA Rt. 55 crossing. When we visited that area in the summer of 2011, the river bottom and water conditions were drastically changed from a decade earlier. There was abundant 'snot weed' and a lack of grasses that were previously in the river. The river bottom was not visible and what was previously an attractive river for wading was not at all inviting. What should have been a pleasant outing remembering good times from earlier years turned out to be very disappointing and left us concerned that future generations will not realize what a lovely resource the Shenandoah one was."

C91 - Preston Lazer (8/27/14)
"I was both embarrassed and disgusted back in mid July when I took a guest for a first trip on the section of the South Fork from Karo Rapids to Front Royal landing. I thought this would be a great chance to show what a gem Virginia has! Instead, what I had talked up as "one of the top things to do in Virginia" turned into bewilderment at what had happened to our river. For much of the trip, it was just an exercise in frustration to fish because every time we retrieved a fly, it was covered in algae snot. Also, the stench was overpowering at times. . . . I am sorry to say that when one of my friends called to ask for advice on unique things to do in Virginia with his visitors from Denmark, I told them to go visit the New River in West Virginia rather than to float the Shenandoah so they don't embarrass themselves like I did."

C97 - Robert Forbes (9/17/14)
"After one South Fork fishing trip in July, 2014, when I got in my car, I noticed an overpowering odor of rotting material and thought the odor must have come from something decaying in my car. Then I realized the odor came from my shorts that had been immersed in the Shenandoah River while I was fishing!"
C99 - Rodney Miner (8/27/14)
"My most recent outing was July 12th when I floated and fished from Island Ford to Elkton. My friend and I saw lots of algae and the fishing was absolutely terrible. We saw dead fish lying on the bottom of the river and caught very few fish which is very unusual on this stretch of river. I caught two smallmouth bass with lesions on their sides. . . . I had planned to float the river

numerous times this summer but, when one sees these conditions you have to wonder how healthy it is to be in water when you see high levels of algae and dead fish."

C103 & 104 - Stan Ikonen (8/23/14 & 4/12)
"My group of two canoeists and one kayaker encountered a fairly significant bloom just last Sunday, 8/17, on the same stretch [the main stem of the Shenandoah between Shepherds Ford and the bridge at Rt 7. It was nasty enough that our teenaged female guest asked that we get out of the river as she was not comfortable with the floating algae. We stopped about halfway through our float. I hitched a ride to the takeout point to retrieve my truck and a nice day was ruined as the result of the algae in the water."

"Last June I canoed the South Fork of the Shenandoah from Bentonville to Front Royal with a group of friends. It was one of the most unpleasant experiences of the year. Worse than the record-breaking heat was the appearance, smell, and an almost slimy feeling of the water. It was disgusting. We stayed overnight on a sand bar. I usually take a swim before I bed down to remove the day's dirt and sweat. Not that night. I choose not to expose myself to the water anymore than needed."

C107 - Steven R. Adams (9/7/14)
"Just this past July 2014, on a float trip on the South Fork of the Shenandoah River, from Alma to White House near Luray, I encountered numerous stretches of the south fork with large amounts of algae. The algae smelled like something was rotting, it was slimy, and stuck to everything on my kayak and fishing gear. The algae also made the bottom very slippery and dangerous in places. Just trying to get in and out of my kayak was problematic."

III. Expert Observations and Opinions

In addition to experience as river users, 9 of the commenters listed in Appendix A have expertise in areas pertinent to the issues addressed in this report. The first group has extensive professional experience and expertise in fishing and river recreation and in the preferences of stream users. The second has expertise in water quality science, pollution investigations, water monitoring, and comparison of data to water goals.

Fishing and River Recreation Experts - The following listed commenters have many years of experience in outfitting and guiding fishermen and boaters on the Shenandoah watershed streams and on other waters.

C21 - Brian Trow
C26 - Colby Trow
C58 - Jacob Russo
C78 - L.E. Rhodes
C100 - Ron Evans
C102 - Scott Osborne
C114 - Trace Noel
Section X of this Report - Jeff Kelble

Because their livelihoods have depended on knowledge of the natural conditions in these streams, as those conditions relate to fishing success and the enjoyment of their clients, the expert opinions of these persons must receive extra weight regarding the streams' abilities to meet certain water quality goals.

They are qualified to give expert testimony about objective questions regarding the presence or absence of color, turbidity, floating materials, the extent of algal growths, odors, and the integrity and balance of the ecosystems in which they have worked. Subjective questions regarding the levels of algal cover and extent of other effects which rise to the level of nuisance or undesirable conditions and which have and/or will impair clients' enjoyment of their experiences are also within their areas of special expertise. As stated by Kelble (Section X):

> *What is MOST IMPORTANT about my life's fishing history and my professional career as a fishing guide was the fact that I made a living selecting the very best body of water in the Mid-Atlantic to take people fishing. This required that I have access to multiple sections of river, on multiple rivers in multiple states. My reputation and my success hinged on my ability to evaluate the physical conditions of the river including flow, water clarity and seasonal movements of fish to determine where I would take my clients through the ten month full-time season. (underlining added)*

Included below are some quotes from these experts' comments that are especially pertinent to the degrees to which the Shenanandoah and the North and South Forks provide pleasurable conditions for recreationists and the effects of algae on those experiences:

C21 - Brian Trow
"Half of the beauty of floating the rivers of our state is underwater. Looking into a river and seeing nothing but green water, brown and green rocks, and smelling the awful smells of rotting algae is very discouraging. We already have to deal with poor water quality that takes trophy bass from us every year, and now we can't even enjoy the beauty of looking into the river. . . . I guide and fish on many other rivers in the state including the James in central Virginia, The Rappahannok, the Cowpasture, and the New. All of these drainages have algae, but not nearly to the degree that the Shenandoah does."

C26 - Colby Trow
"We are on the river 12 months a year and almost daily in the spring and summer. We have 7 boats and run more float fishing trips on this river than any other fishing guide service. We do target the James River and New River for smallmouth bass and musky, however we consider the Shenandoah River our home water. Unfortunately claiming the Shenandoah our home water is becoming more and more embarrassing each year as we see constant algae blooms, fish kills, disease, foul smelling water, experience waterborne infections, and more. Some of our guests will not return to fish the Shenandoah or our area again as a result of what they see on the water."

C58 - Jacob Russo
"I fish and guide on the North Fork, South Fork and Main Stem of the Shenandoah. However, for much of the year, large sections of each river seems to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers. . . . Over the course of the year I use the entire river system and have seen this on all three rivers from Port Republic down to Front Royal, from Broadway to Front Royal and from Front Royal to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to swim or fish. When I do fish I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero."

C78 - L.E. Rhodes
"Over the years I have enjoyed spending time with family and friends as well as customers on the river. The algae problem has gotten bad enough that I am hesitant to take trips during the time of the algae blooms. It has a musky smell that takes the pleasure out of what would have been a great day on the water. Plus when fishing it is forever fouling in your hooks. I refuse to allow anyone to get in the water to wade or swim."

C102 - Scott Osborne
"I use the river extensively throughout the spring, summer and fall months for recreational fishing as well as professionally guided fly fishing services. Typically, I use the river 2-4 times a week during these months as flows allow for successful navigation of the river. . . . There have been numerous days that my clients were relatively disgusted by the incredible amounts of algae in the river and all of us knew it was the culprit for the slow day of fishing as well as the terrible smell. They did not even want to get in the river to cool off on the hottest of days. . . . I have fished all over the world, and the Shenandoah is one of my favorite, but only when it is not choked by algae."

C114 - Trace Noel
"As a retired outfitter on the South Fork of the Shenandoah with more than 20 years of daily and first hand experience I can speak directly to the impact that both phases of the algal bloom has on the river. . . . During the Spring and Summer large clots of algae break loose and head downstream. Resembling tumbling and floating human waste, these algal turds gross out urban guests, exasperate anglers, collect in slow moving water and leave a vomitus stench that diminish the experience by both private landowner and thousands recreational users. . . . The impact to the watersport recreation industry in the Shenandoah Valley – read economic loss to struggling rural communities - is substantial From float tubers to anglers with tangled lines our operation suffered diminished participation from urban guests who chose other ways to spent discretionary income."

The most detailed comments from an expert in the field of river recreation and fisheries come from Jeff Kelble (labeled C120 in Appendix A). Kelble's testimony is at Section X. of this report.

Water Quality Expert

The author of this report submits this document, attachments, and appendices as expert testimony on the matters addressed herein and has included information, including a resume, to support his status as an expert in the fields of water quality assessments, stream ecology, and pollution impacts.

Agencies

Because resource agency personnel have special expertise in the issues examined in this report, we cite two examples of agency opinions that bear on our assertions.

Virginia Department of Game and Inland Fisheries ("VDGIF") - On its web site, the VDGIF provides descriptions of certain segments of Virginia streams and of the fishing opportunities in these locations. One item on the web site reads as follows:

> *The North Fork is a relatively small, shallow river and is very accessible to wade angling.* ***Excessive nutrients in the watershed promote the growth of algae and aquatic plants.*** *The vegetation* ***can become very dense during the summer/fall months and impede fishing and boating****. (VDGIF 2014)(emphasis added)*

Virginia Department of Environmental Quality - In response to a citizen who reported the possible dumping of cow manure in the North Fork Shenandoah River, Don Kain, Water Monitoring and Assessment Manager in DEQ's Valley Regional Office investigated and responded to the complainant, in part, as follows:

> *I just returned from the river. The material in your photo was indeed still there. . . .* ***based on the appearance and odor (both definitely nasty), I think what we are seeing is decaying blue-green algae mats****. I took a trip down the river 2 weeks ago from Deer Rapids to Strasburg with Jeff Kelble specifically to evaluate nuisance algae problems. The material at Black Bear crossing looks the same as the mats we observed on that section of the river. . . .* ***By the way, these blue-green algae mats are quite often mistaken for sewage, due to both appearance and odor****." (Kain 2012) (emphasis added)*

IV. Photographs

Attached to this report are more than 1,000 photographs and 15 videos. These pictures show a great variety of different kinds of excessive algal growth, including planktonic species, algae attached to substrates and to vascular plants, and floating algal mats and decaying materials. The photos and videos are listed in Appendix B. Each has a designated number (A1, A2, etc.) and in almost all cases is identified by date, stream, and river mile. Through the photographers, the photos can be verified to be true representations of the actual conditions at the sites and times named. The electronic records for each photo shows that the images have not been altered. All of the photos and videos are included as attachments to this report.

Below are examples of certain types of conditions depicted by the photographs. The significance of these views in relation to water quality goals is discussed in Section VII below.

Highly Colored Waters

As described in citizen comments, at times stream segments in the Shenandoah and major tributaries appear to have a bright or dark green color throughout the water column - "like green paint," according to numerous witnesses. Examples of such conditions are shown in Figures 1 - 8. These eight photographs range in time from 2007 through 2014 and represent widespread segments from each of the three streams cover in this report.

Floating Materials

Figures 9 - 15 show scenes of floating masses in the streams, including algae and other plant-related materials, some in different stages of decomposition. Again, the photos range from 2007 to 2014. Most of these Figures are taken from those on the North Fork Shenandoah to show the wide array of appearances that occur in this one stream. Additional examples of floating materials in a variety of forms are shown in photos of the South Fork Shenandoah, which can be found in the attachments to this report, at: photos A659 (river mile 92), A634 (river mile 70), A595 (river mile 38), and A573 (river mile 18). Likewise, photos in the attachments show portions of the mainstem Shenandoah River with various forms of plant-related floating matter at: A453 (river mile 39) and A454 (river mile 38).

Stream Bottom Coverage

Many of the photographs attached to this report show benthic algal growths in the Shenandoah River and its tributaries. These photos show a variety of types of algae that are attached to bottom substrates, from filamentous forms to various low-growing brown and green forms that coat the rocks. Attached form of blue-green algae are present in very substantial amounts and in a wide range of locations. Figures 16 - 21 show the variety of forms and the density and extent of these growths at a number of sites. Overall, the photographs submitted with this report show hundreds of views of excessive algal growth spread throughout the lengths of the North and South Forks of the Shenandoah and on the mainstem. While still photographs are only capable of showing limited fields of visions, the videos show that the algae covering certain portions of the stream bottoms stretch over long distances.

In combination with the pictures and videos taken on these streams, from above and below the surface of the water, SRK submits many more aerial photographs which also show heavy growths at virtually every one of the hundreds of miles of streams photographed.

Algae and Vascular Plants

In some areas in the Shenandoah streams there have historically been healthy growths of underwater grasses and other vascular plants. In Figures 22 through 24 are photos of heavy algal growths on the surface of these vascular plants and throughout these plant beds. In some cases, it is evident from the photographs that dead and dying vascular plants have been covered by algal growths.

More discussion of this issue is presented in Sections VII.F. and X.

V. Transect Data

During 2012, personnel working with Riverkeeper conducted a systematic study to characterize stream bottom conditions in the Shenandoah River, the North and South Forks of the Shenandoah, South River and North River. This study revealed extremely high substrate coverage by periphytic algae in many areas during the months of June and July of 2012. These data are representative of patterns throughout stream segments where the transects were sampled and, given similarities in environmental conditions and observations between these segments and larger segments of the streams represented, are arguably indicative of the wider stream conditions.

The SRK researchers used a square frame of fixed area (see photograph A1027) at every sampling point and placed this frame at, generally, ten evenly distributed locations across a stream transect. They visually assessed the percent coverage of attached algae within the frame at each sampling point. The exceptions to the sampling of tens sites were made when, for example, the water depth prohibited sampling at a particular spot on a certain occasion. For each of the stream segments, which ranged in length from 3 - 6 river miles and wherever possible, given physical conditions, divided into transects 0.25 miles apart.

This method of transect sampling is similar to ones outlined in documents such as the Stream Periphyton Monitoring Manual (Biggs and Kilroy 2000) (See also: . The transect sampling program fits into the category described as a resource survey, which is designed to "establish general patterns of periphyton biomass and composition in time and/or space. Such data can then be used for desk-top assessments in discussions of possible changes to water resources/landuse management regimes, classification of waterways according to degree or type of human impact, etc."

Because the primary objective of this sampling was to see whether algae growths would qualify as nuisances or be termed "undesirable" by recreational river users, many of the aspects that might be important for other studies of benthic algae were not needed in this case. For example, while taxonomic descriptions of the types of algae present would certainly be necessary to meet the objectives of some studies, such information would be of little use here. The SRK study aimed to determine the overall nature and percent coverage of stream transects and the use of general descriptive terms such as "filamentous" or "thick mat" and notations of the color of algae, as contained in the monitoring reports (Appendix C), are fully sufficient.

Biggs and Kilroy (2000) list transect sampling under what they term as "rapid assessment protocols" and note that some such programs are "specifically designed for assessing compliance with the periphyton guidelines for cover to protect aesthetic, recreational and fishing values." (citing: Biggs 2000a). The following description of "rapid assessment protocol 1" by Biggs and Kilroy (2000) is a relatively accurate description of the methods used in the SRK study:

> *This method involves setting up transects across a site and recording the percentage cover of filamentous algae > 3 cm in length for a given number of quadrat points. Percentage cover values for the individual points are then averaged to obtain an estimate of the average cover of the site by*

filamentous green/brown algae. These individual records can also be used to later construct a map of the distribution of filamentous algae and, if repeated sampling is performed, then changes in the distribution of mats or patches of these algae can be traced over time. Such analyses, if combined with some physical measurements (e.g., shading, water velocities, depths and/or substrate composition), can provide useful insights into the primary factors controlling the local development of proliferations.

Aside from limiting the types of algae to filamentous forms of > 3 cm in length, the SRK program is entirely consistent with that described.

Figures 25, 26, and 27 show locations of the stream segments where the sampled transects are located within the Shenandoah River watershed. Researchers sampled three segments on the South Fork and two on the North Fork of the Shenandoah. The North Fork segments covered areas within river miles 11 - 17 and 83 - 86. The South Fork segments covered areas within river miles 18 - 21, 32 - 37, and 75 - 80. For the mainstem Shenandoah one segment stretching from river mile 22 to river mile 27 was monitored and for the North and South Rivers the segments covered river miles 0 - 4 and 1 - 4, respectively.

The results of the transect surveys are contained in Appendix C to this report and Appendix D shows spatial representations of the algal % cover results. Table 1 shows the mean values for percent cover by benthic algae for each stream segment and date monitored.

The results show particularly high mean values on the most upstream segment on the North Fork [NF RMs 83-87], with values of 31% and 35.1% algal coverage for all transects in late June and early July of 2012, respectively. Figure 28 shows a representation of these observations and reveals that, of nine transects where algal cover was measured in the July 12, 2012 sample run, almost all transects had very high percent cover across 60 - 100% of the stream's width. For transect 3, all measurements showed at least 70% coverage.

Table 1 also shows that in at least one sampling period for each segment, two areas on the South Fork [RMs 18-21 and 32-37] had especially high mean values. Likewise, the segment near the mouth of South River had an overall mean coverage of 30.8% on June 16, 2012.

While these mean values are of some value in characterizing conditions in these streams at certain times, more detailed views of the distributions of results are required, because mean calculations are not fully appropriate for situations like those we are trying to represent here. Mean values are most useful in understanding the nature of a sample population where the data are normally distributed, however the variability in stream substrates and other factors that affect plant growth in streams causes an inherent "patchiness" in distribution and a large degree of variability through time.

As explained by Hynes (1966):

A notable feature of plant communities is that they do not occur everywhere; there are nearly always bare areas due to scour, periodic drought or other factors, and the individual patches of plants expand and contract and move around. . . . This sort of impermanence is one of the

reasons for the rapid and often spectacular changes shown by the plant communities of running water. Undoubtedly the general stability of the river bed and the amount of fluctuation in current play and important part in the life of river weeds. . . . The algal community of rivers is essentially sessile, it grows on solid bodies and can develop only where these are present; in places where the substratum is soft mud it can grow only on weeds or hard parts of the bank.

As explained by Biggs and Kilroy 2000, "the degree to which our 'sample' represents the 'whole' of what we are interested in is a function of the number of samples we take in relation to the degree of variability (or patchiness) of communities or populations." To gain a true picture of the effects of algal growth on the Shenandoah streams, a more detailed examination of growth patterns than that which can be gained by looking at averages for entire segments is necessary.

For example, while the overall percent coverage in the most upstream segment in the South Fork on July 17, 2012 was only 18.1%, Figure 29 shows that certain transects within that segment had extraordinarily high degrees of bottom growth. Transect 6 has coverages of from 50 - 90% in 9 of the 10 samples taken that day, for an average of 63% coverage in this section of the stream. Also, in the North Fork on June 29, 2012, the overall percent coverage is 21.1% but in a number of the transects coverage was much higher (Figure 30).

Table 1

Stream Segment	Date	Segment Mean % Cover
Shenandoah R. [MS RMs 22-27]	June 20, 2012	2.9
" "	July 11, 2012	6.5
" "	July 25, 2012	2.5
North Fork Shen. [NF RMs 11-17]	June 15, 2012	23.2
" "	June 29, 2012	21.9
" "	July 16, 2012	25.2
North Fork Shen. [NF RMs 83-86]	June 26, 2012	31.0
" "	July 12, 2012	35.1
" "	July 26, 2012	0.4
South Fork Shen. [SF RMs 18-21]	June 21, 2012	39.0
" "	July 10, 2012	13.0
South Fork Shen. [SF RMs 32-37]	June 14, 2012	42.2
" "	June 27, 2012	10.5
" "	July 15, 2012	11.5
South Fork Shen.[SF RMs 75-80]	June 13, 2012	4.1
" "	June 28, 2012	10.2
" "	July 17, 2012	18.2
North River [NR RMs 0-4]	June 23, 2012	15.1
" "	July 9, 2012	11.5
" "	July 23, 2012	9.3
South River [SR RMs 1-4]	June 16, 2012	30.8
" "	July 2, 2012	16.7
" "	July 14, 2012	4.1

VI. Water Quality Goals

The water quality requirements set by the Clean Water Act ("CWA" or "the Act"), though necessarily interpreted in legal contexts, also reflect qualities by which scientists routinely judge the health of water bodies. Though terms used in the scientific literature may differ in some aspects from those used in the law, the concepts behind the terms used in the Act are consistent with those used by water quality scientists and ecologists.

Congress stated that the Act's objective is "to restore and maintain the chemical, physical, and biological integrity of the Nation's waters." This focus on the "integrity" of water bodies emphasizes the fact that for environments to be truly functional and sustainable they must be maintained so they continue to work as integrated systems. Those systems that most closely approximate "un-impacted" conditions (where there has been very little or no anthropogenic disturbance) are likely to have the highest levels of integrity. Biological integrity, for example, has been defined as "the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of the natural habitat of the region" (Karr and Dudley 1981).

The CWA also requires that uses, both human and ecological, must be fully supported and sustained to fulfill the law's purposes. As one authority has stated, "drawing a sharp line between the human and natural realms serves no purpose when our imprint is as ancient as it is pervasive" (Western 2001). Humans have evolved alongside natural features such as streams and can use streams to supply basic as well as recreational and aesthetic needs in ways that do not destroy the basic nature and structure of the systems. A healthy ecosystem will support reasonable, beneficial human uses and the impairment of such uses indicates that the integrity of that system is also likely to be impaired (Carlisle et al. 2013).

In sum, if a stream doesn't fulfill its purposes - as a sustainable home for plants and animals; a resilient whole, designed by time and ever-evolving to handle natural changes; and a resource suitable for beneficial human uses - then it lacks those characteristics that make it a "healthy" body of water. Virginia's water quality standards contain both narrative statements and, for some parameters, numeric measures of required quality. In this report we compare the narrative guidelines in the standards to conditions in Shenandoah River watershed streams and, thereby, decide whether these streams meet the kinds of technical measures that make them "healthy" streams.

The foundation of water quality standards is the designation of reasonable and beneficial uses, including the maintenance of healthy communities of plants and animals, that must be possible in each water body. Where necessary to support those uses, officials must develop specific measurable "criteria" to make it easier to know when there's a problem but, regardless of such technical analyses, the bottom line is the same - that the streams still be useable for reasonable purposes. The criteria must be measurable in some way (either quantitatively or qualitatively - or usually both) to be meaningful. Both types evidence are routinely and necessarily collected and used by scientists to assess water body health and each is fully valid, when applied in the correct context.

Virginia's water quality standards regulation contains a number of requirements that are pertinent to our study of excessive algal growth in the Shenandoah River system.

A. **Uses designated for all streams in Virginia include**: "**recreational uses**, e.g., swimming and boating; **the propagation and growth of a balanced, indigenous population of aquatic life**, including game fish, which might reasonably be expected to inhabit them; wildlife; and the production of edible and marketable natural resources, e.g., fish and shellfish." 9VAC25-260-10 (emphasis added).

B. "**State waters** . . . **shall be free from substances** attributable to sewage, industrial waste, or other waste **in concentrations, amounts, or combinations which** contravene established standards or **interfere directly or indirectly with designated uses of such water** or which are inimical or harmful to human, animal, plant, or aquatic life." 9VAC25-260-20.A. (emphasis added).

C. "**Specific substances to be controlled include**, but are not limited to: floating debris, oil, scum, and other **floating materials**; toxic substances (including those which bioaccumulate); **substances that produce color**, tastes, turbidity, **odors**, or settle to form sludge deposits; **and substances which nourish undesirable or nuisance aquatic plant life**." 9VAC25-260-20.A. (emphasis added).

D. "All surface waters of the Commonwealth shall be provided" a level of protection which maintains and protects "existing instream water uses and the level of water quality necessary to protect the existing uses." 9VAC25-260-30.A.1. (emphasis added).

To make a technical determination as to whether Shenandoah watershed streams meet these required levels of quality, the following questions are answered in this report.

✦Do these waters exhibit unnatural colors?
✦Do these waters exhibit unnatural odors?
✦Are there unusual floating materials present in these waters?
✦Are there forms of undesirable or nuisance plant growths in these waters?
✦Does the quality of these waters interfere with the recreational uses, including aesthetic enjoyment?
✦ Does the quality of these waters interfere with the maintenance of balanced, healthy aquatic communities?

VII. Comparison of Data to Water Quality Goals

The following sections A. through F. discuss the evidence of conditions occurring in Shenandoah valley streams, to answer each of the questions posed in Section VI. After the individual categories are addressed, the temporal and areal coverage of conditions is described.

In discussing its response to citizen complaints regarding excessive algae growth in the Shenadoah in 2012, the Virginia Department of Environmental Quality ("DEQ") has stated its views as to the nature of data that will be sufficient to determine whether a water quality impairment exists or not (VADEQ 2014b):

> *Waters that do not meet Water Quality Standards due to a pollutant(s) may be listed as impaired. "Pollutant" is defined in Federal law and either narrative or numeric water quality standards may be used to list waters as impaired. However, an "impaired" designation can only be made based on specific and objective monitoring data, in terms of location, extent, and duration, as well as an accepted, scientifically valid assessment method that compares monitoring data to water quality standards or criteria.*

While this statement acknowledges that violation of narrative standards may qualify a water body for an "impaired" designation, the Department's approach to the data citizens have submitted for excess algal growths seems to betray an unwillingness to do so. The assertion that "objective monitoring data" is required, when there are subjective aspects to the criteria in the WQS regulation, substitutes the staff's judgement for that of the State Water Control Board, the body empowered to establish these regulatory requirements. This failure to take the narrative requirements seriously is especially problematic, since the DEQ has so far refused to adopt the kind of objective (numeric) criteria they claim are necessary to control nutrients and algal problems.

Further, the Department's stance, that citizen observations of stream features that are readily and accurately assessed by human senses fail as "scientifically valid assessment method(s)," is simply technically and practically wrong. The agency derogates public comments as "largely anecdotal," despite the fact that many of those comments include specific descriptions of the problems encountered and the ways those problems interfered with human uses (and in some cases aquatic life uses-such as algal growths covering and replacing vascular plant beds).

In many cases, including a number of those quoted in this report, the citizens name exact locations where they've observed problems. (e.g.: C1 - "floating globs of algae nearly fill the river at my house," C68 - "slime that clung to the rocks was clearly evident and . . . noxious odors" at "the low water bridge at Bentonville"). In many other cases comments name a particular stretch that is commonly traversed by boaters and describe conditions with a significant degree of detail. Some commenters cite exact dates (see e.g.: C24 - "water [that] has an odd dark greenish color that seems almost like it could glow in the dark . . . on 4-15-12," C69 - the "weekend of July 19th and 20th [2014] . . . smelled the algae and saw the slimy floating clumps"), while others described longer periods of time within which they had observed algae nuisances on numerous occasions. Finally, some to the people who submitted testimony cited and quoted from the detailed fishing logs they maintain, in a demonstration of systematic data gathering (e.g.: C47).

While these types of citizen reports might be dismissed as merely anecdotal if there were only one or several of them and the commenters attempted to claim a specific sighting represented the conditions of entire streams, such is not the case here. Along with dozens of descriptions of specific problems at specific places and times, the statements include those by river users who describe long-term observations and are able to describe changes over time in some detail. Further, as noted above, the observations were primarily statements of fact, not assertions that commenters' scattered perceptions qualified them to make scientific conclusions for which they are unqualified.

It is very important to recognize that the type of monitoring that is most appropriate for any situation is determined by the nature of the subject under study and the degrees of precision necessary to make valid and usable findings. Virginia DEQ officials seem to assert that only persons with scientific training in the use of specialized equipment, in sampling of water, sediments, or fish, or in the conduct of benthic macroinvertebrate studies may contribute useful and necessary information for use in determining the quality and status of waterbodies. This position is not supportable.

Visual assessments of water bodies are used by all scientists, including those at the DEQ, and often provide data that are as or more important than the concentrations of pollutants or taxonomic identifications. As rightly noted by the DEQ, and as confirmed by the author's experience, when identifying the cause of a fish kill, "notations on conditions at a kill site and the affected species may often be as helpful to the diagnostician as samples sent to the lab." (VA DEQ 2002). The exact types of data that are pertinent to assess compliance with most the narrative criteria are ones that any water user can provide.

The existence of unusual color, odor, or floating materials in a stream do not require special expertise and the testimony of dozens of people, many who have frequented these streams for decades and with great frequency, is sound evidence of these conditions. Whether these same river users have avoided use of these waters or have had their uses impaired is a question that only they can answer. Whether algal growths are undesirable or reach "nuisance" levels are subjective questions but the evidence shows that there is close agreement amongst citizens of various regions who have been surveyed, authors in the scientific literature, regulators in other states and nations - and the dozens of frequent Shenandoah River users who have given their opinions to the DEQ.

The one question that does require scientific expertise to determine whether a portion of the water quality goals is violated, is whether "the quality of these waters interfere with the maintenance of balanced, healthy aquatic communities." Lay observations are of value here even here, especially when made systematically, but must be interpreted, along with other data, by experts before sound conclusions can be made. That recreational users' observations can be valid sources of information upon which to base scientific findings is clearly demonstrated by the common use of creel surveys by fisheries experts or census reports from birdwatchers by avian researchers.

As explained below, an examination of the evidence available to the State of Virginia in 2012, in light of the scientific literature on the nature of streams that exhibit the kinds of growth

described, supported a positive response to the question about the biological integrity of these streams as well as the other questions posed. There is no evidence, however, in the public record that the DEQ conducted such an analysis.

Over two-thirds of the comments accompanying this report were also submitted to the DEQ for consideration in the 2012 Integrated Report's preparation. As discussed in regard to each of the subject areas below in this Section, those reports were fully adequate to assess compliance with all but one of the water quality goals we identified in Section VI.

Because the evidence provided in 2012 supported designation of the Shenandoah River and its tributaries as "impaired" in 2012, the Department's failure to do so then and EPA's failure to override Virginia's decision are not supported by the technical record. Despite the fact that the citizen testimony should have met the threshold test for designation as "impaired" for recreational and aesthetic uses and should meet it even more strongly now, with additional statements in the record, SRK decided to provide the additional and extensive evidence contained in and submitted with this report. The expert opinions, photographs, videos, and results of transect analyses only amplify and make even more overwhelming the scientific and technical case, proving that excessive algal growths cause violation of at least six separate provisions of the Virginia WQS regulations.

The determinations for each of the conditions examined below depend on either objective or subjective evidence. Of course, scientists depend heavily on objective standards to assess the quality of streams and in almost all of the categories discussed here there are measurable, reproducible methods for making these determinations. Only in one of the categories, whether excessive plant growths produce "undesirable" or "nuisance" conditions, are subjective standards used. Even in this category, however, scientists routinely make such determinations, as demonstrated by the published literature.

This kind of common understanding and definition of terms displayed by water quality experts, even on matters where precise measurements may not be easily made, is not only possible - it is common. While any one individual's perceptions of what is undesirable or is a nuisance is subjective, the opinions of a group of people, such as water quality scientists, who are very familiar with a range of situations and who regularly exchange information and opinions within their field of expertise can be relied upon and used to make substantive decisions as to when problems exist and action is needed to address them.

Water pollution experts have recognized for more than fifty years that subjective terms were necessary to the definition of problems and protection of our water bodies. In the foundational 1963 work, "Water Quality Criteria," McKee and Wolf (1963) defined parameters used to determine when certain human uses were supported. Among their definitions:

> *To be acceptable to the public and the regulatory authorities, waters that are used for swimming and bathing . . . must be esthetically enjoyable, i.e., free from obnoxious floating and suspended substances, objectionable color, and foul odors. . . .*

Conditions of water quality that affect boating and esthetic enjoyment are . . . heavy growths of attached plants or animals; blooms or high concentrations of plankton; discoloration or excessive turbidity . . . .

McKee and Wolf (1963) also offer descriptions of the ways that so-called "inferential" or "circumstantial" information from citizens has historically been valued in making important decisions about water quality. When "non-technical" assessments are credible and pertinent, judges and citizen juries have often valued the opinions of "non-technical" people in such cases. One such case from 1937 is especially pertinent to the kinds of problems faced in the Shenandoah. As recounted by McKee and Wolf (1963):

In Albough v. Mt. Shasta Power Corporations (1937) 9 Cal. (2d) 751, 73 Pac (2d) 217, the circumstantial evidence of the growth of weeds, the foul odor that emanated from a pool, and the preferences of cattle and horses for other bodies of water were sufficient to cause the jury to conclude that the water was in fact polluted.

As to the effects of these changes in condition of the water body, the California Supreme Court, as quoted in McKee and Wolf (1963), noted that "[t]wo chemists were produced who testified that from a chemical analysis the water in the pool was fit to drink" but the Court also observed that "[v]arious witnesses for respondents testified as to the preference of cattle and horses for other fresh and nonstagnant water" and "[s]everal witnesses living on the pool testified that in the years since the diversion they have never seen cattle drink from the pool." The Court upheld the juries factual interpretation of the evidence.

Thus, as to matters of fact about whether real conditions in a water body in fact caused users (in this case cattle and horses) to avoid using the water for beneficial and desirable purposes, the subjective opinions of the users (the animals) as manifested in their behavior was determinative for the jury. That the Court upheld the factual findings of the jury in this case over the chemical evidence is not a rejection of sound, scientific methods. This decision simply shows that both the jury and the Court recognized that subjective qualities may be as or more important than those we can measure objectively, when suitability for certain uses is decided.

The authors of EPA's Water Quality Criteria, upon which Virginia's narrative standards are based, forcefully expressed the importance of those attributes the DEQ and the EPA have been asked to acknowledge and protect in this case. "Aesthetic qualities provide the general rules to protect water against environmental insults: they provide minimal freedom requirements from pollution; they are essential properties to protect the Nation's waterways." (U.S. EPA 1986, emphasis added).

After all, it is exactly these kinds of problems that motivated citizens to rise up and demand better protections and that led to adoption of the Clean Water Act. People complained not of parts per million of phosphorus or nitrogen but of water that smelled bad and was ugly; conditions where they were afraid or too repulsed by conditions to swim or boat.

In light of the high priority EPA apparently placed on these factors and the importance they hold for the general public, it is not credible to suppose that the Agency would have set a

criterion that was unusable - whose implementation would be "unscientific" and betray the dedication they'd shown to the scientific methods scientists had used in developing the many numeric criteria established in the same document.

Of course, water quality experts are not the only people who form common understandings about the subjective nature of resources and use common language as to the desirability of water bodies for recreational and aesthetic uses. The opinions of experienced fishermen, boaters, and guides and the ways they characterize conditions are part of a common understanding. The fact that dozens of river users quickly adopted the term "rock snot" to describe algal growths in the Shenandoah streams, shows that their perceptions could easily be summed-up in a term that could be understood by all.

As noted by Kain 2012, "blue-green algae mats" such as DEQ officials had seen floating in the North Fork Shenandoah River, "are quite often mistaken for sewage, due both to appearance and odor," both of which Kain described as "definitely nasty" People readily use descriptions of known entities and sensations to describe things they cannot quantify or precisely label. At a minimum, it seems that a water body where conditions are described as "nasty" by DEQ officials or one where citizens "often mistake" the products of heavy algal growth for sewage must meet anyone's definition of "undesirable" or as a "nuisance."

A. Unnatural Colors

Do these waters exhibit unnatural colors?

The perception of color is central to the basic human sense of sight and the vast majority of humans can readily perceive when the waters near where they live and on which they recreate are relatively "normal" or not. This is a question that can be answered objectively and, while certain types of electronic instruments can provide quantitative measures of color, the human eye is the most appropriate instrument for measurement when the uses to be protected are human recreational and aesthetic uses.

Whether the color present in the water column obscures the bottom and makes wading and swimming dangerous or scary is a question that the eye of the potential user must answer. Likewise, whether the water's color deters a potential user from fishing, because he or she cannot see a lure or the locations of habitat or fish underwater, is not a complex scientific question but one people who fish must answer. And people have answered these and other questions about how color affects their use of Shenandoah watershed streams. Forty of the commenters whose submittals are attached to this report specifically cited unusual colors in the streams as deterrents to their uses.

Kelble (Section X) notes that "when the planktonic/pelagic algae blooms in the river it turns a thick pea green color and fish become lethargic, they don't' find food effectively because they can't see and they reduce their feeding" and "when a planktonic bloom colored the water and decreased visibility there was no chance to see fish and narrow your search, observe their habitat or even to sight fish specifically to an individual fish."

Both scientists and members of the general public naturally compare the color of a stream they encounter to that in another part of the same stream or to a similar stream that is known to be in a relatively un-impacted state. The comparison of water body conditions with those in "reference" streams is a widespread and accepted method of assessing water quality. (See e.g.: U.S. EPA 2000; Dodds and Welch 2000) When conditions in a certain location are worse than those in one of these "reference" or "un-impacted" waters, then pollution problems may be assumed to be present - as long as the reference water body is truly close enough in type and underlying conditions to make the comparison valid.

It is true that, in some waters, organic materials or naturally-occurring minerals produce distinct colors. However, under natural conditions the water column of streams such as those in the Shenandoah valley streams have little or no color. The author of this report is aware of no stream in Virginia, or indeed in any part of the Southeastern or Mid-Atlantic regions of the U.S., where the kinds of colors shown in Figures 1 through 8 could possibly be considered to be "reference" conditions. In fact that these colors found in numerous locations in the Shenandoah watershed are not just marginally different from those in other streams in the region, they are startlingly different.

As stated in Section II, at least 40 of the comments received cited color in the water column as a problem that affected their use of the Shenandoah streams. That such colors exist, and over a wide range of areas, is easily determined by looking at Figures 1 - 8, which show conditions on the mainstem, at river miles 0, 22, and 39; on the North Fork at river miles 10 and 84; and on the South Fork at river miles 48 and 82.

Kelble's expert testimony describes color problems in "one of the worst sections of the North Fork . . . between Broadway and Timberville [NF RMs 83-86]" where he states: "Repeatedly our observations in this section of river has shown extremely off-color water, green from a nearly continuous planktonic/pelagic algae bloom."

That the descriptions of colored water given by commenters and those shown in the photographs submitted with this report match those from many other sources describing and warning of planktonic blue-green algae blooms, in the scientific literature, in news media, and in communications from government agencies (See Part F of this Section), can only lend added credibility and weight to complaints that were fully proven in 2012.

B. Unnatural Odors

Do these waters exhibit unnatural odors?

This is another question which can easily be answered with objective evidence and for which human senses are the best instrument of measurement. Note that, when detectable levels of odor are tested for in water and wastewater, a premier authority in such procedures cites "difficulties in testing for odor, including the fact that most odors are too complex and are

detectable at concentrations too low to permit their definition by isolating and determining the odor-producing chemicals." (APHA 2012)

This same authoritative reference, Standard Methods for the Examination of Water and Wastewater, proposes Method 2150B, "the threshold odor test," for determining odor thresholds in drinking water. Thus, in preferring human olfactory powers over laboratory methods, APHA (2012) verifies that people's noses can meet the definition of "an accepted, scientifically valid assessment method that compares monitoring data to water quality standards or criteria." (VA DEQ 2014b)

Sixty commenters specifically mentioned and described the odors they had encountered in using one or more of the Shenandoah streams. Many of the comments submitted with this report confirm the note in Kain (2012) that floating algal mats in the Shenandoah streams are often mistaken for sewage, due both to the odor and appearance of the mats. Some examples:

* "the clumps had a sewage-like stench" (C7);
* "numerous clumps of foul-smelling algae—this section of the river smelled like an outhouse" (C31);
* "this algae smells like sewage or rotting broccoli" (C16);
* "The algae had started to rot and the odor was horrible. It smelled like a combination of untreated human waste and a decaying body. The smell carried 1/3 of the way across the river; it took a long time for the smell to get out of my nostrils" (C60);
* "this algae piles up into giant greenish brown mats. The smell is horrendous as if a dead animal carcass was encased in it." (C26).

Some of those who have complained of such odors described specific ways in which their uses of the waters had been impaired or prevented:

* "I have a Labrador retriever that absolutely loves the water. He'll go in the river all year long to swim and drink. Many times, he'll stink afterwards from getting algae in his fur. I always have to give him a bath after taking him to the river. Sometimes he'll also throw up from drinking the river water." (C40);
* "the algae presents a foul odor (somewhere between sewage and a dead animal) such that you do not want to be on the river in a canoe or along the banks. . . . The older boys asked what was wrong with the river, when they smelled the algae and saw the slimy floating clumps during a brief canoe trip" (C69);
* "During the summer one unfortunately has to check first for the presence of green algae clumps to determine if the river experience will be worth pursuing. These clumps smell terrible and are a strong indicator for my family and me to avoid recreating on or in the river. (C74)

Apparently DEQ and EPA did not consider such a compilation of reports from river users submitted in 2012 to constitute valid or sufficient data against which to compare that part of the narrative WQS, which states "Specific substances to be controlled include . . . substances that produce . . . odors." As stated above, the presence or absence of odors is an objective matter. Dozens of citizens and a number of river recreation experts have complained of the odors and their complaints are supported by a DEQ official (Kain 2012). There are no evident reasons to

question the honesty of these many commenters nor are there reasons to think their senses of smell are defective. Therefore, violation of the WQS against odor in Virginia's water bodies is clearly proven.

C. Presence of Floating Materials

Are there unusual amounts or types of floating materials present in these waters?

This is another simple, objective question that is answered routinely by scientists performing stream studies or investigating pollution complaints. Both qualitative and quantitative measures can be used in this analysis and non-scientists who are avid and frequent river users are just as able to make conclusions, in many cases, as are technical experts.

The citizen testimony on this issue shows that commenters have observed unusual floating masses in the Shenandoah watershed streams on many occasions and in many locations. The expert opinions, both recreational and scientific, agree with the information provided by other river users. The photographs strong support the citizen testimony.

Further discussion or exhaustive presentation and analysis of these sources is unnecessary. **The answer to the question "Are there unusual amounts or types of floating materials present in these waters?" is a strong and unequivocal "Yes."**

D. Undesirable or Nuisance Plant Growths

Are there forms of undesirable or nuisance plant growths in these waters?

As stated above, and as noted by the Virginia DEQ (DEQ 2014b), findings of undesirable or nuisance conditions do depend on subjective judgements by humans. However, the DEQ's refusal to make judgements as to the presence or absence of such conditions cannot be based on a lack of reliable and defensible guidance and information in the scientific or regulatory literature.

The Department states in the current draft Integrated Report (DEQ 2014b), that "an 'impaired' designation can only be made based on specific and objective monitoring data." This assertion is clearly wrong. Virginia law sets a criterion that is subjective. To assert that a measure of quality against which conditions are to judged, whether legally or scientifically derived, may be set in subjective terms but that decisions as to whether water bodies meet that measure cannot validly be based on subjective evidence is nonsensical. Researchers are continually striving to develop standards of ecosystem health and water quality that are more easily measurable and reproducible. However, these efforts will always require subjective judgements.

The DEQ further states that "the terms 'undesirable and nuisance' . . . require interpretation" and implies that without "numeric thresholds" such interpretations may not be made in a way that is scientifically valid and defensible. "The fact that there is no widely

accepted, objective threshold by which "nuisance" conditions caused by excessive algae may be judged has certainly not deterred respected authorities in the field from using the term and declaring that "nuisance" algal growths exist under certain circumstances. An abundance of journal articles and contributions to scientific treatises demonstrate as much.

The following references are just a small sampling of published sources using the term and confidently defining or describing conditions that meet that threshold: Neil 1957; Horner et al. 1983; Lembi et al. 1988; Welch et al. 1988; Berlind 1992; Dodds and Welch 2000; Paerl et al. 2007; and Matheson et al. 2012; Stevenson et al. 2012. One representative definition, from Berlind (1992): "Algae levels can be considered a nuisance if the algae interferes with some aspect of recreational, commercial, or natural use of the river. This interference can be purely aesthetic or have some more tangible physical effect."

Regulatory bodies in numerous jurisdictions also have not shown the kind of timidity that the Virginia DEQ has exhibited. The Saskatchewan Ministry of the Environment has stated that "certain aquatic plants and animals can be called 'aquatic nuisances' when they become present in sufficient numbers to pose problems for people or animals using a water body or its surrounding environment." (Saskatchewan Min. of Envir. 2002)

By commissioning a study to assess the levels of algal coverage in stream beds that the public found unacceptable (Responsive Mgt. 2012), the West Virginia Department of Environmental Protection clearly signaled that the Department felt and accepted the responsibility of making regulatory decisions as to the levels of algal growth that were undesirable or rose to "nuisance" status." Likewise, personnel from the Montana Department of Environmental Quality cited the body of scientific literature seeking to define "undesirable or nuisance level[s] of aquatic life in a water body" and decided that "some type of assessment of the public's opinion on the matter is clearly warranted." (Suplee et al. 2009)

New Zealand's Ministry for the Environment released "guidelines for the control of undesirable biological growths in water (MfE 1992). These guidelines included nuisance plants (phytoplankton, benthic algae (periphyton) and macrophytes) and were provided for different waterbody types including lakes, rivers/streams and estuaries" in 1992 and again in 2000.

Having dispensed with the idea that subjective decisions as to whether Virginia's narrative criterion prohibiting levels of pollutants in waterbodies "which nourish undesirable or nuisance aquatic plant life" cannot be validly made, the judgement as to whether such conditions exist in the Shenandoah River and other major streams in the watershed is relatively easy to make. Using the body of evidence presented in Sections II, III, IV, and V of this report the answer to the question "Are there forms of undesirable or nuisance plant growths in these waters?" is clearly and undeniably "Yes." The analyses in parts A., B., C., E., and F. of this section (VI) support this conclusion in an overwhelming fashion.

To reinforce this conclusion even further, we refer to transect analyses described in Section V. of this report. As noted, this type of survey of stream bottom coverage by algae has been conducted by numerous parties.

After determining the percent coverage of various stream stretches, parties working on behalf of the West Virginia Department of Environmental Protection surveyed about one thousand individuals, for a population determined to provide a valid representation of all West Virginians 18 years old or older. (Responsive Mgt. 2012) While the West Virginia study broke responses down into categories, based on the types of activities for which respondents used rivers and other factors, the survey report found that, over the entire population of respondents, views with 26 percent bottom coverage were "unacceptable" to nearly half of respondents (49%) and concluded: "This suggests that waters with any more than a quarter coverage will be unacceptable to a majority of residents. (Ibid.) As one would expect, at higher percent cover levels those finding conditions unacceptable was also higher. Seventy-one percent (71%) of those surveyed found 39% coverage unacceptable; 87% found 47% unacceptable; and 90% found 65% bottom coverage by algae to be unacceptable.

The results of the West Virginia study are particularly suitable for comparison with conditions in the Shenandoah Valley, addressing streams and stream users from the same region of the country and with many similarities in environment, culture, and preferences. Therefore, the overall threshold derived by the West Virginia surveyors is appropriately compared to the transect sampling results obtained by SRK.

Given that the most precise level of bottom coverage averages that can be applied across all segments is likely at the individual transect level or at an even smaller scale due to variability in stream conditions, as discussed in Section V, the mean values for each of these transects have been examined to see how many are equal to or greater than a threshold value of 26% and of the higher percentage coverage levels. The results of this comparison show that at many points the stream bottom coverage greatly exceeds the 26% level.

Table 2 shows results by stream segment and date sampled and reveals that only one of the eight stream segments sampled for percent algal coverage, on the mainstem Shenandoah, failed to exceed West Virginia's lowest threshold level. In fact, for every other segment, on the North and South Forks as well as North River and South River, the higher percentage threshold of 47% coverage (at which 87% found views unacceptable) was exceeded a least once. These results, combined with the data discussed above, indicates that undesirable or "nuisance" conditions are present throughout the Shenandoah watershed. The fact that heavy coverage was not found in the mainstem during June and July sampling does not indicate that high percent coverage does not occur here, though, because the photographic and witness evidence proves otherwise. This absence, as well as the absence of high cover in some other segments sampled, seems more likely to be related to the time of year and/or other factors. For example, that the South Fork segment between river miles 32 and 37 had drastically different results between samplings on June 14, 2012 and June 27, 2012.

Table 2 - Stream Bottom Algae Coverage

Stream Segment	Date	# of Transects Sampled	# ≥ 26%	# ≥ 39%	# ≥ 47%
North Fork Shen.					
(RMs 11 - 17)	6/15/12	22	10	2	1
	6/29/12	23	6	5	3
	7/16/12	21	10	7	6
(RMs 83 - 86)	6/26/12	10	6	4	2
	7/12/12	10	6	4	4
	7/26/12	5	0	0	0
South Fork Shen.					
(RMs 18 -21)	6/21/12	9	4	4	3
	7/10/12	10	3	2	1
(RMs 32 -37)	6/14/12	9	7	6	5
	6/27/12	12	1	1	0
	7/15/12	11	2	1	0
(RMs 75 - 80)	6/13/12	5	1	0	0
	6/28/12	11	0	0	0
	7/17/12	12	4	3	1
Main Stem Shen.					
(RMs 22 - 27)	6/20/12	17	0	0	0
	7/11/12	9	0	0	0
	7/25/12	2	0	0	0
North River					
(RMs 0 - 4)	6/23/12	18	4	4	1
	7/9/12	19	2	1	1
	7/23/12	7	0	0	0
South River					
(RMs 1 - 4)	6/16/12	14	5	4	3
	7/2/12	16	4	3	1
	7/14/12	13	1	0	0

E. Interference with Recreational Uses

Does the quality of these waters interfere with the recreational uses, including aesthetic enjoyment?

When river users say they have decided not to use a river or that conditions interfere with their traditional and habitual uses of the waters, then their testimony must be respected as a statement of fact, unless there is reason to believe their representations are untrue. More than 120 users, 8 of whom are river recreation experts, have testified that stream conditions related to excessive algal growth have interfered with their uses or eliminated them altogether.

All of the evidence discussed in section A. through D. above must also be considered in answering this question and must compel a positive response. "Noxious" and "nasty" odors, colors that make it impossible to see the river bottom to wade or fish, and benthic coverage by attached algae that greatly exceeds criteria, based on scientific surveys, to term waters unacceptable for use, floating masses of decaying algae - there is no rational basis to dispute that these are conditions that would deter most people from using and enjoying a river.

Further, these are exactly the kinds of algae-related problems that have been universally described in the scientific literature. While the author could quote from reference after reference from the list in Section IX of this report, such an exercise seems unnecessary.

However, one additional issue that has not been previously discussed is pertinent here and important to address. Heavy amounts of blue-green algae have been found throughout the Shenandoah River, both in phytoplankton and in attached algae. Just one source of evidence is found in Appendix H to this report. The images included there are satellite images in which spectral reflective signatures of several substances in the North Fork Shenandoah River are shown. These images indicate concentrations of chlorophyll and phycocyanin (the pigment in blue-green algae or cyanobacteria).

The results of the spectral imaging show that, not only were the blue-green algae/ cyanobacteria present throughout the 70 miles of the North Fork we evaluated, it was present at high levels. In comparison to the chlorophyll analysis we did, the values for phycocyanin, which is the surrogate for blue-green algae/cyanobacteria were often higher than chlorophyll. Blue-Green algae/cyanobacteria negatively affect the ecosystem, present a potential danger to river users if they are developing toxins, and diminish peoples' use and enjoyment, because they almost always lead to the kinds of results described in parts A. through D. above.

Beyond these physical and ecological impacts, blue-green algae are a deterrent to use of water bodies where they are found to "bloom," because people rightly fear that toxins may be present. While not all forms of blue-green algae produce toxins and even where those that do produce them high levels are not necessarily found at any one time, the threat exists, and the uncertainties make it even harder for citizens and officials to react safely and appropriately to blue-green blooms.

SRK has obtained lab results for samples collected from the Shenandoah River and both Forks in April and May of 2014 (Appendix I), showing that at least two types of potentially toxicity-producing cyanobacteria are present in the Shenandoah watershed. The laboratory reports for these samples state, in part:

> *Microscopic observation of the . . . Farmers Mill sample collected on 4/18/2014 revealed the dominance of the filamentous cyanobacteria Phormidium cf. favosum. Phormidium autumnale and P. favosum share many morphological traits and are mainly separated based on habitat, slight differences in average trichome width and frequency of sheath formation. P. autumnale is described from mesotrophic to eutrophic streams and rivers, and P. favosum mostly from cold, flowing waters on limestone substrates. The trichomes observed in this sample fit the description for P. favosum. Phormidium autumnale and Phormidium favosum are both potential anatoxin producers. Recommendations: Toxin analysis for anatoxin is recommended at this time.*

Based upon the laboratory's recommendation, samples were analyzed for toxins but found no detectable concentrations. Subsequently, testing has been done by personnel from the U.S. Geological Survey ("USGS") and detectable amounts of microcystin toxins have been found.

F. Interference with Aquatic Life Uses

Does the quality of these waters interfere with the maintenance of balanced, healthy aquatic communities?

The determination as to whether stream conditions in the Shenandoah River and its tributaries meet the requirement of supporting "the propagation and growth of a balanced, indigenous population of aquatic life, including game fish, which might reasonably be expected to inhabit them" is exactly the kind of scientific inquiry that stream ecologists make on a routine basis.

The Virginia DEQ has recognized that excessive algal growths may lead to certain impairments such as low dissolved oxygen and fish kills. However, the Department has failed to acknowledge a widely-recognized fact - that the presence of excessive and unusual growths of aquatic plants, including algae, represent an imbalance in the local ecosystem even if the known follow-on impacts are not present or measurable. The nature of algal populations in these streams can be compared to those in streams that are minimally affected or unaffected by high nutrient inputs and the extreme densities of certain types, such as those presented with this report, are not typical of "normal," or "un-impacted" streams in the region where the Shenandoah watershed lies.

"Blooms" of planktonic algae or very large populations of attached or floating algae are often the first step in producing the severe chemical and biological results. Whether the subsequent steps in degradation of water quality will result cannot necessarily be predicted based on the present of the blooms or excessive growths alone, because many other factors affect these outcomes. In fact, Voshell et al. 2000 stated, after they performed benthic macroinvertebrate

sampling throughout the watershed, that while conditions in the larger rivers were not yet affected in the same ways as numerous smaller streams by heavy nutrient loads that those larger streams would be so impacted if nutrient pollution continued.

That the scientific literature is replete with descriptions of the progressions that can occur, from heavy nutrient pollution, to excessive algal growth, to a plethora of outcomes is indisputable. Once of the prominent changes that are intimately connected with the changing populations of algae, in both density and diversity, is the change in vascular plant health and populations, which can have cascading effects on benthic animals, on nutrient cycling, and on sediment washout patterns in-stream.

Example sources discussing this type of effect are:

Balls et al. (1989), explaining that in response to "very large" crops of phytoplankton "submerged plant growth may dwindle, with subsequent loss of the plant beds" and noting that "this represents a major change of structure in the ecosystem;"
Irvine et al. (1989) noting that great increases in nutrient inputs to freshwater systems frequently lead to "a switch from dominances by submerged plant communities to dominance by phytoplankton and that "the mechanism of this switch is generally seen in terms of a set of relationships between nutrient availability and competition between the plants and the algae" (internal citations omitted); and
Brönmark and Vermaat (1998) "Eutrophication of shallow freshwater . . . ecosystems has often resulted in a drastic decline in the areal extension and biomass of submerged macrophytes and a concomitant increase in the biomass of phytoplankton. Light availability is usually the most important factor determining the distribution pattern, biomass, and production of submerged macrophytes and it has been suggested that increasing phytoplankton biomass due to higher nutrient input results in a reduction of available light to a level at which net photosynthesis by submerged macrophytes is impossible)." Other researchers suggest "that macrophytes may disappear even when the bottom is within the euphotic zone" but "increasing nutrient levels stimulate epiphyton growth, which has a negative effect on the macrophyte host through shading and competition for nutrients." (internal citations omitted)

Temporal Extent of WQS Violations

The problems with excessive algal growths and the consequences have persisted in the large streams within the Shenandoah River watershed on a yearly basis since 2007 or before. This window matches the stated coverage period for Virginia's 2014 Integrated Report.

Criteria which are designed to protect against negative impacts should have three dimensions:

* level of severity of a condition to avoid problems (e.g. concentration of a pollutant),
* length of occurrence allowed (how long can the condition exist for any one period?), and
* frequency of occurrence (how many times can this recur over a period of time without uses being impaired?)

The narrative criteria/general standards set in Virginia's WQS do not specify time components (either length of one occurrence or recurrence of key conditions). Therefore, these features of the WQS must be interpreted such that the conditions named are prohibited - "nuisance" or "undesirable" conditions due to algae may not be created, unnatural odors and colors may not be caused, etc.

The lengths of time that any problem algae growths exist and the locations where they are found are extremely hard to predict, because changes in stream flow, temperature, sunlight, and any number of other factors can cause accumulations to form and be dispersed. The key is that, if these excessive growths occur even once, the baseline conditions (amounts of nitrogen, phosphorus, etc.) that were needed to produce that "bloom" are very unlikely to disappear without intervention. Therefore, if algal problems severe enough to produce the kinds of narrative violations described herein have occurred at any time and are proven, then a designation of impairment is appropriate. Further, the creation of a situation where people are unable to use their wasters, even once, must be investigated and measures must be taken to prevent additional episodes.

In light of this analysis, the frequent and long-recurring excessive growths of various types of algae in many places in the Shenandoah watershed definitely meet the temporal requirements for an "impaired" designation.

Areal Extent of WQS Violation

The complex matrix of various algae-related problems identified on various sections of the Shenandoah River, as well as the North and South Forks, and other tributaries presents a picture of extremely heavy infestations of the mainstem, the North and South Forks, and North and South Rivers. While not all segments are plagued to the same degrees or at the same times by any one of the problems described and proven herein, more than one of the seven areas of violation of Virginia WQS is shown to reach nearly every river mile of the three larger streams. Because significant data is presented here for only the 4 miles nearest each of the mouths of

North River and South River, a judgment as to the degrees to which the remainders of these two Rivers are in violation of WQS would be premature.

To substantiate the extremely wide coverage of these streams by the variety of problems cited above (1-colors, 2-odors, 3-floating materials, 4-undesirable or nuisance growths, 5-interference with recreational uses, and 6-interference with aquatic life uses), series of maps have been prepared to represent the areal extent of just a limited sampling of the findings from the various assessment methods.

Although the evidence submitted in this report and attachments does show an extraordinarily widespread occurrence of algal problems on the stream segments where impairments have been found, it should be noted that standard proving the areal extent of problems required to designate larger stream segments applied to this survey effort is much more demanding than that applied in the DEQ's and the EPA's normal process for making "impaired" designations. Given that the excessive algal growths are biological indicators of stream health, in a way that can be compared to the representative nature of benthic macroinvertebrate surveys, similar rationales for setting the boundaries of "impaired" segments should be sufficient here. Benthic sampling is generally conducted on just one or a few sites within a relatively large stream reach and are held to represent conditions well beyond those sites. For example,

The Department's method for designating "Nutrient Enriched Waters," which the DEQ has followed in some circumstances, is just as strongly supported in these waters. Section 9VAC25-260-350. of the VA WQS regulations designated four waterbodies as "nutrient enriched" and in three of these four named waters, the dowstream bounday of the segments is set while the upstream extent of the waters affected is listed as reaching "all tributaries to their headwaters." Such an approach is technically and practically jjstified in the regulation and is just as well supported for the waters draining to the Shenandoah River. At least some of the contributors of the condtions causing excess plant growths can be expected to reach to any upstream waters where the nature of the streams and the influences exerted on those streams are similar.

Findings as to the factors producing the excessive growths in Shenandoah watershed streams are beyond the capabilities of the studies so far completed. Therefore, measures to solve theses problems cannot possibly be designed at this time. Such determinations cannot be made with any degree of scientific validity and are not properly addressed at this stage of the regulatory process in any case. Despite this fact, Virginia officials have asserted that pollutant allocationns and controls mandated under the Chesapeake Bay TMDL may be adequate to address problems in these local waters. Such as assertion is unsound for a number of reasons.

First, and most obvious, is the fact that those Bay-related allocations were derived for the major tributaries to the Bay are made to address conditions in the estuarine waters of the Bay and those tributaries. The allocations that are applied to the various upstream waters in each of these major stream basins were then applied to upstream waters in a way that takes no account of the characteristics of upland and headwaters streams. In some instances, these basin allocations were then translated into required load reductions on a county-by-county basis in Virginia, based on the relative estimation of inputs from the various local areas and on the perceived

opportunities for reducing those pollutant amounts, based on known and estimated pollution sources. It is simply scientifically unsound to propose that such methods, which are based on large-scale modeling, could necessarily result in any significant improvement in specific headwaters streams, such as those we address in this report.

Second, it is universally acknowledged that the suite of factors needed tol protect or restore waters subject to excessive growth will require examination of the particular characteristics of those streams. This is exactly the rationale used to avoid the setting of criteria for nutrients and sediments in the free-flowing waters of Virginia. The necessity of setting criteria with due consideration of regional conditions, including typical "background" conditions, hydrologic conditions, soils, stream flows, and other parameters has led EPA to recommend the development of criteria on ecoregion, or even sub-ecoregion bases, with the understanding that only such suitably tailored criteria are scientifically valid for local waters. (EPA ecoregion doc.)

Likewise, the Academic Panel tasked with recommending criteria for nutrients in Virginia's upland waters has recommended measures for finding waters to be impaired or un-impaired, suggested different levels for each of four hydrogeologic provinces of the state upstream of the coastal plain. (Academic Panel report). Streams in the Shenandoah watershed arise in and flow through three of these provinces: with streams arising from the Blue Ridge on the east, the Appalachian Plateau on the west, and the central part of the watershed, which lies in the Valley and Ridge province. Given that such variability exists across the Shenandoah watershed, reliance upon allocations from the Bay TMDL, which fail to account for these differences in any detailed manner would be irresponsible and scientifically unsupportable. If Virginia officials though otherwise, it would seem that the State would be confident in seting numeric criteria based upon the Bay allocations but, of course, this has not been the case.

Third, the Bay TMDL and Virginia's implementation plans allow for permitting of discharges for facilities that exceed Bay-protective allocations to meet their goals through pollutant trading. In this way, facitities or activities may exceed allocations in one part of the Bay watershed where credits from load reductions in other parts of the watershed are to be achieved. (VA imple plans) This aspect of the Bay cleanup plan invariably leave some loal streams without the protections supposed to result from the Bay TMDL. In fact, SRK has identified local streams where high pollutant loadings will continue unabated, because dischargers have bought credits from supposed load-reducers far away from the local environments we seek to protect.

VIII. Conclusions

A. The level of information provided by citizens and SRK is, and was in 2012, more than sufficient and technically valid for making conclusions about the nature of impairments related to excessive algae growth in the Shenandoah watershed.

B. The failure of the DEQ to develop methods to measure whether a water quality goal, such as the prohibition of discharges that result in nuisance conditions is inexplicable and does not conform to professional standards. These water quality standards, with the subject language, have been in force for approximately forty years - the author must ask what, if any, specific measures the DEQ has contemplated during that long period and why the State has failed to act before now.

C. The DEQ's reluctance to value and make decisions based on evidence, such as citizen observations of issues well within the ability of the general public and the Department's failure to take any account of odor and color evidence, of which even the Department's personnel are well aware, is not a defense of valid scientific methods, as officials seem to suggest. Rather, it is a rejection of valid and appropriate assessment methods that are perfectly, and sometimes uniquely, suited to find the answers that are being sought.

D. The evidence shows that the conditions that are prohibited in Virginia WQS which are analyzed for the Shenandoah watershed streams (listed in Section VI.A.- D.) , every one is exceeded, frequently and over large areas in the major streams. By any one of these measures, the Shenandoah River, the North Fork Shenandoah River, the South Fork Shenandoah River, and 4-mile segments of each the North and South Rivers are impaired and should be designated as such by Virginia and the U.S. EPA.

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X. Expert Testimony from Jeff Kelble

Statement of Jeff Kelbe
January 28, 2015

I am submitting this document for inclusion in the comments presented to the Virginia Department of Environmental Quality (DEQ) regarding the 2014 Draft Integrated Report, on behalf of Potomac Riverkeeper and Shenandoah Riverkeeper. I will include my observations about some particular problems I've observed in the watershed, because I believe some of them may not have been as well explained as some other problems by algae. I also believe that there I am better equiped to address these issues than anyone else I know of. After those descriptions and opinions, I describe my background and the way I came to be an expert on fishing, fish behaviours, and the environment of the streams in the Shenandoah, as well as many other streams. Because I made my living by knowing about these things, I depended on all of the training and information I'd received, both from others and through my determination to teach myself.

During my career as a heavy user of the Shenandoah and other mid-atlantic rivers, with a pattern of use heavier than any other known individual, and as a professional fishing guide for nine years, I became extremely familiar with the seasonal rhythms of our rivers. The quality of the fishing trips I was able to provide hinged on the river that I chose. One of the primary factors for choosing both the river I fished and the stretch of that river was the physical condition of the river. Smallmouth bass are residents of our Mid-Atlantic rivers. They are always there. They are also generalist predators so for much of the year they occupy most or the river from bank to bank, along nearly every mile. They also feed almost every day between March and November. So the biggest driving force to catching fish was the ability of the fish to see your lure/bait, and the ability for you to make an unimpeded presentation.

First though I want to make and overall statement. The environment for fish and for people in most parts of the Shenandoah Mainstem and the North and South Forks has deteriorated greatly since I started fishing here and the effects of an explosion of algae are very serious and destructive. As DEQ and EPA officials, I have been petitioning to have these rivers listed as impaired for some years now and I am frustrated that the agencies have not used the information I and others have given them. I renew my request that the listing be made now.

Comparisons with Other Streams

As a professional fishing guide I used four stretches of the Potomac River, three stretches of the North Fork, three stretches of the South Fork, three stretches of the main stem, three stretches of the Rappahannock, one stretch of the Rapidan, two stretches of the James River, three stretches of the Susquehanna River and five stretches of the New River. It is widely accepted that my guiding business was unique in that I made a living as a smallmouth bass guide through all four seasons. There was no other person who spent as much time on these various bodies of water.

I can say, without question that there is no other river, stream, or lake I have observed which even approaches the Shenandoah River in the temporal and spatial coverage of algae, nor has any

river ever achieved the severity of bloom that the Shenandoah Rivers experience. I have even fished in rivers with heavy populations of people like the Rappahannock River and Potomac River downstream of towns and cities like Fredericksburg, Culpeper, Warrenton. The Rappahannock and Rapidan, while heavily affected by sediment pollution has never exhibited heavy algae growth of any kind.

The Potomac River, with the exception of the waters downstream of where the Shenandoah pours into it, has occasional algae blooms. Except in the areas downstream of the Shenandoah influence these algae blooms are light and sporadic. The grasses in these areas still predominate and are healthy and lush. I have never observed the algae interfering with fishing. However in the areas downstream of the Shenandoah the algae can be extremely prolific and does affect fishing. I have observed and other guides have corroborated that when the Shenandoah has algea blooming in it the fish in the Shenandoah-influenced water are lethargic or absent. The affects of this can be observed down to Swains Lock Virginia downstream of Violetts Lock. In these areas the same colonized algaes form in the summer after one or two months of planktonic/pelagic bloom.

The New River downstream of Radford and the Arsenal have occasional light blooms of colonized algae but I would estimate that the river sees 1-2% of what the Shenandoah River sections see.

The Susquehanna Downstream of Harrisburg and the farming areas of Lancaster has the most algae I've seen on any other Mid-Atlantic River, but doesn't approach the degree or depth of algae that the Shenandoah produces.

<u>Concerns with the Upstream Reaches of the North Fork</u>
Access is very limited on the Upper North Fork but we spent a significant amount of time observing sections we had access to. One day, day for example, I followed the algae bloom upstream into Broch's Gap, up the North Fork, up Fulks Run and up to Hopkins Gap. Algae was heavy all the way upstream until I found a place where the stream flowed out form between the cobblestones just downstream from a poultry operation. There was heavy algae that high up the river and filled the water column.

One of the worst sections of the North Fork is that between Broadway and Timberville. Repeatedly our observations in this section of river has shown extremely off-color water, green from a nearly continuous planktonic/pelagic algae bloom. During low flows in the summer this stretch is literally choked with algae. It's so heavy that the turtle carapaces are often completely covered with algae. River users under the Route 42 Bridge have complained to us during our investigations and have thought the blue green clumps on the survace were actually raw sewage from the Cargill/Pilgrims/ Broadway discharge.

<u>Algae's Impacts on Underwater Grasses in the Shenandoah Watershed</u>
I have taken specific interest in these kinds of impacts, because the process has been quite visible and disturbing. I know that a number of commenters mentioned such concerns and that quite a few of the photos in our collection show dead and dying grasses covered by thick algae coatings.

For example, for the section of river between Route 50 and Lockes Landing on the Main Stem Shenandoah, grasses predominated during the period between 1994 when I started using this float and 2002 which is the last season any substantial grasses were observed. During 2002, the river was so lush with grass which grew in nearly the entire 16 mile length from bank to bank. That year the majority of flow of the river existed under the shade of the tree canopy along the bank.

During those years small fish and bugs found tremendous refuge in the grasses. The spaces in between the grasses were full of the predatory fish. Very little algae was ever present during the lush periods of grass growth. I did notice during the very last year of 2002, that during the lowest flows many of the grasses began to be covered by algae growth during the end of the summer and very low flow. But the preponderance of growth was grass. However, that year marked the last time grass was observed in any quantity and now what we have from year to year are small vestige patches at times miles apart. These grasses emerge in late May only to be overwhelmed by algae which grow on top of the grasses causing the leaves to fall off. What remains are grass stems.

I have made the exact same observations regarding the North Fork between Deer Rapids and Strasburg except the algae took over earlier. During the peak of the drought in 1999 I recall the VDGIF predicting that there would be a full wipeout of the fish population in the North Fork if the algae in the river died before a substantial flushing event. I fished the North fork a number of times during that period and could not believe the extent of algae growth. There were places where the algae was three feet thick and it filled up the water column in the slowest areas. Blue Green Algae's predominated but there were also filamentous greens that both covered the bottom AND filled the water column in the slowest flowing backwaters. Fish went nearly dormant intheir feeding. On approximately September 7th a tropical storm flushed the river with a 7 foot rise. I fished three days later and the river was clear of algae but there were huge piles of it on the bridge pilings and on everything stationary along the banks including rocks, trees and tree limbs. The fish were literally ravenous and had begun feeding again. I was with professional guide Lou Giusto who specialized in the North Fork Smallmouth and we noted that every cast for several hours we hooked a fish and there would be up to a dozen starving fish following the hooked fish to the boat hoping to pick up regurgitated scraps.

Since then this section of the North Fork has been one that I spent a lot of time on fishing, guiding and observing. Each year the grasses and the algae engage in a battle for space and dominance. Most years now the algae wins and the grasses are stunted, die or never emerge.

The same exact pattern has emerged on the South Fork, the area I have frequent most as a professional has been the section of river from Andy Guest State Park downriver to Karo Landing.

<u>Personal History</u>

My history with fishing began at the age of 5 during a trip to the finger lakes. Like many kids I experienced an immediate attraction to fishing but it seemed like my interest went way beyond normal and when I look back at the patterns, I tended to orchestrate most of the rest of my life

around fishing in some way. When I was in second grade I moved to a house in rural Massachusetts surrounded by a network of streams connected to natural and man-made ponds.

By fourth grade I was fishing three to four days a week May through October. I learned how to catch minnows, worms, frogs, salamanders, crayfish, and just about every other possible live bait by the time I was nine, and used all of them. I sold golden shiners to my friends when I was ten after I taught myself how to catch more than I needed for my own fishing. I used my skateboard to transport myself and my bait from pond to pond, stream to stream, identifying and fishing the water body that was in the best condition and was fishing best. I learned this from a young age.

In fourth grade I proposed a school project which constituted taking time during the school week to travel all over the state to find access to, paddle and fish most of the rivers, ponds and lakes in half of the State of Massachusetts. My project included authoring a book called "Fishing In Massachusetts." My best friend and I pooled our lawn-mowing money and bought a canoe which we learned to transport, with our parents permission and help, on top of their cars. My mother and my teacher took their time to work with my friend and I for over a year researching for the book which was published by fifth grade and carried by most of the region's fishing and tackle stores.

Additionally, our family acquired a saltwater boat when I was approximately 8 years old and our weekends through the summer were spent traveling down the Charles River, through the Locks into the Boston Harbor. We spent hundreds of days through high school exploring the islands, feeder streams to the bays and harbors of the New England coast, sleeping on our boat, fishing, and swimming where we went.

By the time I graduated high school I estimate I had fished over 1000 days on more than 200 bodies of water around inland Massachusetts as well as the coasts from Salem to Block Island. I had fished rivers from the Penobscot in Maine to tributaries of the Connecticut River in Western Massachusetts, all the way down to rivers and streams in Connecticut and Rhode Island. There wasn't a stream that was safe or private property we weren't willing to cross to get to our fishing destinations. I cringe at the thought, but am thankful for understanding landowners. We left no trace. I also fished and explored rivers and streams in Montana, Colorodo and Wyoming, all the way up to and through Calgary Canada.

In college I set up carp fishing tournaments on the incredibly impaired Mystic River. I was determined to fish. In our tournaments our goal was not to catch the biggest or the most fish, it was to see how many different things we could use to catch carp. Our fishing was limited to the region around college but we explored the mostly polluted rivers and water storage reservoirs north of Boston.

After graduating college my roommate and I moved to Virginia from Massachusetts and our stated reason for the move to our parents was that the fishing season was longer. We ogled at the idea that we might be able to fish through the winter some years. Living in Arlington I built relationships with the local fishing and flyfishing communities, fishing stores and rod/reel repair shops and began exploring and fishing the waters of the Mid-Atlantic with . Over twenty years

this exploration continues and I have walked, fished and seen hundreds of bodies of water as a result. Here are some details:

In 1995 I joined the 200+ member Potomac River Smallmouth Club. By 2001 I had won every fishing contest in the club for several years running, had served as Newsletter Editor, Vice President and President.

Along the way I was invited to guide for Mark Kovach Fishing Services in Harper's Ferry, learned how to row an oar rig, and guided my first year in 1999 approximately 60 days. By the end of 2000 I had built a full time business and guided March through November, 5 days a week through 2005, a total of seven years. I spent three years guiding part time even after starting the Shenandoah Riverkeeper program during 2006 as I continued guiding six weeks a year for several more years.

Another example of the breadth of my experience on Virginia waters was my participating in the development of the "Flyfishers Guide to Virginia". author David Hart got a contract to write the "Flyfishers Guide to Virginia" and he asked me to be his primary companion in exploring most of the fishable streams, rivers, and reservoir in the state ranging from Goose Creek in Loudon County to the South Holston Reservoir on Virginia's Southern border and the mountain and valley streams in between. We fished for trout, bass and everything with fins and a mouth, at times camping along the way and at other times sustenance fishing.

In 2000 I helped LL Bean open their first retail store outside of Maine by setting up a flyfishing shop in Tysons Corner, and I also worked with their staff to start up their first flyfishing school outside of Maine. I taught their 1, 2 and 3 day classes for the next five years until my guide schedule became so heavy I had no time left to teach. During my time as an instructor I took hundreds of students to the water and into their first fishing experiences in multiple locations in the Shenandoah Valley centered near Front Royal.

By 2003 I had moved to the Shenandoah Valley to tend to my full guiding schedule which meant over 150 guided days per year. In addition to those 150 days I spent another 50 days with other professional guides exploring new water, learning existing water and working on new fishing techniques. My wife and I completed renovation of our old home in 2005 and opened a bed and breakfast to cater to our fishermen. We integrated the bed and breakfast with my guide business.

When we lost 80% of the smallmouth and sunfish population in the Shenandoah during 2005 and 2006 I was invited to join Virginia's Fish Kill Task Force as a fishing guide. This task force convened for five years and engaged in an extremely robust series of studies to determine why fish were sick in the Shenandoah and why we lost huge numbers of fish during 2005 and 2006. Looking back, while no single cause has been identified, most of the theories that had evidence to support them related to water quality. We considered the role of ammonia from high nutrient loads and decomposition of nutrients, we considered toxic algae, we considered the role of pesticides and herbicides, we looked at a range of other factors.

During this time I provided countless hours of witness and testimony to the poor health of the Shenandoah River fish even during the years before the fish kills. Every published scientific study

from the Fish Kill Task Force shows a correlation with many factors that have a link to algae growth. 1) Herbicides have been found in high concentrations which studies show would hold back the growth of native grasses and favor algae growth 2) High nutrient loads lead to heavy algae growth which causes daily spikes in water PH, which leads to increased toxicity from existing ammonia 3) High parasite load mainly due to the extreme proliferation of the Leptoxis Snail which hosts parasites that prey on the same species of sick which are stick. The snails themselves feed on algae and proliferate due to the extreme algae levels. One study showed that the Leptoxis snail constituted the majority of the entire biomass alive in the river. This is tremendously informative when looking at the algae issue.

I testified that starting in May of every year, smallmouth bass lose a tremendous amount of weight, and muscle fitness. Smallmouth in the Shenandoah come July when the algae blooms are the heaviest have become thin and lethargic. Their fins droop and they don't fight when you pick them up. In the late 90's before I expanded my fishing out to other rivers in the state I thought that all smallmouth got sickly looking in the summer. We were also used to finding relatively high numbers of sick and dead fish in the Shenandoah even outside of the "normal" fish kill season in April – June. The fish were sick in the presence of a tremendously rich food base made up of legions of crayfish, schools of minnows and heavy terrestrial life. Smallmouth on all the other rivers I eplored were robust, thick and healthy during the summer months. This was left unexplained by the scientists who didn't have the time to study it. I noted a very clear correlation between the level of algae growth in the river and the lethargy level of the fish, and their overall health..

What is MOST IMPORTANT about my life's fishing history and my professional career as a fishing guide was the fact that I made a living selecting the very best body of water in the Mid-Atlantic to take people fishing. This required that I have access to multiple sections of river, on multiple rivers in multiple states. My reputation and my success hinged on my ability to evaluate the physical conditions of the river including flow, water clarity and seasonal movements of fish to determine where I would take my clients through the ten month full time season.

This becomes very important in the context of our efforts to get the Shenandoah River listed as impaired due to loss of recreational use. Algae has a deep impact on both of those factors so fishing often hinged on whether or not algae was blooming in the Shenandoah. When the planktonic/pelagic algae blooms in the river it turns a thick pea green color and fish become lethargic, they don't' find food effectively because they cant' see and they reduce their feeding. Often fish have sores when you catch them. There is strong inverse correlation between the murkiness of the water and the number of fish that can be caught in a day. Murky water from an algae bloom meant poor fishing, every time.

The planktonic/pelagic algae has a deep affect on the enjoyment of fishermen beyond the drop in the quality of fishing. Fishermen were acutely aware when the algae was blooming due to the unpleasant look of the river, poor visibility, fish behavior/health and often odor as well. As a guide I would not purposefully guide a river that had a heavy planktonic/pelagic bloom and would spend my time working to avoid these conditions because it damaged the quality of the fishing day.

Additionally, when a planktonic bloom colored the water and decreased visibility there was no chance to see fish and narrow your search, observe their habitat or even to sight fish specifically to an individual fish.

An even greater threat to fisherman enjoyment on the Shenandoah are the colonial algae, which colonizes on the bottom substrate of the river and makes the river completely un-fishable. Our research shows the majority of these are toxin producing blue-green algaes. This algae begins to colonize in April every year on the tops of rocks and rock ledges and as flows fall to normal summer levels they literally cover over between 50% and 100% of the bottom of the North Fork, South Fork and Main Stem Shenandoah. The algae grows a thick slimy layer which is dangerous and unpleasant to walk or wade on.

When algae has colonized the bottom of the river fishermen would complain that every single cast into the river end up fouled with the algae, the hook would gather frustrating clumps on your hook/lure/bait that literally had to be picked clean with your fingernails between casts. Fish literally will not eat your offering if there was so much as a tiny speck of algae on the hook/lure/bait. It has always been our belief that the algae makes the fish sick so they literally avoid getting it in their mouths. When the algae gets heavy fish will literally abandon vast areas of the river in favor of areas without algae growing. This greatly diminishes the amount of fishable miles of the river and confuses anglers when they literally can't find fish in their favorite holes any more. Many conclude the fish are dead. Sometimes they are dead. Fishermen often become depressed at the idea that there are only a few places they can catch fish in the river and don't understand

In addition, even for anglers using flies/lures/bait that floats or doesn't touch the bottom the colonized blue green algaes still impede fishing. Every day when the algaes photosynthesize they produce prolific gases which form bubbles on the surface of the algae and underneath the mats. Eventually the bubbles will lift a nearly infinite number of these chunks from the bottom and flaot them to the surface. On a bright sunny day it would not be unusual for this floating action to sour water clarity, but the worst part is that these floating mats cover the surface of the river. They look like human or animal feces. People mistake them for the this.

<u>Riverkeeper Experiences</u>
As Riverkeeper, we have spent significant time educating the public about the algae in the river. What we found when we surveyed users with an official survey form (attached) was that users had no idea what algae was. However they were very bothered by what they called snot grass, grass or in many cases manure balls. The users were deeply troubled by the coating of blue green algae on the bottom of the river and with few exceptions, mistook the masses of floating algae which had broken off the bottom and were floating to be manure, sewage our poultry litter. They noted the foul odor, the unsightliness and the interference with swimming and particularly fishing.

Many other users (approximately 25 complaints) complained to us about sewage odors and raw sewage spills or seeps. At first we would investigate the claims by visiting the river in the affected areas. The complaints almost always sounded like this "we were floating from point A to B, and when we passed X tributary or Y housing complex or Z poultry farm we began to see clumps of

feces on the surface of the river. The users always related the clumps of feces to a physical stationary feature on the bank. Noone knew in the beginning of our education campaign that the feces was actually blue-green algae.

What we learned upon investigation and eventually concluded is that the presence of those floating globs of feces were not related to a specific source. What was happening is that the algae would only begin to dislodge from the bottom to float to the surface during the noon/ afternoon period of the day when the algae was photosynthesizing oxygen. The river was saturated with oxygen during that narrow part of the day causing oxygen bubbles to form which floated these decaying mats of algae to the surface. Every user told us they were disgusted by the odor and alarmed by the idea that this substance threatened their health.

Since then I would estimate that we have received over 200 personal complaints about the algae.

XI. **Qualifications of David Sligh**

A. Statement

My name is David Sligh and I am qualified to present analyses and opinions on matters related to water quality monitoring and assessments, quality controls for monitoring data, water pollution, stream ecology, and investigative methods. My resume is included below at part B. of this Section but I offer some more specific information here about my background and abilities as they relate to the review performed in this Technical Report.

During my time in college, I worked for the Virginia State Water Control Board ("SWCB" or "the Board"), a predecessor agency to the Department of Environmental Quality, for two summers. This began a long series of jobs and advancements within the Water Board and the DEQ, where I was trained in many of the skills and began acquiring the knowledge I bring to this technical review.

After receiving my undergraduate degree in Environmental Science from the University of Virginia, I worked for the the SWCB in Roanoke on an EPA-funded monitoring study to assess runoff pollution problems and relative impacts from urban, suburban, agricultural, and forested watersheds. I helped plan and coordinate the sampling program, managed the data, performed a range of analyses, and co-wrote the final report for this study.

Next, I took a postion in the Roanoke office where I compiled and analyzed all of the ambient water quality data for the region covered by our office. I wrote portions of the narrative for the agency's 305(b) report and helped assemple the "priority water bodies list" - what is now generally known as the "impaired water list - under section 303(d) of the Clean Water Act.

I then received a promotion to a job where I conducted the first comprehensive review of all surface water monitoring activities by the Roanoke office. I documented the purposes of each sampling type and the individual locations and designed new protocols for monitoring parameters, schedules, and reporting. For this work, Ron Gregory, the Director of the Office of Water Quality Assessments for the Board, wrote that I had "pioneered the modernization of ambient water quality monitoring networks in Virginia" and noted that the methods I had developed were a model for changes made by regional offices around the state. Mr. Gregory also praised me for my "high level of competency" in the areas of aquatic ecology and limnology and for my knowledge of surface water monitoring programs and techniques, including quality assurance.

Finally, I served as a Senior Engineer for the SWCB and the DEQ, overseeing all aspects of permitting in the Roanoke region for NPDES facilities and land application operations. In this role I worte requirements for stream studies for permitted parties and reviewed their proposals and results.

Since leaving the DEQ, I have worked in several non-profit organizations, where my knowledge of stream ecology and water quality studies has been very important. I was the representative in the Southeast U.S. for American Rivers. In this role I worked in six states on

both state and federal regulatory matters, served on technical advisory teams for river studies undertaken in relation to hydropower dam relicensing cases in North Carolina, Tennessee, Alabama, South Carolina, and Georgia. Later, I was Executive Director of the a local watershed group in Northeast Georgia. I supervised and helped conduct a watershed-wide water quality study with EPA funds. I also served as Upper James Riverkeeper and most recently have worked as a consultant for many non-profit groups. Among the projects I have completed are: reviews of NPDES permitted facilities in Maryland, reviewed and commented on the District of Columbia's bacterial TMDL, and designed a monitoring program to assess impacts on a watershed on the Eastern Shore of Maryland, where major poultry operations and other farming activities were present.

Throughout my state agency work and time with non-profit groups I have investigated many pollution complaints and sampled hundreds of streams, many in the Ridge and Valley, Blue Ridge, and Appalachian regions. I have testified in court and administrative hearings for the SWCB and DEQ as an expert on the types of issues addressed in this Technical Report. As well, I testified in a number of court proceedings for the Georgia River Network and Altamaha Riverkeeper in Georgia.

Two of the areas in which I believe my trainng and expertise are most applicable to the Shenandoah algae question are my familiarity with:

* proper data gathering, quality control, and analyses and
* my long experience applying Water Quality Standards, including those in Virginia and the southeaster states mentioned above, as well as in Vermont, Pennsylvania, D.C., and Maryland

I well understand the need for high quality data for the State of Virginia Integrated Report and listing of impaired waters and it is with that understanding that I comment upon the nature and quality of data that are presented by SRK in this case.

B. Resume

David Sligh
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Charlottesville, Virginia 22901
434-964-7455
davidwsligh@yahoo.com

Education

Vermont Law School, South Royalton, VT - J.D. degree - 1999

Pertinent Courses: watershed protection (CWA), water resources law, law of toxic and hazardous substances (RCRA & CERCLA), air pollution law (CAA), general environmental law, land use planning, administrative law, legislation

Independent Study: analysis of states' applications of water quality standards provisions - submitted as comments in response to EPA NOIRA

McNeese State University, Lake Charles, LA - Graduate course work in Biology - 1984

Pertinent Courses: ecology (focus on fish ecology in estuarine habitats), biochemistry

University of Virginia, Charlottesville, VA - B.A. degree in Environmental Science - 1982

Pertinent Courses: coastal and fluvial environments, hydrology, geology (field work in shoreline processes), fundamentals of ecology, applied ecology, forest ecology, aquatic chemistry, biology of fishes, tropical ecology

Independent Study: effects of low-flow conditions on the chemical, physical, and biological integrity, Roanoke River below Leesville Dam

Independent Study: trophic adaptations of marine benthic animals

Professional Qualification

Member of District of Columbia Bar

Employment

Environmental Consultant, Self-employed, Charlottesville, VA

Have completed projects including NPDES permit reviews, technical reviews of TMDLs, s. Clients include: Earthjustice, Gunpowder Riverkeeper, the Environmental Integrity Project, Miles-Wye Riverkeeper, and Shenandoah Riverkeeper.

Special Research Faculty, Virginia Tech,

Was assigned to the Virginia DEQ, to help develop and manage Annual Standards and Specifications program for compliance with Erosion & Sediment Control law and Stormwater Protection law, mandated by 2012 statutory changes. Conducted analysis of statute and regulations to ensure that requirements and fees are set appropriately for covered parties. Developed guidance for document preparation and conformance with legal requirements. Reviewed submitted documents for compliance.

Upper James Riverkeeper, James River Assoc., Charlottesville, VA

Protected the James River, its tributaries, and watershed through patrolling and monitoring, enforcement, involvement in regulatory matters, and education/motivation of citizens to act to improve and preserve their waters. Advocated and helped achieve improved regulation of poultry waste, industrial stormwater runoff, and construction stormwater pollution.

Executive Director, Soque River Watershed Assoc., Clarkesville, GA

Managed all programs, including a comprehensive, 3-year watershed study funded by the U.S. EPA and the State of Georgia. Supervised and conducted stream water sampling, benthic macroinvertebrate sampling, flow measurements, physical habitat assessments, and analyses of data.

Southeast Regional Representative, American Rivers, Chattanooga, Tennessee

Established regional office and led campaigns in TN, NC, SC, AL, GA, and VA. Advocated for river protection and restoration, through state and federal regulatory programs, news media, and education. Coordinated with and awarded/managed pass-through grants to state and local partners. Regularly served on technical and legal advisory committes, wrote and filed comments on studies and regulatory proposals. Consulted on technical and legal matters with partner environmental groups. Served as an expert witness on behalf of the Georgia River Network and the Altamaha Riverkeeper.

Adjunct Faculty Member, Univ. of Tennessee at Chattanooga

Taught environmental science.

Water Quality Assessment Assistant, Dept. of Environmental Conservation, Waterbury, Vermont (temporary job during law school)

Researched agency cases and files for data on pollution problems and conformance of programs with statutory and administrative mandates. Presented findings in state water quality assessment, impaired waterbodies listing, and legal and technical analyses of various programs.

Researcher, ARCS, Inc., Roanoke, Virginia

Researched energy trends and aerial pesticide spraying of power lines in West Virginia and Virginia and drafted formal submissions to Virginia State Corporation Commission. Prepared comments for U.S. Forest Service NEPA process regarding water quality and other issues. Lobbied state legislators to support citizen interests in state proceedings.

Senior Environmental Engineer, Virginia Dept. of Environmental Quality, Roanoke, Virginia,

Supervised division of engineers in: preparation of NPDES and Virginia Pollution Abatement permits (for land application of sludge and animal waste); analysis of environmental data and compliance records and preparation of enforcement documents; representation of agency at public hearings, negotiations, and in legal proceedings. Oversaw inspections of treatment facilities and land application operations, reviewed plans for special stream studies submitted by permit holders or applicants, completed stream models. Instructed environmental engineers under my supervision in technical, procedural, and legal matters associated with permitting processes.

Environmental Specialist, Virginia State Water Control Board

Coordinated all water quality research and monitoring activities in West Central region of state and designed new ambient monitoring system; prepared annual water quality reports on lakes program; conducted field surveys for benthic macro-invertebrates and water sampling; investigated pollution complaints and fish kills; prepared enforcement cases. Was the lead investigator in a landfill case, for which I testified in federal, state, and formal administrative court proceedings. Succeeded in closing the landfill, obtaining a judgement of $1.4 million for damages and penalties, and provided evidence for criminal prosecution of owners.

Environmental Technician, Virginia State Water Control Board

Compiled and analyzed regional water quality monitoring data and co-authored Virginia Water Quality Assessment (CWA section 305(b) report); analyzed data and wrote portions of water quality and water supply plans.

Environmental Technician, Virginia State Water Control Board

Planned and coordinated year-long EPA-funded research program to assess water quality impacts from non-point source pollution/storm water runoff. Conducted interest group meetings and public meetings. Co-wrote final report.

Intern, Summers of 1980 and 1981, Virginia State Water Control Board

Conducted water quality studies and pollution investigations; compiled and analyzed facility compliance data.

Other Activities and Positions

Technical Advisory Committee to Tennessee Clean Water Network, 2000-2002
Legal Advisory Committee to Dogwood Alliance Board of Directors, 2002 - 2004
Steering Committee Member, Southeastern Imperiled Fish Network

Speaker at numerous conferences on water quality issues, including:
Chesapeake Watershed Forum, Shepherdstown, WV, 2011, 2012.
Waterkeeper Alliance Conferences, 2009, 2013.
When the Water Runs Dry, New Orleans, LA, 2003 (speaker and session leader).
The Future of Flows, Morgantown, WV, 2002.
National River Rally - River Network, 2001, 2002, 2013, 2014.
Georgia River Network Conferences, Milledgeville, GA, 2002 & 2003.
Alabama Rivers Alliance, Annual Conferences 2000, 2001.

DEQ's Response to the Technical Report prepared by David Sligh on behalf of the Shenandoah Riverkeeper

Comment:

> While this statement acknowledges that violation of narrative standards may qualify a water body for an "impaired" designation, the Department's approach to the data citizens have submitted for excess algal growths seems to betray an unwillingness to do so. The assertion that "objective monitoring data" is required, when there are subjective aspects to the criteria in the WQS regulation, substitutes the staff's judgement for that of the State Water Control Board, the body empowered to establish these regulatory requirements. This failure to take the narrative requirements seriously is especially problematic, since the DEQ has so far refused to adopt the kind of objective (numeric) criteria they claim are necessary to control nutrients and algal problems.

Response:

DEQ takes the position that all impaired water listings must be based on high quality data, since a listing decision is a regulatory action and can thus have far-reaching impacts across multiple sectors of the community. The integrity of all of DEQ's water quality assessments rests on this policy. There is always the concern that if anecdotal reports are used to deem one waterbody "impaired", DEQ will be obligated to use them to assess another waterbody as "fully supporting". The use of objective monitoring data ensures that assessment decisions are always unbiased and defensible, and that resources for restoration are directed at those waterbodies needing them the most.

DEQ also takes the position that narrative criteria are as objective as numeric criteria. Numeric criteria consist of numeric limitations on specific pollutants, while narrative criteria are more general statements that apply to a wide set of pollutants or conditions. While it is true that narrative criteria are subject to more interpretation than numeric criteria, both are implemented through peer-reviewed, systematic, validated procedures. For instance, since 2003 DEQ has used the Virginia Stream Condition Index (VSCI) to interpret the narrative criteria of the aquatic life use. The VSCI was developed with benthic data collected across the entire state over seven years, and the analysis involved extensive collaboration among DEQ, EPA, and Tetra Tech, Inc. To ensure that the VSCI is consistently and accurately implemented, DEQ's biologists undergo routine training, certification, and internal auditing. Citizens who submit benthic data to DEQ for use in assessments are treated with a similar level of scrutiny. Thus, it is incorrect to assume that narrative criteria are somehow more lenient or subjective than numeric criteria. Since the ramifications of listing decisions are the same for both types of criteria, the data quality and assurance requirements for assessment are the same.

Comment:

> It is very important to recognize that the type of monitoring that is most appropriate for any situation is determined by the nature of the subject under study and the degrees of precision necessary to make valid and usable findings. Virginia DEQ officials seem to assert that only persons with scientific training in the use of specialized equipment, in sampling of water, sediments, or fish, or in the conduct of benthic macroinvertebrate studies may contribute useful and necessary information for use in determining the quality and status of waterbodies. This position is not supportable.
>
> Visual assessments of water bodies are used by all scientists, including those at the DEQ, and often provide data that are as or more important than the concentrations of pollutants or taxonomic identifications. As rightly noted by the DEQ, and as confirmed by the author's experience, when identifying the cause of a fish kill, "notations on conditions at a kill site and the affected species may often be as helpful to the diagnostician as samples sent to the lab." (VA DEQ 2002). The exact types of data that are pertinent to assess compliance with most the narrative criteria are ones that any water user can provide.

Response:

DEQ values the information gathered by its citizen monitors. For more than ten years, DEQ has been at the national forefront in terms of funding and utilizing citizen monitoring data for water quality assessments. It would be inaccurate to imply that DEQ rejects citizen data wholesale. DEQ just requires citizen data to be under the same scrutiny as DEQ-collected data when that information can lead to regulatory actions.

As you note, DEQ does use visual assessments to characterize water bodies. Fish kills are an example of an "observed effect" that is recorded in the Assessment Database, allowing a waterbody to be prioritized for follow-up monitoring so that any water quality-related causes can be identified. Fish kills can be the result of water quality impairments (e.g., low dissolved oxygen or high concentrations of ammonia), but they can also result from weather phenomena or disease completely independent of pollutant loadings. If a fish kill is traced to a specific water quality cause via follow-up monitoring, that waterbody will subsequently be listed on the 303(d) Impaired Waters List for that cause. Thus, while DEQ uses a visual assessment to trigger an investigation, the agency considers it insufficient information for making a determination of impairment.

Currently, DEQ has the Shenandoah River (including North and South Forks) categorized as having an "observed effect" based on citizen reports of excessive algae. DEQ's Academic Advisory Committee (AAC) is developing an approach that could be implemented in the Shenandoah (and other free-flowing streams) to identify nutrient pollutants when a visual assessment or another observed effect suggests eutrophication. The AAC is a group composed of academics and scholars from Virginia colleges and universities. The AAC's progress with this work is detailed on the DEQ Water Quality Standards webpage:

http://www.deq.virginia.gov/Programs/Water/WaterQualityInformationTMDLs/WaterQualityStandards/NutrientCriteriaDevelopment.aspx#Nutrient_Criteria_Streams_Rivers.

Comment:

The existence of unusual color, odor, or floating materials in a stream do not require special expertise and the testimony of dozens of people, many who have frequented these streams for decades and with great frequency, is sound evidence of these conditions. Whether these same river users have avoided use of these waters or have had their uses impaired is a question that only they can answer. Whether algal growths are undesirable or reach "nuisance" levels are subjective questions but the evidence shows that there is close agreement amongst citizens of various regions who have been surveyed, authors in the scientific literature, regulators in other states and nations - and the dozens of frequent Shenandoah River users who have given their opinions to the DEQ.

Response:

DEQ does not argue that algae growth can never be a nuisance, but the subjectivity involved in making this determination is not a trivial concern. It is sometimes difficult for even the well-trained eye to readily differentiate filamentous algae from native macrophytes. Native macrophyte biomass can snare fishing lines and clog boat propellers, and it can cause bad odors when it senesces. But native macrophytes are associated with healthy waters, not degraded waters.

Furthermore, some citizens do not have a full understanding of what constitutes a natural condition for a waterbody, mistakenly assuming that if a stream is not crystal clear and completely free from algae, it must be impaired. DEQ does not believe that citizens registering complaints about the Shenandoah are necessarily operating under this assumption. But this is a concern that must be addressed before making any listing decision based on subjective citizen complaints.

Comment:

Of course, water quality experts are not the only people who form common understandings about the subjective nature of resources and use common language as to the desirability of water bodies for recreational and aesthetic uses. The opinions of experienced fishermen, boaters, and guides and the ways they characterize conditions are part of a common understanding. The fact that dozens of river users quickly adopted the term "rock snot" to describe algal growths in the Shenandoah streams, shows that their perceptions could easily be summed-up in a term that could be understood by all.

Response:

Rock snot is the common name of the invasive diatom *Didymo geminata*. The Shenandoah is not among the three rivers that the Department of Game and Inland Fisheries (DGIF) has determined to be impacted by *Didymo*. The presence of *Didymo* would not signify water quality problems, since its growth appears to be limited to cool, shallow, clear waters more than waters affected by pollutants. The following link contains information on how recreational users can help keep *Didymo* under control: http://www.dgif.virginia.gov/fishing/didymo.asp.

Comment:

> Beyond these physical and ecological impacts, blue-green algae are a deterrent to use of water bodies where they are found to "bloom," because people rightly fear that toxins may be present. While not all forms of blue-green algae produce toxins and even where those that do produce them high levels are not necessarily found at any one time, the threat exists, and the uncertainties make it even harder for citizens and officials to react safely and appropriately to blue-green blooms.

Response:

Citizens are becoming increasingly aware of harmful algal blooms (HABs), particularly in light of the recent shut-down of the public water supply in Toledo, OH due to a severe *Microcystis* bloom. The Virginia Department of Health (VDH) has developed provisional guidance for the inter-agency response to suspected HABs in recreational waters. The guidance can be found here: http://www.vdh.virginia.gov/epidemiology/dee/habs/documents/VDHMicrocystisGuidance.pdf. Recreational users are an important component of this response plan, since users are usually the first to notice blooms. Citizens are urged to contact their regional DEQ office or local health district if they suspect an HAB in any waterbody.

VDH uses the thresholds of microcystin and *Microcystis* recommended by the World Health Organization for issuing notices. The public should be advised that it is safe to swim or conduct other recreational water activities when sampling results show microcystin levels are below 6 *µg/L*. There is no evidence that concentrations of microcystin or other cyanotoxins have ever been high enough to warrant an advisory for the Shenandoah River system.

Comment:

The determination as to whether stream conditions in the Shenandoah River and its tributaries meet the requirement of supporting "the propagation and growth of a balanced, indigenous population of aquatic life, including game fish, which might reasonably be expected to inhabit them" is exactly the kind of scientific inquiry that stream ecologists make on a routine basis.

The Virginia DEQ has recognized that excessive algal growths may lead to certain impairments such as low dissolved oxygen and fish kills. However, the Department has failed to acknowledge a widely-recognized fact - that the presence of excessive and unusual growths of aquatic plants, including algae, represent an imbalance in the local ecosystem even if the known follow-on impacts are not present or measurable. The nature of algal populations in these streams can be compared to those in streams that are minimally affected or unaffected by high nutrient inputs and the extreme densities of certain types, such as those presented with this report, are not typical of "normal," or "un-impacted" streams in the region where the Shenandoah watershed lies.

Response:

DEQ uses a wide range of metrics to assess the attainment status of the aquatic life use, and like any major river in the state, the Shenandoah River is continually monitored and assessed for these metrics. Water chemistry data (dissolved oxygen, pH, and toxics) indicate that the conditions are acceptable for a "balanced, indigeneous population of aquatic life" in most areas of the watershed. However, DEQ has assessed as impaired approximately 72 stream miles of the Shenandoah watershed based on low VSCI scores. Sedimentation (burial) and phosphorus have been identified as the stressors responsible for most of the impaired benthic communities, but in some areas toxics such as polyaromatic hydrocarbons have also been implicated. DEQ has developed 15 TMDLS to address the benthic impairments in the Shenandoah watershed. It is important to note that the VSCI applies only to wadeable streams, not boatable areas. DEQ's Academic Advisory Committee is developing a means of assessing fish communities in non-wadeable streams, in conjunction with its work on statewide nutrient criteria. The following link is a presentation of this work: http://www.deq.virginia.gov/Portals/0/DEQ/Water/WaterQualityStandards/NutrientCriteria/Stakeholder_Briefing_Nutrient_Garman_JUNE2008.pdf. DEQ hopes that a fish index of biological integrity will help provide a more comprehensive characterization of the aquatic life use.

Comment #: "C_"	Name(s)	Date Submitted	Stream(s)[1]				
			MS	NF	SF	Other	Name of Other
1	Alan Lehman	9/3/14	x	x	x		
2	Alan Lehman	4/10/12	x	x	x		
3	Allan Thomson	1/1/12	x	x			
4	Amy Mrstik	9/16/14			x		
5	Andrea Young	9/7/14			x		
6	Andrew Riccobono	4/13/12			x		
7	Andrew Thayer	1/1/12	x	x			
8	Anthony Morris	4/2/12		x			
9	B. Peter Yarrington	4/9/12	x				
10	Beau Morgan	1/1/12		x	x		
11	Ben Lienard	8/31/14					

12	**Bernard C. Nagelvoort**	4/13/12	x		
13	**Bernard C. Nagelvoort**	8/5/12			
14	**Bernard Griswald**	4/12/12		x	
15	**Bill and Duncan McGrath**	4/13/12			
16	**Bill Millhouser**	4/6/12	x	x	x
17	**Bill Minarik**	4/27/12			
18	**Bill Prokopchak**	4/18/12	x		
19	**Bill Tanger**	8/19/14	x		
20	**Boyd W. Post**	4/6/12			
21	**Brian Trow**	8/26/14	x	x	x
22	**Carl Cisky**	4/8/12	x	x	x
23	**Carl Onesty**	4/5/12	x	x	
24	**Charles V. Loudermilk II**	4/18/12	x		x
25	**Charles V. Loudermilk II**	1/1/14	x	x	x

26	**Colby Trow**	8/25/14		x	x		
27	**Cory M. Miller**	4/23/12	x				
28	**Craig Bishop**	4/11/12			x		
29	**Craig Serrels**	10/14/14		x	x		
30	**Dietrich Maune**	1/1/14	x	x	x		
31	**Douglas Lees**	8/31/14	x		x		
32	**Dr. Hays B. Lantz, Jr.**	4/16/12		x		x	Stony Creek
33	**Dr. Hays B. Lantz, Jr.**	4/14/12		x		x	Stony Creek
34	**Dr. John R. Cook**	3/29/12					
35	**Dr. Mark Zimmerman**	4/8/12	x	x	x		
36	**Drew Morrison**	1/1/14	x				
37	**Drs. Donna and Zahrl Schoeny**	8/27/14			x		
38	**Drs. Donna and Zahrl Schoeny**				x		

39	Dusty Wissmath	9/3/14	x	x	x
40	Dylan Cooper	4/15/12	x	x	x
41	Elwyn “Chip” Comstock	4/10/12			x
42	Ernesto J. Rojas	4/17/12	x		
43	Francis C. Steinbauer	4/15/12	x	x	x
44	Frank Filipy	8/29/14			x
45	Frank Polito	9/2/14			x
46	George “HookUp” Thurston	3/27/12	x	x	x
47	George Moran	4/12/12	x	x	x
48	George Paine	4/12/12		x	
49	Harold Allen	4/14/12			x
50	Henry J. Staudinger	4/15/12		x	

51	**Herschel L. Finch**	9/5/14			
52	**Herschel L. Finch**	1/1/12	x	x	
53	**J. Mark Pullen**	4/14/12	x		x
54	**J. Seth Coffman**	8/29/14		x	
55	**Jack Chapman**	4/13/12		x	
56	**Jack Dalby**	8/15/14		x	
57	**Jack McAllister**	4/19/12	x	x	x
58	**Jacob Russo**	9/3/14	x	x	x
59	**James Nashed MD**	4/17/12	x	x	x
60	**Jamie Gold**	8/28/14	x	x	x
61	**Jay Eiche**	4/18/12	x	x	x
62	**Jay Lovring**	4/6/12			
63	**Jeff Browne**	8/25/14			
64	**Jeff Little**	4/9/12		x	
65	**John Cabala**	12/18/13	x		
66	**John Cabala**	4/17/12			

67	**John D. Lipetz**	3/27/12	x	x	x
68	**John F. Ehrlich**	4/6/12			x
69	**John Holmes**	8/27/14		x	
70	**John Holmes**	4/15/12		x	
71	**John Politz**	1/1/12	x	x	x
72	**John R. Durbin**	4/17/12	x	x	
73	**Jonathan Hashisaki**	1/1/14			x
74	**Jonathan N. Turkel**	4/10/12	x		
75	**Kaitlan Potts**				
76	**Kara Rice**	4/14/12			x
77	**Kirk Comer**	3/27/12			x
78	**L.E. Rhodes**	1/1/14	x	x	x
79	**Lawrence DiJoseph**	4/2/12			
80	**Leslie D. Mitchell**	4/12/12		x	

81	**Mark J. Frondorf**	4/13/12	x	x	x
82	**Mark R. Myers**	4/5/12	x	x	x
83	**Mark Zimmermand PhD**	1/1/14	x	x	x
84	**Mary Beth Martin**	8/31/14	x		x
85	**Mary Gessner**	4/20/12		x	
86	**Mathew Frondorf**	4/19/12	x	x	x
87	**Michael E. Dunn**	4/15/12	x		x
88	**Nick Elgas**	3/27/12	x	x	x
89	**Orv Lehman**	4/11/12			
90	**Peter Pfotenhauer**	4/10/12			
91	**Preston Lazer**	8/27/14			x
92	**Ricardo Lianez**	1/1/14	x		x
93	**Richard Foust**	1/1/14		x	x

94	**Richard Fox**	1/1/14	x		x
95	**Richard Mrstik**	9/14/14			x
96	**Robert Abrams**	4/5/12	x	x	x
97	**Robert E. Forbes**	9/17/14	x	x	x
98	**Robert E. Forbes**	4/16/12	x		
99	**Rodney Miner**	8/27/14			x
100	**Ron Evans**	4/12/12	x	x	x
101	**Sam Berenstain**	4/13/12			
102	**Scott Osborne**	9/2/14			
103	**Stan Ikonen**	8/23/14	x		x
104	**Stan Ikonen**	1/1/12			
105	**Steve Kimm**	9/1/14			
106	**Steve Kimm**	4/11/12	x		x
107	**Steven R. Adams**	9/7/14	x	x	x

108	**Stuart C. Harding**	4/14/12			x		
109	**Terrence Cooney**	8/26/14					
110	**Terrence Cooney**	3/30/12	x	x	x		
111	**Thomas A. Bahleda MD**	4/21/12			x		
112	**Tim England**	1/1/12					
113	**Tommy Everett**	9/3/14	x	x	x		
114	**Trace Noel**	4/19/12			x		
115	**Urbie Nash, P.E.**	3/27/12	x	x	x		
116	**Vernon and Eileen Mann**	4/9/12	x				
117	**W.W. Harvey**	1/1/14			x		
118	**William J. “Bill” Amshey, Jr.**	4/4/12	x	x	x	x	Big Stony Creek
119	**William M. Prokopchak**	8/23/14	x		x		
120	**Jeff Kelble**	1/28/15	x	x	x		
121	**Chris Fordney**	1/27/15					

122	John Mark Pullen	1/14/14			
123	Kara Rice	1/26/15			
124	Mark J. Frondorf	1/25/15			
125	Orv Lehman	1/25/15			
126	Steve DiLoreto	1/15/15			
	126 comments		61	58	70
	111 commenters				
	Notes:				
	[1] MS = mainstem Shenandoah River, NF = North Fork Shenandoah R., SF = South Fork Shenandoah R.				

[2] River Miles are based on the system contained in "The Shenandoah River Atlas" prepared by W.E. Trout, Iii, Revised Edition 2013.

Comment #: "C_"	Name(s)	MS	Stream Miles[2]
1	Alan Lehman	Front Royal to Potomac confl.	[MS RMs 54 to 0]
2	Alan Lehman		
3	Allan Thomson		
4	Amy Mrstik		
5	Andrea Young		
6	Andrew Riccobono		
7	Andrew Thayer	Front Royal to Potomac confl.	[MS RMs 54 to 0]
8	Anthony Morris		
9	B. Peter Yarrington		
10	Beau Morgan		
11	Ben Lienard		

12	Bernard C. Nagelvoort		
13	Bernard C. Nagelvoort		
14	Bernard Griswald		
15	Bill and Duncan McGrath		
16	Bill Millhouser	Front Royal to Potomac confl.	[MS RMs 54 to 0]
17	Bill Minarik		
18	Bill Prokopchak		
19	Bill Tanger		
20	Boyd W. Post		
21	Brian Trow		
22	Carl Cisky	Downstream from Route 50	[MS RM 36]
23	Carl Onesty		
24	Charles V. Loudermilk II	Morgan Ford, Route 7, Route 50, RMs 22 & 36	[MS RMs 22 & 36]
25	Charles V. Loudermilk II	Front Royal to WV	[MS RMs 54 to 19]

26	Colby Trow	Front Royal to Potomac confl.	[MS RMs 54 to 0]
27	Cory M. Miller		
28	Craig Bishop		
29	Craig Serrels		
30	Dietrich Maune		
31	Douglas Lees		
32	Dr. Hays B. Lantz, Jr.		
33	Dr. Hays B. Lantz, Jr.		
34	Dr. John R. Cook		
35	Dr. Mark Zimmerman		
36	Drew Morrison		
37	Drs. Donna and Zahrl Schoeny		
38	Drs. Donna and Zahrl Schoeny		

39	Dusty Wissmath	Front Royal to Potomac confl.	[MS RMs 54 to 0]
40	Dylan Cooper		
41	Elwyn “Chip” Comstock		
42	Ernesto J. Rojas	Front Royal to Potomac confl.	[MS RMs 54 to 0]
43	Francis C. Steinbauer		
44	Frank Filipy		
45	Frank Polito		
46	George “HookUp” Thurston	Route 50 to Potomac confluence	[MS RMs 36 to 0]
47	George Moran		
48	George Paine		
49	Harold Allen		
50	Henry J. Staudinger		

51	Herschel L. Finch		
52	Herschel L. Finch		
53	J. Mark Pullen	Front Royal to Route 50 bridge	[MS RMs 54 to 36]
54	J. Seth Coffman		
55	Jack Chapman		
56	Jack Dalby		
57	Jack McAllister		
58	Jacob Russo	Front Royal to Potomac confl.	[MS RMs 54 to 0]
59	James Nashed MD	Front Royal to Route 7	[MS RMs 54 to 22]
60	Jamie Gold		
61	Jay Eiche	Front Royal to Potomac confl.	[MS RMs 54 to 0]
62	Jay Lovring		
63	Jeff Browne		
64	Jeff Little		
65	John Cabala		
66	John Cabala		

67	John D. Lipetz	Front Royal to Potomac confl.	[MS RMs 54 to 0]
68	John F. Ehrlich		
69	John Holmes		
70	John Holmes		
71	John Politz		
72	John R. Durbin		
73	Jonathan Hashisaki		
74	Jonathan N. Turkel	From Route 50 to WV line	[MS RMs 36 to 19]
75	Kaitlan Potts		
76	Kara Rice		
77	Kirk Comer		
78	L.E. Rhodes		
79	Lawrence DiJoseph		
80	Leslie D. Mitchell		

81	Mark J. Frondorf		
82	Mark R. Myers		
83	Mark Zimmermand PhD		
84	Mary Beth Martin		
85	Mary Gessner		
86	Mathew Frondorf		
87	Michael E. Dunn	Near Harpers Ferry	[MS RM 0]
88	Nick Elgas	Front Royal to Potomac confl.	[MS RMs 54 to 0]
89	Orv Lehman		
90	Peter Pfotenhauer		
91	Preston Lazer		
92	Ricardo Lianez		
93	Richard Foust		

94	**Richard Fox**	Front Royal to Castlemans Ferry	[MS RMs 54 to 22]
95	**Richard Mrstik**		
96	**Robert Abrams**	Front Royal to Potomac confl.	[MS RMs 54 to 0]
97	**Robert E. Forbes**		
98	**Robert E. Forbes**	Route 50 to Lockes Landing	[MS RMs 36 to 27]
99	**Rodney Miner**		
100	**Ron Evans**	Route 50 to WV line	[MS RMs 36 to 19]
101	**Sam Berenstain**		
102	**Scott Osborne**		
103	**Stan Ikonen**		
104	**Stan Ikonen**		
105	**Steve Kimm**		
106	**Steve Kimm**		
107	**Steven R. Adams**		

108	Stuart C. Harding		
109	Terrence Cooney		
110	Terrence Cooney		
111	Thomas A. Bahleda MD		
112	Tim England		
113	Tommy Everett		
114	Trace Noel		
115	Urbie Nash, P.E.		
116	Vernon and Eileen Mann		
117	W.W. Harvey		
118	William J. “Bill” Amshey, Jr.		
119	William M. Prokopchak	Front Royal to Route 7	[MS RMs 54 to 22]
120	Jeff Kelble		
121	Chris Fordney		

122	John Mark Pullen	
123	Kara Rice	
124	Mark J. Frondorf	
125	Orv Lehman	
126	Steve DiLoreto	
	126 comments	
	111 commenters	
	Notes:	
	[1] MS = mainstem Shenandoah River, NF = North Fork Shenandoah R., SF = South Fork Shenandoah R.	

[2] River Miles are based on the system contained in "The Shenandoah River Atlas" prepared by W.E. Trout, Iii, Revised Edition 2013.

Comment #: "C_"	Name(s)	Location(s)	
		NF	Stream Miles
1	Alan Lehman	Fulks Run to Front Royal	[NF RMs 93 to 0]
2	Alan Lehman		
3	Allan Thomson		
4	Amy Mrstik		
5	Andrea Young		
6	Andrew Riccobono		
7	Andrew Thayer	Fulks Run to Front Royal	[NF RMs 93 to 0]
8	Anthony Morris	Upstream and downstream from Elkton	[NF RM 80]
9	B. Peter Yarrington		
10	Beau Morgan	New Market to Front Rouyal	[NF RMs 77 to 0]
11	Ben Lienard		

12	Bernard C. Nagelvoort		
13	Bernard C. Nagelvoort		
14	Bernard Griswald	Edinburg to Strasburg	[NF RMs 59 to 12]
15	Bill and Duncan McGrath		
16	Bill Millhouser		
17	Bill Minarik		
18	Bill Prokopchak		
19	Bill Tanger		
20	Boyd W. Post		
21	Brian Trow		
22	Carl Cisky	Near Edinburg	[NF RM 59]
23	Carl Onesty		
24	Charles V. Loudermilk II		
25	Charles V. Loudermilk II		

26	Colby Trow		
27	Cory M. Miller		
28	Craig Bishop		
29	Craig Serrels	Timberville to New Market	[NF RMs 83 to 77]
30	Dietrich Maune		
31	Douglas Lees		
32	Dr. Hays B. Lantz, Jr.	Near Bergton	[NF RM 103]
33	Dr. Hays B. Lantz, Jr.		
34	Dr. John R. Cook		
35	Dr. Mark Zimmerman		
36	Drew Morrison		
37	Drs. Donna and Zahrl Schoeny		
38	Drs. Donna and Zahrl Schoeny		

39	Dusty Wissmath	Broadway to Front Royal	[NF RMs 86 to 0]
40	Dylan Cooper		
41	Elwyn “Chip” Comstock		
42	Ernesto J. Rojas		
43	Francis C. Steinbauer		
44	Frank Filipy		
45	Frank Polito		
46	George “HookUp” Thurston		
47	George Moran		
48	George Paine		
49	Harold Allen		
50	Henry J. Staudinger		

51	**Herschel L. Finch**		
52	**Herschel L. Finch**		
53	**J. Mark Pullen**		
54	**J. Seth Coffman**	Edinburg to Woodstock, Meems Bottom to Red Banks	[NF RMs 59 to 45, 71 to 66]
55	**Jack Chapman**	Near Edinburg	[NF RM 59]
56	**Jack Dalby**		
57	**Jack McAllister**		
58	**Jacob Russo**	Broadway to Front Royal	[NF RMs 86 to 0]
59	**James Nashed MD**	South of Strasburg	[NF RM 12]
60	**Jamie Gold**		
61	**Jay Eiche**	Broadway to Front Royal	[NF RMs 86 to 0]
62	**Jay Lovring**		
63	**Jeff Browne**		
64	**Jeff Little**	Near Pughs Run	[NF RM 36]
65	**John Cabala**		
66	**John Cabala**		

67	**John D. Lipetz**	Broadway to Front Royal	[NF RMs 86 to 0]
68	**John F. Ehrlich**		
69	**John Holmes**	from 10 miles upstream to 15 miles downstream of Burnshire Dam	[NF RMs 51 to 26]
70	**John Holmes**	From Artz Rd. bridge to Ridgely Rd.	[NF RMs 36 to 31
71	**John Politz**		
72	**John R. Durbin**		
73	**Jonathan Hashisaki**		
74	**Jonathan N. Turkel**		
75	**Kaitlan Potts**		
76	**Kara Rice**		
77	**Kirk Comer**		
78	**L.E. Rhodes**		
79	**Lawrence DiJoseph**		
80	**Leslie D. Mitchell**		

81	Mark J. Frondorf		
82	Mark R. Myers		
83	Mark Zimmermand PhD		
84	Mary Beth Martin		
85	Mary Gessner		
86	Mathew Frondorf		
87	Michael E. Dunn		
88	Nick Elgas	Broadway to Front Royal	[NF RMs 86 to 0]
89	Orv Lehman		
90	Peter Pfotenhauer		
91	Preston Lazer		
92	Ricardo Lianez		
93	Richard Foust		

94	Richard Fox		
95	Richard Mrstik		
96	Robert Abrams	Broadway to Front Royal	[NF RMs 86 to 0]
97	Robert E. Forbes	Near Edinburg	[NF RM 59]
98	Robert E. Forbes		
99	Rodney Miner		
100	Ron Evans		
101	Sam Berenstain		
102	Scott Osborne		
103	Stan Ikonen		
104	Stan Ikonen		
105	Steve Kimm		
106	Steve Kimm		
107	Steven R. Adams		

108	Stuart C. Harding	
109	Terrence Cooney	
110	Terrence Cooney	
111	Thomas A. Bahleda MD	
112	Tim England	
113	Tommy Everett	
114	Trace Noel	
115	Urbie Nash, P.E.	
116	Vernon and Eileen Mann	
117	W.W. Harvey	
118	William J. “Bill” Amshey, Jr.	
119	William M. Prokopchak	
120	Jeff Kelble	
121	Chris Fordney	

122	John Mark Pullen	
123	Kara Rice	
124	Mark J. Frondorf	
125	Orv Lehman	
126	Steve DiLoreto	
	126 comments	
	111 commenters	
	Notes:	
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Comment #: "C_"	Name(s)	SF	Stream Miles	Other
1	Alan Lehman	Port Republic to Front Royal	[SF RMs 97 to 0]	
2	Alan Lehman			
3	Allan Thomson			
4	Amy Mrstik	Guest SP to Front Royal	[SF RMs 16 to 0]	
5	Andrea Young	Guest SP	[SF RM 16]	
6	Andrew Riccobono	Near Andy Guest State Park	[SF RM 16]	
7	Andrew Thayer			
8	Anthony Morris			
9	B. Peter Yarrington			
10	Beau Morgan			
11	Ben Lienard			

12	Bernard C. Nagelvoort		
13	Bernard C. Nagelvoort		
14	Bernard Griswald		
15	Bill and Duncan McGrath		
16	Bill Millhouser		
17	Bill Minarik		
18	Bill Prokopchak		
19	Bill Tanger		
20	Boyd W. Post		
21	Brian Trow	Port Republic to Luray	[SF RMs 97 to 46
22	Carl Cisky	Near Bentonville	[SF RM 20]
23	Carl Onesty		
24	Charles V. Loudermilk II	Karo, Newport, Alma	[SF RMs 9, 59, & 57
25	Charles V. Loudermilk II	Elkton to Front Royal	[SF RMs 80 to 0]

26	**Colby Trow**			North River & Middle River
27	**Cory M. Miller**			
28	**Craig Bishop**			
29	**Craig Serrels**	Shenandoah to Luray	[SF RMs 73 to 46]	
30	**Dietrich Maune**			
31	**Douglas Lees**			
32	**Dr. Hays B. Lantz, Jr.**			
33	**Dr. Hays B. Lantz, Jr.**			
34	**Dr. John R. Cook**			
35	**Dr. Mark Zimmerman**			
36	**Drew Morrison**			
37	**Drs. Donna and Zahrl Schoeny**			
38	**Drs. Donna and Zahrl Schoeny**	Near Bentonville	[SF RM 20]	

39	**Dusty Wissmath**	Port Republic to Front Royal	[SF RMs 97 to 0]
40	**Dylan Cooper**		
41	**Elwyn “Chip” Comstock**	Luray to Front Royal	[SF RMs 46 to 0]
42	**Ernesto J. Rojas**		
43	**Francis C. Steinbauer**		
44	**Frank Filipy**	Newport to Whitehouse bridge	[SF RMS 59 to
45	**Frank Polito**	Island Ford to Elkton; Newport to Alma	[SF RMs 87 to 80]
46	**George “HookUp” Thurston**	Luray to Front Royal	[SF RMs 46 to 0]
47	**George Moran**		
48	**George Paine**		
49	**Harold Allen**	Bentonville area	[SF RM 20]
50	**Henry J. Staudinger**		

51	Herschel L. Finch		
52	Herschel L. Finch		
53	J. Mark Pullen	Luray to Front Royal	[SF RMs 46 to 0]
54	J. Seth Coffman		
55	Jack Chapman		
56	Jack Dalby		
57	Jack McAllister		
58	Jacob Russo	Port Republic to Luray	[SF RMs 97 to 46]
59	James Nashed MD	Luray to Front Royal	[SF RMs 46 to 0]
60	Jamie Gold		
61	Jay Eiche	Luray to Front Royal	[SF RMs 46 to 0]
62	Jay Lovring		
63	Jeff Browne		
64	Jeff Little		
65	John Cabala		
66	John Cabala		

67	John D. Lipetz	Port Republic to Front Royal	[SF RMs 97 to 0]
68	John F. Ehrlich	Bentonville to Hazard Mill	[SF RMs 20 to 19]
69	John Holmes		
70	John Holmes		
71	John Politz		
72	John R. Durbin		
73	Jonathan Hashisaki	Near town of Shenandoah	[SF RM 73]
74	Jonathan N. Turkel		
75	Kaitlan Potts		
76	Kara Rice		
77	Kirk Comer	Town of Shenandoah to Luray	[SF RMS 73 to 46]
78	L.E. Rhodes		
79	Lawrence DiJoseph		
80	Leslie D. Mitchell		

81	Mark J. Frondorf		
82	Mark R. Myers		
83	Mark Zimmermand PhD		
84	Mary Beth Martin		
85	Mary Gessner		
86	Mathew Frondorf		
87	Michael E. Dunn	Luray to Front Royal	[SF RMs 46 to 0]
88	Nick Elgas	Port Republic to Front Royal	[SF RMs 97 to 0]
89	Orv Lehman		
90	Peter Pfotenhauer		
91	Preston Lazer	Karo Landing to Front Royal	[SF RMs 9 to 0]
92	Ricardo Lianez	Port Republic to Front Royal	[SF RMs 97 to 0]
93	Richard Foust		

94	**Richard Fox**	Port Republic to Front Royal	[SF RMs 97 to 0]
95	**Richard Mrstik**	Karo Landing to Front Royal	[SF RMs 9 to 0]
96	**Robert Abrams**	Port Republic to Front Royal	[SF RMs 97 to 0]
97	**Robert E. Forbes**	Downstream from Burners Bottom	[SF RM 25]
98	**Robert E. Forbes**		
99	**Rodney Miner**		
100	**Ron Evans**		
101	**Sam Berenstain**		
102	**Scott Osborne**		
103	**Stan Ikonen**	Shepherds Ford to Rt. 7	
104	**Stan Ikonen**		
105	**Steve Kimm**		
106	**Steve Kimm**		
107	**Steven R. Adams**	Alma to Whitehouse	[SF RMs 57 to

108	**Stuart C. Harding**	Near Front Royal	[SF RM 0]
109	**Terrence Cooney**		
110	**Terrence Cooney**		
111	**Thomas A. Bahleda MD**		
112	**Tim England**		
113	**Tommy Everett**		
114	**Trace Noel**		
115	**Urbie Nash, P.E.**		
116	**Vernon and Eileen Mann**		
117	**W.W. Harvey**		
118	**William J. “Bill” Amshey, Jr.**		
119	**William M. Prokopchak**	Elkton to Front Royal	[SF RMs 80 to 0]
120	**Jeff Kelble**		
121	**Chris Fordney**		

122	John Mark Pullen	
123	Kara Rice	
124	Mark J. Frondorf	
125	Orv Lehman	
126	Steve DiLoreto	
	126 comments	
	111 commenters	
	Notes:	
	[1] MS = mainstem Shenandoah River, NF = North Fork Shenandoah R., SF = South Fork Shenandoah R.	

[2] River Miles are based on the system contained in "The Shenandoah River Atlas" prepared by W.E. Trout, Iii, Revised Edition 2013.

Comment #: "C_"	Name(s)	Uses Impaired				
		Aesthetics	Fishing	Primary Contact Rec.	Boating	Wading
1	Alan Lehman	x		x	x	x
2	Alan Lehman	x		x	x	
3	Allan Thomson	x				x
4	Amy Mrstik					
5	Andrea Young		x	x	x	
6	Andrew Riccobono	x	x			
7	Andrew Thayer	x			x	
8	Anthony Morris		x			x
9	B. Peter Yarrington	x	x			x
10	Beau Morgan	x	x		x	x
11	Ben Lienard		x			x

12	Bernard C. Nagelvoort	x	x			
13	Bernard C. Nagelvoort		x			
14	Bernard Griswald	x	x	x	x	
15	Bill and Duncan McGrath		x		x	
16	Bill Millhouser		x	x	x	
17	Bill Minarik		x			
18	Bill Prokopchak		x	x	x	
19	Bill Tanger			x	x	
20	Boyd W. Post	x			x	
21	Brian Trow	x	x			
22	Carl Cisky		x	x		x
23	Carl Onesty		x			
24	Charles V. Loudermilk II	x	x	x	x	
25	Charles V. Loudermilk II	x	x	x	x	x

26	Colby Trow	x		x		
27	Cory M. Miller		x		x	x
28	Craig Bishop	x	x			
29	Craig Serrels		x	x		
30	Dietrich Maune	x	x	x		
31	Douglas Lees	x	x			
32	Dr. Hays B. Lantz, Jr.					
33	Dr. Hays B. Lantz, Jr.	x	x		x	
34	Dr. John R. Cook					
35	Dr. Mark Zimmerman	x	x			x
36	Drew Morrison		x	x	x	
37	Drs. Donna and Zahrl Schoeny	x	x	x		x
38	Drs. Donna and Zahrl Schoeny		x	x	x	

39	Dusty Wissmath	x	x	x		
40	Dylan Cooper		x		x	x
41	Elwyn “Chip” Comstock		x		x	
42	Ernesto J. Rojas		x		x	
43	Francis C. Steinbauer	x	x			x
44	Frank Filipy		x	x	x	x
45	Frank Polito		x			
46	George “HookUp” Thurston	x	x	x	x	
47	George Moran		x			x
48	George Paine		x			
49	Harold Allen		x			
50	Henry J. Staudinger	x	x	x	x	x

51	Herschel L. Finch		x			
52	Herschel L. Finch		x	x		x
53	J. Mark Pullen		x			
54	J. Seth Coffman		x	x	x	
55	Jack Chapman	x	x		x	x
56	Jack Dalby	x	x			x
57	Jack McAllister		x			
58	Jacob Russo					
59	James Nashed MD	x	x	x		x
60	Jamie Gold		x		x	
61	Jay Eiche		x		x	
62	Jay Lovring		x			
63	Jeff Browne		x			x
64	Jeff Little		x	x		x
65	John Cabala		x		x	
66	John Cabala	x	x			

67	John D. Lipetz		x	x	x	
68	John F. Ehrlich	x	x			
69	John Holmes	x	x	x	x	x
70	John Holmes		x		x	x
71	John Politz		x			
72	John R. Durbin	x	x			
73	Jonathan Hashisaki		x			x
74	Jonathan N. Turkel		x	x	x	
75	Kaitlan Potts		x	x	x	
76	Kara Rice	x			x	
77	Kirk Comer		x			x
78	L.E. Rhodes	x	x	x		x
79	Lawrence DiJoseph	x	x		x	x
80	Leslie D. Mitchell	x			x	

81	Mark J. Frondorf		x			
82	Mark R. Myers	x	x		x	x
83	Mark Zimmermand PhD		x			
84	Mary Beth Martin		x	x		
85	Mary Gessner		x		x	
86	Mathew Frondorf	x	x	x	x	
87	Michael E. Dunn		x		x	x
88	Nick Elgas	x	x		x	
89	Orv Lehman	x			x	
90	Peter Pfotenhauer		x	x		
91	Preston Lazer	x	x			
92	Ricardo Lianez	x	x	x	x	
93	Richard Foust		x		x	

94	Richard Fox	x		x	x	x
95	Richard Mrstik		x	x	x	x
96	Robert Abrams	x	x			
97	Robert E. Forbes		x			x
98	Robert E. Forbes					x
99	Rodney Miner	x	x		x	
100	Ron Evans					
101	Sam Berenstain		x			x
102	Scott Osborne		x			
103	Stan Ikonen	x	x	x	x	
104	Stan Ikonen	x	x	x	x	
105	Steve Kimm		x			
106	Steve Kimm		x			
107	Steven R. Adams	x	x		x	x

108	Stuart C. Harding	x	x	x	x	
109	Terrence Cooney	x	x			
110	Terrence Cooney	x	x		x	
111	Thomas A. Bahleda MD	x		x	x	x
112	Tim England		x			
113	Tommy Everett	x	x	x		
114	Trace Noel	x	x	x	x	x
115	Urbie Nash, P.E.		x			
116	Vernon and Eileen Mann	x	x	x		
117	W.W. Harvey		x			
118	William J. “Bill” Amshey, Jr.	x	x			x
119	William M. Prokopchak	x	x	x		
120	Jeff Kelble	x	x	x	x	x
121	Chris Fordney					

122	John Mark Pullen					
123	Kara Rice					
124	Mark J. Frondorf					
125	Orv Lehman					
126	Steve DiLoreto					
	126 comments	57	102	44	55	40
	111 commenters					
	Notes:					
	[1] MS = mainstem Shenandoah River, NF = North Fork Shenandoah R., SF = South Fork Shenandoah R.					

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Comment #: "C_"	Name(s)	Description of Problems/Conce				
		Odor	Algae - general	Filamentous	Periphyton - General	Health Concerns: Toxicity and/ or pathogens
1	Alan Lehman		x		x	x
2	Alan Lehman		x			
3	Allan Thomson		x		x	
4	Amy Mrstik	x	x			
5	Andrea Young		x			x
6	Andrew Riccobono	x	x	x		
7	Andrew Thayer	x	x			
8	Anthony Morris	x	x	x		x
9	B. Peter Yarrington		x	x		
10	Beau Morgan		x	x		
11	Ben Lienard		x			

12	Bernard C. Nagelvoort		x		x	
13	Bernard C. Nagelvoort		x	x		
14	Bernard Griswald	x	x	x		
15	Bill and Duncan McGrath		x			
16	Bill Millhouser	x	x	x	x	
17	Bill Minarik		x			
18	Bill Prokopchak		x			
19	Bill Tanger		x			
20	Boyd W. Post		x	x		
21	Brian Trow	x	x	x	x	
22	Carl Cisky		x	x	x	x
23	Carl Onesty	x	x	x		
24	Charles V. Loudermilk II	x	x	x		
25	Charles V. Loudermilk II	x	x	x		

26	Colby Trow	x	x		x	x
27	Cory M. Miller		x	x	x	
28	Craig Bishop		x		x	
29	Craig Serrels	x	x			
30	Dietrich Maune		x	x	x	
31	Douglas Lees	x	x			
32	Dr. Hays B. Lantz, Jr.		x			
33	Dr. Hays B. Lantz, Jr.	x	x			
34	Dr. John R. Cook		x	x		
35	Dr. Mark Zimmerman	x	x	x		
36	Drew Morrison		x			x
37	Drs. Donna and Zahrl Schoeny		x		x	
38	Drs. Donna and Zahrl Schoeny		x			x

39	Dusty Wissmath		x			
40	Dylan Cooper	x	x	x	x	x
41	Elwyn “Chip” Comstock		x	x		
42	Ernesto J. Rojas	x	x			
43	Francis C. Steinbauer	x	x	x		
44	Frank Filipy	x	x	x		
45	Frank Polito		x			
46	George “HookUp” Thurston	x	x	x		
47	George Moran		x	x		
48	George Paine		x			
49	Harold Allen		x			
50	Henry J. Staudinger		x			

51	Herschel L. Finch		x	x		x
52	Herschel L. Finch	x	x	x	x	x
53	J. Mark Pullen		x			
54	J. Seth Coffman		x	x		
55	Jack Chapman		x	x	x	
56	Jack Dalby	x	x	x		
57	Jack McAllister		x			
58	Jacob Russo	x	x	x		
59	James Nashed MD		x	x	x	x
60	Jamie Gold	x	x			
61	Jay Eiche	x	x	x		x
62	Jay Lovring	x	x			x
63	Jeff Browne	x	x			
64	Jeff Little		x	x		
65	John Cabala		x			
66	John Cabala		x			

67	John D. Lipetz	x	x	x	
68	John F. Ehrlich	x	x	x	x
69	John Holmes	x	x	x	
70	John Holmes		x		x
71	John Politz		x	x	
72	John R. Durbin	x	x	x	x
73	Jonathan Hashisaki		x	x	x
74	Jonathan N. Turkel	x	x		
75	Kaitlan Potts		x		
76	Kara Rice		x		
77	Kirk Comer		x		x
78	L.E. Rhodes	x	x	x	
79	Lawrence DiJoseph	x	x		
80	Leslie D. Mitchell		x		x

81	Mark J. Frondorf	x	x		x	
82	Mark R. Myers		x		x	
83	Mark Zimmermand PhD		x	x		
84	Mary Beth Martin	x	x			x
85	Mary Gessner	x	x	x		
86	Mathew Frondorf	x	x	x		
87	Michael E. Dunn	x	x			
88	Nick Elgas	x	x	x		
89	Orv Lehman		x		x	
90	Peter Pfotenhauer	x	x	x		
91	Preston Lazer	x	x	x		
92	Ricardo Lianez	x	x			
93	Richard Foust		x			

94	Richard Fox		x			
95	Richard Mrstik	x	x			
96	Robert Abrams	x	x	x	x	
97	Robert E. Forbes	x	x			
98	Robert E. Forbes		x		x	
99	Rodney Miner		x			x
100	Ron Evans	x	x			
101	Sam Berenstain		x			
102	Scott Osborne	x	x			
103	Stan Ikonen	x	x		x	
104	Stan Ikonen	x	x			
105	Steve Kimm	x	x	x		
106	Steve Kimm	x	x	x		
107	Steven R. Adams	x	x		x	

108	Stuart C. Harding	x	x	x		
109	Terrence Cooney	x	x			
110	Terrence Cooney		x			
111	Thomas A. Bahleda MD	x	x	x	x	
112	Tim England		x			
113	Tommy Everett	x	x	x		
114	Trace Noel	x	x	x		
115	Urbie Nash, P.E.		x	x		
116	Vernon and Eileen Mann		x			
117	W.W. Harvey		x			x
118	William J. “Bill” Amshey, Jr.		x	x	x	x
119	William M. Prokopchak	x	x			x
120	Jeff Kelble	x	x	x	x	x
121	Chris Fordney		x			

122	John Mark Pullen		x			
123	Kara Rice		x			
124	Mark J. Frondorf		x			
125	Orv Lehman		x			
126	Steve DiLoreto		x			
	126 comments	60	126	55	31	19
	111 commenters					
	Notes:					
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Comment #: "C_"	Name(s)	rns			Time Period (s)	Contrast w/ other watersheds
		Plankton and/or Floating Masses	Color	Turbidity		
1	Alan Lehman	x	x		Yearly	x
2	Alan Lehman	x	x	x		
3	Allan Thomson					
4	Amy Mrstik				Jul 4, 2014	
5	Andrea Young					
6	Andrew Riccobono				decline began before 2005	x
7	Andrew Thayer	x				
8	Anthony Morris					
9	B. Peter Yarrington	x			Past five years	
10	Beau Morgan	x				
11	Ben Lienard				recent years	

12	**Bernard C. Nagelvoort**			x	2011	
13	**Bernard C. Nagelvoort**				2012	
14	**Bernard Griswald**	x	x		Recent years	
15	**Bill and Duncan McGrath**	x	x		Past several years	
16	**Bill Millhouser**	x	x		Each of past six years	
17	**Bill Minarik**				2010, 2011	
18	**Bill Prokopchak**				2011	
19	**Bill Tanger**		x		Yearly	
20	**Boyd W. Post**	x			Last several years	
21	**Brian Trow**	x			Yearly, 75 days per year	x
22	**Carl Cisky**			x		
23	**Carl Onesty**	x	x			
24	**Charles V. Loudermilk II**	x	x			
25	**Charles V. Loudermilk II**		x	x		x

26	Colby Trow	x	x		12 months/ year, 20 years	x
27	Cory M. Miller	x				
28	Craig Bishop					
29	Craig Serrels	x		x		
30	Dietrich Maune	x	x			
31	Douglas Lees	x				
32	Dr. Hays B. Lantz, Jr.					
33	Dr. Hays B. Lantz, Jr.					
34	Dr. John R. Cook	x				
35	Dr. Mark Zimmerman	x	x		Last five years	x
36	Drew Morrison					
37	Drs. Donna and Zahrl Schoeny					x
38	Drs. Donna and Zahrl Schoeny					

39	Dusty Wissmath		x		Each of past six years	x
40	Dylan Cooper	x	x			
41	Elwyn “Chip” Comstock				Last five years	
42	Ernesto J. Rojas					
43	Francis C. Steinbauer	x	x			
44	Frank Filipy	x			Last six or seven years	
45	Frank Polito				2014 worst conditions	x
46	George “HookUp” Thurston	x	x			
47	George Moran	x		x		
48	George Paine					
49	Harold Allen					
50	Henry J. Staudinger					

51	**Herschel L. Finch**	x	x		Last five years	
52	**Herschel L. Finch**	x			Last few years	x
53	**J. Mark Pullen**					
54	**J. Seth Coffman**				Summers, 2012 -2014	x
55	**Jack Chapman**	x				
56	**Jack Dalby**					x
57	**Jack McAllister**					
58	**Jacob Russo**	x	x		Past six years	x
59	**James Nashed MD**	x	x		Past few summers	x
60	**Jamie Gold**				Past several years	x
61	**Jay Eiche**	x	x		Past six years	
62	**Jay Lovring**		x	x	Past year	
63	**Jeff Browne**		x			
64	**Jeff Little**					
65	**John Cabala**				Since 2004	
66	**John Cabala**				Last five years	

67	John D. Lipetz	x	x	Past six years	
68	John F. Ehrlich			2008	
69	John Holmes	x			
70	John Holmes	x			
71	John Politz				
72	John R. Durbin				x
73	Jonathan Hashisaki		x	Each year	x
74	Jonathan N. Turkel				
75	Kaitlan Potts	x			
76	Kara Rice			2010, 2011	
77	Kirk Comer				
78	L.E. Rhodes				
79	Lawrence DiJoseph				x
80	Leslie D. Mitchell	x		2010, 2011	

81	**Mark J. Frondorf**		x		Last five years	
82	**Mark R. Myers**			x	Past several years	
83	**Mark Zimmermand PhD**		x			
84	**Mary Beth Martin**					
85	**Mary Gessner**					
86	**Mathew Frondorf**	x	x		Past six years	
87	**Michael E. Dunn**			x		
88	**Nick Elgas**	x	x			
89	**Orv Lehman**	x				
90	**Peter Pfotenhauer**	x	x		Past six years	x
91	**Preston Lazer**					x
92	**Ricardo Lianez**		x		Recent years	
93	**Richard Foust**					x

94	Richard Fox				
95	Richard Mrstik	x			x
96	Robert Abrams		x	Past six years	
97	Robert E. Forbes			Yearly	
98	Robert E. Forbes		x		
99	Rodney Miner			Past seven to eight years	
100	Ron Evans				
101	Sam Berenstain				x
102	Scott Osborne				
103	Stan Ikonen				
104	Stan Ikonen		x		
105	Steve Kimm				
106	Steve Kimm	x	x	Last five years	
107	Steven R. Adams				x

108	**Stuart C. Harding**	x	x		Past six years	
109	**Terrence Cooney**	x	x			
110	**Terrence Cooney**					
111	**Thomas A. Bahleda MD**	x				x
112	**Tim England**					x
113	**Tommy Everett**	x	x			
114	**Trace Noel**	x				
115	**Urbie Nash, P.E.**	x				x
116	**Vernon and Eileen Mann**					
117	**W.W. Harvey**					x
118	**William J. “Bill” Amshey, Jr.**	x	x		Last six years	
119	**William M. Prokopchak**	x	x		Yearly since late 1990s	x
120	**Jeff Kelble**	x	x	x		x
121	**Chris Fordney**					

122	John Mark Pullen				
123	Kara Rice				
124	Mark J. Frondorf				
125	Orv Lehman				
126	Steve DiLoreto				
	126 comments	50	40	10	30
	111 commenters				
	Notes:				
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Comment #: "C_"	Name(s)	Expert
1	Alan Lehman	x
2	Alan Lehman	x
3	Allan Thomson	
4	Amy Mrstik	
5	Andrea Young	
6	Andrew Riccobono	
7	Andrew Thayer	
8	Anthony Morris	
9	B. Peter Yarrington	
10	Beau Morgan	
11	Ben Lienard	

12	Bernard C. Nagelvoort	
13	Bernard C. Nagelvoort	
14	Bernard Griswald	
15	Bill and Duncan McGrath	
16	Bill Millhouser	
17	Bill Minarik	
18	Bill Prokopchak	
19	Bill Tanger	
20	Boyd W. Post	
21	Brian Trow	x
22	Carl Cisky	
23	Carl Onesty	
24	Charles V. Loudermilk II	
25	Charles V. Loudermilk II	

26	Colby Trow	x
27	Cory M. Miller	
28	Craig Bishop	
29	Craig Serrels	
30	Dietrich Maune	
31	Douglas Lees	
32	Dr. Hays B. Lantz, Jr.	x
33	Dr. Hays B. Lantz, Jr.	
34	Dr. John R. Cook	
35	Dr. Mark Zimmerman	
36	Drew Morrison	
37	Drs. Donna and Zahrl Schoeny	
38	Drs. Donna and Zahrl Schoeny	

39	Dusty Wissmath	
40	Dylan Cooper	
41	Elwyn “Chip” Comstock	
42	Ernesto J. Rojas	
43	Francis C. Steinbauer	
44	Frank Filipy	
45	Frank Polito	
46	George “HookUp” Thurston	
47	George Moran	
48	George Paine	
49	Harold Allen	
50	Henry J. Staudinger	

51	Herschel L. Finch	
52	Herschel L. Finch	
53	J. Mark Pullen	
54	J. Seth Coffman	
55	Jack Chapman	
56	Jack Dalby	
57	Jack McAllister	
58	Jacob Russo	x
59	James Nashed MD	
60	Jamie Gold	
61	Jay Eiche	
62	Jay Lovring	
63	Jeff Browne	
64	Jeff Little	
65	John Cabala	
66	John Cabala	

67	John D. Lipetz	
68	John F. Ehrlich	
69	John Holmes	
70	John Holmes	
71	John Politz	
72	John R. Durbin	
73	Jonathan Hashisaki	
74	Jonathan N. Turkel	
75	Kaitlan Potts	
76	Kara Rice	
77	Kirk Comer	
78	L.E. Rhodes	x
79	Lawrence DiJoseph	
80	Leslie D. Mitchell	

81	Mark J. Frondorf	
82	Mark R. Myers	
83	Mark Zimmermand PhD	
84	Mary Beth Martin	
85	Mary Gessner	
86	Mathew Frondorf	
87	Michael E. Dunn	
88	Nick Elgas	
89	Orv Lehman	
90	Peter Pfotenhauer	
91	Preston Lazer	
92	Ricardo Lianez	
93	Richard Foust	

94	Richard Fox	
95	Richard Mrstik	
96	Robert Abrams	
97	Robert E. Forbes	
98	Robert E. Forbes	
99	Rodney Miner	
100	Ron Evans	x
101	Sam Berenstain	
102	Scott Osborne	x
103	Stan Ikonen	
104	Stan Ikonen	
105	Steve Kimm	
106	Steve Kimm	
107	Steven R. Adams	

108	Stuart C. Harding	
109	Terrence Cooney	
110	Terrence Cooney	
111	Thomas A. Bahleda MD	
112	Tim England	
113	Tommy Everett	
114	Trace Noel	x
115	Urbie Nash, P.E.	
116	Vernon and Eileen Mann	
117	W.W. Harvey	
118	William J. “Bill” Amshey, Jr.	
119	William M. Prokopchak	
120	Jeff Kelble	x
121	Chris Fordney	

122	John Mark Pullen	
123	Kara Rice	
124	Mark J. Frondorf	
125	Orv Lehman	
126	Steve DiLoreto	
	126 comments	11
	111 commenters	
	Notes:	
	[1] MS = mainstem Shenandoah River, NF = North Fork Shenandoah R., SF = South Fork Shenandoah R.	

[2] River Miles are based on the system contained in "The Shenandoah River Atlas" prepared by W.E. Trout, Iii, Revised Edition 2013.

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
662	**Aug 15, 2013**	Main Stem	0	x	x				
663	**Aug 15, 2013**	Main Stem	2	x	x				
664	**Aug 15, 2013**	Main Stem	3	x					
665	**Aug 15, 2013**	Main Stem	4	x					
666	**Aug 15, 2013**	Main Stem	5	x					
667	**Aug 15, 2013**	Main Stem	6	x					
668	**Aug 15, 2013**	Main Stem	7	x					
669	**Aug 15, 2013**	Main Stem	8	x					
670	**Aug 15, 2013**	Main Stem	9	x					
671	**Aug 15, 2013**	Main Stem	11	x					
672	**Aug 15, 2013**	Main Stem	12	x					
673	**Aug 15, 2013**	Main Stem	13	x					
674	**Aug 15, 2013**	Main Stem	14	x					
675	**Aug 15, 2013**	Main Stem	16	x					
676	**Aug 15, 2013**	Main Stem	18	x					
677	**Aug 15, 2013**	Main Stem	19	x					
678	**Aug 15, 2013**	Main Stem	20	x					
880	**Jul 25, 2014**	Main stem	20	x					
679	**Aug 15, 2013**	Main Stem	21	x					
881	**Jul 25, 2014**	Main stem	21	x					
680	**Aug 15, 2013**	Main Stem	22	x					
681	**Aug 15, 2013**	Main Stem	23	x					
451	**Sep 5, 2014**	Main stem	23				x		
682	**Aug 15, 2013**	Main Stem	24	x					

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
452	**Sep 5, 2014**	Main stem	24				x		
683	**Aug 15, 2013**	Main Stem	25	x					
684	**Aug 15, 2013**	Main Stem	26	x					
882	**Jul 25, 2014**	Main stem	26	x					
685	**Aug 15, 2013**	Main Stem	27	x					
883	**Jul 25, 2014**	Main stem	27	x					
686	**Aug 15, 2013**	Main Stem	29	x					
687	**Aug 15, 2013**	Main Stem	31	x					
688	**Aug 15, 2013**	Main Stem	32	x					
689	**Aug 15, 2013**	Main Stem	34	x					
690	**Aug 15, 2013**	Main Stem	35	x					
691	**Aug 15, 2013**	Main Stem	36	x					
8	**Jul 11, 2012**	Main stem	36				x	x	
9	**Jul 11, 2012**	Main stem	36				x		
692	**Aug 15, 2013**	Main Stem	37	x					
693	**Aug 15, 2013**	Main Stem	38	x					
453	**Aug 28, 2014**	Main stem	38		x			x	
694	**Aug 15, 2013**	Main Stem	39	x					
454	**Aug 28, 2014**	Main stem	39		x			x	
695	**Aug 15, 2013**	Main Stem	40	x					
696	**Aug 15, 2013**	Main Stem	41	x					
697	**Aug 15, 2013**	Main Stem	42	x					
698	**Aug 15, 2013**	Main Stem	43	x					
699	**Aug 15, 2013**	Main Stem	44	x					

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
700	**Aug 15, 2013**	Main Stem	45	x					
701	**Aug 15, 2013**	Main Stem	46	x					
702	**Aug 15, 2013**	Main Stem	47	x					
884	**Jul 25, 2014**	Main stem	48	x					
703	**Aug 15, 2013**	Main Stem	49	x					
705	**Aug 15, 2013**	Main Stem	52	x	x				
706	**Aug 15, 2013**	Main Stem	53	x					
707	**Aug 15, 2013**	Main Stem	54	x					
704	**Aug 15, 2013**	Main Stem		x					
1	**Jun 20, 2012**	Main stem				x			
2	**Jun 20, 2012**	Main stem			x				
3	**Jun 20, 2012**	Main stem			x				
4	**Jun 20, 2012**	Main stem			x				
5	**Jun 20, 2012**	Main stem			x				
6	**Jun 20, 2012**	Main stem							x
7	**Jun 20, 2012**	Main stem							x
10	**Jul 30, 2012**	Main stem			x				
11	**Jul 30, 2012**	Main stem					x		
455	**Aug 8, 2014**	North Fork	0				x		
456	**Aug 7, 2014**	North Fork	0				x		
708	**Aug 15, 2013**	North Fork	0	x					
457	**Aug 8, 2014**	North Fork	1				x		
458	**Aug 8, 2014**	North Fork	1				x		
500	**Sep 2, 2014**	North Fork	1			x	x	x	

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
709	**Aug 15, 2013**	North Fork	1	x					
459	**Aug 8, 2014**	North Fork	2				x		
710	**Aug 15, 2013**	North Fork	2	x					
460	**Aug 8, 2014**	North Fork	3				x		
711	**Aug 15, 2013**	North Fork	3	x					
461	**Aug 8, 2014**	North Fork	4				x		
462	**Jul 18, 2014**	North Fork	4				x		
712	**Aug 15, 2013**	North Fork	4	x					
463	**Jul 18, 2014**	North Fork	5				x		
713	**Aug 15, 2013**	North Fork	5	x					
885	**Jul 25, 2014**	North Fork	5	x					
714	**Aug 15, 2013**	North Fork	6	x					
464	**Jul 18, 2014**	North Fork	7				x		
465	**Jul 18, 2014**	North Fork	7				x		
715	**Aug 15, 2013**	North Fork	7	x					
466	**Jul 18, 2014**	North Fork	8				x		
467	**Jul 18, 2014**	North Fork	8				x		
468	**Sep 17, 2014**	North Fork	8				x		
469	**Sep 17, 2014**	North Fork	8				x		
470	**Jul 18, 2014**	North Fork	8				x		
716	**Aug 15, 2013**	North Fork	8	x					
471	**Jul 18, 2014**	North Fork	9				x		
472	**Jul 18, 2014**	North Fork	9				x		
473	**Jul 18, 2014**	North Fork	9				x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
474	**Jul 18, 2014**	North Fork	9				x		
475	**Jul 18, 2014**	North Fork	10				x		x
476	**Jul 18, 2014**	North Fork	10				x		
477	**Sep 17, 2014**	North Fork	10				x		
478	**Jul 18, 2014**	North Fork	10			x	x		
717	**Aug 15, 2013**	North Fork	10	x					
479	**Jul 18, 2014**	North Fork	11				x		
480	**Jul 18, 2014**	North Fork	11				x		
481	**Sep 17, 2014**	North Fork	11				x		
482	**Sep 17, 2014**	North Fork	11				x		
483	**Jul 18, 2014**	North Fork	11				x		
718	**Aug 15, 2013**	North Fork	11	x					
87	**Jun 15, 2012**	North Fork	12				x		
88	**Jun 15, 2012**	North Fork	12				x		
89	**Jun 15, 2012**	North Fork	12				x		
90	**Jun 15, 2012**	North Fork	12				x		
91	**Jun 15, 2012**	North Fork	12				x		
120	**Jun 29, 2012**	North Fork	12				x		
121	**Jun 29, 2012**	North Fork	12				x		
122	**Jun 29, 2012**	North Fork	12				x		
123	**Jun 29, 2012**	North Fork	12				x		
124	**Jun 29, 2012**	North Fork	12				x		
125	**Jun 29, 2012**	North Fork	12				x		
137	**Jun 29, 2012**	North Fork	12			x	x	x	

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
174	**Jul 16, 2012**	North Fork	12				x		
175	**Jul 16, 2012**	North Fork	12				x		
176	**Jul 16, 2012**	North Fork	12				x		
177	**Jul 16, 2012**	North Fork	12				x		
178	**Jul 16, 2012**	North Fork	12				x		
484	**Aug 7, 2014**	North Fork	12				x		
485	**Aug 7, 2014**	North Fork	12				x		
486	**Aug 7, 2014**	North Fork	12				x		
487	**Aug 7, 2014**	North Fork	12				x		
488	**Sep 2, 2014**	North Fork	12				x		
489	**Aug 7, 2014**	North Fork	12				x		
719	**Aug 15, 2013**	North Fork	12	x					
886	**Jul 25, 2014**	North Fork	12	x					
887	**Jul 25, 2014**	North Fork	12	x					
888	**Jul 25, 2014**	North Fork	12	x					
82	**Jun 15, 2012**	North Fork	13				x		
83	**Jun 15, 2012**	North Fork	13				x		
84	**Jun 15, 2012**	North Fork	13				x		
85	**Jun 15, 2012**	North Fork	13				x		
86	**Jun 15, 2012**	North Fork	13				x	x	
116	**Jun 29, 2012**	North Fork	13				x		
117	**Jun 29, 2012**	North Fork	13				x		
118	**Jun 29, 2012**	North Fork	13				x		
119	**Jun 29, 2012**	North Fork	13				x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
163	**Jul 16, 2012**	North Fork	13				x		
164	**Jul 16, 2012**	North Fork	13				x		
165	**Jul 16, 2012**	North Fork	13				x		
166	**Jul 16, 2012**	North Fork	13				x		
167	**Jul 16, 2012**	North Fork	13				x		
168	**Jul 16, 2012**	North Fork	13				x		
169	**Jul 16, 2012**	North Fork	13				x		
170	**Jul 16, 2012**	North Fork	13				x		
171	**Jul 16, 2012**	North Fork	13				x		
172	**Jul 16, 2012**	North Fork	13				x		
173	**Jul 16, 2012**	North Fork	13				x		
490	**Sep 2, 2014**	North Fork	13				x		
491	**Sep 2, 2014**	North Fork	13				x		
720	**Aug 15, 2013**	North Fork	13	x					
70	**Jun 15, 2012**	North Fork	14				x		
71	**Jun 15, 2012**	North Fork	14				x		
72	**Jun 15, 2012**	North Fork	14				x	x	
73	**Jun 15, 2012**	North Fork	14				x		
75	**Jun 15, 2012**	North Fork	14				x		
75	**Jun 15, 2012**	North Fork	14				x		
76	**Jun 15, 2012**	North Fork	14				x		
77	**Jun 15, 2012**	North Fork	14				x		
78	**Jun 15, 2012**	North Fork	14				x		
79	**Jun 15, 2012**	North Fork	14				x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
80	**Jun 15, 2012**	North Fork	14				x		
81	**Jun 15, 2012**	North Fork	14				x		
111	**Jun 29, 2012**	North Fork	14				x		
112	**Jun 29, 2012**	North Fork	14				x		
113	**Jun 29, 2012**	North Fork	14				x		
114	**Jun 29, 2012**	North Fork	14				x		
115	**Jun 29, 2012**	North Fork	14				x		
161	**Jul 16, 2012**	North Fork	14				x	x	
162	**Jul 16, 2012**	North Fork	14				x	x	
492	**Apr 16, 2014**	North Fork	14				x		
493	**Sep 2, 2014**	North Fork	14				x		
721	**Aug 15, 2013**	North Fork	14	x					
889	**Jul 25, 2014**	North Fork	14	x					
64	**Jun 15, 2012**	North Fork	15				x		
65	**Jun 15, 2012**	North Fork	15				x		
66	**Jun 15, 2012**	North Fork	15				x		
67	**Jun 15, 2012**	North Fork	15				x		
68	**Jun 15, 2012**	North Fork	15			x			
69	**Jun 15, 2012**	North Fork	15			x			
131	**Jun 29, 2012**	North Fork	15			x	x		
132	**Jun 29, 2012**	North Fork	15			x	x		
133	**Jun 29, 2012**	North Fork	15			x	x		
134	**Jun 29, 2012**	North Fork	15			x	x		
135	**Jun 29, 2012**	North Fork	15			x	x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
136	**Jun 29, 2012**	North Fork	15			x	x		
150	**Jul 16, 2012**	North Fork	15				x		
151	**Jul 16, 2012**	North Fork	15				x		
152	**Jul 16, 2012**	North Fork	15				x		
153	**Jul 16, 2012**	North Fork	15				x		
154	**Jul 16, 2012**	North Fork	15				x		
155	**Jul 16, 2012**	North Fork	15				x		
156	**Jul 16, 2012**	North Fork	15				x		
157	**Jul 16, 2012**	North Fork	15				x		
158	**Jul 16, 2012**	North Fork	15			x	x		
159	**Jul 16, 2012**	North Fork	15				x		
160	**Jul 16, 2012**	North Fork	15				x		
181	**Jul 16, 2012**	North Fork	15			x	x		
494	**Sep 2, 2014**	North Fork	15				x		
495	**Sep 2, 2014**	North Fork	15				x		
722	**Aug 15, 2013**	North Fork	15	x					
890	**Jul 25, 2014**	North Fork	15	x					
891	**Jul 25, 2014**	North Fork	15	x					
892	**Jul 25, 2014**	North Fork	15	x					
36	**Jun 15, 2012**	North Fork	16				x		
37	**Jun 15, 2012**	North Fork	16				x		
38	**Jun 15, 2012**	North Fork	16				x		
39	**Jun 15, 2012**	North Fork	16				x		
40	**Jun 15, 2012**	North Fork	16				x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
41	**Jun 15, 2012**	North Fork	16				x		
42	**Jun 15, 2012**	North Fork	16				x		
43	**Jun 15, 2012**	North Fork	16				x		
44	**Jun 15, 2012**	North Fork	16					x	
45	**Jun 15, 2012**	North Fork	16				x		
46	**Jun 15, 2012**	North Fork	16				x		
47	**Jun 15, 2012**	North Fork	16				x		
48	**Jun 15, 2012**	North Fork	16				x		
49	**Jun 15, 2012**	North Fork	16				x		
50	**Jun 15, 2012**	North Fork	16				x		
51	**Jun 15, 2012**	North Fork	16				x		
52	**Jun 15, 2012**	North Fork	16					x	
53	**Jun 15, 2012**	North Fork	16				x	x	
54	**Jun 15, 2012**	North Fork	16			x	x		
55	**Jun 15, 2012**	North Fork	16				x		
56	**Jun 15, 2012**	North Fork	16				x	x	
57	**Jun 15, 2012**	North Fork	16				x		
58	**Jun 15, 2012**	North Fork	16				x		
59	**Jun 15, 2012**	North Fork	16				x		
61	**Jun 15, 2012**	North Fork	16				x	x	
62	**Jun 15, 2012**	North Fork	16				x	x	
63	**Jun 15, 2012**	North Fork	16				x		
102	**Jun 29, 2012**	North Fork	16				x	x	
103	**Jun 29, 2012**	North Fork	16				x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
104	**Jun 29, 2012**	North Fork	16				x	x	
105	**Jun 29, 2012**	North Fork	16				x	x	
106	**Jun 29, 2012**	North Fork	16				x	x	
107	**Jun 29, 2012**	North Fork	16				x	x	
108	**Jun 29, 2012**	North Fork	16				x	x	
109	**Jun 29, 2012**	North Fork	16				x	x	
110	**Jun 29, 2012**	North Fork	16				x	x	
126	**Jun 29, 2012**	North Fork	16				x	x	
127	**Jun 29, 2012**	North Fork	16				x	x	
128	**Jun 29, 2012**	North Fork	16				x		
129	**Jun 29, 2012**	North Fork	16			x	x		
130	**Jun 29, 2012**	North Fork	16				x		
138	**Jul 16, 2012**	North Fork	16			x	x		
139	**Jul 16, 2012**	North Fork	16				x		
140	**Jul 16, 2012**	North Fork	16				x		
141	**Jul 16, 2012**	North Fork	16				x		
142	**Jul 16, 2012**	North Fork	16				x		
143	**Jul 16, 2012**	North Fork	16				x		
144	**Jul 16, 2012**	North Fork	16				x		
145	**Jul 16, 2012**	North Fork	16				x		
146	**Jul 16, 2012**	North Fork	16				x		
147	**Jul 16, 2012**	North Fork	16				x		
148	**Jul 16, 2012**	North Fork	16				x		
149	**Jul 16, 2012**	North Fork	16				x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
179	**Jul 16, 2012**	North Fork	16			x	x		
180	**Jul 16, 2012**	North Fork	16			x	x		
496	**Sep 2, 2014**	North Fork	16				x		
497	**Sep 2, 2014**	North Fork	16				x	x	
498	**Sep 2, 2014**	North Fork	16			x	x		
499	**Sep 2, 2014**	North Fork	16			x	x	x	
501	**Sep 2, 2014**	North Fork	16			x	x	x	
502	**Sep 2, 2014**	North Fork	16				x		
893	**Jul 25, 2014**	North Fork	16	x					
894	**Jul 25, 2014**	North Fork	16	x					
503	**Aug 7, 2014**	North Fork	17				x		
504	**Aug 7, 2014**	North Fork	17				x		
723	**Aug 15, 2013**	North Fork	17	x					
895	**Jul 25, 2014**	North Fork	17	x					
505	**Nov 11, 2014**	North Fork	19			x	x		
724	**Aug 15, 2013**	North Fork	19	x					
506	**Nov 11, 2014**	North Fork	20			x	x		
507	**Nov 11, 2014**	North Fork	20			x	x		
725	**Aug 15, 2013**	North Fork	20	x					
508	**Nov 11, 2014**	North Fork	21				x		
726	**Aug 15, 2013**	North Fork	21	x					
509	**Nov 11, 2014**	North Fork	22				x		
510	**Nov 11, 2014**	North Fork	22				x		
727	**Aug 15, 2013**	North Fork	22	x					

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
511	**Nov 11, 2014**	North Fork	23				x		
512	**Nov 11, 2014**	North Fork	23				x		
728	**Aug 15, 2013**	North Fork	23	x					
513	**Nov 11, 2014**	North Fork	24				x		
514	**Nov 11, 2014**	North Fork	24				x		
729	**Aug 15, 2013**	North Fork	24	x					
896	**Jul 25, 2014**	North Fork	24	x					
515	**Nov 11, 2014**	North Fork	25				x		
730	**Aug 15, 2013**	North Fork	25	x					
516	**Nov 11, 2014**	North Fork	26				x		
517	**Nov 11, 2014**	North Fork	26				x		
731	**Aug 15, 2013**	North Fork	26	x					
518	**Nov 11, 2014**	North Fork	27				x		
732	**Aug 15, 2013**	North Fork	27	x					
519	**Aug 7, 2014**	North Fork	28				x		
520	**Nov 11, 2014**	North Fork	28				x		
521	**Nov 11, 2014**	North Fork	28				x		
522	**Nov 11, 2014**	North Fork	28				x		
733	**Aug 15, 2013**	North Fork	28	x					
523	**Jul 29, 2014**	North Fork	29				x		
524	**Jul 29, 2014**	North Fork	29				x		
525	**Jul 29, 2014**	North Fork	29				x		
526	**Jul 29, 2014**	North Fork	29				x		
527	**Jul 29, 2014**	North Fork	29				x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
528	**Jul 29, 2014**	North Fork	30				x		
529	**Jul 29, 2014**	North Fork	30				x		
530	**Jul 29, 2014**	North Fork	30				x		
734	**Aug 15, 2013**	North Fork	30	x					
897	**Jul 25, 2014**	North Fork	30	x					
531	**Jul 29, 2014**	North Fork	31				x		
532	**Jul 29, 2014**	North Fork	31				x		
735	**Aug 15, 2013**	North Fork	31	x					
533	**Aug 7, 2014**	North Fork	32				x		
534	**Jul 29, 2014**	North Fork	32				x	x	
535	**Jul 29, 2014**	North Fork	32				x	x	
536	**Jul 29, 2014**	North Fork	32				x		
736	**Aug 15, 2013**	North Fork	32	x					
737	**Aug 15, 2013**	North Fork	33	x					
898	**Jul 25, 2014**	North Fork	34	x					
738	**Aug 15, 2013**	North Fork	35	x					
899	**Jul 25, 2014**	North Fork	35	x					
900	**Jul 25, 2014**	North Fork	35	x					
537	**Aug 7, 2014**	North Fork	36				x		
739	**Aug 15, 2013**	North Fork	36	x					
740	**Aug 15, 2013**	North Fork	37	x					
901	**Jul 25, 2014**	North Fork	37	x					
741	**Aug 15, 2013**	North Fork	38	x					
902	**Jul 25, 2014**	North Fork	38	x					

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
742	**Aug 15, 2013**	North Fork	39	x					
743	**Aug 15, 2013**	North Fork	40	x					
744	**Aug 15, 2013**	North Fork	41	x					
903	**Jul 25, 2014**	North Fork	41	x					
904	**Jul 25, 2014**	North Fork	41	x					
538	**Jul 27, 2014**	North Fork	43				x	x	
745	**Aug 15, 2013**	North Fork	43	x					
539	**Jul 27, 2014**	North Fork	44				x	x	
746	**Aug 15, 2013**	North Fork	44	x					
540	**Sep 4, 2014**	North Fork	45			x	x		
747	**Aug 15, 2013**	North Fork	45	x					
905	**Jul 25, 2014**	North Fork	45	x					
906	**Jul 25, 2014**	North Fork	45	x					
907	**Jul 25, 2014**	North Fork	45	x					
748	**Aug 15, 2013**	North Fork	46	x					
908	**Jul 25, 2014**	North Fork	46	x					
541	**Oct 21, 2014**	North Fork	48				x		
542	**Oct 21, 2014**	North Fork	48				x		
750	**Aug 15, 2013**	North Fork	48	x					
909	**Jul 25, 2014**	North Fork	48	x					
910	**Jul 25, 2014**	North Fork	48	x					
911	**Jul 25, 2014**	North Fork	48	x					
912	**Jul 25, 2014**	North Fork	48	x					
543	**Oct 21, 2014**	North Fork	49				x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
544	**Oct 21, 2014**	North Fork	49				x		
751	**Aug 15, 2013**	North Fork	49	x					
913	**Jul 25, 2014**	North Fork	49	x					
545	**Oct 21, 2014**	North Fork	50				x		
752	**Aug 15, 2013**	North Fork	50	x	x				
753	**Aug 15, 2013**	North Fork	51	x					
914	**Jul 25, 2014**	North Fork	51	x					
546	**Oct 21, 2014**	North Fork	52				x		
754	**Aug 15, 2013**	North Fork	52	x					
915	**Jul 25, 2014**	North Fork	52	x					
547	**Sep 4, 2014**	North Fork	53				x		
755	**Aug 15, 2013**	North Fork	53	x					
756	**Aug 15, 2013**	North Fork	54	x					
757	**Aug 15, 2013**	North Fork	55	x					
758	**Aug 15, 2013**	North Fork	56	x	x				
916	**Jul 25, 2014**	North Fork	56	x					
917	**Jul 25, 2014**	North Fork	56	x	x				
759	**Aug 15, 2013**	North Fork	57	x					
759	**Aug 15, 2013**	North Fork	58	x					
760	**Aug 15, 2013**	North Fork	58	x					
548	**Aug 7, 2014**	North Fork	59				x	x	
549	**Aug 7, 2014**	North Fork	59				x		
761	**Aug 15, 2013**	North Fork	59	x					
550	**Aug 7, 2014**	North Fork	60				x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
551	**Aug 7, 2014**	North Fork	60				x	x	
762	**Aug 15, 2013**	North Fork	60	x					
918	**Jul 25, 2014**	North Fork	60	x					
919	**Jul 25, 2014**	North Fork	60	x					
552	**Nov 30, 2014**	North Fork	61				x		
763	**Aug 15, 2013**	North Fork	61	x					
920	**Jul 25, 2014**	North Fork	61	x					
553	**Nov 30, 2014**	North Fork	62				x		
764	**Aug 15, 2013**	North Fork	62	x					
554	**Nov 30, 2014**	North Fork	63			x	x		
555	**Nov 30, 2014**	North Fork	63				x		
765	**Aug 15, 2013**	North Fork	63	x					
92	**Jun 26, 2012**	North Fork	64				x	x	
93	**Jun 26, 2012**	North Fork	64				x	x	
182	**Jul 25, 2012**	North Fork	64			x	x		
556	**Nov 30, 2014**	North Fork	64				x		
766	**Aug 15, 2013**	North Fork	64	x					
94	**Jun 26, 2012**	North Fork	65				x	x	
95	**Jun 26, 2012**	North Fork	65				x	x	
557	**Nov 30, 2014**	North Fork	65				x		
558	**Nov 30, 2014**	North Fork	65				x		
767	**Aug 15, 2013**	North Fork	65	x					
96	**Jun 26, 2012**	North Fork	66				x		
768	**Aug 15, 2013**	North Fork	66	x	x				

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
97	**Jun 26, 2012**	North Fork	67				x		
769	**Aug 15, 2013**	North Fork	67	x					
98	**Jun 26, 2012**	North Fork	68				x		
559	**Nov 5, 2014**	North Fork	68				x		
770	**Aug 15, 2013**	North Fork	68	x					
99	**Jun 26, 2012**	North Fork	69				x		
560	**Nov 5, 2014**	North Fork	69				x		
771	**Aug 15, 2013**	North Fork	69	x					
100	**Jun 26, 2012**	North Fork	70				x		
772	**Aug 15, 2013**	North Fork	70	x					
101	**Jun 26, 2012**	North Fork	71				x		
561	**Nov 5, 2014**	North Fork	71				x		
562	**Nov 5, 2014**	North Fork	71				x		
773	**Aug 15, 2013**	North Fork	71	x					
563	**Nov 5, 2014**	North Fork	72				x		
774	**Aug 15, 2013**	North Fork	73	x					
775	**Aug 15, 2013**	North Fork	74	x					
776	**Aug 15, 2013**	North Fork	75	x					
777	**Aug 15, 2013**	North Fork	76	x					
564	**Aug 7, 2014**	North Fork	77				x		
778	**Aug 15, 2013**	North Fork	77	x					
921	**Jul 25, 2014**	North Fork	77	x					
922	**Jul 25, 2014**	North Fork	77	x					
923	**Jul 25, 2014**	North Fork	77	x					

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
924	**Jul 25, 2014**	North Fork	77	x					
565	**Aug 7, 2014**	North Fork	78				x		
566	**Aug 7, 2014**	North Fork	78				x		
779	**Aug 15, 2013**	North Fork	78	x					
925	**Jul 25, 2014**	North Fork	78	x					
926	**Jul 25, 2014**	North Fork	78	x					
927	**Jul 25, 2014**	North Fork	78	x					
780	**Aug 15, 2013**	North Fork	79	x					
781	**Aug 15, 2013**	North Fork	80	x					
782	**Aug 15, 2013**	North Fork	81	x					
783	**Aug 15, 2013**	North Fork	82	x					
567	**Aug 7, 2014**	North Fork	83				x		
784	**Aug 15, 2013**	North Fork	83	x					
928	**Jul 25, 2014**	North Fork	83	x					
929	**Jul 25, 2014**	North Fork	83	x					
930	**Jul 25, 2014**	North Fork	83	x					
24	**Jul 12, 2012**	North Fork	84					x	
25	**Jul 12, 2012**	North Fork	84				x	x	
26	**Jul 12, 2012**	North Fork	84					x	
27	**Jul 12, 2012**	North Fork	84					x	
785	**Aug 15, 2013**	North Fork	84	x					
931	**Jul 25, 2014**	North Fork	84	x					
16	**Jul 12, 2012**	North Fork	85		x				
17	**Jul 12, 2012**	North Fork	85		x				

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
18	**Jul 12, 2012**	North Fork	85		x				
19	**Jul 12, 2012**	North Fork	85		x				
20	**Jul 12, 2012**	North Fork	85		x				
21	**Jul 12, 2012**	North Fork	85		x				
22	**Jul 12, 2012**	North Fork	85					x	
23	**Jul 12, 2012**	North Fork	85					x	
32	**Jul 12, 2012**	North Fork	85				x		
33	**Jul 12, 2012**	North Fork	85				x		
34	**Jun 22, 2012**	North Fork	85		x				x
35	**Jun 22, 2012**	North Fork	85						
786	**Aug 15, 2013**	North Fork	85	x					
12	**Jul 12, 2012**	North Fork	86				x		
13	**Jul 12, 2012**	North Fork	86				x		
14	**Jul 12, 2012**	North Fork	86				x		
15	**Jul 12, 2012**	North Fork	86				x	x	
28	**Jul 12, 2012**	North Fork	86		x	x	x		x
29	**Jul 12, 2012**	North Fork	86				x		
30	**Jul 12, 2012**	North Fork	86				x		
31	**Jul 12, 2012**	North Fork	86				x		
787	**Aug 15, 2013**	North Fork	86	x					
788	**Aug 15, 2013**	North Fork	87	x					
789	**Aug 15, 2013**	North Fork	88	x					
790	**Aug 15, 2013**	North Fork	89	x					
393	**Jun 4, 2007**	North Fork					x	x	

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
394	**Jun 4, 2007**	North Fork					x	x	
395	**Jun 4, 2007**	North Fork					x	x	
1001	**Jun 26, 2012**	North Fork	11						
1002	**Jun 26, 2012**	North Fork	11						
1003	**Jun 26, 2012**	North Fork	11						
1004	**Jun 26, 2012**	North Fork	11						
1005	**Jun 26, 2012**	North Fork	11						
1006	**Jun 26, 2012**	North Fork	12						
1007	**Jun 26, 2012**	North Fork	12						
1008	**Jun 26, 2012**	North Fork	12						
1009	**Jun 26, 2012**	North Fork	12						
1010	**Jun 26, 2012**	North Fork	12						
1011	**Jun 26, 2012**	North Fork	12						
1012	**Jun 26, 2012**	North Fork	13						
1013	**Jun 26, 2012**	North Fork	13						
1014	**Jun 26, 2012**	North Fork	14						
1015	**Jun 26, 2012**	North Fork	14						
1016	**Jun 26, 2012**	North Fork	14						
1017	**Jun 26, 2012**	North Fork	15						
1018	**Jun 26, 2012**	North Fork	15						
1019	**Jun 26, 2012**	North Fork	15						
1020	**Jun 26, 2012**	North Fork	15						
1021	**Jun 26, 2012**	North Fork	16						
1022	**Jun 26, 2012**	North Fork	16						

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
1023	**Jun 26, 2012**	North Fork	16						
1029	**Jun 22, 2010**	North Fork							
1030	**Jun 22, 2010**	North Fork							
1031	**Jul 1, 2011**	North Fork			x				
1332	**Jul 1, 2011**	North Fork			x				
203	**Jul 9, 2012**	North River	1				x		
204	**Jul 9, 2012**	North River	1				x		
205	**Jul 9, 2012**	North River	1			x	x		
206	**Jul 9, 2012**	North River	1			x	x		
207	**Jul 9, 2012**	North River	1			x	x		
208	**Jul 9, 2012**	North River	1			x	x		
209	**Jul 9, 2012**	North River	1			x	x		
190	**Jun 23, 2012**	North River	2			x	x		
191	**Jun 23, 2012**	North River	2			x	x		
194	**Jun 23, 2012**	North River	2						x
213	**Jul 9, 2012**	North River	2				x		
214	**Jul 9, 2012**	North River	2				x		
215	**Jul 9, 2012**	North River	2				x		
187	**Jun 23, 2012**	North River	3				x		
188	**Jun 23, 2012**	North River	3				x		
189	**Jun 23, 2012**	North River	3				x		
202	**Jul 9, 2012**	North River	3				x		
218	**Jul 9, 2012**	North River	3				x		
219	**Jul 9, 2012**	North River	3			x	x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
220	**Jul 9, 2012**	North River	3			x	x		
221	**Jul 9, 2012**	North River	3			x	x		
222	**Jul 9, 2012**	North River	3			x	x		
183	**Jun 23, 2012**	North River	4				x		
184	**Jun 23, 2012**	North River	4				x		
185	**Jun 23, 2012**	North River	4				x		
186	**Jun 23, 2012**	North River	4						
192	**Jun 23, 2012**	North River	4			x	x		
193	**Jun 23, 2012**	North River	4			x	x		
195	**Jul 9, 2012**	North River	4				x		
210	**Jul 9, 2012**	North River	4				x		
211	**Jul 9, 2012**	North River	4			x	x		
212	**Jul 9, 2012**	North River	4			x	x		
216	**Jul 9, 2012**	North River	4				x		
217	**Jul 9, 2012**	North River	4				x		
196	**Jul 9, 2012**	North River	4				x		
197	**Jul 9, 2012**	North River	4				x		
198	**Jul 9, 2012**	North River	4				x		
199	**Jul 9, 2012**	North River	4			x	x		
200	**Jul 9, 2012**	North River	4				x		
201	**Jul 9, 2012**	North River	4			x	x		
444	**Nov 23, 2010**	Potomac					x	x	
445	**Nov 23, 2010**	Potomac					x	x	
446	**Nov 23, 2010**	Potomac					x	x	

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
447	**Nov 23, 2010**	Potomac					x	x	
448	**Nov 23, 2010**	Potomac					x	x	
449	**Nov 23, 2010**	Potomac					x	x	
450	**Nov 23, 2010**	Potomac					x		x
791	**Aug 15, 2013**	South Fork	0	x					
932	**Jul 25, 2014**	South Fork	0	x					
792	**Aug 15, 2013**	South Fork	1	x					
793	**Aug 15, 2013**	South Fork	2	x					
568	**Aug 7, 2014**	South Fork	3				x	x	
569	**Aug 7, 2014**	South Fork	3					x	
570	**Aug 7, 2014**	South Fork	3					x	
794	**Aug 15, 2013**	South Fork	3	x					
933	**Jul 25, 2014**	South Fork	3	x					
571	**Aug 7, 2014**	South Fork	4				x		
572	**Aug 7, 2014**	South Fork	4				x		
934	**Jul 25, 2014**	South Fork	4	x					
795	**Aug 15, 2013**	South Fork	5	x					
796	**Aug 15, 2013**	South Fork	7	x					
797	**Aug 15, 2013**	South Fork	8	x					
798	**Aug 15, 2013**	South Fork	9	x					
935	**Jul 25, 2014**	South Fork	9	x					
799	**Aug 15, 2013**	South Fork	11	x					
936	**Jul 25, 2014**	South Fork	11	x					
1333	**Dec 1, 2014**	South Fork	11				x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
800	**Aug 15, 2013**	South Fork	12	x					
937	**Jul 25, 2014**	South Fork	12	x					
938	**Jul 25, 2014**	South Fork	12	x					
1334	**Dec 1, 2014**	South Fork	12						
801	**Aug 15, 2013**	South Fork	13	x					
939	**Jul 25, 2014**	South Fork	13	x					
1336	**Dec 1, 2014**	South Fork	13						
802	**Aug 15, 2013**	South Fork	14	x					
940	**Jul 25, 2014**	South Fork	14	x					
1335	**Dec 1, 2014**	South Fork	14						
804	**Aug 15, 2013**	South Fork	15	x					
941	**Jul 25, 2014**	South Fork	15	x					
942	**Jul 25, 2014**	South Fork	15	x					
943	**Jul 25, 2014**	South Fork	15	x					
944	**Jul 25, 2014**	South Fork	15	x					
1337	**Dec 1, 2014**	South Fork	15						
805	**Aug 15, 2013**	South Fork	16	x					
945	**Jul 25, 2014**	South Fork	16	x					
946	**Jul 25, 2014**	South Fork	16	x					
806	**Aug 15, 2013**	South Fork	17	x					
947	**Jul 25, 2014**	South Fork	17	x					
316	**Jul 15, 2012**	South Fork	18			x	x		
317	**Jul 15, 2012**	South Fork	18			x	x		
320	**Jul 15, 2012**	South Fork	18			x	x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
573	**Aug 7, 2014**	South Fork	18					x	
574	**Aug 7, 2014**	South Fork	18					x	
575	**Aug 7, 2014**	South Fork	18			x	x		
807	**Aug 15, 2013**	South Fork	18	x					
948	**Jul 25, 2014**	South Fork	18	x					
949	**Jul 25, 2014**	South Fork	18	x					
289	**Jun 27, 2012**	South Fork	19				x		
290	**Jun 27, 2012**	South Fork	19				x		
291	**Jun 27, 2012**	South Fork	19				x		
292	**Jun 27, 2012**	South Fork	19				x		
293	**Jun 27, 2012**	South Fork	19				x		
294	**Jun 27, 2012**	South Fork	19			x	x		
295	**Jun 27, 2012**	South Fork	19			x	x		
296	**Jun 27, 2012**	South Fork	19			x	x		
297	**Jun 27, 2012**	South Fork	19				x		
298	**Jun 27, 2012**	South Fork	19				x		
299	**Jun 27, 2012**	South Fork	19				x		
300	**Jun 27, 2012**	South Fork	19			x	x		
301	**Jun 27, 2012**	South Fork	19			x	x		
302	**Jun 27, 2012**	South Fork	19			x	x		
303	**Jun 27, 2012**	South Fork	19			x	x		
304	**Jun 27, 2012**	South Fork	19			x	x		
310	**Jul 15, 2012**	South Fork	19			x	x		
311	**Jul 15, 2012**	South Fork	19			x	x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
312	**Jul 15, 2012**	South Fork	19			x	x		
313	**Jul 15, 2012**	South Fork	19			x	x		
314	**Jul 15, 2012**	South Fork	19			x	x		
315	**Jul 15, 2012**	South Fork	19			x	x		
319	**Jul 15, 2012**	South Fork	19			x	x		
576	**Oct 7, 2014**	South Fork	19				x	x	
577	**Oct 7, 2014**	South Fork	19				x		
950	**Jul 25, 2014**	South Fork	19	x					
951	**Jul 25, 2014**	South Fork	19	x					
952	**Jul 25, 2014**	South Fork	19	x					
268	**Jun 14, 2012**	South Fork	20				x		
269	**Jun 14, 2012**	South Fork	20				x		
270	**Jun 14, 2012**	South Fork	20		x		x		
271	**Jun 14, 2012**	South Fork	20				x		
272	**Jun 14, 2012**	South Fork	20				x		
273	**Jun 14, 2012**	South Fork	20				x		
274	**Jun 14, 2012**	South Fork	20			x	x		
275	**Jun 14, 2012**	South Fork	20				x		
276	**Jun 14, 2012**	South Fork	20				x		
277	**Jun 14, 2012**	South Fork	20				x		
278	**Jun 14, 2012**	South Fork	20				x		
279	**Jun 14, 2012**	South Fork	20				x		
280	**Jun 14, 2012**	South Fork	20			x	x		
281	**Jun 14, 2012**	South Fork	20			x	x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
282	**Jun 14, 2012**	South Fork	20			x	x		
283	**Jun 14, 2012**	South Fork	20				x		
284	**Jun 14, 2012**	South Fork	20				x		
285	**Jun 14, 2012**	South Fork	20			x	x		
286	**Jun 14, 2012**	South Fork	20			x	x		
287	**Jun 14, 2012**	South Fork	20		x				
288	**Jun 14, 2012**	South Fork	20		x				
305	**Jul 15, 2012**	South Fork	20			x	x		
306	**Jul 15, 2012**	South Fork	20			x	x		
307	**Jul 15, 2012**	South Fork	20			x	x		
308	**Jul 15, 2012**	South Fork	20				x		
309	**Jul 15, 2012**	South Fork	20				x		
318	**Jul 15, 2012**	South Fork	20			x	x		
579	**Oct 7, 2014**	South Fork	20				x		
580	**Oct 7, 2014**	South Fork	20			x	x		
953	**Jul 25, 2014**	South Fork	20	x					
581	**Oct 7, 2014**	South Fork	21				x		
583	**Aug 15, 2014**	South Fork	21			x	x	x	
954	**Jul 25, 2014**	South Fork	21	x					
955	**Jul 25, 2014**	South Fork	21	x					
584	**Aug 15, 2014**	South Fork	22			x	x	x	
585	**Aug 15, 2014**	South Fork	22				x	x	
808	**Aug 15, 2013**	South Fork	22	x					
956	**Jul 25, 2014**	South Fork	22	x					

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
957	**Jul 25, 2014**	South Fork	22	x					
586	**Aug 15, 2014**	South Fork	24				x		
809	**Aug 15, 2013**	South Fork	24	x	x				
587	**Aug 7, 2014**	South Fork	25				x		
810	**Aug 15, 2013**	South Fork	25	x					
958	**Jul 25, 2014**	South Fork	25	x					
588	**Aug 15, 2014**	South Fork	26				x		
811	**Aug 15, 2013**	South Fork	26	x					
959	**Jul 25, 2014**	South Fork	26	x					
589	**Aug 15, 2014**	South Fork	27				x		
812	**Aug 15, 2013**	South Fork	27	x					
813	**Aug 15, 2013**	South Fork	28	x					
590	**Aug 7, 2014**	South Fork	29				x		
591	**Aug 7, 2014**	South Fork	29			x	x		
814	**Aug 15, 2013**	South Fork	29	x					
960	**Jul 25, 2014**	South Fork	29	x					
961	**Jul 25, 2014**	South Fork	29	x					
815	**Aug 15, 2013**	South Fork	30	x					
816	**Aug 15, 2013**	South Fork	31	x					
328	**Jun 21, 2012**	South Fork	32				x		
329	**Jun 21, 2012**	South Fork	32				x		
817	**Aug 15, 2013**	South Fork	32	x					
962	**Jul 25, 2014**	South Fork	32	x					
818	**Aug 15, 2013**	South Fork	33	x					

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
963	**Jul 25, 2014**	South Fork	33	x					
964	**Jul 25, 2014**	South Fork	33	x					
819	**Aug 15, 2013**	South Fork	34	x					
965	**Jul 25, 2014**	South Fork	34	x					
966	**Jul 25, 2014**	South Fork	34	x					
967	**Jul 25, 2014**	South Fork	34	x					
330	**Jun 22, 2012**	South Fork	35			x	x		
339	**Jul 10, 2012**	South Fork	35				x		
340	**Jul 10, 2012**	South Fork	35			x	x		
341	**Jul 10, 2012**	South Fork	35			x	x		
342	**Jul 10, 2012**	South Fork	35			x	x		
343	**Jul 10, 2012**	South Fork	35			x	x		
820	**Aug 15, 2013**	South Fork	35	x					
968	**Jul 25, 2014**	South Fork	35	x					
321	**Jun 21, 2012**	South Fork	36			x	x		
322	**Jun 21, 2012**	South Fork	36			x	x		
323	**Jun 21, 2012**	South Fork	36			x	x		
324	**Jun 21, 2012**	South Fork	36			x	x		
325	**Jun 21, 2012**	South Fork	36			x	x		
326	**Jun 21, 2012**	South Fork	36			x	x		
327	**Jun 21, 2012**	South Fork	36			x	x		
331	**Jul 10, 2012**	South Fork	36			x	x		
332	**Jul 10, 2012**	South Fork	36			x	x		
333	**Jul 10, 2012**	South Fork	36			x	x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
334	**Jul 10, 2012**	South Fork	36				x		
335	**Jul 10, 2012**	South Fork	36				x		
336	**Jul 10, 2012**	South Fork	36				x		
337	**Jul 10, 2012**	South Fork	36				x		
338	**Jul 10, 2012**	South Fork	36				x		
344	**Jul 10, 2012**	South Fork	36				x		
345	**Jul 10, 2012**	South Fork	36				x		
346	**Jul 10, 2012**	South Fork	36			x	x		
347	**Jul 10, 2012**	South Fork	36			x	x		
348	**Jul 10, 2012**	South Fork	36			x	x		
349	**Jul 10, 2012**	South Fork	36			x	x		
350	**Jul 10, 2012**	South Fork	36				x		
821	**Aug 15, 2013**	South Fork	36	x					
592	**Aug 7, 2014**	South Fork	37			x	x		
593	**Sep 30, 2014**	South Fork	37			x	x		
594	**Aug 7, 2014**	South Fork	37				x		
822	**Aug 15, 2013**	South Fork	37	x					
969	**Jul 25, 2014**	South Fork	37	x					
970	**Jul 25, 2014**	South Fork	37	x					
595	**Sep 30, 2014**	South Fork	38				x	x	
823	**Aug 15, 2013**	South Fork	38	x					
596	**Sep 30, 2014**	South Fork	39				x	x	
824	**Aug 15, 2013**	South Fork	39	x					
971	**Jul 25, 2014**	South Fork	39	x					

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
825	**Aug 15, 2013**	South Fork	40	x					
597	**Sep 30, 2014**	South Fork	41				x	x	
598	**Sep 30, 2014**	South Fork	42				x	x	
599	**Sep 30, 2014**	South Fork	42				x	x	
826	**Aug 15, 2013**	South Fork	42	x					
972	**Jul 25, 2014**	South Fork	42	x					
600	**Jun 20, 2012**	South Fork	43					x	
827	**Aug 15, 2013**	South Fork	43	x					
601	**Jun 15, 2012**	South Fork	44				x		
602	**Sep 30, 2014**	South Fork	44				x		
603	**Aug 7, 2014**	South Fork	44			x	x		
828	**Aug 15, 2013**	South Fork	44	x					
973	**Jul 25, 2014**	South Fork	44	x					
604	**Sep 30, 2014**	South Fork	45				x		
605	**Oct 23, 2014**	South Fork	45				x		
829	**Aug 15, 2013**	South Fork	45	x					
974	**Jul 25, 2014**	South Fork	45	x					
975	**Jul 25, 2014**	South Fork	45	x					
606	**Oct 23, 2014**	South Fork	46				x		
830	**Aug 15, 2013**	South Fork	46	x					
607	**Oct 23, 2014**	South Fork	47				x		x
831	**Aug 15, 2013**	South Fork	47	x	x				
608	**Oct 23, 2014**	South Fork	48				x		
609	**Aug 7, 2014**	South Fork	48				x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
832	**Aug 15, 2013**	South Fork	48	x	x				
610	**Jun 30, 2014**	South Fork	49				x		
611	**Jun 30, 2014**	South Fork	49				x		
612	**Jun 30, 2014**	South Fork	49		x		x		x
833	**Aug 15, 2013**	South Fork	49	x	x				
613	**Jun 30, 2014**	South Fork	50				x		
614	**Jun 30, 2014**	South Fork	50				x		
615	**Jun 30, 2014**	South Fork	50				x		
834	**Aug 15, 2013**	South Fork	51	x					
976	**Jul 25, 2014**	South Fork	51	x					
835	**Aug 15, 2013**	South Fork	52	x					
977	**Jul 25, 2014**	South Fork	52	x					
978	**Jul 25, 2014**	South Fork	52	x					
616	**Jun 18, 2014**	South Fork	53				x		
836	**Aug 15, 2013**	South Fork	53	x					
979	**Jul 25, 2014**	South Fork	53	x					
837	**Aug 15, 2013**	South Fork	54	x					
838	**Aug 15, 2013**	South Fork	55	x					
980	**Jul 25, 2014**	South Fork	55	x					
839	**Aug 15, 2013**	South Fork	56	x					
981	**Jul 25, 2014**	South Fork	56	x					
617	**Oct 28, 2014**	South Fork	57			x	x		
840	**Aug 15, 2013**	South Fork	57	x					
618	**Oct 28, 2014**	South Fork	58				x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
619	**Oct 28, 2014**	South Fork	58				x		
620	**Jun 15, 2012**	South Fork	58				x		
621	**Oct 28, 2014**	South Fork	58				x		
841	**Aug 15, 2013**	South Fork	58	x					
982	**Jul 25, 2014**	South Fork	58	x					
622	**Oct 28, 2014**	South Fork	59				x		
623	**Oct 28, 2014**	South Fork	59				x		
842	**Aug 15, 2013**	South Fork	59	x					
624	**Oct 28, 2014**	South Fork	60				x		
843	**Aug 15, 2013**	South Fork	60	x					
844	**Aug 15, 2013**	South Fork	61	x	x				
845	**Aug 15, 2013**	South Fork	62	x	x				
846	**Aug 15, 2013**	South Fork	63	x					
625	**Oct 28, 2014**	South Fork	64				x		
626	**Oct 28, 2014**	South Fork	64				x		
627	**Oct 28, 2014**	South Fork	64				x		
847	**Aug 15, 2013**	South Fork	64	x					
628	**Oct 28, 2014**	South Fork	65				x		
629	**Jul 17, 2014**	South Fork	65				x		
848	**Aug 15, 2013**	South Fork	65	x					
983	**Jul 25, 2014**	South Fork	65	x					
984	**Jul 25, 2014**	South Fork	65	x					
630	**Jul 17, 2014**	South Fork	66				x		
849	**Aug 15, 2013**	South Fork	66	x					

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
985	**Jul 25, 2014**	South Fork	66	x					
631	**Jul 17, 2014**	South Fork	67				x		
850	**Aug 15, 2013**	South Fork	67	x					
632	**Jul 17, 2014**	South Fork	68				x		
633	**Jul 17, 2014**	South Fork	69				x		
851	**Aug 15, 2013**	South Fork	69	x					
634	**Jul 17, 2014**	South Fork	70			x	x	x	
852	**Aug 15, 2013**	South Fork	70	x					
635	**Jul 17, 2014**	South Fork	71				x		
636	**Jul 17, 2014**	South Fork	71				x		
853	**Aug 15, 2013**	South Fork	71	x					
637	**Jul 17, 2014**	South Fork	72			x	x		x
854	**Aug 15, 2013**	South Fork	72	x					
638	**Jul 17, 2014**	South Fork	73				x		
855	**Aug 15, 2013**	South Fork	73	x					
986	**Jul 25, 2014**	South Fork	73	x	x				
856	**Aug 15, 2013**	South Fork	74	x	x				
987	**Jul 25, 2014**	South Fork	74	x	x				
237	**Jun 13, 2012**	South Fork	75				x		
238	**Jun 13, 2012**	South Fork	75				x		
239	**Jun 13, 2012**	South Fork	75				x		
257	**Jun 13, 2012**	South Fork	75			x	x		
258	**Jun 13, 2012**	South Fork	75			x	x		
639	**Aug 31, 2014**	South Fork	75			x	x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
857	**Aug 15, 2013**	South Fork	75	x					
988	**Jul 25, 2014**	South Fork	75	x					
989	**Jul 25, 2014**	South Fork	75	x					
240	**Jun 13, 2012**	South Fork	76				x		
241	**Jun 13, 2012**	South Fork	76				x		
640	**Aug 31, 2014**	South Fork	76				x	x	
641	**Aug 31, 2014**	South Fork	76				x	x	
858	**Aug 15, 2013**	South Fork	76	x					
990	**Jul 25, 2014**	South Fork	76	x					
991	**Jul 25, 2014**	South Fork	76	x	x				
223	**Jun 13, 2012**	South Fork	77			x	x		
224	**Jun 13, 2012**	South Fork	77			x	x		
225	**Jun 13, 2012**	South Fork	77			x	x		
226	**Jun 13, 2012**	South Fork	77				x		
227	**Jun 13, 2012**	South Fork	77				x		
242	**Jun 13, 2012**	South Fork	77				x		
243	**Jun 13, 2012**	South Fork	77			x	x		
642	**Aug 31, 2014**	South Fork	77				x	x	
859	**Aug 15, 2013**	South Fork	77	x					
992	**Jul 25, 2014**	South Fork	77	x					
993	**Jul 25, 2014**	South Fork	77	x					
994	**Jul 25, 2014**	South Fork	77	x					
228	**Jun 13, 2012**	South Fork	78						
229	**Jun 13, 2012**	South Fork	78						

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
643	**Aug 31, 2014**	South Fork	78						
860	**Aug 15, 2013**	South Fork	78	x					
995	**Jul 25, 2014**	South Fork	78	x					
230	**Jun 13, 2012**	South Fork	79			x	x		
231	**Jun 13, 2012**	South Fork	79			x	x		
244	**Jun 13, 2012**	South Fork	79			x	x		
245	**Jun 13, 2012**	South Fork	79			x	x		
246	**Jun 13, 2012**	South Fork	79				x		
247	**Jun 13, 2012**	South Fork	79				x		
248	**Jun 13, 2012**	South Fork	79				x	x	
249	**Jun 13, 2012**	South Fork	79				x		
259	**Jun 13, 2012**	South Fork	79		x	x	x		
260	**Jun 13, 2012**	South Fork	79		x				
261	**Jun 13, 2012**	South Fork	79				x		
262	**Jun 13, 2012**	South Fork	79			x	x		
263	**Jun 13, 2012**	South Fork	79				x		
264	**Jun 13, 2012**	South Fork	79				x		
265	**Jun 13, 2012**	South Fork	79				x		
266	**Jun 13, 2012**	South Fork	79				x		
267	**Jun 13, 2012**	South Fork	79				x		
644	**Aug 31, 2014**	South Fork	79						
861	**Aug 15, 2013**	South Fork	79	x					
232	**Jun 13, 2012**	South Fork	80			x	x		
233	**Jun 13, 2012**	South Fork	80			x	x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
234	**Jun 13, 2012**	South Fork	80			x	x		
235	**Jun 13, 2012**	South Fork	80			x	x		
236	**Jun 13, 2012**	South Fork	80			x	x		
250	**Jun 13, 2012**	South Fork	80		x				
251	**Jun 13, 2012**	South Fork	80			x	x		
252	**Jun 13, 2012**	South Fork	80		x				
253	**Jun 13, 2012**	South Fork	80			x	x		
254	**Jun 13, 2012**	South Fork	80			x	x		
255	**Jun 13, 2012**	South Fork	80				x		
256	**Jun 13, 2012**	South Fork	80						
862	**Aug 15, 2013**	South Fork	80	x					
863	**Aug 15, 2013**	South Fork	81	x					
396	**Jun 4, 2007**	South Fork	82		x			x	
645	**Aug 18, 2014**	South Fork	82						
646	**Aug 18, 2014**	South Fork	82						
864	**Aug 15, 2013**	South Fork	82	x	x				
647	**Aug 18, 2014**	South Fork	83						
865	**Aug 15, 2013**	South Fork	83	x					
648	**Aug 18, 2014**	South Fork	84						
866	**Aug 15, 2013**	South Fork	84	x					
996	**Jul 25, 2014**	South Fork	84	x					
649	**Aug 18, 2014**	South Fork	85						
867	**Aug 15, 2013**	South Fork	85	x					
650	**Aug 18, 2014**	South Fork	86						

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
651	**Aug 18, 2014**	South Fork	86						
653	**Aug 21, 2014**	South Fork	86						
868	**Aug 15, 2013**	South Fork	86	x					
869	**Aug 15, 2013**	South Fork	87	x					
654	**Aug 21, 2014**	South Fork	88						
870	**Aug 15, 2013**	South Fork	88	x					
997	**Jul 25, 2014**	South Fork	88	x					
655	**Aug 21, 2014**	South Fork	89						
871	**Aug 15, 2013**	South Fork	89	x					
998	**Jul 25, 2014**	South Fork	89	x					
999	**Jul 25, 2014**	South Fork	89	x					
1000	**Jul 25, 2014**	South Fork	89	x					
872	**Aug 15, 2013**	South Fork	90	x					
656	**Aug 21, 2014**	South Fork	91						
657	**Aug 21, 2014**	South Fork	91						
873	**Aug 15, 2013**	South Fork	91	x					
658	**Aug 21, 2014**	South Fork	92						
659	**Aug 21, 2014**	South Fork	92						
874	**Aug 15, 2013**	South Fork	92	x					
660	**Aug 21, 2014**	South Fork	93						
661	**Aug 21, 2014**	South Fork	93						
803	**Aug 21, 2014**	South Fork	93						
875	**Aug 15, 2013**	South Fork	93	x					
876	**Aug 15, 2013**	South Fork	94	x					

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
582	**Aug 21, 2014**	South Fork	95			x	x	x	
877	**Aug 15, 2013**	South Fork	95	x					
578	**Aug 21, 2014**	South Fork	96				x		
878	**Aug 15, 2013**	South Fork	96	x					
879	**Aug 15, 2013**	South Fork	97	x					
351	**Jun 16, 2012**	South River							
352	**Jun 16, 2012**	South River							
353	**Jun 16, 2012**	South River				x	x		
354	**Jun 16, 2012**	South River				x	x		
355	**Jun 16, 2012**	South River					x		
356	**Jul 2, 2012**	South River					x		
357	**Jul 2, 2012**	South River					x		
358	**Jul 2, 2012**	South River					x		
359	**Jul 2, 2012**	South River					x		
360	**Jul 2, 2012**	South River					x		
361	**Jul 2, 2012**	South River					x		
362	**Jul 2, 2012**	South River				x	x		
363	**Jul 2, 2012**	South River					x		
364	**Jul 2, 2012**	South River					x		
365	**Jul 2, 2012**	South River				x	x		
366	**Jul 2, 2012**	South River					x		
368	**Jul 2, 2012**	South River				x	x		
369	**Jul 2, 2012**	South River					x		
370	**Jul 2, 2012**	South River					x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
371	**Jul 2, 2012**	South River					x		
372	**Jul 2, 2012**	South River					x		
373	**Jul 2, 2012**	South River					x		
374	**Jul 2, 2012**	South River					x		
375	**Jul 2, 2012**	South River					x	x	
376	**Jul 2, 2012**	South River					x		
377	**Jul 2, 2012**	South River					x		x
378	**Jul 2, 2012**	South River					x		x
379	**Jul 14, 2012**	South River					x		
380	**Jul 14, 2012**	South River					x		x
381	**Jul 14, 2012**	South River					x		x
382	**Jul 14, 2012**	South River					x		x
383	**Jul 14, 2012**	South River					x		x
384	**Jul 14, 2012**	South River					x		x
385	**Jul 14, 2012**	South River				x	x		
386	**Jul 14, 2012**	South River					x		
387	**Jul 14, 2012**	South River					x		
388	**Apr 30, 2007**	South Fork				x	x		
389	**Apr 30, 2007**	South Fork				x	x		
390	**Apr 30, 2007**	South Fork					x		
391	**Apr 30, 2007**	South Fork					x		
392	**Apr 30, 2007**	South Fork					x		
397	**Jul 30, 2007**	South Fork				x	x		x
398	**Jul 30, 2007**	South Fork					x	x	

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
399	**Jul 30, 2007**	South Fork						x	
400	**Jul 30, 2007**	South Fork				x	x		
401	**Jul 30, 2007**	South Fork			x				
402	**Jul 30, 2007**	South Fork					x		
403	**Jul 27, 2006**	South Fork					x		
404	**Jul 27, 2006**	South Fork					x		
405	**Jul 27, 2006**	South Fork					x		
406	**Apr 30, 2007**	South Fork					x		
407	**Apr 30, 2007**	South Fork					x		
408	**Apr 30, 2007**	South Fork					x		
409	**Apr 30, 2007**	South Fork					x		
410	**Apr 30, 2007**	South Fork					x		
418	**Apr 7, 2008**	South Fork				x	x		x
419	**Apr 7, 2008**	South Fork				x	x		
420	**Apr 9, 2008**	South Fork					x		
421	**Apr 7, 2008**	South Fork					x		
422	**Apr 9, 2008**	South Fork					x		
423	**Apr 7, 2008**	South Fork				x	x		
424	**Apr 7, 2008**	South Fork					x		
425	**Mar 17, 2008**	South Fork					x		
426	**Mar 17, 2008**	South Fork					x		
427	**Mar 17, 2008**	South Fork					x		
428	**Mar 17, 2008**	South Fork					x		
429	**Mar 17, 2008**	South Fork					x		

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
430	Mar 17, 2008	South Fork					x		
431	Mar 17, 2008	South Fork					x		
652	Aug 18, 2014	South Fork							
367	Jul 2, 2012	South River				x	x		
411	May 25, 2010						x		
412	May 25, 2010						x		
413	May 25, 2010						x		
414	May 25, 2010						x		
415	May 25, 2010						x		
416	May 25, 2010						x		
417	May 25, 2010						x		x
432	Nov 4, 2009					x	x		
433	Nov 4, 2009						x	x	
434	Oct 30, 2009					x	x		
435	Oct 30, 2009					x	x		
436	Oct 30, 2009					x	x		
437	Oct 30, 2009						x	x	
438	Oct 30, 2009						x		
439	Oct 30, 2009					x	x		
440	Oct 30, 2009					x	x		
441	Oct 30, 2009					x	x		
442	Oct 30, 2009						x		
443	Sep 24, 2010					x	x		
1024	Aug 26, 2014								

Photo # (A_)	Date		River Mile(s)	Aerial	Highly colored water	Vascular w/ Periphyton	Periphyton	Floating Materials	Turbidity
1025	Aug 26, 2014								
1026	Aug 29, 2014								
1027	Aug 29, 2014								
1028	Aug 29, 2014								
1333	Jan 1, 2014								

Videos (Vid#)				
1	Oct 30, 2009	13:14		
2	Oct 30, 2009	15:48		
3	Oct 30, 2009	16:23		
4	Oct 30, 2009	15:45		
5	Oct 30, 2009	13:15		
6	Oct 30, 2009	13:17		
7	Oct 30, 2009	12:48		
8	Oct 30, 2009	12:53		
9	Oct 30, 2009	13:09		
10	Oct 30, 2009	12:50		
11	Jul 29, 2014			
12	Jul 29, 2014			
13	Oct 7, 2014			
14	Oct 7, 2014			
15	Oct 7, 2014			

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Observations

	Stream Width	Distance From Start	Time	Avg. Stream Depth	Stream Shading	Stream Sun	Organic	Mud	Sand	Gravel	Cobble	Bedrock
1	76	0	7:58	1.5	0	100				40–70	10–40	10–40
2	55	0.25	8:14	1.5	20	80				10–40	10–40	10–40
3	48	0.5	8:25	2.5	30	70				10–40	10–40	10–40
4	53	0.75	8:37	2.5	90	10				1–10	10–40	10–40
5	55	1	8:50	1	50	50				1–10	10–40	40–70
6	50	1.25	9:06	1.25	30	70				40–70		40–70
7	50	1.5	9:37	1.5	20	80				40–70		10–40
8	40	1.75	9:51	1	40	60				10–40	10–40	10–40
9	40	2	10:00		20	80				40–70		40–70
10	52	2.25	10:28	1.5	20	80						>70
11	40	2.5	10:41	<1	50	50						>70
12	56	2.75	10:54		20	80				40–70		
13	52	3	11:12	3	20	80		10–40		10–40	1–10	
14	38	3.25	11:32	2	50	50				40–70	10–40	10–40
15	47	3.5	11:38	1.5	50	50				>70		
16	47	3.75	11:46	1	20	80				>70	10–40	
17	50	4	11:56	2.5	50	50					10–40	40–70
18	55	4.25	12:14	3	40	60				10–40	10–40	10–40
19	50	4.5	12:25	1.5	10	90				10–40		40–70
20	53	4.75	12:33	1	30	70				40–70		40–70
21	57	5	12:45	1.5	15	85				10–40		>70
22	56	5.25	12:56	1.5	40	60				10–40	10–40	40–70

Algae Community

ansect	lgae Typ	Color	ample #	ample #2	Sample #3	ample #4	ample #5	ample #6	ample #7	Sample #8	Sample #9	Sample #10
1	Filamentous	Brown										
	Filamentous	Dark	0	0	0	0	0	0	0	0	10	0
1		Green	0	0	10	30	10	0	10	10	10	0
2	Filamen	Dark										

	tous	Green										
	Thick	Dark	10	10	30	70	10	10	100	100	0	0
2	Mat	Green	0	0	0	0	0	0	50	100	0	0
3	Filament	Dark Gre	0	30	70	70	70	70	70	70	30	10
4	Filament	Dark Gre	10	10	40	50	70	70	40	10	10	0
5	Filament	Dark Gre	10	10	10	10	10	30	50	50	30	0
6	Filament	Dark Gre	70	70	100	100	50	10	10	30	30	10
7	Filament	Dark Gre	10	10	10	10	10	10	50	50	30	10
8	Filament	Dark Gre	30	30	30	30	30	30	30	30	30	30
9	Thick Ma	Dark Gre	0	0	0	0	0	0	0	0	0	90
9	Filament	Dark Gre	10	10	10	10	10	10	10	10	10	90
10	Filament	Dark Gre	0	10	30	40	50	50	70	70	30	30
11	Filament	Dark Gre	10	10	10	20	30	30	10	10	0	0
12	Filament	Dark Gre	0	0	10	0	10	0	10	30	30	0
13	Filament	Dark Gre	0	0	0	0	0	0	0	0	0	0
14	Filament	Dark Gre	10	10	10	30	50	10	10	0	0	0
15	Filament	Dark Gre	0	0	0	0	10	70	70	70	30	10
16	Filament	Dark Gre	30	70	70	70	50	50	0	0	0	0
17	Filament	Dark Gre	10	10	10	10	10	20	20	10	0	0
18	Filament	Dark Gre	70	70	0	0	10	10	10	10	0	0
19	Filament	Dark Gre	10	10	30	50	50	50	0	0	0	0
20	Filament	Dark Gre	0	0	30	30	50	50	50	50	10	0
21	Filament	Dark Gre	0	0	10	10	20	20	20	30	50	10
22	Filament	Dark Gre	0	10	30	30	50	50	50	30	10	0
			11.7	15.7	21.7	24.3	27.4	27.4	24.8	23.9	13.9	4.8

Plant Community

Trans	Plant Typ	Sample #1	ample #	ample #3	Sample #4	ample #5	Sample #6	mple #7	ample #8	Sample #9	Sample #10
1	Submerg	0	0	0	0	0	0	10	0	0	0
2											
3											
4											
5	Submerg	0	0	0	10	10	10	10	0	0	0
6	Submerg	10	0	0	0	0	0	0	0	0	0
7	Submerg	0	10	90	90	70	30	10	10	10	10
8	Submerged		30	30	30	30	30	0	0	0	0
9	Submerg	0	0	10	10	0	0	0	0	0	0
10											
11											
12											
13											
14											
15											
16											
17											
18											
19											
20											
21											
22											

Effect of Algae on Plants												
Trans	lgae Typ	Color	ample #	ample #2	Sample #3	ample #4	ample #5	mple #6	ample #7	Sample #8	Sample #9	Sample #10
1												
2												
3												
4												
5	Filament	Dark Green				30	30	30	30			
6	Film	Dark Gre	10									
7												
8	Film	Dark Green		50	50	50	50	50				
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
Other Wildlife												
Trans	Wildlife	Stressors										
1												
2												
3												
4	No fish											
5	Heron											
6												

7	Turtle											
8	Deer											
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22		Water pipe coming in										

0.0	–	
0.0	9.0	9.0
0.0	–	49.0

0.0	49.0	49.0
49.0	49.0	31.0
31.0	31.0	21.0
21.0	21.0	48.0
48.0	48.0	20.0
20.0	20.0	30.0
30.0	30.0	27.0
0.0	–	38.0
0.0	27.0	13.0
38.0	38.0	9.0
13.0	13.0	0.0
9.0	9.0	13.0
0.0	0.0	26.0
13.0	13.0	34.0
26.0	26.0	10.0
34.0	34.0	18.0
10.0	10.0	20.0
18.0	18.0	27.0
20.0	20.0	17.0
27.0	27.0	26.0
17.0	17.0	
26.0	26.0	
19.6		

median	21.0	
mean	24.3	
std dev.	14.1	
95% conf.	5.9	

Observations

	Stream Width	Distance From Start	Time	Avg. Stream Depth	Stream Shading	Stream Sun	Organic	Mud	Sand	Gravel	Cobble	Bedrock
1	73	0	8:02	1	0	100				40-70		10-40
2		0.25	8:13	1	15	85				40-70		40-70
3		0.5	8:27	2	10	90				1-10	40-70	40-70
4		0.75	8:36	1	20	80					40-70	40-70
5	38	1	8:59	1	40	60				10-40	1-10	>70
6	36	1.25	9:17	1	40	60				40-70		40-70
7	45	1.5	9:32	2	10	90				1-10		>70
8	30	1.75	9:43	1.5	20	80					40-70	10-40
9	35	2	9:57	1	20	80				10-40	10-40	40-70
10	57	2.25	10:19	1	10	90				1-10	1-10	>70
11	49	2.5	10:36	1	50	50					10-40	>70
12		2.75	10:53	1	20	80						>70
13		3	11:04	1.5	20	80				40-70		40-70
14		3.25	11:27	1	30	70				10-40		>70
15	40	3.5	11:35	1.5	50	50					40-70	40-70
16	38	3.75	11:41	1	40	60					40-70	40-70
17	49	4	11:54	2	40	60				10-40		>70
18	55	4.25	12:.05	1.5	60	40					10-40	>70
19	50	4.5	12:16	1	0	100				40-70		10-40
20	52	4.75	12:28	1	10	90				40-70		>70
21	42	5	12:35	1	10	90				10-40		>70
22	60	5.25	12:42	1.5	20	80				1-10	1-10	>70
23	48	5.5	12:50	1	30	70				10-40		>70

Algae Community

ansec	Algae Type	Color	ample #	Sample #2	Sample #3	ample #5	ample #5	ample #6	ample #7	Sample #8	Sample #9	Sample #10
1	Thick Mat	Dark Green	0	50	30	10	10	10	S	S	10	10
2	Filamentous	Dark Green	0	0	0	0	0	10	90	0	0	0
2	Thick Mat	Dark Green	0	0	50	70	50	90	0	70	70	50

3	Thick Mat	Dark Green	100	100	70	70	90	70	50	90	20	10
4	Filamentous	Dark Green	0	0	10	0	0	20	0	0	0	0
4	Thick Mat	Dark Green	0	20	20	40	70	40	50	70	90	30
5	Filamentous	Dark Green	0	0	0	0	0	0	0	30	30	0
5	Thick Mat	Dark Green	S	S	0	0	0	S	10	0	10	10
6	Thick Mat	Dark Green	0	0	S	10	30	20	40	10	20	40
7	Thick Mat	Dark Green	0	0	20	30	30	30	50	70	0	0
8	Thick Mat	Dark Green	S	S	0	0	10	10	10	10	10	0
9	Thick Mat	Dark Green	0	20	30	40	10	10	20	S	S	S
10	Thick Mat	Dark Green	10	10	10	10	40	10	10	20	20	90
11	Thick Mat	Dark Green	S	S	10	10	S	0		S	S	0
12	Thick Mat	Dark Green	S	0	0	0	0	0	0	S	10	10
13	Thick Mat	Brown	0	0	0	0	0	30	30	30	50	50
14	Thick Mat	Dark Green	10	10	10	0	0	0	0	0	0	0
15	Filamentous	Dark Green	0	0	0	10	10	0	0	0	0	0
15	Thick Mat	Dark Green	20	20	10	10	10	10	10	20	0	0
16	Filamentous	Dark Green	0	0	0	10	10	0	0	0	0	0
16	Thick Mat	Dark Green	30	10	0	40	30	30	20	0	0	0
17	Thick Mat	Dark Green	40	40	30	30	10	10	10	0	0	0
18	Filamentous	Dark Green	0	0	0	0	0	0	10	10	10	10
19	Thick Mat	Dark Green	40	40	20	20	20	50	50	50	30	10
20	Thick Mat	Dark Green	50	50	50	50	70	70	70	50	90	0
21	Thick Mat	Dark Green	30	30	30	0	0	0	0	10	10	0
22	Thick Mat	Dark Green	10	50	50	50	50	70	70	70	10	0
23	Thick Mat	Dark Green	0	10	20	20	20	20	20	10	0	0
			14	18	17	19	21	23	24	26	19	12

Plant Community												
Tran	Plant Types	Sample #1	ample #	Sample #3	Sample #4	ample #5	Sample #6	mple #7	mple #8	Sample #9	Sample #10	
1												
2												
3												
4												
5												
6												
7	Submerged			50	50	50	0	10	10			
8	Submerged			30	90	30	30	50	20			
9	Submerged			20			10					
10												
11	Submerged			20		10	0	30	40	0	0	
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												

Effect of Algae on Plants												
Tran	Algae Type	Color	ample #	Sample #2	Sample #3	ample #4	ample #5	mple #6	mple #7	Sample #8	Sample #9	Sample #10
1												
2												
3												
4												
5												
6												
7	Thick Mat	Dark Green			10	10	10					
8												
9	Film	Dark Green			50							
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
Other Wildlife												
Tran	Wildlife	Stressors										
1												
2												
3												
4												
5												

6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												

16	16	16	
10	–	55	
45	55	67	

67	67	46	Almost dea
3	–	10	
43	46	19	
6	–	23	
4	10	6	
19	19	19	
23	23	23	
6	6	5	
19	19	3	
23	23	13	
5	5	2	
3	3	18	
19	19	17	
3	3	4	
2	–	33	
11	13	55	
2	–	11	
16	18	43	
17	17	12	1st was dea
4	4		
33	33		
55	55		Dead and A
11	11		
43	43		Dying and
12	3		Alive and d
19			
#	24		

mean	21.8		
min.	2		
max.	67		
median	17.5		Stinks
std. dev.	18.9		
95% conf.	7.6		
			Stinks

Blobs

Observations												
	Stream Width	Distance From Start	Time	Avg. Stream Depth	Stream Shading	Stream Sun	Organic	Mud	Sand	Gravel	Cobble	Bedrock
1		0	8:18	1.5	0	100				10-40	10-40	10-40
2		0.25	8:30	1.5	20	80				40-70		10-40
3		0.5	8:39		30	70					10-40	40-70
4		0.75	8:48	2	30	70					>70	
5		1	9:08	1.5	20	80			10-40	40-70	10-40	
6		1.25	9:20	1.5	10	90				10-40	10-40	10-40
7		1.5	9:40	2	10	90				10-40	1-10	>70
8		1.75	9:50	2	10	90					10-40	>70
9		2	10:03	1	20	80				10-40	10-40	10-40
10		2.25	10:21	2	10	90			10-40			40-70
11		2.5	10:37	1.5	50	50					10-40	>70
12		2.75	10:55	2	20	80			40-70	10-40		10-40
13		3	11:17	3	10	90			10-40	10-40		>70
14		3.25	11:41	1.5	50	50			10-40			40-70
15			11:53	2						10-40	10-40	
16		3.75	12:10	1	20	80				10-40		>70
17		4	12:19	3	20	80			10-40	10-40		1-10
18		4.25	12:25	2	20	80				10-40	10-40	10-40
19		4.5	12:33	1	0	100				>70		
20			12:40	1								>70
21		5	12:47	1						10-40		40-70
22			12:56	1.5	20	80				>70		
23			1:06	1	30	70				10-40		40-70
Algae Community												
ransect	Algae Type	Color	ample #	Sample #2	Sample #3	Sample #4	Sample #5	ample #	ample #7	Sample #8	Sample #9	Sample #10
1	Filamento	Dark Gre	0	10	10	10	30	40	30	10	0	0
2	Filamento	Dark Green		20	10	10	10	10	30	S	S	0
3	Filamento	Dark Gre	50	20	20	20	20	20	30	50	50	10

4	Filamento	Dark Gre	40	50	50	50	50	40	40	50	30	0
5	Filamento	Dark Gre	30	80	30	30	30	30	30	30	50	0
5	Filamento	Bright Gr	20	10	10	0	0	0	0	0	0	0
6	Filamento	Dark Gre	30	0	0	0	0	0	30	30	0	50
7	Filamento	Dark Gre	0	0	0	30	30	30	30	30	30	90
8												
9	Filamento	Dark Gre	0	0	0	S	0	0	0	0	0	0
10	Filamento	Dark Gre	10	0	0	0	0	0	0	0	S	0
11												
12	Filamento	Dark Gre	10	0	0	S	0	S	0	0	0	0
13	Filamento	Dark Gre	S	0	0	0	0	0	0	0	0	0
14	Filamento	Dark Gre	0	0	0	0	0	0	0	0	0	30
14	Film	Dark Gre	10	0	0	0	0	0	0	0	0	0
15	Filamento	Dark Gre	30	0	20	50	40	10	10	0	0	0
16	Filamento	Dark Gre	10	100	0	0	0	0	0	0	0	0
17	Filamento	Dark Green		S	20	20	50	30	10	0	0	0
17	Filamento	Bright Gr	50	50	50	20	50	70	20	30	0	0
18	Filamento	Dark Gre	30	0	0	0	30	50	0	20	0	0
19	Filamento	Dark Gre	100	90	90	90	80	10	0	0	0	0
19	Filamento	Bright Gr	0	0	0	0	0	0	50	10	10	0
20	Filamento	Dark Gre	100	90	10	100	90	100	90	0	0	0
20	Filamento	Bright Gr	0	0	0	0	0	0	0	30	0	0
21	Filamento	Dark Gre	0	10	10	30	60	60	50	30	0	0
21	Filamento	Bright Gr	S	70	90	70	40	10	0	0	30	50
22	Filamento	Dark Gre	0	10	10	0	0	0	10	30	30	30
22	Filamento	Bright Gr	30	90	90	100	100	100	90	70	70	0
23	Filamento	Dark Gre	30	30	30	50	40	70	80	20	0	0
23	Filamento	Bright Gr	0	0	0	0	0	30	20	80	90	0
			23	26	19	25	26	25	22	19	14	9

Plant Community

Transe	Plant Typ	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	mple #7	mple #8	mple #9	mple #10
1	Submerge	0	0	10	0	0	30	0	0	0	0
2											0
3		0	0	0	0	0	0	0	10	0	0
4											
5	Submerge	0	0	0	40	60	20	20	0	0	0
6	Submerge	50	100	90	50	50	70	70	70	50	0
7	Submerge	0	10	0	30	60	70	50	10	30	0
8	Submerge	0	50	70	70	50	30	30	30	30	0
9	Submerge	0	0	0	10	10	0	30	0	0	0
10											
11	Submerge	0	0	0	10	30	50	0	0	0	0
12											
13											
14	Submerge	0	0	0	0	0	10	0	10	0	0
15											
16											
17											
18											
19											
20											
21											
22											
23											

Effect of Algae on Plants												
Transe	Algae Type	Color	ample #	Sample #2	Sample #3	Sample #4	Sample #5	nple #6	ample #7	ample #8	ample #9	Sample #10
1	Filamento	Dark Green			70			90				
2	Filamento	Dark Green								90		
3												
4												
5	Filamento	Dark Green				90	90	90	90			
6	Filamento	Dark Gre	50	90	90	90	90	90	90	90	50	
7	Filamento	Dark Green				50	50	50	50	50	50	50
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
Other Wildlife												
Transe	Wildlife	Stressors										
1												
2												
3												
4												
5												

6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												

14	14	14
13	13	13
29	29	29

40	40	40
34	–	38
4	38	14
14	14	27
27	27	0
		1
0	0	1
1	1	0
		4
1	1	16
0	0	11
3	–	50
1	4	13
16	16	53
11	11	61
16	–	65
34	50	86
13	13	57
46	–	
7	53	
58	–	
3	61	
25	–	
40	65	
12	–	
74	86	
35	–	
22	57	

#	21	
mean	28.2	
min	0	
max	86	
median	15.0	
std. dev.	25.5	
95% conf.	10.9	

Observations									
Transect #	Stream Wid	Distance Fr	Time	Avg. Stream	ream Shadir	Stream Sun	Organic	Mud	Sand
1	45	0	7:50	3	50	50	0	0	10-40
2	60	0.25	8:10	2	0	100	0	0	0
3	70	0.5	8:25	4	20	80	0	1-10	0
4	70	0.75	8:35	3	10	90	0	0	0
5	62	1	8:46		10	90	0	1-10	0
6	70	2.00	9:40	4	30	70	0	1-10	0
7	63	2.25	9:50	2.5	20	80	0	0	0
8	72	2.5	10:07		10	90	0	1-10	0
9	78	2.75	10:27		10	90	1-10	1-10	0
10	58	3	10:45	3	0	100	0	0	1-10
11	100	4	11:39	3	5	95	0	1-10	1-10
12	67	4.25	12:24	NA	0	100	0	10-40	1-10
13	104	5.25	1:11	3.5	15	85	1-10	1-10	0
14	90	5.5	1:30	NA	20	80	0	40-70	1-10
15									
Algae Community									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1	Thick Mat	Dark Green	0	0	NA	NA	0	0	0
2									
3								NA	NA
4									
4									
5									
6									
7	Filamentous	Dark Green	50	0	0	0	0	0	0
8									
9									
9									
10									
11	Filamentous	Brown	30	0	0	0	0	0	0

12	Filamentous	Brown	50	5	0	0	0	0	0
13									
14	Filamentous	Brown							
15									
			32.5	0	0	0	0	0	0
Plant Community									
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1	Emergent	0	0	NA	NA	0	0	0	0
2	Submerged	5	0	0	5	10	10	10	10
2	Emergent	0	0	0	0	0	0	0	0
3	Submerged	0	0	30	30	30	NA	NA	40
4	Emergent	50	40	0	0	0	0	0	0
4	Submerged	0	0	0	10	10	10	50	90
5	Submerged	0	0	NA	NA	NA	NA	NA	70
6	Submerged	0	0	0	NA	NA	NA	0	5
6	Emergent	10	0	0	NA	NA	NA	0	0
7	Emergent	10	0	0	0	0	0	0	0
8	Submerged	10	30	40	70	0	0	90	70
9	Submerged	0	10	40	40	40	40	40	10
9	Emergent	0	0	0	0	0	0	0	0
10	Emergent	100	0	0	0	0	0	0	0
11	Emergent	70	0	0	0	0	0	0	0
11	Submerged	0	30	0	40	70	0	90	0
12	Submerged	0	NA	NA	NA	NA	NA	NA	0
13	Submerged	0	0	40	100	10	40	100	100
14	Submerged	0	100	80	100	NA	NA	NA	NA
15									
Effect of Algae on Plants									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									

2									
3									
4									
5									
6									
7	Filamentou	Dark Green	10						
8									
9									
10									
11									
12	Filamentou	Brown	50						
13									
14									
15									
Other Wildlife									
Transect #	Wildlife		Stressors						
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
Transect Landmarks									
Transect #	Landmarks								
1									

2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									

Gravel	Cobble	Bedrock			
1-10	10-40	1-10			
10-40	40-70	0			
10-40	10-40	1-10			
10-40	40-70	0			
1-10	40-70	0			
1-10	40-70	1-10			
10-40	40-70	0			
10-40	40-70	0			
1-10	40-70	0			
1-10	10-40	40-70			
10-40	10-40	0			
0	1-10	10-40			
1-10	10-40	10-40			
1-10	1-10	10-40			
Sample #8	Sample #9	Sample #10			
0	0	0	0	0	0
					8
					3
					5
				0	
0	0	30	8		
				3	
0	0	0	3	5	

0	0	0	5		
	S	S			
0	0	7.5			
			#	4	
			mean	4	
			min	0	
Sample #9	Sample #10		max	8	
0	30		median	0.0	
10	0		std. dev.	2.3	
30	30		95% conf.	2.3	
40	0				
0	0				
70	10				
30	10				
0	0				
0	0				
0	0				
30	10				
5	0				
0	100				
0	0				
0	0				
50	10				
0	5				
10	0				
0	0				
Sample #8	Sample #9	Sample #10			

Observations							
Transect #	Stream Wid	Distance Fr	Time	Avg. Stream	ream Shadir	Stream Sun	Organic
1	42	0	7:30	3	30	70	0
2	82	0.25	7:42	2	10	90	0
3	75	0.5	7:57	4	20	80	0
4	84	0.75	8:19	2	10	90	0
5	60	1	8:36	2.5	20	80	
6	68	2	9:35	3.5	20	80	0
7	62	2.25	9:48	1.5	10	90	0
8		2.5					
9	72	2.75	10:16	2.5	10	90	0
10	76	3	10:35	3	5	95	0
11	52	4	10:54	1.5	0	100	0
12	86	4.25	11:54	2	5	95	0
13	43	4.5	12:42	3	5	95	0
14	111	5.25	1:14	2.5	10	90	0
15	81	5.5	1:27	4	20	80	0
Algae Community							
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
1							
2	Filamentous	Dark Green					
3	Filamentous	Brown	10				
4	Filamentous	Brown	10	10			
5	Thick Mat	Dark Green	10				
5	Filamentous	Brown					
5	Film	Dark Green					
6	Thick Mat	Dark Green	10				
7	Filamentous	Dark Green	0	0	0	0	0
8							
9							
10	Thick Mat	Dark Green					
10	Filamentous	Brown	10				
11							
12	Thick Mat	Dark Green	0	20	20	0	0
13	Filamentous	Brown	50	0	0	0	0
14	Thick Mat	Dark Green				10	10
15	Thick Mat	Dark Green					
Plant Community							
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6
1	Emergent	0	0	0	0	0	0
1	Submerged	0	0	0	0	10	10

2	Submerged	10	20	20	20	20	20
2	Emergent	0	0	0	0	0	0
3	Submerged	0	30	50	50	40	40
4	Submerged	0	0	10	30	30	50
5	Submerged	10	70	70	30	30	30
6	Submerged	0	10	0	0	0	0
7	Submerged	10	10	0	0	0	0
9	Submerged	20	50	30	0	30	30
10	Submerged	50	50	50	50	40	40
11	Submerged	S	S	S	S	S	0
12	Emergent	100	0	0	0	0	0
12	Submerged	0	10	30	70	70	10
13	Submerged	10	10	10	10	0	0
14	Submerged	10	30	10	10	30	100
15	Submerged	0	0	10	10	70	50
Effect of Algae on Plants							
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
Other Wildlife							
Transect #	Wildlife		Stressors				
1							
2							
3							
4							
5							
6							
7							
8							

9							
10							
11							
12							
Transect Landmarks							
Transect #	Landmarks						
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							

Mud	Sand	Gravel	Cobble	Bedrock			
0	0	0	>70	0			
0	0	0	>70	0			
0	0	0	>70	0			
0	0	0	>70	0			
0	0	0	40-70	10-40			
0	0	0	>70	0			
0	0	0	40-70	40-70			
0	0	0	>70	0			
0	0	0	40-70	40-70			
0	0	0	>70	0			
0	0	0	10-40	>70			
0	0	1-10	40-70	40-70			
0	0	10-40	40-70	0			
Sample #6	Sample #7	Sample #8	Sample #9	Sample #10			
	10	10			10	10	
					10	10	
					10	10	
					10	30	
				10	10	10	
				10	10	7	
					10		
0	0	10	50	10	7		
						17	
		20	20	10	16.7	13.3	
						5	
						10	
30	30	0	20	S	13.3	10.0	
0	0	0	0	0	5		
					10		
				10	10		
					#	10	
					mean	12.0	
Sample #7	Sample #8	Sample #9	Sample #10		min	5.0	
0	0	0	50		max	30.0	
10	10	20	0		median	10.0	

20	10	0	0		std. dev.	4.5	
0	0	100	100		95% conf.	2.8	
30	10	10	0				
70	50	30	30				
10	10	10	0				
0	0	0	0				
10	10	10	0				
30	30	50	40				
40	30	30	0				
0	0	0	10				
0	0	0	0				
90	100	50	50				
0	0	10	10				
100	100	30	10				
50	50	10	0				
Sample #6	Sample #7	Sample #8	Sample #9	Sample #10			

Observations									
Transect #	Stream Wid	Distance Fr	Time	Avg. Stream	ream Shadir	Stream Sun	Organic	Mud	Sand
1	42	0	8:40	4	20	80	0	0	0
2	82	0.25	8:51	1.5	10	90	0	0	0
3	75	0.5	9:02	4	30	70	0	0	0
4	84	0.75	9:10	2.5	20	80	0	0	0
5	60	1	9:18	4	20	80	0	0	0
6	68	2	9:57	4	30	70	0	0	0
7	62	2.25	10:19	1.5	10	90	0	0	0
8	72	2.5	10:31	2.5	10	90	0	0	0
9	72	2.75	10:42	3	10	90	0	0	0
10	76	3	10:47	2	0	100	0	0	0
11	52	4	11:18	2.5	10	90	0	0	0
12	86	4.25	11:53	3	10	90	0	0	0
13	43	4.5	12:00	3.5	5	95	0	10-40	0
14	111	5.25	12:23	3	10	90	1-10	0	0
15	81	5.5	1:27	4	20	80	0	0	0
Algae Community									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1	Filamentous	Dark Green				40	40	NA	NA
2	Filamentous	Dark Green	90	90	70	40	10	10	0
3									
4	Filamentous	Dark Green	0	80	80	80	70	0	0
5	Filamentous	Dark Green					50		
6	Filamentous	Dark Green	10	10	10	0	0	0	0
7	Filamentous	Dark Green	80	90	70	50	50	50	90
8	Filamentous	Dark Green	0	10	40	60	40	10	0
9	Filamentous	Dark Green					20		
10	Filamentous	Dark Green	90	10	10	0	0	0	0
11									
12	Filamentous	Dark Green		20					
13	Filamentous	Dark Green	60	0	0	0	0	0	0

14									
15	Filamentous	Dark Green	40						
Plant Community									
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1	Emergent	0	0	0	0	0	NA	NA	NA
2	Submerged	10	10	10	10		10	10	0
2	Emergent	0	0	0	0	0	0	0	0
3	Submerged	30	50	90	90	90	40	40	0
4	Submerged	0	0	20	20	30	90	70	70
5	Submerged	40	70	70	90	50	50	NA	NA
6	Submerged	0	0	10	10	0	10	30	30
7	Submerged	0	0	0	0	0	0	10	0
8	Submerged	40	50	40	40	30	30	90	70
9	Submerged	40	70	70	40	30	70	60	30
10	Submerged	10	10	10	10	10	10	0	10
11	Emergent	30	0	0	0	0	0	0	0
11	Submerged	0	10	30	40	100	30	90	100
12	Submerged	30	0	10	10	10	0	0	10
12	Emergent	0	0	0	0	0	0	0	0
13	Submerged	0	70	0	0	30	0	50	30
14	Submerged	0	0	20	50	90	70	100	100
15	Submerged	0	20	30	50	10	40	50	90
Effect of Algae on Plants									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									
2									
3	Thick Mat	Dark Green		10					
4									
5	Thick Mat	Dark Green	10						
6									
7									

8									
9									
10									
11									
12									
13									
14	Thick Mat	Bright Green							
15	Thick Mat	Bright Green							
Other Wildlife									
Transect #	Wildlife		Stressors						
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
Transect Landmarks									
Transect #	Landmarks								
1									
2									
3									
4									
5									
6									
7									

8									
9									
10									
11									
12									

Gravel	Cobble	Bedrock			
0	>70	0			
0	>70	0			
40-70	40-70	0			
0	>70	0			
0	>70	0			
0	>70	0			
0	>70	0			
0	>70	10-40			
0	>70	0			
0	40-70	10-40			
0	>70	0			
0	10-40	>70			
0	1-10	>70			
0	0	>70			
10-40	40-70	0			
Sample #8	Sample #9	Sample #10			
NA	NA		40	40	
0	50	90	45	45	
0	0	0	31	31	
			50	50	
10	10	0	5	5	
90	50	10	63	63	
0	0	0	16	16	
			20	20	
0	10	0	12	12	
			20	20	
20	0	50	13	13	

			40	40	
		#	12		
Sample #9	Sample #10	mean	29.6		
NA	50	min	5		
0	0	max	63		
0	S	median	20.0		
0	0	std. dev.	19.0		
40	0	95% conf.	10.8		
0	0				
0	0				
0	0				
30	10				
30	0				
0	50				
0	0				
90	0				
10	10				
0	30				
20	10				
70	0				
100	0				
Sample #8	Sample #9	Sample #10			

50					
	10				

Observations							
Transect #	Stream Wi	Distance F	Time	Avg. Strea	ream Shadi	Stream Sun	Organic
1	51	0	7:56	3.5	10	90	0
2	54	0.25	8:07	3	10	90	0
3	54	0.5	8:15	3	10	90	0
4	55	0.75	8:23	3	5	95	0
5	45	1	8:32	1.5	10	90	0
6	58	1.25	8:45	2.5	0	100	0
7	40	1.5	9:00		10	90	0
8	41	1.75	9:13	7			0
9	58	2	9:26	7	30	70	0
10	57	2.25	9:34	4	30	70	0
11	67	2.5	9:42	3	30	70	0
12	62	2.75	9:53	2.5	10	90	0
13	78	3	10:04	2	10	90	0
14	66	3.25	10:18	3	10	90	0
15	58	3.5	10:34	4	10	90	0
16	84	3.75	10:52	4	20	80	0
17	96	4	11:00	2.5	5	95	0
18	84	4.25	11:09	1.5	10	90	0

Algae Community							
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
1	Filamentous		0	0	0	0	0
2	Filamentous		0	0	0	0	0
3	Filamentous		0	0	0	0	0
4	Filamentous	Dark Green	10	10	0	0	0
5	Filamentous	Dark Green	0	0	0	0	30
6	Filamentous	right Green	90	80	40	50	50
7	Filamentous		0	0	0	0	0
8	Film	Brown	100	100	100	NA	NA
8	Filamentous	Dark Green	100	100	100	NA	NA
9	Filamentous	Brown	0	0	0	NA	NA
10	Filamentous		0	0	0	0	0
11	Filamentous		0	0	0	0	0
12	Filamentous		0	0	0	0	0
13	Filamentous	Dark Green	10	10	0	0	0
13	Filamentous	Brown	0	0	0	0	0
14	Filamentous	Brown	10	10	10	10	10
15	Filamentous	Brown	10	10	10	10	10
16	Filamentous	Brown	10	10	10	10	10
17	Filamentous	Brown	30	30	30	40	40
18	Filamentous	Dark Green	0	0	0	0	0
18	Filamentous	Brown	40	40	40	40	40

Plant Community							
Transect #	Plant Type	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6
1	Submerge	10	20	0	0	0	NA
2	Submerge	0	10	0	0	40	40
3	Submerge	10	20	90	90	90	50
4	Submerge	0	10	30	50	50	70
4	Emergent	70	0	0	0	0	0
5	Submerge	0	10	10	10	0	0
6	Submerge	0	30	100	100	100	100
6	Emergent	100	100	0	0	0	0
7	Submerge	10	0	0	10	0	0
7	Emergent	0	0	0	0	0	0
8	Emergent	100	0	100	0	0	0
9	Submerge	0	0	0	90	90	0
9	Emergent	0	0	0	0	0	0
10	Submerge	0	0	0	0	50	50
11	Submerge	0	0	50	0	50	70
12	Submerge	0	10	10	10	10	10
12	Emergent	10	10	10	0	0	0
13	Submerge	0	0	10	20	50	50
13	Emergent	70	50	0	0	0	0
14	Submerge	0	0	100	100	100	70
15	Submerge	0	0	40	40	80	80
16	Submerge	0	0	0	60	60	20
17	Submerge	S	10	60	70	80	100
18	Submerge	0	0	10	10	0	10
Effect of Algae on Plants							
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
1							
2							
3							
4	Filamentous	Brown	10				
5							
6	Filamentous	Bright Green	10	10	10	20	20
7							
8	Filamentous	Dark Green	70		70		
9							
10							
11							
12							
13	Filamentous	Dark Green	100	100			

14	Filamentous	Brown	10	10	10	10	10
15	Filamentous	Brown	10	10	10	10	10
16	Filamentous	Brown	10	10	10	10	10
17	Filamentous	Brown	20	20	30	30	30
18	Filamentous	Brown	40	40	40	40	40
Other Wildlife							
Transect #	Wildlife		Stressors				
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
Transect Landmarks							
Transect #	Landmarks						
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

15							
16							
17							
18							

Mud	Sand	Gravel	Cobble	Bedrock			
0	0	0	10-40	10-40			
0	0	10-40	0	10-40			
1-10	0	10-40	0	40-70			
0	0	1-10	1-10	>70			
0	0	0	0	>70			
0	0	0	1-10	>70			
1-10	0	0	0	>70			
10-40	0	0	0	>70			
1-10	0	0	0	>70			
10-40	0	0	0	>70			
0	10-40	0	0	>70			
0	10-40	0	0	>70			
0	0	0	40-70	40-70			
0	0	1-10	0	>70			
1-10	1-10	0	0	>70			
1-10	1-10	0	0	>70			
1-10	0	0	10-40	>70			
0	0	1-10	1-10	>70			
Sample #6	Sample #7	Sample #8	Sample #9	Sample #10			
0	0	0	0	0	0	0	
0	0	0	0	0	0	0	
0	0	0	0	0	0	0	
0	0	0	0	0	2	2	
50	50	50	10	0	19	19	
50	50	30	20	20	48	48	
0	0	0	0	0	0	0	
NA	NA	NA	NA	100	100	-	
NA	NA	NA	NA	100		100	
NA	NA	0	0	0	0	0	
0	0	0	0	0	0	0	
0	0	0	0	0	0	0	
0	0	0	0	0	0	0	
0	0	0	0	0	2	-	
0	0	0	0	10	1	3	
10	10	10	10	10	10	10	
10	10	10	10	10	10	10	
10	10	10	10	10	10	10	
40	40	40	40	40	37	37	
0	0	30	40	40	11	-	
40	40	40	40	40	40	51	

						#	18
						mean	16.1
Sample #7	Sample #8	Sample #9	Sample #10			min	0.0
NA	NA	NA	50	13		max	100.0
40	40	NA	NA	21		median	2.0
70	70	30	30	55		std. dev.	26.4
70	NA	NA	NA	40	-	95% conf.	12.2
0	0	0	0	7	47		
0	0	0	0	3			
100	20	10	5	57	-		
0	0	0	0	20	77		
0	0	0	0	2	-		
10	10	0	0	2	4		
0	0	0	0	20			
0	0	50	50	28			
0	80	0	0	8			
30	30	0	0	16			
70	30	0	0	27			
10	0	0	S	6			
0	0	0	0	3			
50	0	10	10	20			
0	0	0	10	13			
70	100	80	0	62			
90	40	90	30	49			
30	40	50	50	31			
90	100	60	40	61			
20	10	0	0	6			
Sample #6	Sample #7	Sample #8	Sample #9	Sample #10			
20	20	20	10	10			

10	10	10	10	10			
10	10	10	10	10			
10	10	10	10	10			
30	40	40	30	20			
40	40	40	40	40			

Observations									
Transect #	Stream Width	tance From St	Time	g. Stream Dep	Stream Shading	Stream Sun	Organic	Mud	Sand
1	54	0	8:03	4	10	90			
2	51	0.25	8:15	3	10	90			1-10
3	58	0.5	8:26	3	10	90			
4	50	0.75	8:39	3	10	90			
5	44	1	8:49	1	10	90			
6	69.5	1.25	9:05	1	20	80		<10	<10
7	30.5	1.5	9:18	3	20	80			
8	40.5	1.75	9:30	2.5	20	80			
9	63.5	2	9:51		20	80		<10	
10	59.5	2.25	10:00	2.5	30	70		1-10	
11	75	2.5	10:11	2.5	5	95		10-40	
12	66	2.75	10:25	2.5	5	95			
13	75	3	10:33	2	0	100			
14	61	3.25	10:48	3.5	10	90			
15	54.5	3.5	10:58	2.5	20	80			<10
16	77	3.75	11:05	3.5	10	90			
17	82	4	11:13	1	10	90			
18	74	4.25	11:23		10	90		1-10	
Algae Community									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1	Filamentous	Dark Green	10	20	20	0	0	NA	NA
2									
3									
4	Filamentous	Dark Green	90	10	0	0	0	0	0
5	Filamentous	Dark Green	0	0	0	0	0	0	90
6	Filamentous	Dark Green	0	80	90	70	70	70	70
7	Filamentous	Dark Green	10	20	10	10	10	S	S
8	Filamentous	Dark Green	40	30	40				
9	Filamentous	Dark Green	S	S	0	0	0	30	30
10									

11	Filamentous	Dark Green	0	0	0	0	0	10	10
12	Filamentous	Dark Green	90	90	40				
13	Filamentous	Dark Green	90	50	20	20	0	0	0
14									
15	Thick Mat	Dark Green	S	S	S	S	0	0	0
16	Filamentous	Dark Green	50	60	70	0	0	0	0
17	Filamentous	Brown	10	0	0	0	0	0	0
18	Film	Brown	30	20	30	0	0	0	0
18	Filamentous	Dark Green	0	0	0	0	0	0	70
Plant Community									
Transect	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1	Submerged	20			10	0	NA	NA	NA
2	Submerged	20	100	10	20	50	90	60	80
3	Submerged	90	90	90	90	90	90	90	90
4	Submerged	10	0	70	70	50	50	50	10
5	Submerged		10	20	20	50	70	10	10
6	Submerged			80	90	60	100	100	90
7	Submerged				10	10	S	S	S
8	Submerged	S	S	S	80	90	100	S	S
9	Submerged			S	S	80	90	S	S
10	Submerged			S	S	S	60	60	
11	Submerged		10	20	70	0	70	70	70
12	Submerged	0	10	20	10	10	10	30	10
13	Submerged		50	70	0	0	20	10	10
14	Submerged	10	50	50	90	90	100	90	20
15	Submerged				S	70	80	90	100
16	Submerged		S	S	50	80	S	80	90
17	Submerged	S	S	30	50	50	S	S	
17	Emergent								80
18	Submerged			S	S			S	S

Effect of Algae on Plants									
Transect	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									
2									
3									
4									
5	Film	Dark Green					10	50	
6	Filamentous	Dark Green		50	60	60	60	60	70
7									
8	Filamentous	Brown	50	40	50	50			
9	Filamentous	Dark Green							20
10									
11	Thin Mat	Dark Green						20	20
12									
13									
14	Filamentous	Dark Green				20		10	10
15	Filamentous	Dark Green	10	10	10	S	S		
16									
17	Filamentous	Dark Green				20	20	20	
18	Filamentous	Dark Green							70
Other Wildlife									
Transect	Wildlife	Stressors							
1									
2									
3									
4									
5	some snails								
6									
7	bass, carp								
8									
9	bass								

10									
11									
12									
13	lots of snails on algae								
14									
15									
16									
17									
18									

Gravel	Cobble	Bedrock			
	10-40	>70			
1-10		>70			
10-40		40-70			
1-10		>70			
		>70			
		>70			
		>70			
		>70			
		>70			
		>70			
		>70			
1-10		>70			
	10-40	40-70			
10-40		>70			
		>70			
		>70			
		>70			
	1-10	>70			
Sample #8	Sample #9	Sample #10			
NA	NA	0	8	8	
0	0	0	10	10	
90	90	90	36	36	
80	70	60	66	66	
S			12	12	
			37	37	
20	S	S	8	8	

10	20	30	8	8	
			73	73	
0	0	0	18	18	
0	0	0	0	0	
0	0	0	18	18	
0	0	0	1	1	
0	0	0	8	-	
80	0	60	21	29	
			#	14	
			mean	23.1	
			min	0	
Sample #9	Sample #10		max	73	
NA	90		median	12.0	
50	0		std. dev.	22.8	
90	40		95% conf.	11.9	
10	0				
10					
S	S				
	S				
S	S				
S					
S					
0					
0	0				
10					
10	50				
100	80				
S					

Sample #8	Sample #9	Sample #10			
70	80	70			
20	20				
20			Top of grass		
80					

Observations							
Transect #	Stream Wid	Distance Fr	Time	Avg. Stream	ream Shadir	Stream Sun	Organic
1	51	0	7:40	4	10	90	0
2	54	0.25	7:52	3.5	20	80	0
3	54	0.5	7:57	3	10	90	0
4	55	0.75	8:08	3	20	80	
5	45	1	8:16	2	20	80	0
6	58	1.25	8:27	1.5	20	80	0
7	40	1.5	8:40	4	30	70	0
8	41	1.75	8:55	3.5	20	80	0
9	58	2	9:03	2.5	30	70	0
10	57	2.25	9:08	4	20	80	0
11	67	2.5	9:16	3	10	90	0
12	62	2.75	9:27	2.5	0	100	0
13	78	3	9:33	2	0	100	0
14	66	3.25	9:45	3	10	90	0
15	58	3.5	9:54	3.5	20	80	0
16	84	3.75	10:03	3	10	90	0
17	96	4	10:14	2	10	90	0
18	84	4.25	10:23	2	20	80	0

Algae Community							
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
1							
2							
3							
4							
5							
6							
7	Filamentous	Brown	10	10	0	0	0
8	Filamentous	Dark Green	40	40	20	30	30
9							
10							
11							
12	Filamentous	Dark Green	40	40	20	0	0
13	Filamentous	Dark Green	90	0	0	0	0
14							
15							
16	Thick Mat	Dark Green	20	20	20	0	0
16	Filamentous	Dark Green	50	50	50	0	0
17							
18	Filamentous	Brown	10	10	0	0	0

Plant Community							
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6
1	Submerged	10	30	0	0	0	NA
2	Submerged	20	30	50	30	0	20
3	Submerged	0	20	20	70	90	70
4	Submerged	10	0	40	90	90	90
5	Submerged	0	20	30	70	70	70
6	Submerged	S	10	40	60	50	60
7	Submerged	S	0	S	20	20	S
8	Emergent	30	0	60	70	50	S
9	Submerged	0	0	S	S	60	30
10	Submerged	0	S	S	20	50	50
11	Submerged	0	20	40	10	100	90
12	Submerged	0	10	10	20	0	50
13	Submerged	0	70	100	10	0	70
14	Submerged	0	0	70	90	90	90
15	Submerged	0	S	0	S	40	40
16	Submerged	0	S	0	50	S	60
17	Submerged	0	S	30	40	60	40
17	Emergent	0	0	0	0	0	0
18	Submerged	0	0	S	S	20	0
Effect of Algae on Plants							
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
1							
2							
3							
4							
5							
6							
7	Filamentous	Dark Green	10				
8	Filamentous	Dark Green	40		40	50	
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							

Other Wildlife							
Transect #	Wildlife		Stressors				
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
Transect Landmarks							
Transect #	Landmarks						
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							

Mud	Sand	Gravel	Cobble	Bedrock			
1-10	1-10	0	1-10	40-70			
0	10-40	10-40	0	10-40			
0	0	10-40	1-10	40-70			
0	0	0	0	>70			
0	10-40	10-40	0	10-40			
0	0	0	0	>70			
1-10	1-10	0	0	>70			
0	1-10	1-10	0	>70			
1-10	0	1-10	0	>70			
10-40	0	0	10-40	10-40			
0	10-40	0	0	>70			
0	0	0	10-40	>70			
0	0	0	10-40	40-70			
1-10	0	1-10	10-40	>70			
1-10	0	1-10	0	>70			
1-10	0	1-10	0	>70			
0	0	1-10	10-40	>70			
Sample #6	Sample #7	Sample #8	Sample #9	Sample #10			
NA	NA	NA	NA				
0	0	0	0	0	2	2	
0	0	0	0	0	16	16	
0	0	0	0	10	11	11	
0	0	0	0	0	9	9	
0	0	0	0	0	6	-	
0	0	0	0	0	15	21	
0	0	0	20	20	6	6	
					#	6	

					mean	10.8	
					min	2.0	
Sample #7	Sample #8	Sample #9	Sample #10		max	21.0	
NA	NA	NA	70		median	9.0	
70	50	70	0		std. dev.	7.5	
70	50	90	50	ess healthy	95% conf.	6.0	
90	50	0	0				
30	0	0	0				
60	70	0	0				
S	S	0	0				
S	0	0	0				
S	S	50	50				
S	0	0	0				
70	50	10	10				
20	10	0	0				
0	10	20	0				
50	100	100	50				
40	60	60	0				
70	60	S	0				
20	0	0	0				
50	60	70	0				
S	S	0	0				
Sample #6	Sample #7	Sample #8	Sample #9	Sample #10			
NA	NA	NA	NA				

Observations									
Transect #	Stream Wid	Distance Fr	Time	Avg. Stream	ream Shadir	Stream Sun	Organic	Mud	Sand
1	190	0	7:43	3	5	95	0	0	10-40
2	97	0.25	8:07	2.5	10	90	1-10	0	>70
3	88	0.5	8:17	2	10	90	0	0	0
4	90	0.75	8:30	2	10	90	0	0	40-70
5	95	1	8:46	3	5	95	0	0	40-70
6	97	1.25	8:53	3.5	0	100	0	0	10-40
7	90	1.5	9:08	3.5	5	95	0	0	0
8	120	1.75	9:21	3	5	95	0	0	0
9	150	2	9:31	3.5	5	95	0	0	0
10	125	2.25	9:39	2.5	5	95	0	0	10-40
11	155	2.5	9:53						
12	150	2.75	10:02	3.5	5	95	0	0	0
13	132	3	10:14	2.5	10	90	0	0	10-40
14	125	3.25	10:29	3	10	90	0	0	0
15	125	3.5	11:02	2.5			0	0	0
16	108	3.75	11:18	2.5	15	85	0	0	0
17	103	4	11:22	2.5	10	90	0	0	0
18	110	4.25	11:34	3.5	5	95	0	0	0
19	125	4.5	11:51	2	10	90	0	0	1-10
20	134	4.75	12:07	2.5	5	95	0	0	0
21	109	5	12:17	3.5	5	95	0	0	0
Algae Community									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1	Thick Mat	Dark Green	0	0	0	0	0	10	0
2	Film	Dark Green	10	0	0	10	0	0	0
3									
4									
5	Thick Mat	Dark Green	0	0	0	0	0	0	0
6									
7	Thick Mat	Dark Green	0	0	0	0	0	0	0

8	Thick Mat	Dark Green	20	10	0	0	0	0	0
9	Thick Mat	Dark Green	S	S	S	S	S	S	S
10	Thick Mat	Dark Green	0	0	0	0	0	0	S
11	Thick Mat	Dark Green	0	0	0	0	0	0	0
12	Thick Mat	Dark Green	0	0	0	0	10	30	10
13	Thick Mat	Dark Green	0	0	0	0	S	S	0
14	Filamentous	Dark Green	0	0	0	0	S	0	0
15	Film	Bright Green	0	0	0	0	0	0	0
15	Thick Mat	Dark Green	0	0	0	S	S	S	S
16	Thick Mat	Dark Green	0	0	0	0	S	S	S
17	Thick Mat	Dark Green	0	0	0	S	S	S	S
18	Thick Mat	Dark Green	0	0	0	0	0	S	S
19	Thick Mat	Dark Green	0	0	0	10	10	10	10
19	Filamentous	Brown	0	0	0	0	0	0	0
20	Thick Mat	Dark Green	0	0	0	10	10	S	S
20	Thick Mat	Brown	0	0	0	0	0	0	0
21									
Plant Community									
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1	Submerged	0	0	0	0	10	0	0	0
2									
3									
4									
5									
6	Submerged	0	0	0	0	0	0	0	NA
7									
8									
9									
10									
11	Submerged	0	0	20	S	10	S	S	10
12									
13									

14									
15									
16									
17									
18									
19									
20									
21									
Effect of Algae on Plants									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									

Other Wildlife									
Transect #	Wildlife		Stressors						
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
Transect Landmarks									
Transect #	Landmarks								
1									
2									

3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									

Gravel	Cobble	Bedrock			
10-40	1-40	0			
0	0	0			
1-10	1-10	>70			
10-40	0	10-40			
40-70	0	0			
40-70	10-40	0			
10-40	10-40	10-40			
1-10	>70	1-10			
1-10	1-10	>70			
0	0	40-70			
1-10	40-70	40-70			
10-40	0	40-70			
10-40	40-70	10-40			
0	10-40	>70			
0	40-70	40-70			
0	10-40	>70			
40-70	40-70	1-10			
40-70	40-70	0			
10-40	40-70	0			
1-10	40-70	40-70			
Sample #8	Sample #9	Sample #10			
0	0	0	1	1	
0	0	0	2	2	
0	S	S	0	0	
NA	NA	NA			
S	S	0	0	0	

0	0	0	3	3	
20	0	0	20	20	
S	0	0	0	0	
10	0	0	1	1	
0	0	30	8	8	
0	0	0	0	0	
0	0	0	0	0	
0	20	20	4	4	
0	0	0	0	0	
0	0	0	0	2	
S	10	0	2	0	
S	0	0	0	10	
10	10	0	6	12	
0	20	20	4		
20	20	0	8		
20	20	0	4		
			#	17	
			mean	3.7	
Sample #9	Sample #10		min	0	
0	0		max	20	
			median	1.0	
			std. dev.	5.5	
			95% conf.	2.6	
NA	NA				
S	0				

Sample #8	Sample #9	Sample #10			

Observations									
Transect #	Stream Wid	Distance Fr	Time	Avg. Stream	ream Shadir	Stream Sun	Organic	Mud	Sand
1	180	0	8:43	3	5	95	0	0	0
2	97	0.25	8:53	2.5	10	90	1-10	1-10	0
3	26	0.5	9:02	2	10	90	0	0	0
4	87	0.75	9:13	3.5	10	90	0	0	0
5	97	1	9:25	2.5	10	90	0	0	>70
6	98	1.25	9:33	2	5	95	0	0	0
7	125	1.5	9:50	4	5	95	0	0	0
8	97	1.75	9:59	1.5	0	100	0	0	0
9	138	2	10:08	3.5	5	95	0	0	10-40
10	125	2.25	10:18	1.5	5	95	0	0	10-40
11	158	2.5	10:36	2	0	100	0	0	0
12	150	2.75	10:49	3	10	90	0	0	0
13	125	3	10:59	2.5	10	90	0	0	0
14									
15	125	3.5	11:10	3			0	0	0
16	123	3.75	11:23	2	10	90	0	0	0
17	110	4	11:31	2	10	90	0	0	0
18	100	4.25	11:51	1.5	10	90	0	0	0
19	135	4.5	12:12	2	10	90	0	1-10	1-10
20	125	4.75	12:22	2	0	100	0	0	0
21	135	5	12:31	2	10	90	0	0	0
Algae Community									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									
2									
3	Thick Mat	Dark Green	30	10	0	0	0	0	0
4	Filamentous	Dark Green	0	0	0	0	50	NA	NA
5	Thick Mat	Dark Green	0	0	0	0	0	0	S
6									
6	Filamentous	Brown	0	0	0	0	0	0	0

7	Filamentous	Dark Green	0	NA	NA	NA	NA	NA	NA
8									
9	Thick Mat	Dark Green	0	0	0	0	0	0	S
10	Filamentous	Brown	0	0	0	0	0	0	0
11	Filamentous	Dark Green	0	0	0	0	0	0	0
12									
13									
14									
15									
16									
17									
18									
19	Thick Mat	Dark Green	0	0	0	0	0	0	0
20									
21									
Plant Community									
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1	Submerged	0	0	0	0	10	10	0	S
2	Emergent	0	0	0	0	0	0	0	0
3	Submerged	0	10	0	0	0	0	0	0
4									
5									
6	Emergent	0	0	0	0	0	0	0	0
7									
8	Submerged	0	10	0	0	0	0	0	0
9	Submerged	0	0	0	0	0	0	0	0
10									
11	Submerged	0	30	30	30	10	10	20	10
12									
13									

14									
15									
16	Emergent	0	0	0	0	0	0	0	0
17									
18	Submerged	0	0	0	0	0	10	0	0
19									
20	Submerged	0	0	0	0	0	10	10	0
20	Emergent	100	100	0	0	0	0	0	0
21	Submerged	10	0	0	0	0	0	0	0
Effect of Algae on Plants									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									
2									
3	Thick Mat	Dark Green							
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									

Other Wildlife									
Transect #	Wildlife		Stressors						
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
Transect Landmarks									
Transect #	Landmarks								
1									
2									

3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									

Gravel	Cobble	Bedrock			
0	10-40	40-70	L>R		
0	0	>70			
10-40	0	>70			
0	0	>70			
0	0	10-40			
0	>70	0			
0	40-70	40-70			
0	10-40	40-70			
0	0	>70			
0	40-70	10-40			
0	>70	10-40			
0	10-40	40-70;			
0	10-40	40-70			
0	>70	0			
0	10-40	>70			
0	10-40	40-70			
0	10-40	>70			
0	10-40	40-70			
0	>70	0			
10-40	>70	0	L>R		
Sample #8	Sample #9	Sample #10			
0	0	0	4	4	
NA	NA	NA	10	10	
0	0	0	0	0	
NA	NA			6	
NA	NA	50	6	25	

NA	NA	50	25		
				0	
0	0	0	0	3	
0	0	30	3	8	
70	10	0	8		
				2	
10	10	0	2		
			#	9	
			mean	6.4	
			min	0	
Sample #9	Sample #10		max	25	
S	0		median	3.5	
0	50		std. dev.	7.6	
50	S		95% conf.	5.0	
0	50				
10	0				
S	0				
10	S				

0	100				
10	0				
0	0				
0	0				
0	0				
Sample #8	Sample #9	Sample #10			
	10				

Observations									
Transect #	Stream Wid	Distance Fr	Time	Avg. Stream	ream Shadir	Stream Sun	Organic	Mud	Sand
1		0	8:11	3	10	90	0	0	0
2		0.25	8:19	3	10	90	0	0	0
3		0.5	8:28	2.5	20	80	0	0	0
4		0.75	8:41	NA	20	80	NA	NA	NA
5		1	8:53	NA	10	90	NA	NA	NA
6		1.25	9:07	2	0	100			
7		1.5	9:28	NA	10	90	NA	NA	NA
8		1.75	9:40	2.5	0	100	0	0	0
9		2	9:59	3	10	90	NA	NA	NA
10		2.25	10:20	2	10	90	0	0	0
11		2.5	10:32	2	0	100	0	0	0
12		2.75	10:40	2.5	10	90	0	0	0
13		3	10:49	3	10	90	0	1-10	0
14		3.25	10:54		10	90	0	0	10-40
15		3.5	11:04	2	10	90	0	0	0
16									
17		4	11:22	2	10	90	0	0	0
18		4.25	11:35	NA	20	80	NA	NA	NA
19		4.5	11:47	2	10	90	0	0	1-10
20		4.75	11:53	2	0	100	0	0	0
21		5	12:00	3	10	90	0	0	0
Algae Community									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									
2									
3									
4									
5									
6	Filamentous	Dark Green	0	0	0	0	0	0	0
7									

8									
9									
10									
11	Filamentous	Dark Green	10	0	0	0	0	0	0
12									
13									
14									
15									
15									
16									
17									
18									
19									
19									
20									
20									
21									
Plant Community									
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1	Submerged	NA	NA	NA	50	50	30	20	20
2	Submerged	50	NA	NA	NA	NA	NA	NA	NA
3	Submerged	0	30	50	30	50	70	70	70
4		NA	NA	NA	NA	NA	NA	NA	NA
5		NA	NA	NA	NA	NA	NA	NA	NA
6	Submerged	0	10	20	30	30	30	0	0
6	Emergent	0	0	0	0	0	0	0	0
7	Submerged	NA	NA	NA	NA	NA	NA	NA	NA
8	Submerged	10	20	10	0	0	0	NA	10
9	Submerged	NA	NA	NA	NA	NA	NA	NA	NA

10	Submerged	0	0	10	10	S	10	S	S
11	Submerged	0	0	90	50	50	30	30	30
12	Submerged	0	0	0	0	0	0	0	0
13							NA	NA	NA
14	Submerged	S	0	0	0	S	NA	NA	NA
15	Submerged	0	0	0	10	0	0	0	0
15	Emergent	0	0	0	0	0	0	0	0
16									
17									
18		NA	NA	NA	NA	NA	NA	NA	NA
19	Submerged	0	0	0	0	0	0	0	10
20	Emergent	100	100	10	0	0	0	10	10
21	Submerged	0	0	0	0	0	0	0	0
Effect of Algae on Plants									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									
2									
3									
4									
5									
6									
7	Filamentou	Dark Green							
8									
9									
10									
11									
12									
13									
14									
15									
16									

17									
18									
19									
20									
21									
Other Wildlife									
Transect #	Wildlife		Stressors						
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									

Transect Landmarks									
Transect #	Landmarks								
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									

Gravel	Cobble	Bedrock			
0	>70	0	L>R		
0	10-40	>70			
10-40	0	>70			
NA	NA	NA			
NA	NA	NA			
NA	NA	NA			
0	40-70	10-40			
NA	NA	NA			
0	>70	0			
0	40-70	40-70			
10-40	10-40	40-70			
0	40-70	10-40			
0	40-70	0			
10-40	1-10	>70			
0	40-70	40-70			
NA	NA	NA			
10-40	10-40	40-70			
0	>70	10-40			
10-40	>70	0	L>R		
Sample #8	Sample #9	Sample #10			
0	0	40	4	4	

0	0	0	1	1	
			#	2	
			mean	2.5	
			min	1	
			max	4	
Sample #9	Sample #10		median	1.0	
10	10		std. dev.	2.1	
NA	NA		95% conf.	2.9	
10	0				
NA	NA				
NA	NA				
0	0				
0	40				
NA	10				
S	S				
10	0				

S					
50	0				
S	0				
NA					
10	0				
S	0				
0	100				
0	0				
10	10				
S	0				
Sample #8	Sample #9	Sample #10			
		50			

Observations									
Transect #	Stream Wid	Distance From Start	Time	Avg. Stream	ream Shadir	Stream Sun	Organic	Mud	Sand
1	22	0	7:55	3.5	10	90	0	0	0
2	28	0.25	8:10	3	10	90	0	0	0
3	23	0.5	8:33	NA	30	70	NA	NA	NA
4	20	0.5+	8:38	3	40	60	0	0	0
5	50	1	9:18	2.5	0	100	0	1-10	0
6	83	1.25	10:26	2.5	0	100	0	1-10	0
7	28	1.5	10:45	1.5	10	90	0	0	0
8	55	1.75	11:12	2	10	90	0	1-10	0
9	31	2	11:28	2	20	80	0	0	0
10	51	2.25	11:38	2.5	10	90	0	10-40	0
11	52	2.5	12:02	2.5	10	90	0	10-40	0
Algae Community									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1	Filamentous	Brown	0	0	0	0	0	0	0
2	Filamentous	Brown	0	0	0	0	0	0	0
3	Filamentous	Brown	100	100	NA	NA	NA	NA	NA
4	Filamentous	Brown	10	10	10	10	10	10	10
5									
6	Filamentous	Dark Green	0	0	0	0	0	50	50
6	Filamentous	Brown	0	0	50	50	0	0	0
7	Filamentous	Brown	30	10	0	0	0	0	30
8	Thick Mat	Dark Green	0	S	0	0	0	0	0
8	Filamentous	Brown	30	30	30	30	30	100	90
9	Filamentous	Dark Green	0	0	30	30	0	0	0
9	Filamentous	Brown	20	30	0	0	30	30	30
10	Filamentous	Brown	90	90	90	90	90	90	90
11	Film	Dark Green	10	0	0	0	0	0	0
11	Filamentous	Brown	10	10	10	10	10	10	10

Plant Community									
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1									
2									
3									
4	Emergent	0	0	0	0	0	0	0	0
5	Submerged	0	0	0	0	10	10	10	10
5	Emergent	10	0	0	0	0	0	0	0
6	Emergent	50	0	0	0	0	0	0	0
7									
8									
9									
10	Submerged	10	20	30	0	0	10	0	0
11	Emergent	10	0	0	0	10	10	0	0
Effect of Algae on Plants									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									
2									
3									
4	Filamentou	Brown							
5	Filamentou	Brown					50	50	50
6	Filamentou	Brown	50						
7									
8									
9									
10	Filamentou	Brown	90	90	90			90	
11	Filamentou	Brown	90				90	90	

Other Wildlife									
Transect #	Wildlife		Stressors						
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
Transect Landmarks									
Transect #	Landmarks								
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									

Gravel	Cobble	Bedrock				
0	1-10	>70				L>R
0	40-70	40-70				
NA	NA	NA				
0	40-70	10-40				
0	>70	0				
0	40-70	10-40				
0	>70	1-10				
0	>70	0				
0	10-40	40-70				
10-40	10-40	0				
0	10-40	10-40				
Sample #8	Sample #9	Sample #10				L>R
0	70	70	14	14	14	
50	50	50	15	15	14	
NA	100	100	100	100	100	
100	100	0	27	27	27	
50	50	50	25	-	45	
0	50	50	20	45	21	
40	50	50	21	21	61	
0	0	0	0	-	26	
90	90	90	61	61	90	
0	0	0	6	-	12	
30	30	0	20	26		
90	90	90	90	90		
0	0	10	2	-		
10	10	10	10	12		

			#	10		
			mean	41.1		
			min	12		
			max	100		
Sample #9	Sample #10		median	26.0		
			std. dev.	33.0		
			95% conf.	20.5		
0	100					
10	0					
0	50					
0	0					
0	0					
0	0					
Sample #8	Sample #9	Sample #10				
		70				
50	50	100				

Observations							
Transect #	Stream Wid	Distance Fr	Time	Avg. Stream	ream Shadir	Stream Sun	Organic
1	22	0	2:32	3	0	100	0
2	27	0.25	2:48	3	20	80	0
3	26	0.5	3:10	3	30	70	0
4	60	0.75	3:18	3	0	100	0
5	50	1	3:42	2.5	5	95	0
6	87	1.25	4:14	3	10	90	0
7	35	1.5	4:31	1.5	10	90	0
8	50	1.75	4:50	2.5	20	80	0
9	30	2	5:10	2	10	90	0
10	50	2.25	5:20	3	10	90	0

Algae Community							
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
1							
2	Thick Mat	Dark Green	10	0	0	0	0
2	Filamentous	Dark Green	0	0	50	0	0
2	Filamentous	Brown	0	0	0	0	0
3	Thick Mat	Dark Green	10	0	0	0	0
3	Filamentous	Brown	70	70	70	70	70
4	Thick Mat	Dark Green	70	30	10	0	0
4	Filamentous	Dark Green	0	0	0	0	0
5	Thick Mat	Dark Green	0	50	30	30	0
6	Filamentous	Dark Green	70	0	0	0	0
6	Filamentous	Bright Green	0	50	50	50	50
7	Thick Mat	Dark Green	90	50	30	0	0
7	Filamentous	Brown	0	0	0	0	0
8	Film	Dark Green	50	0	0	0	0
8	Thick Mat	Dark Green	0	0	0	0	0
8	Filamentous	Brown	50	70	70	70	70
9	Filamentous	Dark Green	50	50	50	50	0
9	Filamentous	Brown	30	30	30	30	30
10	Thick Mat	Dark Green	30	0	0	0	0

Plant Community							
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6
1	Submerged	0	0	0	0	0	0
2	Submerged	0	10	0	0	0	0
3	Emergent	0	0	0	0	0	0
4	Submerged	0	0	0	0	0	0

5	Submerged	0	0	0	0	10	0
6	Submerged	0	0	0	0	0	0
7	Submerged	0	0	0	0	0	0
8	Submerged	0	0	0	0	0	0
9	Submerged	20	0	0	0	0	0
10	Submerged	0	0	0	0	0	50
Effect of Algae on Plants							
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
1							
2							
3							
4							
5	Thick Mat	Dark Green					
6							
7							
8							
9							
10	Thick Mat	Dark Green					
Other Wildlife							
Transect #	Wildlife		Stressors				
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
Transect Landmarks							
Transect #	Landmarks						
1							
2							
3							
4							

5							
6							
7							
8							
9							
10							
11							
12							

Mud	Sand	Gravel	Cobble	Bedrock			
0	0	0	10-40	40-70			
0	0	0	10-40	40-70			
0	0	0	>70	0			
0	0	0	>70	10-40			
0	0	0	>70	0			
0	0	0	40-70	40-70			
0	0	0	>70	0			
0	1-10	0	10-40	40-70			
0	0	0	10-40	>70			
0	0	0	>70	0			
Sample #6	Sample #7	Sample #8	Sample #9	Sample #10			
							1
0	0	0	0	0	1	1	10
0	0	0	0	0	5	-	72
0	0	0	20	20	4	10	48
10	0	0	0	0	2	-	16
70	70	70	70	70	70	72	57
0	0	0	0	0	11	-	37
90	90	90	90	10	37	48	54
0	0	10	50	50	16	16	38
50	50	50	50	0	17	-	3
50	50	50	50	0	40	57	
0	0	0	0	0	17	-	
0	50	50	50	50	20	37	
0	0	0	0	0	5	-	
0	0	10	10	0	2	-	
70	70	0	0	0	47	54	
0	0	0	0	0	20	-	
30	0	0	0	0	18	38	
0	0	0	0	0	3	3	
					#	9	
					mean	37.2	
					min	3	
Sample #7	Sample #8	Sample #9	Sample #10		max	72	
0	0	0	0		median	37.5	
0	0	0	0		std. dev.	25.0	
0	0	0	100		95% conf.	16.3	
0	0	0	0				

30	0	70	10				
0	0	0	0				
0	0	0	0				
0	0	0	0				
0	0	0	0				
70	90	30	10				
Sample #6	Sample #7	Sample #8	Sample #9	Sample #10			
			30	30			
	10	10	10	10			

Floating chu

mostly dead

Observations									
Transect #	Stream Wid	Distance Fr	Time	Avg. Stream De	eam Shadir	Stream Sun	Organic	Mud	Sand
1		0	1:22	4	10	90	0	0	0
2		0.25	1:27	NA	10	90	NA	NA	NA
3		0.5	1:39	NA	50	50	NA	NA	NA
4		0.75	1:43	NA	10	90	NA	NA	NA
5		1	2:03	3	10	90	0	40-70	0
6		1.25	2:15	3.5	10	90	0	0	0
7		1.5	2:28	3.5	10	90	0	0	0
8		1.75	2:34	4	10	90	0	0	0
9		2	2:43	4			0	0	0
10		2.25	2:50	4	10	90	0	0	0

Algae Community									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									
2	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	Filamentous	Dark Green	NA	NA	NA	0	0	NA	NA
5	Thick Mat	Dark Green	0	0	0	0	0	0	0
6	Thick Mat	Dark Green	0	0	0	0	0	S	0
7									
8									
9									
10									

Plant Community									
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1	Submerged	0	0	0	0	0	0	0	0

2	Submerged	0	0	0	0	0	0	0	0
3	Emergent	NA	NA	NA	NA	NA	NA	NA	NA
4	Submerged	0	0	0	0	0	0	0	0
5	Submerged	0	0	50	10	10	30	10	30
5	Emergent	0	0	0	0	0	0	0	0
6	Emergent	50	0	0	0	0	0	0	0
7	Emergent	0	0	0	0	0	0	0	0
8	Submerged	0	0	0	0	0	0	0	0
9	Submerged	0	0	0	0	0	0	0	0
10	Submerged	0	0	0	0	S	S	20	0
10	Emergent	0	0	0	0	0	0	0	0
Effect of Algae on Plants									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
Other Wildlife									
Transect #	Wildlife		Stressors						
1									
2									
3									
4									

5									
6									
7									
8									
9									
10									
11									
12									
Transect Landmarks									
Transect #	Landmarks								
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									

Gravel	Cobble	Bedrock		
0	40-70	10-40		
NA	NA	NA		
NA	NA	NA		
NA	NA	NA		
0	10-40	0		
0	10-40	>70		
0	10-40	>70		
0	10-40	>70		
10-40	>70	0		
1-10	>70	0		
Sample #8	Sample #9	Sample #10		
NA	0	0	0	0
NA	0	0	0	0
NA	NA	0	0	0
0	0	20	2	2
0	0	0	0	0
			#	5
			mean	0.4
			min	0
Sample #9	Sample #10		max	2
0	0		median	0.0

0	0		std. dev.	0.8
NA	100		95% conf.	0.7
0	0			
0	0			
0	50			
0	0			
70	70			
0	0			
0	0			
0	0			
0	30			
Sample #8	Sample #9	Sample #10		

Observations									
Transect #	Stream Wid	Distance Fr	Time	Avg. Stream	ream Shadir	Stream Sun	Organic	Mud	Sand
1	113	0	2:10	2.5	5	95	0	1-10	0
2	95	0.25	2:26	2	10	90	1-10	0	0
3	78	0.5	2:45	2	10	90	0	1-10	0
4	82	0.75	2:55	2	5	95	1-10	0	0
5	90	1	3:01	5	5	95	0	0	0
6	80	1.25	3:18	4	30	70	0	0	0
7	90	1.5	3:37	3	5	95	0	0	0
8	100	1.75	4:03	3	5	95	0	0	0
9	150	2	4:16	2	10	90	0	0	0
10	140	2.25	4:30	2	0	100	0	0	0
11	95	2.5	4:43	3.5	0	100	0	1-10	1-10
12									
Algae Community									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1	Filamentous	Brown	0	70	70	70	70	70	70
2									
3	Filamentous	Dark Green	0	0	0	0	0	50	50
4									
5	Filamentous	Dark Green	0	0	10	0	NA	NA	NA
5	Filamentous	Brown	0	30	50	50	NA	NA	NA
6	Filamentous	Dark Green	10	10	10	10	10	10	10
6	Filamentous	Brown	90	90	90	0	0	0	10
7	Filamentous	Brown	90	90	90	90	90	90	90
8	Filamentous	Dark Green	10	0	0	0	0	0	0
8	Filamentous	Brown	90	90	90	90	90	90	90
9	Filamentous	Brown	50	50	50	50	50	50	50
10	Filamentous	Brown	0	30	30	30	30	80	0
11	Filamentous	Brown	50	50	50	50	50	50	50
12									

Plant Community									
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1	Submerged	0	0	0	0	0	40	40	20
2	Submerged	0	50	50	50	50	50	50	10
3	Submerged	0	50	50	50	50	40	40	50
4	Submerged	0	40	50	100	70	70	10	0
5	Submerged	0	0	0	0	0	0	0	0
6	Submerged	0	0	0	0	10	10	30	10
7	Submerged	0	10	30	10	70	100	30	10
8	Submerged	0	10	10	10	30	30	10	10
9	Submerged	50	50	50	50	50	50	50	50
10	Submerged	100	70	70	70	70	20	100	80
11	Submerged	0	40	40	40	40	40	40	40
12	Submerged								
Effect of Algae on Plants									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1	Filamentou	Brown							
2									
3									
4									
5									
6									
7	Film	Brown		10	10	10	10	10	10
8	Filamentou	Brown							
9	Filamentou	Brown	10	10	10	10	10	10	10
10	Filamentou	Brown	10	10	10	10	10	10	10
11	Film	Brown	10	10	10	10	10	10	10
12									

Other Wildlife									
Transect #	Wildlife		Stressors						
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
Transect Landmarks									
Transect #	Landmarks								
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									

Gravel	Cobble	Bedrock			
0	40-70	40-70			
10-40	40-70	0			
0	40-70	40-70			
40-70	40-70	1-10			
10-40	10-40	40-70			
1-10	10-40	40-70			
10-40	10-40	40-70			
10-40	0	>70			
40-70	1-10	40-70			
>70	0	10-40			
10-40	0	10-40			
Sample #8	Sample #9	Sample #10			
70	70	0	56	56	
50	10	0	16	16	
10	10	20	7	26	some floati
0	0	0	19	41	
10	10	10	10	81	
10	10	10	31	92	
90	90	0	81	51	
0	0	10	2	31	
90	90	90	90	50	
50	30	80	51		
20	30	60	31		
50	50	50	50		

			#	9	
			mean	49.3	
			min	16.0	
Sample #9	Sample #10		max	92.0	
10	0		median	45.5	
10	0		std. dev.	28.1	
50	0		95% conf.	18.4	
0	0				
10	0				
10	0				
10	0				
10	20				
70	20				
70	40				
40	50	emerging			
Sample #8	Sample #9	Sample #10			
50	50				
10	10				
		10			
10	10	10			
10	10	10			
10	10	10			

Observations

Transect #	Stream Wid	Distance	Time	Avg. Stream	ream Shadir	Stream Sun	Organic	Mud	Sand	Gravel
1	109	0	2:08	2.5	5	95	0	0	0	0
2	95	0.25	2:18	2	10	90	0	0	0	0
3	78	0.5	2:25	2	10	90	0	0	0	0
4	86	0.75	2:36	2	10	90	0	0	0	0
5	90	1	2:45	4	10	90	0	0	0	0
6	78	1.25	3:00	4	5	95	0	0	0	0
7	90	1.5	3:08	2	5	95	0	0	0	0
8	98	1.75	3:21	3	0	100	0	0	0	0
9	143	2	3:33	2.5	10	90	0	0	0	1-10
10	170	2.25	3:44	1	5	95	0	0	0	10-40
11	136	2.5	3:55	3.5	5	95	0	0	0	1-10
12	98	2.75	4:05	3	5	95	0	0	10-40	0

Algae Community

Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1	Thick Mat	Dark Green	10	10	0	0	0	0	0	0
2	Filamentous	Dark Green	10	10	10	10	10	10	10	10
3	Filamentous	Dark Green	10	10	10	10	10	10	10	10
4	Filamentous	Dark Green	10	10	10	10	10	10	10	10
5	Thick Mat	Dark Green	10	10	10	10	10	10	90	90
5	Filamentous	Dark Green	0	10	0	0	0	0	0	0
6	Filamentous	Dark Green	0	0	0	0	0	10	20	20
7	Thick Mat	Dark Green	0	0	0	0	0	10	10	10
8	Thick Mat	Dark Green	10	10	10	10	10	10	10	20
8	Filamentous	Brown	50	0	0	0	0	0	0	0
9	Thick Mat	Dark Green	10	10	10	10	10	10	10	10
9	Filamentous	Dark Green	10	0	0	0	0	0	0	0
10	Thick Mat	Dark Green	10	10	10	10	10	10	10	0
10	Filamentous	Dark Green	10	0	0	0	0	0	0	0
11	Thick Mat	Dark Green	0	0	0	0	0	0	10	10
12			0	0	0	0	0	0	0	0

Plant Community

Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8	Sample #9
1	Submerged	10	0	0	10	20	30	30	0	0
2	Submerged	30	50	90	90	90	90	50	30	30
3	Submerged	0	40	40	40	80	80	30	30	30
4	Submerged	30	50	50	70	70	70	30	10	10
5	Submerged	10	10	30	30	30	10	10	10	10
6	Submerged	10	30	80	80	80	50	50	10	10
7	Submerged	10	10	10	10	10	0	0	0	10
8	Submerged	10	10	10	10	10	10	10	10	10
9	Submerged	10	20	0	40	40	40	70	80	30
10	Submerged	50	70	90	90	90	90	90	70	30
11	Submerged	100	100	10	70	50	50	50	50	50
12	Submerged	90	100	10	30	30	50	50	30	30

Effect of Algae on Plants

Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1										
2										
3										
4										
5										
6										
7										
8										
9	Thick Mat	Dark Green								10
10										
11										

12										
Other Wildlife										
Transect #	Wildlife		Stressors							
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
Transect Landmarks										
Transect #	Landmarks									
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										

Cobble	Bedrock			
0	>70			
>70	0			
40-70	40-70			
40-70	40-70			
10-40	>70			
40-70	40-70			
10-40	>70			
10-40	>70			
1-10	>70			
0	>70			
0	>70			
1-10	40-70			
Sample #9	Sample #10			
0	0	2	2	
10	10	10	10	
10	10	10	10	
10	10	10	10	
70	70	22	23	
0	0	1	11	
30	30	11	5	
10	10	5	17	
20	10	12	11	massive flo
0	0	5	8	
10	10	10	3	
0	0	1	0	
0	0	7		
0	0	1		
10	0	3		
0	0	0		

		#	9.0	
		mean	9.2	
		min	0.0	
Sample #10		max	23.0	
0		median	10.0	
30		std. dev.	6.7	
0		95% conf.	4.3	
10				
10				
0				
0				
10				
30				
30				
10				
90				
Sample #9	Sample #10			
10				

Observations									
Transect #	Stream Wid	Distance Fr	Time	Avg. Stream	ream Shadir	Stream Sun	Organic	Mud	Sand
1	109	0	2:34	2.5	10	90	0	0	1-10
2	95	0.25	2:46	1.5	10	90	0	0	0
3	78	0.5	2:55	1.5	10	90	0	0	0
4	86	0.75	3:04	2	10	90	0	0	0
5	90	1	3:14	3	10	90	0	0	0
6	78	1.25	3:23	3	5	95	0	0	0
7	90	1.5	3:27	1.5	5	95	0	0	0
8	98	1.75	3:32	2	0	100	0	0	0
9	143	2	4:09	1.5	5	95	0	0	0
10	170	2.25	4:27	1	5	95	0	0	0
11	136	2.5	4:41	1.5	0	100	0	0	0
12	98	2.75	4:49	3	5	95	0	0	0
Algae Community									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1	Thick Mat	Dark Green	0	0	0	0	0	0	0
2									
3									
4	Thick Mat	Dark Green	0	0	0	0	0	0	0
4	Filamentous	Dark Green	0	0	0	0	0	0	0
5	Thick Mat	Dark Green	10	10	50	10	10	30	30
6	Thick Mat	Dark Green	0	0	0	0	0	0	0
7	Thick Mat	Dark Green	0	0	0	0	0	0	0
8	Thick Mat	Dark Green	70	50	10	0	0	10	30
9	Thick Mat	Dark Green	50	50	0	0	0	0	0
9	Filamentous	Dark Green	0	0	0	0	0	0	0
10									
11	Filamentous	Dark Green	0	0	0	0	10	0	0
12	Thick Mat	Dark Green	0	10	10	30	30	30	30

Plant Community									
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1	Submerged	0	0	0	30	30	30	50	50
2	Submerged	70	90	90	100	100	90	70	30
3	Submerged	0	70	70	90	90	100	90	90
4	Submerged	30	50	50	70	50	30	50	50
5	Submerged	30	50	10	30	30	50	50	0
6	Submerged	100	90	90	90	70	50	50	20
7	Submerged	40	20	40	60	60	10	10	40
8	Submerged	30	10	30	30	30	50	30	10
9	Submerged	50	30	30	30	30	30	10	0
10	Submerged	100	100	100	100	100	100	100	90
11	Submerged	100	100	100	100	70	100	70	70
12	Submerged	20	10	10	10	10	10	30	30
Effect of Algae on Plants									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1	Thick Mat	Dark Green							
2									
3									
4									
5	Thick Mat	Dark Green	10						
6									
7	Thick Mat	Dark Green	10						
8	Thick Mat	Dark Green	50						
9	Thick Mat	Dark Green	50	50	50	50	50	50	50
10	Thick Mat	Dark Green							
11									
12	Thick Mat	Dark Green	10	10	10	50			

Other Wildlife									
Transect #	Wildlife		Stressors						
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
Transect Landmarks									
Transect #	Landmarks								
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									

Gravel	Cobble	Bedrock		
0	>70	1-10		
0	>70	0		
0	>70	0		
>70	0	1-10		
0	>70	0		
0	0	>70		
0	40-70	40-70		
0	10-40	>70		
10-40	40-70	10-40		
10-40	0	40-70		
40-70	0	40-70		
40-70	0	10-40		
Sample #8	Sample #9	Sample #10		
30	50	0	8	8
10	10	10	3	10
0	0	70	7	24
90	90	90	24	8
0	30	50	8	6
0	10	50	6	26
30	30	30	26	11
0	0	0	10	
0	0	10	1	1
				15
0	0	0	1	
10	0	0	15	

			#	9
			mean	12.1
			min	1
Sample #9	Sample #10		max	26
0	0		median	9
10	0		std. dev.	8.659613
60	60		95% conf.	5.7
10	0			
0	0			
10	0			
20	0			
10	10			
10	30			
30	50			
100	100			
100	100			
Sample #8	Sample #9	Sample #10		
50				
50	50	50		
		10		

Observations							
Transect #	Stream Wid	Distance Fr	Time	Avg. Stream	ream Shadir	Stream Sun	Organic
1	113	0	8:02	2.5	10	90	0
2	90	0.25	8:17	2.5	5	95	0
3	116	0.5	8:28	2	10	90	0
4	83	0.75	8:37	3	5	95	0
5	87	1	8:47	3.5	10	90	0
6	85	1.25	9:01	3.5	15	85	0
7	84	1.5	9:13	4	20	80	0
8	180	1.75	9:45	2	5	95	0
9	185	2	9:52	2	5	95	0
10	185	2.25	10:15	1.5	10	90	0
11	146	2.5	10:43	2	5	95	0
12	115	2.75	10:56	2.5	10	90	0
13	120	3	11:09	2.5	5	95	0
14	190	3.25	11:21	2	5	95	0
15	210	3.5	11:36	3	5	95	0
16	158	3.75	11:57	2.5	5	95	0
17	165	4	12:10	2	10	90	0
18	109	4.25	12:23	2	5	95	0
19	108	4.5	12:32	2.5	5	95	0
20	115	4.75	12:41	4	10	90	0
Algae Community							
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
1							
2							
3							
4							
5	Thick Mat	Dark Green	10	10	10	10	10
5	Filamentous	Brown	90	90	90	90	90
6	Filamentous	Brown	90	90	90	90	90
6	Thick Mat	Dark Green	S	S	S	S	S
7	Thick Mat	Dark Green	10	10	10	10	10
7	Filamentous	Brown	90	90	90	90	90
8	Thick Mat	Dark Green	0	0	10	10	10
9	Thick Mat	Dark Green	0	10	10	10	10
9	Filamentous	Brown	0	90	90	90	90
10	Filamentous	Dark Green	10	10	10	10	0
11							
12	Thick Mat	Dark Green	0	0	10	10	10
13							
14	Filamentous	Bright Green	0	0	0	0	0
15	Filamentous	Dark Green	0	10	10	10	10
15	Thick Mat	Dark Green	10				

16							
17							
18							
19							
20							
Plant Community							
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6
1	Submerged	0	0	0	10	20	20
2	Submerged	0	0	10	0	30	30
3	Submerged	0	0	10	10	20	10
4	Submerged	0	10	10	10	20	20
5	Submerged	0	0	S	S	S	S
6	Submerged	0	0	0	0	0	0
7	Submerged	0	0	0	0	0	0
8	Submerged	10	10	10	10	10	S
9	Submerged	0	0	0	0	40	40
10	Submerged	20	20	20	20	20	20
11	Submerged	50	50	100	100	50	50
12	Submerged	70	70	70	70	70	70
13	Submerged	70	70	70	50	50	50
14	Submerged	90	90	50	30	30	30
15	Submerged	90	90	50	50	50	50
16	Submerged	10	30	30	70	70	70
17	Submerged	100	100	100	100	100	50
18	Submerged	100	100	50	50	50	50
19	Submerged	100	50	30	30	30	30
20	Submerged	0	0	0	0	50	50
Effect of Algae on Plants							
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5
1							
2							
3							
4							
5							
6							
7							
8	Thick Mat	Dark Green	0	0	0	50	50
9							
10							

11							
12							
13							
14							
15	Thick Mat	Dark Green	0	10	10	10	10
16							
17							
18							
19							
20							
Other Wildlife							
Transect #	Wildlife		Stressors				
1							
2							
3							
4							
5							
6	Brown Strings with Black Eggs						
7	Small Minnows						
8	Largemouth Bass, Crayfish						
9	Trout						
10							
11							
12							
13			Cows on left bank upstream				
14							
15	lots of snails, clean rocks						
16	no snails, covered rocks						
17							
18							
19							
20							
Transect Landmarks							
Transect #	Landmarks						
1	End up rocks						
2	Above riffle						
3							

4							
5	Above riffle						
6	Staircase						
7	Above riffle						
8	Before Riffles						
9							
10	Right island to left channel						
11							
12							
13	Tree opening on left, above powerlines						
14	Just above old mill wheel						
15	Above riffle						
16	Right in front of island						
17							
18							
19							
20							

Mud	Sand	Gravel	Cobble	Bedrock		
0	0	10-40	10-40	40-70		
0	1-10	1-10	0	>70		
0	0	0	0	>70		
0	0	10-40	1-10	40-70		
0	1-10	1-10	10-40	>70		
0	1-10	1-10	0	>70		
0	10-40	0	0	>70		
0	0	0	10-40	>70		
0	0	0	1-10	>70		
0	0	0	1-10	>70		
0	0	0	10-40	>70		
0	0	0	1-10	>70		
0	0	0	0	>70		
0	0	0	0	>70		
0	10-40	0	0	>70		
0	0	0	10-40	>70		
0	0	1-10	1-10	>70		
0	0	1-10	1-10	>70		
0	0	0	10-40	40-70		
1-10	0	0	0	>70		
Sample #6	Sample #7	Sample #8	Sample #9	Sample #10		
					10	100
					90	90
90	90	90	90		90	70
S	S	S	S			6
10	10	0	0	0	7	40
90	90	40	40	40	63	3
10	10	10	20	0	6	
0	0	0	0	0	4	7
0	0	0	0	0	36	
0	0	0	0	0	3	2
						13
10	10	10	10	0	7	
20	20	20	20	20	2	
10	10	10	10	10	3	
					10	

					#	8.0
					mean	39.8
					min	2.0
Sample #7	Sample #8	Sample #9	Sample #10		max	100.0
20	10	S	0		median	7.0
10	10	10	10		std. dev.	33.4
30	40	20	0		95% conf.	23.2
20	80	80	20			
S	S	10	0			
0	0	0	0			
0	0	0	0			
S	S	S	S			
40	10	10	0			
20	20	20	20			
50	10	10	10			
70	70	20	10			
10	10	10	10			
30	30	10	10			
10	10	10	0			
70	70	100	100			
50	30	30	0			
50	50	50	10			
30	30	10	10			
50	50	10	10			
Sample #6	Sample #7	Sample #8	Sample #9	Sample #10		
50						

10	10	10	10	0		

Observations									
Transect #	Stream Wid	Distance Fr	Time	Avg. Stream	ream Shadir	Stream Sun	Organic	Mud	Sand
1	113	0	8:34	1.5	10	90	0	0	0
2	90	0.25	9:04	3.5	5	95	0	0	0
3	116	0.5	9:22	2	5	95	0	0	0
4	83	0.75	9:35	3	5	95	0	0	0
5	87	1	9:46	3	10	90	0	0	0
6	85	1.25	10:00	5	10	90	1-10	0	0
7	84	1.5	10:11	7	0	100	0	1-10	0
8	180	1.75	10:25	1.5	10	90	0	0	0
9	185	2	10:42	3	5	95	0	0	0
10	185	2.25	11:02	2	5	95	0	0	0
11	146	2.5	11:15	1.5	5	95	0	0	0
12	115	2.75	11:26	2.5	5	95	0	0	0
13	120	3	11:36	2	5	95	0	0	0
14	190	3.25	11:46	2.5	5	95	0	0	0
15	210	3.5	12:00	4	5	95	0	0	1-10
16	158	3.75	12:18	2	10	90	0	0	0
17	165	4	12:32	2	5	95	0	0	0
18	109	4.25	12:43	2	10	90	0	0	10-40
19	108	4.5	12:51	2.5	10	90	0	0	0
20	115	4.75	1:01	4	10	90	0	0	10-40
Algae Community									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1	Filamentous	Dark Green		S					
2									
3									
4	Filamentous	Dark Green							
5									
6	Film	Dark Green	40						
7	Film	Dark Green	20						
8									

9									
10									
11									
12	Filamentous	Dark Green	10	30	30	10	0	0	0
13	Filamentous	Dark Green							
14	Filamentous	Bright Green	10	0	10	50	50	10	10
15	Filamentous	Dark Green	0	0	0	10	10	0	0
16									
17	Filamentous	Dark Green	10						
18	Filamentous	Dark Green							
19									
20	Filamentous	Dark Green							
Plant Community									
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1	Submerged	0	10	40	40	50	40	40	40
2	Submerged	10	30	40	40	20	20	0	0
3	Submerged	30	40	10	10	10	10	40	40
4	Submerged	S	S	30	10	40	10	70	10
5	Submerged	S	S	S	S	10	10	10	50
6	Submerged	0	0	0	0	0	0	0	0
7	Submerged	0	0	0	0	0	0	0	0
8	Submerged	10	10	10	10	10	10	20	0
9	Submerged	70	40	40	50	50	30	30	30
10	Submerged	30	50	50	70	70	70	50	50
11	Submerged	100	50	50	70	70	70	90	90
12	Submerged	90	70	70	30	50	50	30	30
13	Submerged	70	50	50	50	50	30	30	30
14	Submerged	50	90	70	50	50	50	70	70
15	Submerged	70	100	70	50	50	70	70	50
16	Submerged	30	100	100	90	90	100	90	100

17	Submerged	10	100	100	90	70	70	100	100
18	Submerged	30	90	90	70	50	50	50	50
19	Submerged	70	30	50	30	10	10	50	50
20	Submerged	0	0	0	0	30	70	0	50
Effect of Algae on Plants									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									
2	Thick Mat	Dark Green		10	10	10			
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13	Film	Dark Green				10	10	10	
14									
15									
16	Thick Mat	Dark Green							
17	Filamentou	Dark Green							
18	Film	Dark Green				10			
19	Filamentou	Dark Green							
20									

Other Wildlife									
Transect #	Wildlife		Stressors						
1									
2									
3									
4									
5									
6	Brown Strings with Black Eggs								
7	Small Minnows								
8	Largemouth Bass, Crayfish								
9	Trout								
10									
11									
12									
13			Cows on left bank upstream						
14									
15	lots of snails, clean rocks								
16	no snails, covered rocks								
17									
18									
19									
20									
Transect Landmarks									
Transect #	Landmarks								
1	End up rocks								
2	Above riffle								
3									
4									
5	Above riffle								

6	Staircase								
7	Above riffle								
8	Before Riffles								
9									
10	Right island to left channel								
11									
12									
13	Tree opening on left, above powerlines								
14	Just above old mill wheel								
15	Above riffle								
16	Right in front of island								
17									
18									
19									
20									

Gravel	Cobble	Bedrock		
0	40-70	40-70		
1-10	40-70	40-70		
0	10-40	40-70		
0	10-40	>70		
0	10-40	>70		
0	0	>70		
1-10	0	>70		
0	0	>70		
0	1-10	>70		
0	1-10	>70		
0	10-40	>70		
0	10-40	>70		
0	0	>70		
0	0	>70		
0	10-40	40-70		
0	10-40	>70		
0	0	>70		
0	10-40	40-70		
10-40	0	40-70		
0	0	40-70		
Sample #8	Sample #9	Sample #10		
10			10	10
			40	40
			20	20

0	0	0	8	8
		90	90	90
10	30	50	7	7
0	70	90	9	9
			10	10
10	10	10	10	10
		30	30	30
			#	10.0
			mean	23.4
			min	7.0
Sample #9	Sample #10		max	90.0
10	10		median	10.0
20	0		std. dev.	25.5
90	40		95% conf.	15.8
100	100			
50	0			
0	0			
10	0			
10	10			
20	0			
50	20			
70	10			
30	10			
10	0			
30	0			
0	0			
100	100			

100	0			
50	30			
90	10			
50	0			
Sample #8	Sample #9	Sample #10		
10	90			
	10			
	10			
	10			

Observations									
Transect #	Stream Widtl	Distance Fro	Time	Avg. Stream	tream Shadin	Stream Sun	Organic	Mud	Sand
1	32	0	10:45	3	40	60		10-40	10-40
2	31	0.25	10:57	1.5	50	50			
3	15	0.5	11:09	1.5	90	10			
4	21	0.75	11:20	3.5	20	80			
5	33	1	11:31	2	50	50			
6	33	1.25	11:48	1.5	30	70			
7	29	1.5	12:11	2	40	60			
8	35	1.75	12:27	3	0	100		1-10	
9									
10	40	2.25	12:51	2	30	70			
11	18	2.5	1:05	2	50	50			
12	28	2.75	1:31	1.5	0	100			
13	29	3	1:45	1	20	80			
14	22	3.25	1:56	2	30	70			
15	22	3.5	2:06	3	30	70			
16	38	3.75	2:17	2	40	60		1-10	
17									
18									
19	32	4	2:31	1	60	40			
20	20	4.25	2:40	3	30	70			10-40
Algae Community									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									
2									
3									
4	Filamentous	Brown	50	0	0	0	0	0	0
5	Filamentous	Brown	30	10	10	10	20	30	30
6	Filamentous	Brown	0	10	10	10	10	10	10
7	Filamentous	Brown	50	50	40	30	30	20	20

8	Filamentous	Brown	100	100	100	100	100	100	100
9									
10	Filamentous	Brown	100	70	50	50	40	50	50
11	Filamentous	Brown	10	10	10	10	10	10	10
12	Filamentous	Brown	50	50	50	50	50	50	50
13	Filamentous	Dark Green	10	10	10	10	10	10	10
14	Filamentous	Brown	10	10	10	10	10	10	10
15	Filamentous	Dark Green	0	0	0	0	0	0	30
15	Filamentous	Brown							
16	Filamentous	Brown	100	100	100	100	100	70	70
17									
18	Filamentous	Brown	10	10	10	10	10	10	10
19	Filamentous	Brown	50	10	10	10	10	10	10
Plant Community									
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1	none								
2	none								
3	none								
4	none								
5	Submerged	0	0	0	0	0	20	0	20
6	Submerged	10	0	0	0	0	10	0	10
7	none								
8	Emergent	0	0	0	0	0	0	0	0
9									
10	Emergent	100	100	0	0	0	100	0	100
11	Submerged	0	0	10	10	0	0	0	0
11	Emergent			20	20				
12	none								
13	Submerged	0	0	50	10	0	50	0	0
14	Submerged	0	0	0	0	0	0	0	10

15	Submerged	0	0	0	0	20	30	0	0
16	Submerged	0	0	0	0	0	10	0	0
16	Emergent								10
16	Other						10		
17									
18	Emergent	0	0	0	0	0	50	0	0
19	Submerged	0	0	0	10	10	10	30	10
Effect of Algae on Plants									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									
2									
3									
4									
5									
6	Filamentous	Brown	10					10	
7									
8	Filamentous	Brown							
9									
10	Filamentous	Brown	100	100					100
11	Filamentous	Brown			0	0			
12									
13									
14									
15									
16	Filamentous	Brown						10	
17									
18									
19	Filamentous	Brown							

Other Wildlife									
Transect #	Wildlife		Stressors						
1									
2									
3									
4									
5									
6	Brown Strings with Black Eggs								
7	Small Minnows								
8	Largemouth Bass, Crayfish								
9	Trout								
10									
11									
12									
13			Cows on left bank upstream						
14									
15	lots of snails, clean rocks								
16	no snails, covered rocks								
17									
18									
19									
Transect Landmarks									
Transect #	Landmarks								
1	End up rocks								
2	Above riffle								
3									
4									
5	Above riffle								
6	Staircase								

7	Above riffle								
8	Before Riffles								
9									
10	Right island to left channel								
11									
12									
13	Tree opening on left, above powerlines								
14	Just above old mill wheel								
15	Above riffle								
16	Right in front of island								
17									
18									
19									

Gravel	Cobble	Bedrock		
	40-70			
	>70			
1-10	>70			
1-10	>70			
1-10	>70			
	>70			
1-10	>70			
1-10	>70			
10-40	40-70			
1-10	>70			
	>100			
40-70	40-70			
1-10	>70			
1-10	>70			
1-10	>70			
	>70			
10-40	10-40			
Sample #8	Sample #9	Sample #10		
0	0	20	7	7
30	30	30	8	8
10	10	10	3	3
30	40	40	18	18

100	100	100	40	40
60	70	70	29	29
10	10	10	4	4
50	50	50	20	20
10	10	10	4	4
10	10	10	4	4
30	50	50	5	
		50	5	10
70	0	0	30	30
10	10	10	4	4
10	10	10	8	8
			#	13.0
			mean	13.8
			min	3.0
Sample #9	Sample #10		max	40.0
			median	7.5
			std. dev.	12.5
			95% conf.	6.8
0	0			
0	0			
0	30			
0	0			
0	0			
0	10			
10	10			

0	0			
0	0			
0	0			
0	30			
Sample #8	Sample #9	Sample #10		
10				
		80	within water	
			within water	
		50		

Observations									
Transect #	Stream Width	ance From S	Time	g. Stream De	ream Shadin	Stream Sun	Organic	Mud	Sand
1	33	0	7:34	2	10	90		1-10	1-10
2	28	0.25	7:42	2	20	80			10-40
3	12	0.5	7:55	1	80	20			
4	21	0.75	8:05	2.5	60	40			
5	36	1	8:16	2	30	70			
6	30	1.25	8:29	2	50	50			1-10
7	34	1.5	8:38	3	60	40			1-10
8	33	1.75	8:53	2	50	50			1-10
9	36	2	9:03	3	50	50			1-10
10		2.25							
11	22	2.5	9:24	1	20	80			1-10
12	20	2.75	9:33	1	10	90			
13	32	3	9:54	1	10	90			
14	25	3.25	10:14	1	10	90			
15	30	3.5	10:30	1.5	60	40			
16	28	3.75	11:06	0.5	70	30			
17	45	4	11:24	0.5	20	80			
18	20.5	4.25	11:51	0.5	50	50			
19	26	4.5	12:05	2	0	100			
Algae Community									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1	Thick Mat	Dark Green	0	0	0	0	0	0	0
2	Thick Mat	Dark Green	10	0	0	0	0	0	0
2	Film	Dark Green	10	0	0	0	0	0	0
2	Filamentous	Brown	10	0	0	0	0	0	0
3	Filamentous	Dark Green	0	0	0	0	0	0	20
4	Filamentous	Brown	30	0	0	0	0	0	0
5	Thick Mat	Dark Green	0	10	0	0	0	0	0
6	Thick Mat	Dark Green	0	0	0	0	0	20	20
6	Filamentous	Brown	50	50	50	50	50	50	50

7	Thick Mat	Dark Green	40	40	40	0	0	0	S
8	Thick Mat	Dark Green	20	20	0	0	0	0	40
8	Filamentous	Brown	0	0	0	0	0	0	0
9	Thick Mat	Dark Green	0	0	0	0	0	0	0
9	Filamentous	Brown	20	20	20	0	0	0	0
10									
11	Filamentous	Brown	40	40	30	30	40	30	40
12	Thick Mat	Dark Green	0	0	0	0	0	0	0
12	Filamentous	Dark Green	20						
13	Thick Mat	Dark Green	10						
14	Filamentous	Dark Green	0	0	0	0	10	20	10
15	Filamentous	Dark Green	0	0	10	10		0	0
16									
17	Thick Mat	Bright Green	0	0	0	0	0	100	100
18	Thick Mat	Dark Green	20	20	20	70	80	S	S
19									
Plant Community									
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1									
2									
3									
4									
5	Submerged	10							
6									
7									
8	Submerged					<10			
9									
10									
11	Emergent	80	90						70
12	Submerged	60	10						

13	Submerged				10				
14	Submerged			10	40				
15	Submerged						10		
16	Submerged	S	S	S			S		
17	Emergent				100	100	60	100	
17	Submerged			5					
18	Emergent				100	100	100		
19	Submerged				60	50	60	<10	<10
Effect of Algae on Plants									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11	Filamentous	Brown	50	50					
12									
13									
14									
15									
16									
17	Filamentous	Dark Green						100	70
17	Thick Mat	Dark Green							100
18									
19									
Other Wildlife									

Transect #	Wildlife	Stressors							
1	Minnows, Fish								
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13	Turtle w/ Algae								
14									
15									
16									
17	Minnows, Fish								
18									
19									

Gravel	Cobble	Bedrock		
	>70			
	>70			
	>70			
	40-70	10-40		
	>70			
1-10	>70			
1-10	>70			
10-40	>70			
1-10	>70			
10-40	40-70			
	>70			
	>70			
	>70			
	>70			
10-40	>70			
<10	>70			
10-40	>70			
10-40	>70			
Sample #8	Sample #9	Sample #10		
0	0	10	1	1
0	0	10	2	6
0	0	10	2	3
0	0	10	2	6
10	0	0	3	1
0	0	30	6	60
0	0	0	1	20
20	20	20	10	23
50	50	50	50	20

S	S	S	20	
10	10	10	11	39
40	40	40	12	23
40	40	60	14	10
0	0	0	6	7
				2
40	50	50	39	
0	20	10	3	30
			20	42
			10	
20	10	0	7	
0	0	0	2	
100	0	0	30	
S	S	S	42	
			#	17.0
			mean	17.8
			min	1.0
Sample #9	Sample #10		max	60.0
			median	10.0
			std. dev.	17.4
			95% conf.	8.3
80	90			

<10				
Sample #8	Sample #9	Sample #10		
50	50	50		
70	70			
100	100			

Observations									
Transect #	Stream Width	ance From S	Time	. Stream De	eam Shadir	Stream Sun	Organic	Mud	Sand
1		0.25	8:35	3.5	10	90			
2		0.5	8:41	2.5	10	90			
3		0.75	8:57	2	80	20			
4		1	9:01	3	40	60			
5		1.25	9:11	2	10	90			
6		1.5	9:18	2.5	20	80		1-10	
7		1.75	9:26	2	20	80		1-10	
8		2	9:44	2	10	90			
9		2.25	9:52	3.5	20	80		1-10	
10		2.5							
11	16	2.75	10:12	0.5	30	70			
12		3	10:20	1.5	0	100			
13		3.25	10:43	1	10	90			
14		3.5	10:56	1.5	30	70			
15		3.75	11:03	2	90	10			
16		4	11:13	0.5	80	20			
17		4.25	11:25	2	10	90			
18		4.5	11:41	1	50	50			
19		4.75	11:47	2	70	30			
Algae Community									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1	Thick Mat	Dark Green	0	0	0	10	0	0	0
2	Thick Mat	Dark Green	10	0	0	0	0	0	0
3	Filamentous	Dark Green	0	0	0	0	0	0	0
4									
5	Thick Mat	Dark Green	10	10	10	0	0	0	0
6	Thick Mat	Dark Green							S
7	Thick Mat	Dark Green	40	40	S	S	0	0	0
8	Thick Mat	Dark Green	20	0	0	S	S	0	S
9	Thick Mat	Dark Green	20	30	0	0	0	0	0

10									
11	Thick Mat	Dark Green	30	10	0	0	0	0	S
12	Thick Mat	Dark Green							
13	Thick Mat	Dark Green	20	0	0	0	0	0	0
14									
15	Thick Mat	Dark Green	30	10	0	0	0	0	0
16									
17	Thick Mat	Dark Green	S	S	20	0	0	0	S
18									
19	Thick Mat	Dark Green	20	20	0	0	0	0	0
Plant Community									
Transect #	Plant Types	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8
1									
2									
3									
4									
5	Submerged			10					
6									
7									
8	Emergent								
9									
10									
11	Emergent	80	60						
12	Submerged	30	30						
13	Submerged								
14	Submerged	10	20	30	10				10
15	Submerged						10	10	
16	Submerged				S	30		60	
17	Submerged	S	S	20				S	S
18	Submerged			S				S	
19	Submerged		S	S	S	40	40	S	70

Effect of Algae on Plants									
Transect #	Algae Type	Color	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
1									
2									
3									
4									
5									
6									
7									
8	Filamentous	Dark Green							
9									
10									
11	Thick Mat	Dark Green	30						
12									
13									
14									
15									
16									
17									
18									
19									
Other Wildlife									
Transect #	Wildlife	Stressors							
1									
2									
3									
4									
5									
6									
7									
8									
9									

10									
11	Largemouth								
12									
13									
14									
15									
16									
17									
18									
19									

Gravel	Cobble	Bedrock		
	>70			
	>70			
	>70			
	>70			
	>70			
10-40	>70			
10-40	>70			
10-40	>70			
10-40	>70			
10-40	>70			
	>70			
	>70			
	>70			
	>70			
10-40	>70			
10-40	>70			
10-40	>70			
1-10	>70			
Sample #8	Sample #9	Sample #10		
0	0	10	2	2.0
0	10	0	1	1.0
10	0	0	0	0.0
0	0	0	3	3.0
S	S	S		
0	0	0	10	10.0
0	0	S	2	2.0
0	20	S	5	5.0

0	S	S	6	6.0
	30	30	15	15.0
0	0	0	2	2.0
0	0	0	4	4.0
S	S	0	4	4.0
0	0	0	4	4.0
			#	13
Sample #9	Sample #10		mean	4.5
			min	1
			max	15
			median	3.5
			std. dev.	4.1
			95% conf.	2.2
	100			
	80			
S	S			
90	10			
S	S			
S				
50	S			

Sample #8	Sample #9	Sample #10		
		40		

	Shenandoah River Mainstem [MS RMs 22-27], June 20, 2012										
	Sample #										
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**
1	0	0	0	0	0	10	0	0	0	0	**1.0**
2	10	0	0	10	0	0	0	0	0	0	**2.0**
3	–	–	–	–	–	–	–	–	–	–	**–**
4	–	–	–	–	–	–	–	–	–	–	**–**
5	0	0	0	0	0	0	0	0	–	–	**0.0**
6	–	–	–	–	–	–	–	–	–	–	**–**
7	0	0	0	0	0	0	0	–	–	0	**0.0**
8	20	10	0	0	0	0	0	0	0	0	**3.0**
9	–	–	–	–	–	–	–	20	0	0	**6.7**
10	0	0	0	0	0	0	–	–	0	0	**0.0**
11	0	0	0	0	0	0	0	10	0	0	**1.0**
12	0	0	0	0	10	30	10	0	0	30	**8.0**
13	0	0	0	0	–	–	0	0	0	0	**0.0**
14	0	0	0	0	–	0	0	0	0	0	**0.0**
15	0	0	0	0	0	0	0	0	20	20	**4.0**
16	0	0	0	0	–	–	–	0	0	0	**0.0**
17	0	0	0	–	–	–	–	–	10	0	**2.0**
18	0	0	0	0	0	–	–	–	0	0	**0.0**
19	0	0	0	10	10	10	10	10	30	20	**10.0**
20	0	0	0	10	10	5	5	40	40	0	**11.0**
						4.6		**6.2**	**6.7**		**2.9**

Shenandoah River Mainstem [MS RMs 22-27], July 11, 2012											
	Sample #										
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**
1	–	–	–	–	–	–	–	–	–	–	**–**
2	–	–	–	–	–	–	–	–	–	–	**–**
3	30	10	0	0	0	0	0	0	0	0	**4.0**
4	0	0	0	0	50	NA	NA	NA	NA	NA	**10.0**
5	0	0	0	0	0	0	–	0	0	0	**0.0**
6	0	0	0	0	0	0	0	–	–	50	**6.3**
7	0	–	–	–	–	–	–	–	–	50	**25.0**
8	–	–	–	–	–	–	–	–	–	–	**–**
9	0	0	0	0	0	0	–	0	0	0	**0.0**
10	0	0	0	0	0	0	0	0	0	30	**3.0**
11	0	0	0	0	0	0	0	70	10	0	**8.0**
12	–	–	–	–	–	–	–	–	–	–	**–**
13	–	–	–	–	–	–	–	–	–	–	**–**
14	–	–	–	–	–	–	–	–	–	–	**–**
15	–	–	–	–	–	–	–	–	–	–	**–**
16	–	–	–	–	–	–	–	–	–	–	**–**
17	–	–	–	–	–	–	–	–	–	–	**–**
18	–	–	–	–	–	–	–	–	–	–	**–**
19	0	0	0	0	0	0	0	10	10	0	**2.0**
20	–	–	–	–	–	–	–	–	–	–	**–**
21	–	–	–	–	–	–	–	–	–	–	**–**

	3.3				**6.3**			**13.3**		**16.3**	**6.5**

Shenandoah River Mainstem [MS RMs 22-27], July 25, 2012											
	Sample #										
Transect #	1	2	3	4	5	6	7	8	9	10	Mean
1	–	–	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–	–	–
6	0	0	0	0	0	0	0	0	0	40	**4.0**
7	–	–	–	–	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–	–	–	–	–
9	–	–	–	–	–	–	–	–	–	–	–
10	–	–	–	–	–	–	–	–	–	–	–
11	10	0	0	0	0	0	0	0	0	0	**1.0**
12	–	–	–	–	–	–	–	–	–	–	–
13	–	–	–	–	–	–	–	–	–	–	–
14	–	–	–	–	–	–	–	–	–	–	–
15	–	–	–	–	–	–	–	–	–	–	–
16	–	–	–	–	–	–	–	–	–	–	–
17	–	–	–	–	–	–	–	–	–	–	–
18	–	–	–	–	–	–	–	–	–	–	–
19	–	–	–	–	–	–	–	–	–	–	–
20	–	–	–	–	–	–	–	–	–	–	–
21	–	–	–	–	–	–	–	–	–	–	–

										20.0	**2.5**

	North Fork Shenandoah River [NF RMs 11-17], June 15, 2012											
	Sample #											
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	
1	0	0	10	30	10	0	10	10	10	0	**8.0**	
2	10	10	30	70	10	10	100	100	0	0	**34.0**	
3	0	30	70	70	70	70	70	70	30	10	**49.0**	**38.0**
4	10	10	40	50	70	70	40	10	10	0	**31.0**	
5	10	10	10	10	10	30	50	50	30	0	**21.0**	
6	70	70	100	100	50	10	10	30	30	10	**48.0**	**48.0**
7	10	10	10	10	10	10	50	50	30	10	**20.0**	
8	30	30	30	30	30	30	30	30	30	30	**30.0**	**30.0**
9	10	10	10	10	10	10	10	10	10	90	**18.0**	
10	0	10	30	40	50	50	70	70	30	30	**38.0**	**38.0**
11	10	10	10	20	30	30	10	10	0	0	**13.0**	
12	0	0	10	0	10	0	10	30	30	0	**9.0**	
13	0	0	0	0	0	0	0	0	0	0	**0.0**	
14	10	10	10	30	50	10	10	0	0	0	**13.0**	
15	0	0	0	0	10	70	70	70	30	10	**26.0**	**30.0**
16	30	70	70	70	50	50	0	0	0	0	**34.0**	
17	10	10	10	10	10	20	20	10	0	0	**10.0**	
18	70	70	0	0	10	10	10	10	0	0	**18.0**	
19	10	10	30	50	50	50	0	0	0	0	**20.0**	
20	0	0	30	30	50	50	50	50	10	0	**27.0**	**27.0**

21	0	0	10	10	20	20	20	30	50	10	**17.0**	
22	0	10	30	30	50	50	50	30	10	0	**26.0**	**26.0**
	13.2	**17.3**	**25.0**	**30.5**	**30.0**	**29.5**	**31.4**	**30.5**	**15.5**	**9.1**	**23.2**	

	North Fork Shenandoah River [NF RMs 11-17], June 29, 2012											
	Sample #											
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	
1	0	50	30	10	10	10	0	0	10	10	**13.0**	
2	0	0	50	70	50	100	90	70	70	50	**55.0**	
3	100	100	70	70	90	70	50	90	20	10	**67.0**	**56.0**
4	0	20	30	40	70	60	50	70	90	30	**46.0**	
5	0	0	0	0	0	0	10	30	40	10	**9.0**	
6	0	0	0	10	30	20	40	10	20	40	**17.0**	
7	0	0	20	30	30	30	50	70	0	0	**23.0**	
8	0	0	0	0	10	10	10	10	10	0	**5.0**	
9	0	20	30	40	10	10	20	0	0	0	**13.0**	
10	10	10	10	10	40	10	10	20	20	90	**23.0**	
11	0	0	10	10	0	0	0	0	0	0	**2.0**	
12	0	0	0	0	0	0	0	0	10	10	**2.0**	
13	0	0	0	0	0	30	30	30	50	50	**19.0**	
14	10	10	10	0	0	0	0	0	0	0	**3.0**	
15	20	20	10	20	20	10	10	20	0	0	**13.0**	
16	30	10	0	50	40	30	20	0	0	0	**18.0**	
17	40	40	30	30	10	10	10	0	0	0	**17.0**	
18	0	0	0	0	0	0	10	10	10	10	**4.0**	
19	40	40	20	20	20	50	50	50	30	10	**33.0**	**44.0**
20	50	50	50	50	70	70	70	50	90	0	**55.0**	
21	30	30	30	0	0	0	0	10	10	0	**11.0**	

22	10	50	50	50	50	70	70	70	10	0	**43.0**	**43.0**
23	0	10	20	20	20	20	20	10	0	0	**12.0**	
	14.8	**20.0**	**20.4**	**23.0**	**24.8**	**26.5**	**27.0**	**27.0**	**21.3**	**13.9**	**21.9**	

	North Fork Shenandoah River [NF RMs 11-17], July 16, 2012											
	Sample #											
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	
1	0	10	10	10	30	40	30	10	0	0	**14.0**	
2	-	20	10	10	10	10	30	-	-	0	**9.0**	
3	50	20	20	20	20	20	30	50	50	10	**29.0**	
4	40	50	50	50	50	40	40	50	30	0	**40.0**	**35.7**
5	50	90	40	30	30	30	30	30	50	0	**38.0**	
6	30	0	0	0	0	0	30	30	0	50	**14.0**	
7	0	0	0	30	30	30	30	30	30	90	**27.0**	**27.0**
8	-	-	-	-	-	-	-	-	-	-	**-**	
9	0	0	0	0	0	0	0	0	0	0	**0.0**	
10	10	0	0	0	0	0	0	0	-	0	**1.0**	
11	-	-	-	-	-	-	-	-	-	-	**-**	
12	10	0	0	0	0	0	0	0	0	0	**1.0**	
13	0	0	0	0	0	0	0	0	0	0	**0.0**	
14	10	0	0	0	0	0	0	0	0	30	**4.0**	
15	30	0	20	50	40	10	10	0	0	0	**16.0**	
16	10	100	0	0	0	0	0	0	0	0	**11.0**	
17	50	55	70	40	100	100	30	30	0	0	**47.5**	**42.0**
18	30	0	0	0	30	50	0	20	0	0	**13.0**	

19	100	90	90	90	80	10	50	10	10	0	**53.0**	
20	100	90	10	100	90	100	90	30	0	0	**61.0**	
21	0	80	100	100	100	70	50	30	30	50	**61.0**	**63.0**
22	30	100	100	100	100	100	100	100	100	30	**86.0**	
23	30	30	30	50	40	70	100	100	90	0	**54.0**	
	25.2	**32.0**	**23.9**	**29.6**	**32.6**	**29.6**	**28.3**	**22.6**	**17.0**	**11.3**	**25.2**	

	North Fork Shenandoah River [NF RMs 83-86], June 26, 2012											
	Sample #											
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	
1	0	0	0	0	0	0	0	0	70	70	14.0	
2	0	0	0	0	0	0	0	50	50	50	15.0	
3	100	100	NA	NA	NA	NA	NA	NA	100	100	40.0	
4	10	10	10	10	10	10	10	100	100	0	27.0	27.0
5											-	
6	0	0	50	50	0	50	50	50	100	100	45.0	
7	30	10	0	0	0	0	30	40	50	50	21.0	
8	30	30	30	30	30	100	90	90	90	90	61.0	48.6
9	20	30	30	30	30	30	30	30	30	0	26.0	
10	90	90	90	90	90	90	90	90	90	90	90.0	
11	20	10	10	10	10	10	10	10	10	10	11.0	
	30.0	28.0	24.4	24.4	18.9	32.2	34.4	51.1	69.0	56.0	35.0	

	North Fork Shenandoah River [NF RMs 83-86], July 12, 2012											
	Sample #											
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	Mean	
1											**0**	
2	10	0	50	0	0	0	0	0	20	20	**10**	
3	80	70	70	70	70	80	70	70	70	70	**72**	60.0
4	70	30	10	0	0	90	90	90	90	10	**48**	
5	0	50	30	30	0	0	0	10	50	50	**22**	
6	70	50	50	50	50	100	100	100	100	0	**67**	49.0
7	90	50	30	0	0	0	50	50	50	50	**37**	
8	100	70	70	70	70	70	70	10	10	0	**54**	
9	80	80	80	80	30	30	0	0	0	0	**38**	
10	30	0	0	0	0	0	0	0	0	0	**3**	
	59	**44**	**43**	**33**	**24**	**41**	**42**	**37**	**43**	**22**	**35.1**	

	North Fork Shenandoah River [NF RMs 83-86], July 26, 2012										
	Sample #										
Transect	1	2	3	4	5	6	7	8	9	10	Mean
1	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	0	0	0.0
3	-	-	-	-	-	-	-	-	0	0	0.0
4	-	-	-	-	-	-	-	-	-	0	0.0
5	0	0	0	0	0	0	0	0	0	20	2.0
6	0	0	0	0	0	S	0	0	0	0	0.0
7	-	-	-	-	-	-	-	-	-	-	
8	-	-	-	-	-	-	-	-	-	-	
9	-	-	-	-	-	-	-	-	-	-	
10	-	-	-	-	-	-	-	-	-	-	
											0.4

	South Fork Shenandoah River [SF RMs 18-21], June 21, 2012											
	Sample #											
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	
1	–	–	–	–	–	–	–	–	–	–	**–**	
2	–	–	–	–	–	–	–	–	–	–	**–**	
3	–	–	–	–	–	–	–	–	–	–	**–**	
4	–	–	–	–	–	–	–	–	–	–	**–**	
5	100	100	100	100	100	–	–	–	–	–	**100**	
6	90	90	90	90	90	90	90	90	90	–	**90**	**91**
7	100	100	100	100	100	100	100	40	40	40	**82**	
8	0	0	10	10	10	10	10	10	20	0	**8**	
9	0	100	100	100	100	0	0	0	0	0	**40**	**36**
10	10	10	10	10	0	0	0	0	0	0	**4**	
11	–	–	–	–	–	–	–	–	–	–	**–**	
12	0	0	10	10	10	10	10	10	10	0	**7**	
13	–	–	–	–	–	–	–	–	–	–	**–**	
14	0	0	0	0	0	20	20	20	20	20	**10**	
15	10	10	10	10	10	10	10	10	10	10	**10**	
16	–	–	–	–	–	–	–	–	–	–	**–**	
17	–	–	–	–	–	–	–	–	–	–	**–**	
18	–	–	–	–	–	–	–	–	–	–	**–**	
19	–	–	–	–	–	–	–	–	–	–	**–**	
20	–	–	–	–	–	–	–	–	–	–	**–**	
	34.4	**45.6**	**47.8**	**47.8**	**46.7**	**30.0**	**30.0**	**22.5**	**23.8**	**10.0**	**39.0**	

	South Fork Shenandoah River [SF RMs 18-21], July 10, 2012											
	Sample #											
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	
1	–	–	–	–	–	–	–	–	–	–	**–**	
2	–	–	–	–	–	–	–	–	–	–	**–**	
3	–	–	–	–	–	–	–	–	–	–	**–**	
4	–	–	–	–	–	–	–	10	–	–	**10**	
5	–	–	–	–	–	–	–	–	–	–	**–**	
6	40	–	–	–	–	–	–	–	–	–	**40**	**40**
7	20	–	–	–	–	–	–	–	–	–	**20**	
8	–	–	–	–	–	–	–	–	–	–	**–**	
9	–	–	–	–	–	–	–	–	–	–	**–**	
10	–	–	–	–	–	–	–	–	–	–	**–**	
11	–	–	–	–	–	–	–	–	–	–	**–**	
12	10	30	30	10	0	0	0	0	0	0	**8**	
13	–	–	–	–	–	–	–	–	–	90	**90**	**90**
14	10	0	10	50	50	10	10	10	30	50	**23**	
15	0	0	0	10	10	0	0	0	70	90	**18**	
16	–	–	–	–	–	–	–	–	–	–	**–**	
17	10	–	–	–	–	–	–	–	–	–	**10**	
18	–	–	–	–	–	–	–	10	10	10	**10**	
19	–	–	–	–	–	–	–	–	–	–	**–**	
20	–	–	–	–	–	–	–	–	–	30	**30**	**30**
	15	**10**	**13**	**23**	**20**				**28**	**45**	**13.0**	

	South Fork Shenandoah River [SF RMs 32-37], June 14, 2012											
	Sample #											
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	Mean	
1	0	70	70	70	70	70	70	70	70	0	56.0	56.0
2	–	–	–	–	–	–	–	–	–	–	–	
3	0	0	0	0	0	50	50	50	10	0	16.0	
4	–	–	–	–	–	–	–	–	–	–	–	
5	0	30	60	50	–	–	–	10	10	20	18.0	
6	100	100	100	10	10	10	20	20	20	20	41.0	57.7
7	90	90	90	90	90	90	90	90	90	0	81.0	
8	100	90	90	90	90	90	90	90	90	100	92.0	
9	50	50	50	50	50	50	50	50	30	80	51.0	
10	0	30	30	30	30	80	0	20	30	60	31.0	
11	50	50	50	50	50	50	50	50	50	50	50.0	
12	–	–	–	–	–	–	–	–	–	–	-	
	43.3	56.7	60.0	48.9	48.8	61.3	52.5	50.0	44.4	36.7	42.2	

	South Fork Shenandoah River [SF RMs 32-37], June 27, 2012											
	Sample #											
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	Mean	
1	10	10	0	0	0	0	0	0	0	0	2.0	
2	10	10	10	10	10	10	10	10	10	10	10.0	
3	10	10	10	10	10	10	10	10	10	10	10.0	
4	10	10	10	10	10	10	10	10	10	10	10.0	
5	10	20	10	10	10	10	90	90	70	70	39.0	39.0
6	0	0	0	0	0	10	20	20	30	30	11.0	
7	0	0	0	0	0	10	10	10	10	10	5.0	
8	60	10	10	10	10	10	10	20	20	10	17.0	
9	20	10	10	10	10	10	10	10	10	10	11.0	
10	20	10	10	10	10	10	10	0	0	0	8.0	
11	0	0	0	0	0	0	10	10	10	0	3.0	
12	0	0	0	0	0	0	0	0	0	0	0.0	
	12.5						15.8	15.8	15.0	13.3	10.5	

	South Fork Shenandoah River [SF RMs 32-37], July 15, 2012											
	Sample #											
Transect	1	2	3	4	5	6	7	8	9	10	Mean	
1	0	0	0	0	0	0	0	30	50	0	8	
2	-	-	-	-	-	-	-	-	-	-	-	
3	-	-	-	-	-	-	-	-	-	-	-	
4	0	0	0	0	0	0	0	10	10	10	3	
5	0	0	0	0	0	0	0	0	0	70	7	
6	10	10	50	10	10	30	30	90	90	90	42	42
7	0	0	0	0	0	0	0	0	30	50	8	
8	0	0	0	0	0	0	0	0	10	50	6	
9	70	50	10	0	0	10	30	30	30	30	26	26
10	50	50	0	0	0	0	0	0	0	0	10	
11	0	0	0	0	0	0	0	0	0	10	1	
12	-	-	-	-	-	-	-	-	-	-	-	
13	0	0	0	0	10	0	0	0	0	0	1	
14	0	10	10	30	30	30	30	10	0	0	15	
	11.8	10.9	6.4	3.6	4.5	6.4	8.2	15.5	20.0	28.2	11.5	

	South Fork Shenandoah River [SF RMs 75-80], June 13, 2012										
	Sample #										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
1	0	0	NA	NA	0	0	0	0	0	0	**0**
2						NA	NA				
3											
4											
5											
6	50	0	0	0	0	0	0	0	0	30	**8**
7											
8											
9											
10	30	0	0	0	0	0	0	0	0	0	**3**
11	50	5	0	0	0	0	0	0	0	0	**6**
12									S	S	
	32.5									**7.5**	**4.1**

	South Fork Shenandoah River [SF RMs 75-80], June 28, 2012										
	Sample #										
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**
1							10	10			**10**
2	10										**10**
3	10	10									**10**
4	10										**10**
5	10										**10**
6	0	0	0	0	0	0	0	10	50	10	**7**
7											
8								20	20	10	**17**
9	0	20	20	0	0	30	30	0	20	S	**13**
10	50	0	0	0	0	0	0	0	0	0	**5**
11				10	10						**10**
12										10	**10**
											10.2

	South Fork Shenandoah River [SF RMs 75-80], July 17, 2012											
	Sample #											
Transects	1	2	3	4	5	6	7	8	9	10	Mean	
1				40	40	NA	NA	NA	NA		40.0	38.7
2	90	90	70	40	10	10	0	0	50	90	45.0	
3	0	80	80	80	70	0	0	0	0	0	31.0	
4					50						5.0	
5	10	10	10	0	0	0	0	10	10	0	5.0	
6	80	90	70	50	50	50	90	90	50	10	63.0	64.0
7	0	10	40	60	40	10	0	0	0	0	16.0	
8					20						2.0	
9	90	10	10	0	0	0	0	0	10	0	12.0	
10											0.0	
11	60	0	0	0	0	0	0	20	0	50	13.0	
12	40										4.0	
	30.8	24.2	23.3	22.5	23.3	5.8	7.5	10.0	10.0	12.5	18.2	

		North River [NR RMs 0-4], June 23, 2012										
		Sample #										
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	
1	0	0	0	0	0	0	0	0	0	0	**0**	
2	0	0	0	0	0	0	0	0	0	0	**0**	
3	10	10	0	0	0	0	0	0	0	0	**2**	
4	0	0	0	0	30	50	50	50	10	0	**19**	
5	90	80	40	50	50	50	50	30	20	20	**49**	**37**
6	100	100	100	NA	NA	NA	NA	NA	NA	100	**41**	
7	100	100	100	NA	NA	NA	NA	NA	NA	100	**41**	
8	0	0	0	NA	NA	NA	NA	0	0	0	**1**	
9	0	0	0	0	0	0	0	0	0	0	**1**	
10	0	0	0	0	0	0	0	0	0	0	**1**	
11	10	10	0	0	0	0	0	0	0	0	**3**	
12	0	0	0	0	0	0	0	0	0	10	**2**	
13	10	10	10	10	10	10	10	10	10	10	**11**	
14	10	10	10	10	10	10	10	10	10	10	**11**	
15	10	10	10	10	10	10	10	10	10	10	**12**	
16	30	30	30	40	40	40	40	40	40	40	**39**	
17	0	0	0	0	0	0	0	30	40	40	**13**	**31**
18	40	40	40	40	40	40	40	40	40	40	**42**	
											15.1	

	North River [NR RMs 0-4], July 9, 2012											
	Sample #											
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	
1	10	20	20	0	0	NA	NA	NA	NA	0	**5**	
2											**0**	
3											**0**	
4	90	10	0	0	0	0	0	0	0	0	**10**	
5	0	0	0	0	0	0	90	90	90	90	**37**	**52**
6	0	80	90	70	70	70	70	80	70	60	**67**	
7	10	20	10	10	10	S	S	S			**7**	
8	40	30	40								**12**	
9	S	S	0	0	0	30	30	20	S	S	**8**	
10											**1**	
11	0	0	0	0	0	10	10	10	20	30	**9**	
12	90	90	40								**23**	
13	90	50	20	20	0	0	0	0	0	0	**19**	
14											**1**	
15	S	S	S	S	0	0	0	0	0	0	**0**	
16	50	60	70	0	0	0	0	0	0	0	**20**	
17	10	0	0	0	0	0	0	0	0	0	**3**	
18	30	20	30	0	0	0	0	0	0	0	**10**	
10	0	0	0	0	0	0	70	80	0	60	**22**	
											11.5	

	North River [NR RMs 0-4], Jull 23, 2012											
	Sample #											
	1	2	3	4	5	6	7	8	9	10	Mean	
1						NA	NA	NA	NA			
2												
3												
4												
5												
6												
7	10	10	0	0	0	0	0	0	0	0	2	
8	40	40	20	30	30	0	0	0	0	0	16	
9												
10												
11												
12	40	40	20	0	0	0	0	0	0	10	11	
13	90	0	0	0	0	0	0	0	0	0	9	
14												
15												
16	20	20	20	0	0	0	0	0	0	0	6	
16	50	50	50	0	0	0	0	0	0	0	15	
17												
18	10	10	0	0	0	0	0	0	20	20	6	
											9.3	

	South River [SR RMs 1-4], June 16, 2012											
	Sample #											
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	
1	–	–	–	–	–	–	–	–	–	–	**–**	
2	–	–	–	–	–	–	–	–	–	–	**–**	
3	–	–	–	–	–	–	–	–	–	–	**–**	
4	50	0	0	0	0	0	0	0	0	20	**7**	
5	30	10	10	10	20	30	30	30	30	30	**23**	
6	0	10	10	10	10	10	10	10	10	10	**9**	
7	50	50	40	30	30	20	20	30	40	40	**35**	**68**
8	100	100	100	100	100	100	100	100	100	100	**100**	
9	–	–	–	–	–	–	–	–	–	–	**–**	**–**
10	100	70	50	50	40	50	50	60	70	70	**61**	**61**
11	10	10	10	10	10	10	10	10	10	10	**10**	
12	50	50	50	50	50	50	50	50	50	50	**50**	**50**
13	10	10	10	10	10	10	10	10	10	10	**10**	
14	10	10	10	10	10	10	10	10	10	10	**10**	
15	0	0	0	0	0	0	30	30	50	100	**21**	
16	100	100	100	100	100	70	70	70	0	0	**71**	**71**
17	–	–	–	–	–	–	–	–	–	–	**–**	
18	10	10	10	10	10	10	10	10	10	10	**10**	
19	50	10	10	10	10	10	10	10	10	10	**14**	
	40.7	**31.4**	**29.3**	**28.6**	**28.6**	**27.1**	**29.3**	**30.7**	**28.6**	**33.6**	**30.8**	

	South River [SR RMs 1-4], July 2, 2012											
	Sample #											
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	
1	0	0	0	0	0	0	0	0	0	10	**1**	
2	30	0	0	0	0	0	0	0	0	30	**6**	
3	0	0	0	0	0	0	20	10	0	0	**3**	
4	30	0	0	0	0	0	0	0	0	30	**6**	
5	0	10	0	0	0	0	0	0	0	0	**1**	
6	50	50	50	50	50	70	70	70	70	70	**60**	
7	40	40	40	0	0	0	–	–	–	–	**12**	
8	20	20	0	0	0	0	40	50	50	50	**23**	
9	20	20	20	0	0	0	0	40	40	60	**20**	
10	–	–	–	–	–	–	–	–	–	–	**–**	
11	40	40	30	30	40	30	40	40	50	50	**39**	
12	20	0	0	0	0	0	0	0	20	10	**5**	
13	10	–	–	–	–	–	–	–	–	–	**10**	
14	0	0	0	0	10	20	10	20	10	0	**7**	
15	0	0	10	10		0	0	0	0	0	**2**	
16	–	–	–	–	–	–	–	–	–	–	**–**	
17	0	0	0	0	0	100	100	100	0	0	**30**	
18	20	20	20	70	80	–	–	–	–	–	**42**	
19	–	–	–	–	–	–	–	–	–	–	**–**	
											16.7	

	South River [SR RMs 1-4], July 14, 2012										
	Sample #										
Transect	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**
1	0	0	0	10	0	0	0	0	0	10	**2.0**
2	10	0	0	0	0	0	0	0	10	0	**2.0**
3	0	0	0	0	0	0	0	10	0	0	**1.0**
4	–	–	–	–	–	–	–	–	–	–	**–**
5	10	10	10	0	0	0	0	0	0	0	**3.0**
6	–	–	–	–	–	–	–	–	–	–	**–**
7	40	40	–	–	0	0	0	0	0	0	**10.0**
8	20	0	0	–	–	0	–	0	0	–	**3.3**
9	20	30	0	0	0	0	0	0	20	S	**7.8**
10	–	–	–	–	–	–	–	–	–	–	**–**
11	30	10	0	0	0	0	–	0	–	–	**5.7**
12									30	30	**30.0**
13	20	0	0	0	0	0	0	0	0	0	**2.0**
14	–	–	–	–	–	–	–	–	–	–	**–**
15	30	10	0	0	0	0	0	0	0	0	**4.0**
16	–	–	–	–	–	–	–	–	–	–	**–**
17	–	–	20	0	0	0	–	–	–	0	**4.0**
18	–	–	–	–	–	–	–	–	–	–	**–**
19	20	20	0	0	0	0	0	0	0	0	**4.0**

	18.2	**10.9**							**5.5**	**4.0**	**4.1**

Photos and videos submitted by David Sligh are available by request: Tish Robertson, (804)698-4309 or tish.robertson@deq.virginia.gov
Citizen complaints submitted by David Sligh can be found in the Appendix.

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Experimental nutrient enrichment causes complex changes in seagrass, microalgae, and macroalgae community structure in Florida Bay

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In press at *Estuaries*

Experimental nutrient enrichment causes complex changes in seagrass, microalgae, and macroalgae community structure in Florida Bay

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Abstract

We examined the spatial extent of nitrogen (N) and phosphorus (P) limitation of each of the major benthic primary producer groups in Florida Bay: seagrass, epiphytes, macroalgae, and benthic microalgae, and characterized the shifts in primary producer community composition following nutrient enrichment. We established 24 permanent 0.25 m^2 study plots at each of six sites across Florida Bay and added N and P to the sediments in a factorial design for 18 months. Tissue nutrient content of the turtlegrass *Thalassia testudinum* revealed a spatial pattern in P limitation, from severe limitation in the eastern bay (N:P > 96:1), moderate limitation in two intermediate sites (~63:1), and balanced with N availability in the western bay (~31:1). P addition increased *T. testudinum* cover by 50-75% and short-shoot productivity by up to 100%, but only at the severely P-limited sites. At sites with an ambient N:P ratio suggesting moderate P limitation (~63:1), few seagrass responses to nutrients occurred. Where ambient *T. testudinum* tissue N:P ratios indicated N and P availability was balanced (~31:1), seagrass was not affected by nutrient addition but was strongly influenced by disturbance (currents, erosion). Macroalgal and epiphytic and benthic microalgal biomass were variable between sites and treatments. In general, there was no algal overgrowth of the seagrass in enriched conditions, possibly due to the strength of seasonal influences on algal biomass or regulation by grazers. N addition had little effect on any benthic primary producers throughout the bay. Overall, the Florida Bay benthic primary producer community was P-limited, but P-induced alterations of community structure were not uniform among primary producers or across Florida Bay and did not always agree with expected patterns of nutrient limitation based on stoichiometric predictions from field assays of *T. testudinum* tissue N:P ratios.

Introduction

Increased nutrient input as a consequence of human land use can cause changes in species composition and primary productivity in terrestrial and aquatic habitats (Borum and Sand-Jensen 1996; Smith et al. 1999; Kennish 2002). Urban and agricultural development in watersheds render coastal systems particularly susceptible to nutrient input (Nixon 1995; Smith et al. 1999), making the prediction of nutrient loading effects on coastal systems a key management goal. Nitrogen (N) is frequently a limiting nutrient in coastal systems, but increasing evidence for phosphorus (P) limitation suggests that both N and P enrichment are of concern in nearshore habitats (Howarth 1988).

Frequently documented responses of tropical and subtropical seagrasses to elevated N or P supply include increases in biomass and productivity (Powell et al. 1989; Tomasko and Lapointe 1991; Lee and Dunton 2000), though consequences of nutrient enrichment vary widely within and among species and regions. Seagrass beds in carbonate sediments are generally considered to be P-limited (Short et al. 1985) and may respond strongly to P enrichment, though N-limitation in carbonate sediments has been documented as well (Udy et al. 1999; Ferdie and Fourqurean 2004). Different nutrient requirements among seagrass species can cause co-occurring species to be limited by different nutrients (Udy and Dennison 1997). Furthermore, different levels of N or P limitation for individual seagrass species can occur along regional nutrient availability gradients (Lee and Dunton 2000; Fourqurean and Zieman 2002; Ferdie and Fourqurean 2004).

Macroalgae are important components of seagrass communities as well, but increases in nutrient supply can cause algal proliferations that overgrow and displace aquatic vegetation (Duarte 1995; Valiela et al. 1997; Hauxwell et al. 2001; McGlathery 2001). Evidence for both P-limitation (Lapointe 1989) and N-limitation (Larned 1998) suggests that tropical macroalgal

responses to nutrient enrichment are highly species-specific and vary among regions. Like seagrasses, tropical macroalgae may also exhibit intraspecific variation in responses to nutrient enrichment along gradients corresponding to background nutrient influence (Fong et al. 2003). However, algae do not necessarily exhibit the same limitation patterns as co-occurring seagrasses (Ferdie and Fourqurean 2004).

Microalgae, both benthic and epiphytic, comprise another important primary producer in seagrass communities (Moncreiff et al. 1992). Microalgal biomass and productivity can increase when enriched with N or P (Nilsson et al. 1991) and can overgrow other aquatic vegetation (Tomasko and Lapointe 1991). However, microalgal responses to nutrient enrichment may also be strongly limited by biotic factors including herbivory (Williams and Ruckelshaus 1993).

Florida Bay is a shallow, semi-enclosed system with extensive seagrass beds dominated by *Thalassia testudinum* (turtle grass). *T. testudinum* tissue in much of Florida Bay has a N:P ratio of >>30:1 with a maximum of 115:1 (Fourqurean and Zieman 2002), suggesting severe P-limitation (Atkinson and Smith 1983). A complex network of shallow carbonate banks within the bay restricts water flow and creates numerous, effectively isolated basins, such that sites in close proximity may have dramatically different stoichiometric patterns. A spatial gradient in N:P of seagrass tissue (Fourqurean et al. 1992) and the water column (Fourqurean et al. 1993) suggest that P availability is highest along the western marine boundary of the Bay and decreases towards the east and north. From this pattern, it has been hypothesized that the marine waters of the Gulf of Mexico are the major P source for Florida Bay (Fourqurean et al. 1992; Fourqurean et al. 1993); these hypotheses have been supported with budgetary calculations (Rudnick et al. 1999). There is evidence, however, that N can limit some components of the pelagic primary producers in the western regions of the Bay (Lavrentyev et al. 1998; Tomas et al. 1999), and

arguments have been made that some of the changes in the Florida Bay ecosystem, including loss of seagrass and increases in phytoplankton abundance, may be a result of increased N loading into western Florida Bay (Brand 2002; Lapointe and Barile 2004). Given the gradient of N:P and the finding of N limitation of benthic primary producers offshore towards the Florida Barrier Reef (Ferdie and Fourqurean 2004), such arguments bear testing experimentally.

Our objectives were to evaluate the spatial extent of nutrient (N or P) limitation of each of the major benthic primary producer groups in Florida Bay: seagrass, epiphytes, macroalgae, and benthic microalgae, and characterize the shifts in primary producer community composition following fertilization of the seagrass community. We hypothesized that N and P enrichment would stimulate acute primary producer responses corresponding to the degree of nutrient limitation as predicted by ambient *T. testudinum* tissue N:P ratios along the gradient of P availability in the Bay. We also predicted that nutrient enrichment would shift primary producer community composition towards micro- and macroalgal species with high turnover rates (sensu Duarte 1995).

Methods

To evaluate the effects of N and P enrichment across a P-availability gradient within Everglades National Park in Florida Bay, we used a three-way split-plot ANOVA design, where the factors were P addition, N addition, and region of the bay. In October 2002 we established six study sites (all depths <2 m), with two sites nested in each of three major regions of the bay: Northeast, Interior/Central, and Gulf, as defined by Zieman et al. (1989) based on macrophyte and sediment characteristics. The two eastern sites (Region "C," Fig. 1) were characterized by a sparse, short *Thalassia testudinum* canopy with some calcareous green macroalgae, primarily *Penicillus* spp., and occurred in an area of severe P-limitation (Fourqurean and Zieman 2002).

The middle two sites (Region "B") occurred in a region of intermediate P-limitation (Fourqurean and Zieman 2002) but varied in their vegetation characteristics. The easternmost of these sites (B2) had vegetation characteristics similar to those in region C, while the westernmost site (B1) featured a dense, tall *T. testudinum* canopy and little macroalgae. The westernmost two sites (Region "A") were located in a region that may experience both N- and P-limitation. The eastern site in region A (A2) had vegetation characteristics similar to site B1. The western site (A1) was characterized by a dense macroalgal-*Syringodium filiforme* (manatee grass)-*T. testudinum* assemblage. At each site we established 24 0.25 m^2 study plots demarcated with a PVC frame secured to the benthos at one meter intervals.

We randomly assigned treatments [control (C), nitrogen only (N), phosphorus only (P), both nitrogen and phosphorus (NP)] to six plots per site (at site A1, n = 3 due to the loss of 12 plots from erosion and boat disturbance over the course of the study). Nitrogen was added in the form of slow release nitrogen fertilizer (Polyon™, Pursell Technologies Inc., 38-0-0, 94% N as urea) and phosphorus as granular phosphate rock (Multifos™, IMC Global, $Ca_3(PO_4)_2$, 18% P). Loading rates of 1.43 g N m^{-2} day^{-1} and 0.18 g P m^{-2} day^{-1} (molar N:P ratio 17.6:1) were selected based on potential sewage loading rates (MCSM 2001) and previous studies in the region (Ferdie and Fourqurean 2004). We began bimonthly applications of fertilizer in October 2002 by sprinkling granular fertilizer evenly on the sediment surface and gently working it into the sediment by hand. Sediment in the control plots was similarly disturbed but no fertilizer was added. Benthic fertilizer applications ensured accessibility of nutrients to both above-ground and benthic primary producers (Ferdie and Fourqurean 2004; Mutchler et al. 2004).

In February 2004 we determined sediment, seagrass tissue, and seagrass epiphyte N and P content to assess the effectiveness of the enrichment treatments. Macroalgal tissue nutrient

content was not determined due to insufficient algal abundance and the occurrence of different species at each site. Sediment cores (1 cm diameter, 5 cm deep) were collected from each plot and dried at 60ºC. Two *T. testudinum* short-shoots were collected from each plot and the epiphytes removed by gently scraping the blades with a razor blade. At site A1, nutrient content of *S. filiforme* tissue was also determined. Nitrogen content of the sediment, epiphytes, and the cleaned seagrass tissue were determined separately using a CHN analyzer (Fisons NA1500). Phosphorus content was determined by a dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis of phosphate concentration of the extract (Fourqurean et al. 1992).

Using methods based on extensive monitoring in this region (Fourqurean et al. 2002), we estimated seagrass (*T. testudinum*, *S. filiforme*, and *Halodule wrightii*) and macroalgal percent cover with a modified Braun-Blanquet (BB) abundance scale: 0 = absent; 0.1 = one individual, <5% cover; 0.5 = few individuals, <5% cover; 1 = many individuals, <5% cover; 2 = 5-25% cover; 3 = 25-50% cover; 4 = 50-75% cover; 5 = 75-100% cover. To measure *T. testudinum* productivity, two haphazardly selected short-shoots in each plot were marked at the base of the leaves with an 18-gauge hypodermic needle and labeled with a cable tie for identification. We collected the shoots after 11-13 days, recorded the increase in leaf lengths, and calculated productivity (mg short-shoot^{-1} d^{-1}). We limited our productivity measurements to two shoots to minimize impact and facilitate future long-term sampling of this experiment

All epiphytes were removed from one short-shoot from each plot as above. Epiphytes were freeze dried and pigments extracted with 90% acetone. The chlorophyll *a* concentration was determined using a Shimadzu RF-Mini 150 fluorometer with low bandwidth filters (Welschmeyer 1994) following calibration with a chlorophyll *a* standard using a Shimadzu UV

Mini 1240 spectrophotometer and the spectrophotometric equations of Jeffrey and Humphrey (1975).

To determine benthic microalgal biomass, we collected a 2.5 cm diameter, 1 cm deep core haphazardly located within each plot. Sediments were freeze dried, pigments extracted with 90% acetone, and chlorophyll *a* concentration determined as for the epiphytes.

The variances of all data were tested for homoscedasticity using the F_{max} test and log transformed if necessary to conform to the assumptions of ANOVA. All data were analyzed with three-way split-plot ANOVA, where the factors were ± P, ± N, and region (A, B, C) with sites (A1, A2, B1, B2, C1, C2) nested within regions. Region, P, and N factors were considered fixed and sites were random for this ANOVA model. The linear model for this design was:

$$(\text{response variable})_{ijklm} = \mu + (\text{region})_i + (\text{P addition})_k + (\text{N addition})_l + (\text{interaction between region and P})_{ik} + (\text{interaction between region and N})_{il} + (\text{interaction between P and N})_{kl} + (\text{interaction between region, P, and N})_{ikl} + (\text{site within region})_{j(i)} + (\text{interaction between site within region and P})_{j(i)k} + (\text{interaction between site within region and N})_{j(i)l} + (\text{interaction between site within region, P, and N})_{j(i)kl} + \varepsilon_{ijklm}$$

where μ is the overall mean, ε is the unexplained error, *i*, *j*, *k*, and *l* represent the levels within each factor, and *m* is the number of observations per site per nutrient treatment (Quinn and Keough 2002). Site A1 was excluded from analyses of *T. testudinum* productivity and epiphyte nutrient content due to insufficient replication, as several plots at that site contained only one *T. testudinum* short-shoot, and we prioritized the epiphyte chlorophyll *a* and seagrass tissue nutrient analyses.

Results

182 Sediment, seagrass, and epiphyte nutrient content reflected nutrient treatments, though the
183 magnitude of the responses varied among regions and sites. Significant region*P and site*P
184 interactions for sediment %P (Table 1) suggested that although sediment P content increased at
185 all sites when P was added, the magnitude of increase varied among sites within regions. All
186 sites had low P content (<0.05%) prior to P addition, but sites A2 and B1 appeared to have the
187 largest increases when P was added (Fig. 2a). Sediment N content was significantly affected by
188 region, site, and N addition. Overall, sediment %N increased when N was added, but the increase
189 was generally less than 50% over control levels (Fig. 2b). Sediment N content was lower in
190 region C than in regions A and B and highest overall in sites A2 and B1.

191 Significant region*P and site*P*N interactions suggested that responses of *T. testudinum*
192 tissue %P content to nutrient treatments varied among sites within regions but that there was no
193 clear regional pattern (Table 1). Overall, *T. testudinum* P content increased by up to 300% when
194 P was added at all sites except A1, where control and enriched levels of P were similar to each
195 other and to enriched P levels at other sites (Fig. 3a). However, at sites B2 and C2, P addition did
196 not increase tissue P content as much when N was also added. Tissue %N content significantly
197 increased in response to N addition, though the change was small (10-20% increase, Fig. 3b),
198 relative to the P responses. Significant site*P and region*P interactions suggested that tissue %N
199 content response to P enrichment varied among sites within regions. Tissue %N content
200 increased when P was also added, but only at sites B2 and C1. In addition, at site A1, N content
201 decreased when P was added. Significant region*P and site*P*N interactions for *T. testudinum*
202 molar N:P ratios closely followed the site-specific but not region-specific tissue %P responses. In
203 control plots, the *T. testudinum* N:P ratio was ~30:1 at site A1, ~60:1 at sites A2 and B1, and
204 ~100:1 at sites B2, C1, and C2 (Fig. 3c). At all sites except A1, P addition lowered *T. testudinum*

N:P ratios to values approaching those at A1, though the largest relative decreases occurred at sites B2 and C2. At those two sites, N:P ratios appeared to be lower when P was added alone than when P and N were added together. *S. filiforme* tissue nutrient content did not vary with nutrient treatment, but did have slightly higher P (mean 0.24 ± 0.01 SE %P) and N (2.45 ± 0.09 %N) content and a lower N:P ratio (22.58 ± 1.27 N:P) than *T. testudinum* at site A1.

The nutrient content of *T. testudinum* epiphytes also showed complex responses to nutrient addition. Significant region*P and site*P interactions for epiphyte P content stemmed from larger increases in %P following P addition at sites B2 and C2 than at the other sites with no distinct regional pattern (Table 1, Fig. 4a). No epiphyte elemental analyses were performed for site A1 due to insufficient *T. testudinum* tissue available for collection. Epiphyte N content was lower in region C than in the other regions and variable among sites, with the highest content at sites A2 and B1, but was not affected by nutrient addition treatment (Fig. 4b). Significant region*P and site*P interactions for epiphyte molar N:P ratios were driven by large P-induced decreases in N:P ratios at all sites except A2 (Fig. 4c). In addition, epiphyte N:P ratios were significantly higher when N was added in all regions, though the magnitude of the N effect was smaller than the P effect.

T. testudinum cover and productivity generally responded positively to P addition but were unaffected by N addition. A significant interaction between site and P for *T. testudinum* cover stemmed from large P-induced increases in cover at sites B2, C1, and C2 (Table 2, Fig 5a). The strong site-specific responses of *T. testudinum* cover to P addition obscured any regional patterns. At sites B2, C1, and C2, control plots had a Braun-Blanquet (BB) score of ~2, which corresponds to about 25% cover. P addition plots had BB scores of 3 or 4, corresponding to 50-75% cover. *T. testudinum* productivity varied significantly with P and N addition but strong site-

228 specific responses masked regional trends. Productivity increased with both N and P addition
229 relative to controls, though productivity tended to be similar between P and NP plots at sites B2,
230 C1, and C2 (Fig. 5b). In addition, productivity tended to be higher at sites A2 and B1 than at the
231 other sites. Site A1 was excluded from the productivity analysis due to insufficient *T. testudinum*
232 tissue available for collection.

233 Two additional species of seagrass occurred at some study sites. *Syringodium filiforme* was
234 found in all plots at site A1 throughout the study period, but percent cover was similar across
235 nutrient treatments (average BB score 3.1 ± 0.3). *Halodule wrightii* colonized one NP plot at
236 both sites A2 and B1 and comprised 25-50% cover in those plots.

237 Macroalgal cover showed group-specific responses to nutrient treatment that varied widely
238 among regions and between sites within regions. Filamentous and other uncalcified, branching
239 red algae (Div. Rhodophyta) and calcified green algae (Div. Chlorophyta) were the most
240 common groups. A significant region*P interaction suggested that cover of red macroalgae
241 (especially the epiphytic species *Polysiphonia binneyi*, *Ceramium brevizonatum* var. *caribicum*,
242 and *Chondria* sp.) increased in response to P addition, but primarily at sites in region C (Table 2;
243 Fig. 5c). Percent cover tended to be highest overall at site A1, and red algae were not detected at
244 site B1 or in the control and N only plots at site B2. A site*N interaction suggested that calcified
245 green macroalgal cover (especially the benthic genus *Penicillus* spp.) response to nutrient
246 addition differed among sites, but high variability among sites obscured regional patterns (Fig.
247 5d). A slight N-induced increase in cover occurred at site C2 and a small N-induced decrease in
248 cover occurred at site A1. Calcareous green algae were not detected at sites A2 or B1.

249 Epiphyte loads were highly variable among regions and sites and exhibited complex
250 responses to nutrient treatments. A significant site*P*N interactions suggested that epiphyte

251 chlorophyll *a* concentration exhibited strong site-specific responses to nutrients that masked
252 regional patterns (Table 2). The highest chlorophyll *a* concentrations occurred at sites A1 and B2
253 (Fig. 5e). There was a strong decrease in chlorophyll *a* in the P and NP treatments at site B2, an
254 increase in the P and NP treatments at site C1, and no nutrient effects at sites A1, A2, B1, or C2.
255 Benthic microalgal biomass, as estimated by benthic chlorophyll *a* concentration, exhibited
256 region-specific but generally weak responses to P addition, as suggested by a significant
257 region*P interaction (Table 2). P addition slightly increased benthic chlorophyll *a* in region C
258 (Fig. 5f). Similar trends occurred in region A, though variability was high at site A1. Benthic
259 microalgal biomass did not respond to P addition treatment at sites in region B, and no N effects
260 were detected throughout the bay. A significant site effect stemmed from higher benthic
261 chlorophyll *a* concentrations at sites A1 and B2 than the other sites.

262 **Discussion**

263 Stoichiometric evidence from seagrass tissue N:P ratios suggests widespread phosphorus
264 limitation in tropical seagrass communities on carbonate sediments (Atkinson and Smith 1983;
265 Fourqurean et al. 1992). This prediction has been corroborated by experimental evidence that
266 demonstrated positive seagrass responses to P addition (Short et al. 1985) and low porewater P
267 concentration in carbonate sediments (McGlathery et al. 2001). P-limitation in these cases may
268 be attributed to the adsorption of phosphate to carbonate sediments (Koch et al. 2001) and the
269 augmentation of N supply through nitrogen fixation in systems like Florida Bay with long water
270 residence times (Howarth 1988). Accordingly, in our study there was little evidence for N
271 limitation for any of the benthic primary producers throughout the bay. This agrees with
272 predictions from studies of seagrass tissue nutrient content, which suggest that %N content above
273 1.8% indicates that N is not limiting (Duarte 1990), and seagrass tissue %N at all of our study

274 sites was ~2%. However, we underestimated the complexity of the benthic primary producer
275 responses to P enrichment. Recent work finds that there is no general pattern of P-limitation on
276 carbonate sediments (Ferdie and Fourqurean 2004), possibly due to an active dissolution of
277 carbonate sediments in the rhizosphere of seagrass beds (Burdige and Zimmerman 2002),
278 making P sorbed to sediments available for root uptake (Jensen et al. 1998). Thus, relative supply
279 of N and P are probably the most important determinants of the limiting nutrient, even in
280 carbonate sediments (Erftemeijer et al. 1994).

281 *T. testudinum* responses to P enrichment exhibited strong site-specific variation that
282 corresponded with the ambient seagrass N:P ratio at each site but did not follow the regional
283 patterns we expected. The eastern bay was severely P-limited, as indicated by the high (~100:1)
284 seagrass N:P ratios in unenriched plots, and P addition caused marked increases in seagrass
285 cover and growth. However, the impacts of P addition were not restricted to the two sites in
286 region C; site B2 had a similarly high ambient N:P ratio and increase in cover and productivity
287 following P enrichment. In contrast, there was little cover or growth response to P addition in the
288 three western sites, despite tissue P content that fell well below the 0.2% DW threshold that
289 suggests P limitation (Duarte 1990) at all sites except A1. Variation in growth responses was
290 introduced by sampling only two shoots per plot in order to preserve the integrity of the canopy
291 and facilitate future observations in this long term study, but relative differences among sites
292 suggest that productivity responses to nutrients were much less pronounced in the western than
293 in the eastern sites. At sites A2 and B1, *T. testudinum* had similar N:P ratios in control plots
294 (~60:1) and exhibited similar responses to nutrient addition, despite the presence of a large,
295 shallow bank between the two sites that limited oceanic and Gulf of Mexico water input to the
296 Interior Bay region (region B). It is not unusual to encounter site-specific patterns of nutrient

limitations in tropical seagrass communities (Agawin et al. 1996; Udy and Dennison 1997), but the spatial complexity we encountered did not follow topographic contours as we expected.

Further complexity in responses to P enrichment was revealed by the generally weak micro- and macroalgal responses to nutrient addition throughout the bay. Epiphytic and macroalgal overgrowth of seagrass frequently occurs in nutrient enriched conditions in both temperate (Valiela et al. 1997; Hauxwell et al. 2001; Cardoso et al. 2004) and tropical (Tomasko and Lapointe 1991; McGlathery 2001) habitats, yet this expected algal proliferation did not occur in our study. In fact, a decrease in epiphyte load occurred in P-addition treatments at site B2. Increased *T. testudinum* leaf productivity may have reduced leaf life span and decreased the amount of time for epiphyte accumulation, a pattern similar to that seen in N-limited seagrass beds offshore from the Florida Keys (Ferdie and Fourqurean 2004). However, differences in *T. testudinum* specific leaf productivity in Florida Bay account for less variation in epiphyte loading than grazer abundance and nutrients (Frankovich and Zieman 2005), suggesting that increased leaf productivity in enriched plots at site B2 only partially explains lower epiphyte loads. Benthic microalgal biomass can also increase in enriched conditions (Nilsson et al. 1991), but we detected little microphytobenthic response. Although we disturbed the sediments every two months during the addition of fertilizer, our activities were unlikely to obscure microalgal responses to nutrient addition because benthic microalgae are often dominated by biraphid pennate diatoms that have well-developed motility, allowing rapid migration back to the sediment surface following small-scale bioturbation events (Admiraal 1984). It is possible that the lack of response of the epiphytic and macroalgal primary producers may have been a result of the mode of nutrient delivery via fertilization of the sediments. It has been suggested that seagrass epiphytes and some macroalgae are unable to utilize sediment nutrient pools

320 (Erftemeijer et al. 1994). In our study, substantial portions of the added nutrients, particularly
321 phosphorus, were retained in the sediment, but we did elicit changes in the nutrient content and
322 N:P ratios of the seagrass and epiphyte assemblages in a pattern consistent with increased N and
323 P availability in fertilized plots. Furthermore, Ferdie and Fourqurean (2004) used an identical
324 fertilization protocol in higher energy sites near the Florida Barrier Reef and detected nitrogen
325 responses in both seagrass and epiphytes, demonstrating that this fertilization technique was
326 effective in enriching both benthic and aboveground producers. Thus, the unexpected lack of
327 responses to P addition by both seagrass and algae in seemingly P-limited habitats was probably
328 not due to inaccessibility of the added nutrients but was the likely result of multiple alternative
329 biotic and abiotic factors.

330 Phosphorus storage in seagrass and algal tissue without subsequent growth or other
331 morphological responses at some sites suggests that another nutrient may have been regulating
332 plant growth. Iron availability may limit seagrasses or algal assemblages, particularly those in
333 carbonate sediments (Duarte et al. 1995). However, experimental Fe additions have yielded few
334 micro- or macroalgal (Kuffner and Paul 2001) or seagrass (Chambers et al. 2001) responses in
335 tropical habitats. Silica availability can control diatom growth when N and P are in excess
336 (Carrick and Lowe 1988). This mechanism may have been important in the benthic microalgal
337 community in our study, which is diatom-dominated (Lewis et al. 2000), but Florida Bay
338 seagrass epiphyte loads are typically dominated by calcium carbonate (coralline algae and
339 adhered sediment) rather than diatoms (Frankovich and Zieman 1994), so silica availability
340 probably did not limit epiphyte responses to N and P addition.

341 Competition for other limiting resources, such as light (Ibarra-Obando et al. 2004) and space
342 (Marbà and Duarte 2003) may have prevented primary producers from responding to P addition

in the western portions of the bay. Sites A2 and B1 were generally less turbid than sites in the eastern bay, but *T. testudinum* shoot densities at those sites (~500-800 short-shoots m^{-2}) approached the maxima recorded in other studies in this region (Zieman et al. 1999), suggesting that the canopy may have been saturated and that self-shading was occurring. In addition, shading from a P-enriched *T. testudinum* canopy may have inhibited the response of benthic microalgae to enriched conditions across all sites.

Grazing can structure temperate and tropical seagrass communities through direct consumption of seagrass (McGlathery 1995; Valentine and Heck 2001), controlling epiphyte growth (Williams and Ruckelshaus 1993; Heck et al. 2000), and regulating benthic microalgal production (Nilsson et al. 1991). Therefore, grazing pressure may have limited *T. testudinum* and algal responses to excess nutrients at the middle bay sites and algal responses throughout the bay. Grazers known to regulate epiphyte assemblages, including snails (van Montfrans et al. 1982) and grass shrimp (Zupo and Nelson 1999) are frequently found in Florida Bay (McClanahan 1992; Matheson et al. 1999; Frankovich and Zieman 2005). Preferential grazing on nutrient-enriched plant tissue, a pattern observed in algal (Boyer et al. 2004) and seagrass assemblages (McGlathery 1995), may compensate for nutrient-induced increases in plant biomass. Though this may have contributed to the control of nutrient-induced micro- or macroalgal growth, the most common seagrass grazer in Florida Bay, the pink urchin *Lytechinus variegatus*, does not exhibit a preference for nutrient-enriched seagrass (McGlathery 1995; Valentine and Heck 2001) and was seldom observed in the study areas (Armitage et al., unpub. data) and was therefore unlikely to mitigate *T. testudinum* response to nutrients.

Species-specific seasonal fluctuations in primary producer biomass and productivity may result in temporally variable responses to excess nutrients. Wintertime peaks in epiphyte

366 (Frankovich and Zieman 1994) and rhodophyte (Collado-Vides et al. 1994) biomass in tropical
367 regions suggest that algal responses to nutrient enrichment might have been most pronounced at
368 the time of year of our sampling effort. Our continued monitoring of this project supports this
369 assertion, as the proliferations of red algae in P addition treatments in the eastern bay had largely
370 senesced by May 2004 (Armitage et al., unpub. data). In addition, we monitored this experiment
371 on a bimonthly basis since its inception and no qualitative algal proliferations were observed at
372 any time in this study until our sampling in February 2004, further suggesting that the macroalgal
373 responses were ephemeral.

374 *T. testudinum* may have responded to nutrient addition in a manner our measurements did not
375 detect because belowground characteristics were not sampled in order to minimize impact and
376 facilitate future long-term sampling of this experiment. Thus, it is possible that nutrient addition
377 was disproportionately allocated to the root/rhizome complex, particularly at sites A2 and B1,
378 where no aboveground response was detected. However, fertilizer-induced changes in shoot:root
379 allocation generally result from an increase in shoot biomass (Lee and Dunton 2000).
380 Alternatively, excess P may have been translocated along the rhizome system to new growth
381 outside the treatment plot (Marbà et al. 2002) and was thus undetected in our sampling protocol,
382 though our qualitative observations suggest that the fertilization effects were strongly localized
383 around the treatment plots.

384 The western site with an ambient *T. testudinum* tissue N:P ratio of 30:1 was not affected by N
385 or P addition, suggesting a balance between N and P supply (Atkinson and Smith 1983).
386 Accordingly, *T. testudinum* tissue phosphorus content at that site was high (Duarte 1990), and
387 the diverse macroalgal assemblage and the abundance of *Syringodium filiforme*, a seagrass
388 species associated with enriched conditions (Ferdie and Fourqurean 2004), reflected the elevated

389 nutrient supply. However, this site was heavily impacted by factors other than nutrient supply,
390 particularly frequent disturbances, both through current and erosion processes as well as boat
391 traffic, illustrating the range of factors that may regulate benthic primary producer assemblages.
392 The replacement of slower growing species with more opportunistic ones in nutrient enriched
393 conditions is a commonly observed occurrence in terrestrial (Bargali 1997), freshwater (Craft
394 and Richardson 1997), and marine habitats (Duarte 1995; Valiela et al. 1997; McGlathery 2001).
395 However, in our study, there was no suggestion of macroalgal replacement of seagrass, possibly
396 due to the seasonal nature of macroalgae in Florida Bay. Shoalgrass *Halodule wrightii* began to
397 colonize some of the NP plots, and though its occurrence was patchy at the time of this study,
398 continued fertilization may eventually lead to *H. wrightii* replacement of *T. testudinum*, a
399 phenomenon observed following two years of increased nutrient supply in a separate study in
400 Florida Bay (Fourqurean et al. 1995).
401 Our results bear directly on the hypothesis that increased freshwater, and therefore N loading
402 from the Everglades ecosystem as a result of restoration efforts could change the nature of
403 seagrass communities of Florida Bay (Brand 2002; Lapointe and Barile 2004). Direct addition of
404 P to eastern Florida Bay seagrass beds caused marked changes in community structure in the
405 short time course of our experiments, but N addition had almost no impact on primary producers
406 in any region of the Bay. These results suggest that an increase in N loading to Florida Bay
407 would have very little direct impact on seagrass communities. However, some bioassay
408 experiments have shown N limitation within the western Bay phytoplankton communities
409 (Lavrentyev et al. 1998; Tomas et al. 1999), so it is still possible that N loading could negatively
410 impact seagrass communities by promoting phytoplankton growth, leading to light limitation of
411 benthic plants. The spatial scale of our experiments was not appropriate for testing this idea, as

412 any phytoplankton bloom stimulated in our small study plots would have quickly been washed
413 away. Further, as the seagrass communities of the adjacent Florida Keys National Marine
414 Sanctuary are N-limited (Ferdie and Fourqurean 2004), increases in N loading to Florida Bay
415 could have an effect on the adjacent, downstream communities without directly altering seagrass
416 communities within the Bay.

417 Stoichiometric ratios are widely used to predict nutrient limitation, where a N:P ratio of 30:1
418 represents the modified Redfield ratio for seagrasses (e.g., Atkinson and Smith 1983; Fourqurean
419 and Zieman 2002). However, our study adds to the growing body of evidence that although very
420 high or very low stoichiometric ratios represent P or N (respectively) limitation, a broad middle
421 range of N:P ratios (~20-60:1) does not yield consistent predictions of patterns of nutrient
422 limitation in seagrasses. Although the breadth of that middle range might be a function of the
423 relatively short time period of this study, several experimental studies provide further support for
424 this conclusion. For example, four species of seagrass in Australia had similar (~20:1) molar N:P
425 ratios, but each species exhibited a unique response to N and P addition: strong N limitation,
426 weak N limitation, N and P balance, and no nutrient response were all observed (Udy and
427 Dennison 1997; Udy et al. 1999). In another study on the ocean side of the Florida Keys,
428 seagrass tissue N:P ratios (~40:1) suggested P-limitation in nearshore seagrass beds (Fourqurean
429 and Zieman 2002). However, experimental nutrient enrichment demonstrated N-limitation in the
430 macro- and microalgae but few effects of N or P enrichment on the seagrass (Ferdie and
431 Fourqurean 2004), illustrating that predictions of nutrient limitation from stoichiometric ratios
432 may not apply uniformly to all primary producers within a community. In addition, other biotic
433 or abiotic factors, such as grazing pressure, space, or the level of disturbance, may exert strong
434 influences on primary producer responses to nutrient enrichment. Supplementing stoichiometric

field assays with experimental manipulations will enable more accurate predictions of the impacts of nutrient enrichment on coastal habitats.

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Table 1: Results from three-way split-plot ANOVA of P, N, and bay region, with site nested within region, on N and P content of the sediment, *Thalassia testudinum* tissue, and epiphytes.

	df	MS	*F*	p	df	MS	*F*	p
	Sediment %P				Sediment %N			
Region	2	373.91	365.20	<0.0001	2	3.83	106.36	<0.0001
P	1	1640.11	1601.92	<0.0001	1	0.01	0.20	0.6580
N	1	1.60	1.57	0.2134	1	0.82	22.71	<0.0001
P*N	1	0.35	0.35	0.5577	1	<0.01	0.01	0.9403
Region*P	2	300.37	293.38	<0.0001	2	0.07	1.87	0.1597
Region*N	2	1.75	1.71	0.1851	2	0.01	0.22	0.8053
Region*P*N	2	0.36	0.35	0.7025	2	0.01	0.35	0.7078
Site(Region)	3	60.95	59.53	<0.0001	3	1.52	42.17	<0.0001
Site(Region)*P	3	19.58	19.12	<0.0001	3	0.04	1.03	0.3821
Site(Region)*N	3	0.81	0.79	0.5002	3	0.08	2.24	0.0874
Site(Region)*P*N	3	0.25	0.24	0.8663	3	0.03	0.85	0.4723
Residual	108	1.02			108	0.04		

Table 1 (cont.):

	df	MS	*F*	p	df	MS	*F*	P	df	MS	*F*	P
	T. testudinum %P				*T. testudinum* %N				*T. testudinum* N:P			
Region	2	0.71	70.98	<0.0001	2	0.03	25.73	<0.0001	2	0.55	60.89	<0.0001
P	1	1.75	175.44	<0.0001	1	<0.01	2.46	0.1196	1	1.61	177.76	<0.0001
N	1	0.11	10.69	0.0014	1	0.01	8.47	0.0044	1	0.18	20.24	<0.0001
P*N	1	0.05	4.67	0.0329	1	<0.01	<0.01	0.9807	1	0.05	5.20	0.0245
Region*P	2	0.25	25.53	<0.0001	2	0.01	5.93	0.0036	2	0.18	19.56	<0.0001
Region*N	2	0.01	1.13	0.3277	2	<0.01	0.15	0.8603	2	0.01	1.56	0.2152
Region*P*N	2	0.02	2.37	0.0985	2	<0.01	0.58	0.5616	2	0.02	2.29	0.1059
Site(Region)	3	0.20	20.13	<0.0001	3	0.05	45.63	<0.0001	3	0.41	45.67	<0.0001
Site(Region)*P	3	0.08	8.45	<0.0001	3	<0.01	2.72	0.0481	3	0.07	7.63	<0.0001
Site(Region)*N	3	0.01	0.96	0.4164	3	<0.01	1.17	0.3231	3	0.01	1.29	0.2832
Site(Region)*P*N	3	0.03	3.33	0.0225	3	<0.01	0.66	0.5811	3	0.03	3.30	0.0233
Residual	107	0.01			107	<0.01			107	0.01		

Table 1 (cont.):

	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p
	Epiphyte %P				Epiphyte %N				Epiphyte N:P			
Region	2	0.55	19.24	<0.0001	2	0.21	16.57	<0.0001	2	0.12	6.23	0.0030
P	1	1.32	45.93	<0.0001	1	0.02	1.72	0.1932	1	1.13	56.86	<0.0001
N	1	0.04	1.38	0.2427	1	0.03	2.02	0.1584	1	0.11	5.45	0.0219
P*N	1	<0.01	0.07	0.7988	1	0.04	2.84	0.0951	1	0.01	0.68	0.4127
Region*P	2	0.13	4.62	0.0124	2	0.01	0.59	0.5578	2	0.16	8.01	0.0006
Region*N	2	<0.01	0.05	0.9466	2	0.01	1.04	0.3577	2	<0.01	0.20	0.8217
Region*P*N	2	<0.01	0.04	0.9638	2	0.01	0.96	0.3858	2	0.01	0.45	0.6368
Site(Region)	2	0.11	3.64	0.0303	2	0.07	5.60	0.0050	2	0.18	9.10	0.0003
Site(Region)*P	2	0.21	7.31	0.0012	2	0.03	1.95	0.1482	2	0.11	5.73	0.0046
Site(Region)*N	2	0.07	2.41	0.0958	2	<0.01	0.23	0.7978	2	0.02	1.20	0.3069
Site(Region)*P*N	2	0.01	0.17	0.8445	2	0.01	0.37	0.6905	2	0.01	0.65	0.5228
Residual	87	0.03			97	0.01			87	0.02		

Table 2: Results from three-way split-plot ANOVA of P, N, and bay region, with site nested within region, on *Thalassia testudinum*, macroalgae, epiphytic microalgal, and benthic microalgal characteristics.

	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p
	T. testudinum Cover				*T. testudinum* Productivity				Rhodophyta Cover			
Region	2	0.77	41.76	<0.0001	2	0.42	9.19	0.0002	2	45.34	83.05	<0.0001
P	1	0.48	26.00	<0.0001	1	1.18	25.85	<0.0001	1	2.04	3.73	0.0560
N	1	<0.01	0.15	0.7027	1	0.31	6.72	0.0110	1	0.43	0.79	0.3767
P*N	1	<0.01	0.21	0.6519	1	<0.01	0.03	0.8515	1	0.54	0.98	0.3234
Region*P	2	0.03	1.67	0.1933	2	0.05	1.06	0.3503	2	3.15	5.77	0.0042
Region*N	2	0.04	2.03	0.1359	2	0.01	0.19	0.8279	2	1.12	2.04	0.1345
Region*P*N	2	0.01	0.60	0.5491	2	0.08	1.81	0.1683	2	0.30	0.56	0.5753
Site(Region)	3	1.78	95.72	<0.0001	2	0.56	12.32	<0.0001	3	15.33	28.09	<0.0001
Site(Region)*P	3	0.22	11.63	<0.0001	2	0.05	1.08	0.3436	3	0.95	1.75	0.1613
Site(Region)*N	3	<0.01	0.17	0.9159	2	<0.01	<0.01	0.9987	3	0.70	1.29	0.2826
Site(Region)*P*N	3	0.02	1.23	0.3023	2	0.01	0.14	0.8724	3	0.35	0.64	0.5894
Residual	108	0.02			98	0.05			108	0.55		

Table 2 (cont.):

	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p
	Chlorophyta Cover				Epiphyte chlorophyll *a*				Benthic chlorophyll *a*			
Region	2	10.33	72.73	<0.0001	2	0.92	34.28	<0.0001	2	0.17	7.97	0.0006
P	1	0.01	0.07	0.7906	1	0.10	3.62	0.0598	1	0.33	15.23	0.0002
N	1	0.15	1.09	0.2988	1	0.02	0.69	0.4073	1	0.01	0.28	0.6000
P*N	1	0.01	0.09	0.7594	1	0.06	2.09	0.1513	1	0.01	0.22	0.6433
Region*P	2	0.43	3.01	0.0532	2	0.25	9.39	0.0002	2	0.09	4.31	0.0159
Region*N	2	0.47	3.27	0.0417	2	0.03	1.08	0.3447	2	0.02	0.91	0.4054
Region*P*N	2	0.11	0.75	0.4772	2	0.03	0.94	0.3921	2	0.02	0.99	0.3760
Site(Region)	3	16.66	117.29	<0.0001	3	4.10	152.69	<0.0001	3	0.72	32.90	<0.0001
Site(Region)*P	3	0.27	1.92	0.1312	3	0.31	11.56	<0.0001	3	0.02	0.79	0.5033
Site(Region)*N	3	0.45	3.20	0.0262	3	0.03	1.03	0.3835	3	0.04	1.78	0.1549
Site(Region)*P*N	3	0.13	0.93	0.4304	3	0.09	3.38	0.0211	3	0.01	0.50	0.6856
Residual	108	0.14			108	0.03			108	0.02		

Figure Captions:

Fig. 1: Map of Florida Bay and study sites. Bay regions are defined based on delineations from Zieman et al. (1989). Region A: Gulf/Atlantic, Region B: Interior/East Central, Region C: Northeast.

Fig. 2: Responses of sediment nutrient content to region and P and N addition: a) P content as a percent of dry weight, b) N content as a percent of dry weight. At site A1, n = 3 per nutrient treatment; at all other sites, n = 6. In all figures, significant p-values are indicated and all bars represent standard error.

Fig. 3: Responses of *Thalassia testudinum* nutrient content to region and P and N addition: a) P content as a percent of dry weight, b) N content as a percent of dry weight, c) N:P molar ratio. At site A1, n = 3 per nutrient treatment; at all other sites, n = 6.

Fig. 4: Responses of *Thalassia testudinum* epiphyte nutrient content to region and P and N addition: a) P content as a percent of dry weight, b) N content as a percent of dry weight, c) N:P molar ratio. n = 6 per site per nutrient treatment. Ψ indicates insufficient replication for analysis.

Fig. 5: Responses of primary producers to region and P and N addition: a) *Thalassia testudinum* percent cover, estimated by Braun-Blanquet (BB) score, b) *T. testudinum* short-shoot (ss) productivity, c) uncalcified red algae (Rhodophyta) cover, d) calcified green algae (Chlorophyta) cover, e) epiphyte chlorophyll *a* concentration, f) benthic chlorophyll *a* concentration. At site A1, n = 3 per site per nutrient treatment; at all other sites, n = 6. Ψ indicates insufficient replication for analysis. § indicates no macroalgae present.

Figure 1

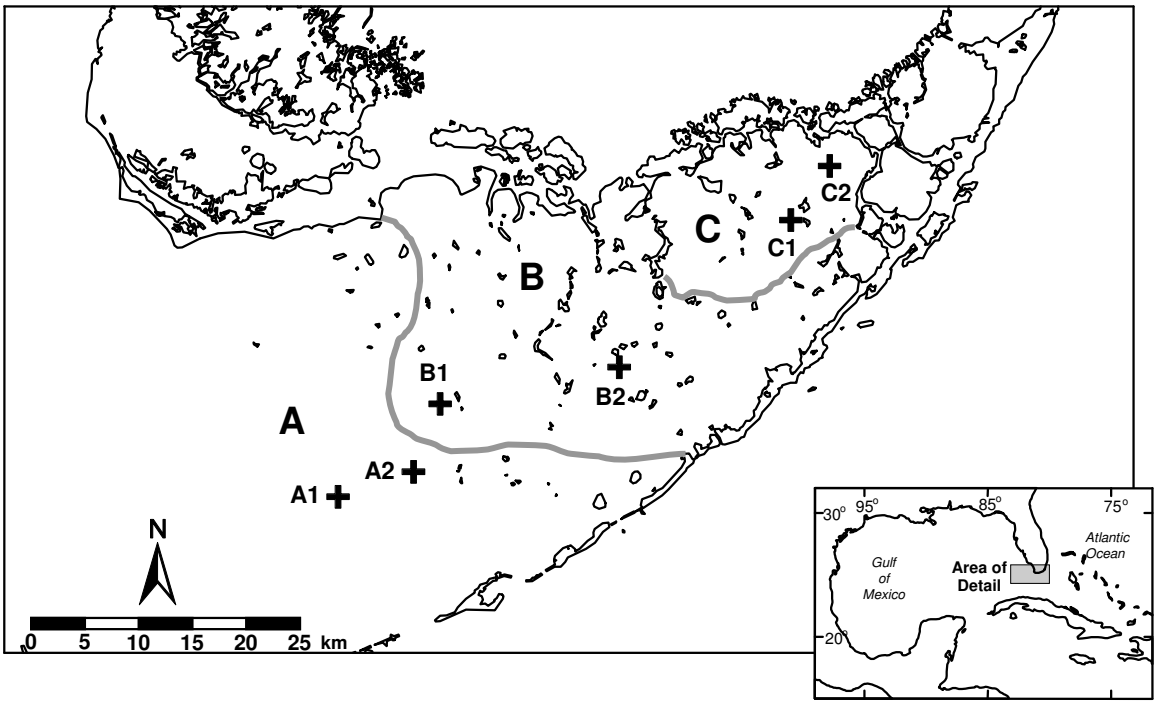

A
B
C
A1
A2
B1
B2
C1
C2
N
0 5 10 15 20 25 km
30°
95°
85°
75°
20°
Gulf of Mexico
Area of Detail
Atlantic Ocean

Figure 2

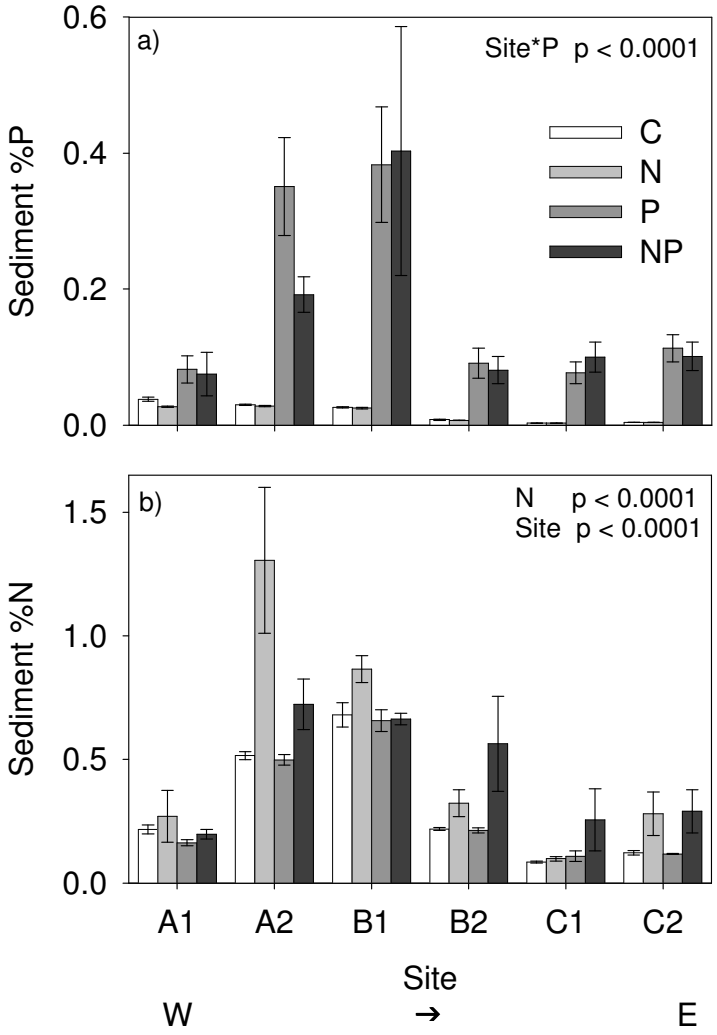

a)
Site*P p < 0.0001
C
N
P
NP
Sediment %P
0.6
0.4
0.2
0.0
b)
N p < 0.0001
Site p < 0.0001
Sediment %N
1.5
1.0
0.5
0.0
A1
A2
B1
B2
C1
C2
Site
W
➔
E

Figure 3

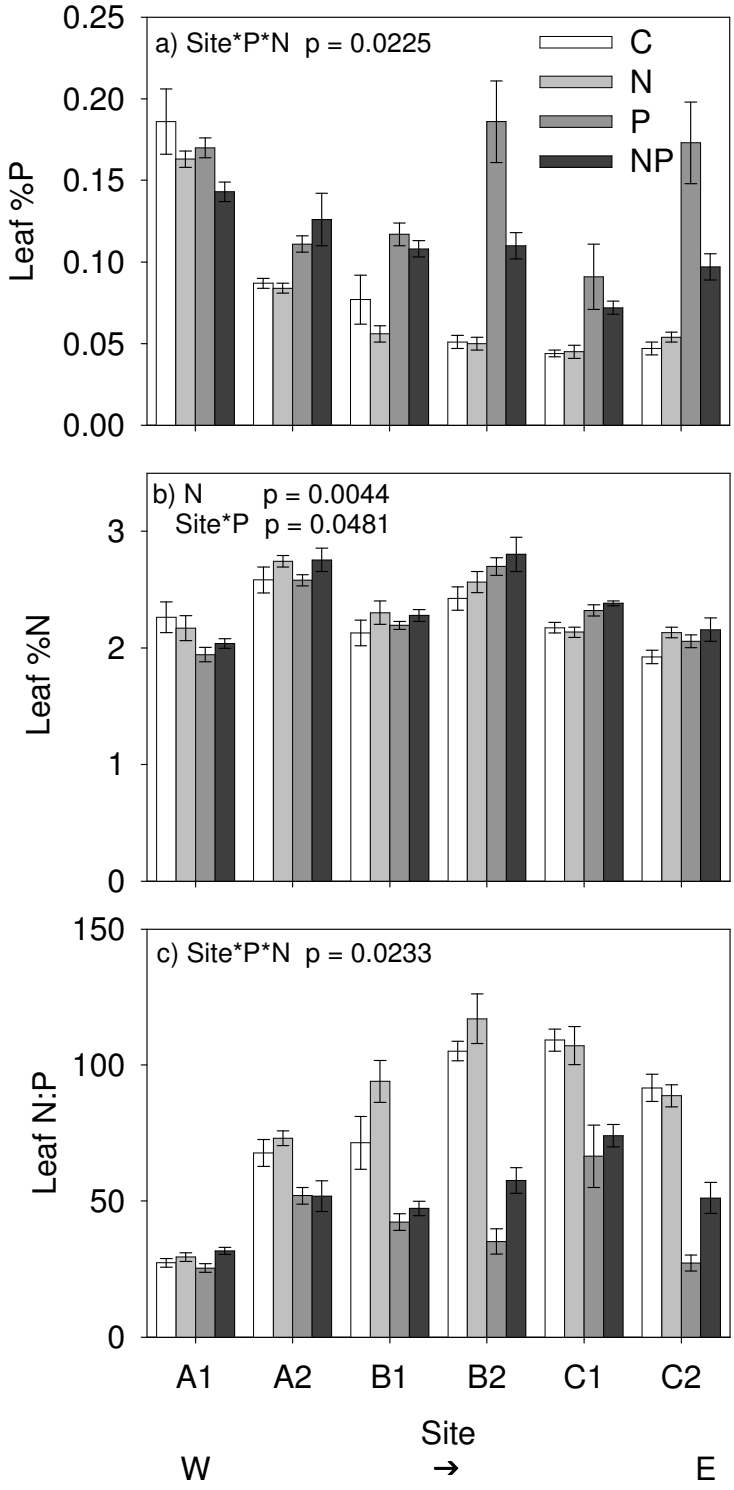

a) Site*P*N p = 0.0225
C
N
P
NP
Leaf %P
0.25
0.20
0.15
0.10
0.05
0.00
b) N p = 0.0044
Site*P p = 0.0481
Leaf %N
3
2
1
0
c) Site*P*N p = 0.0233
Leaf N:P
150
100
50
0
A1
A2
B1
B2
C1
C2
Site
W
→
E

Figure 4

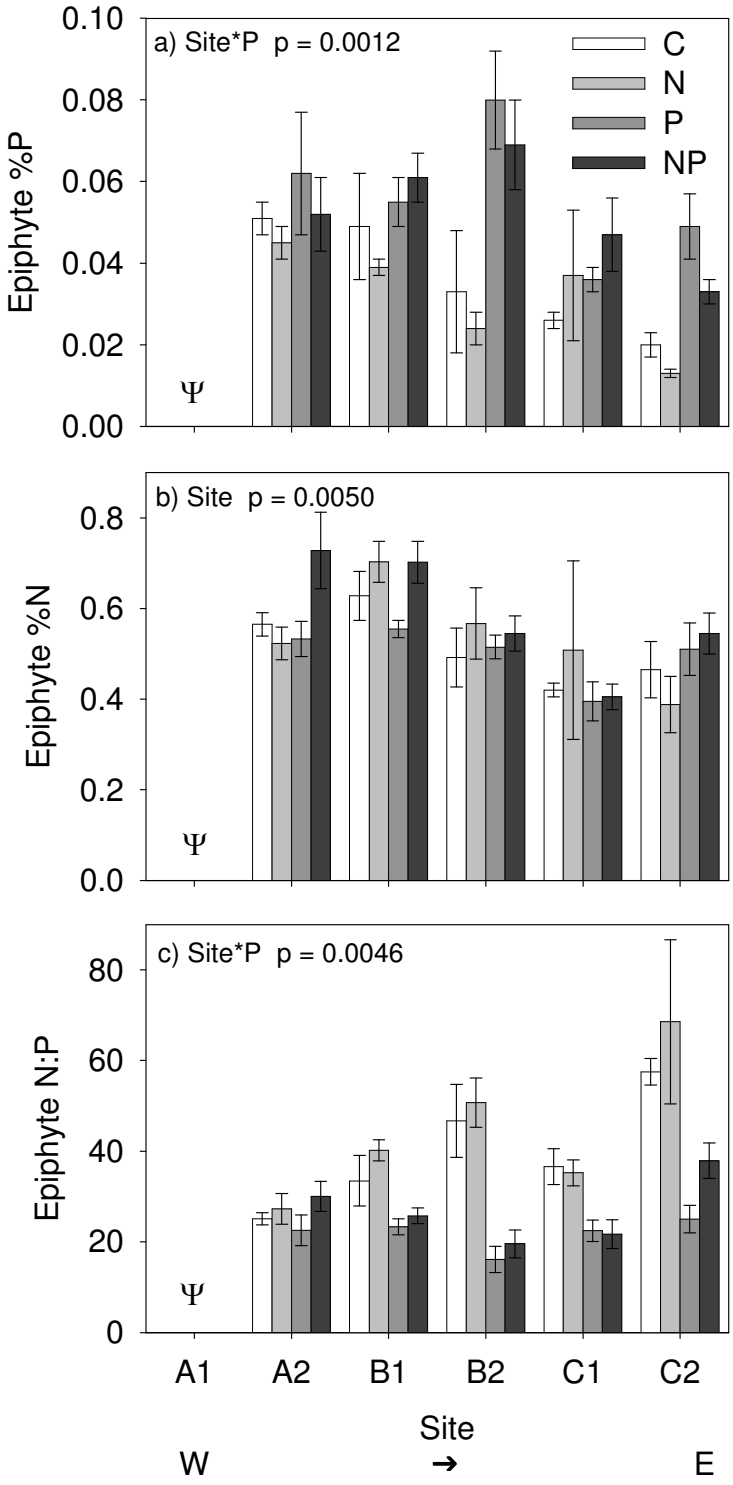
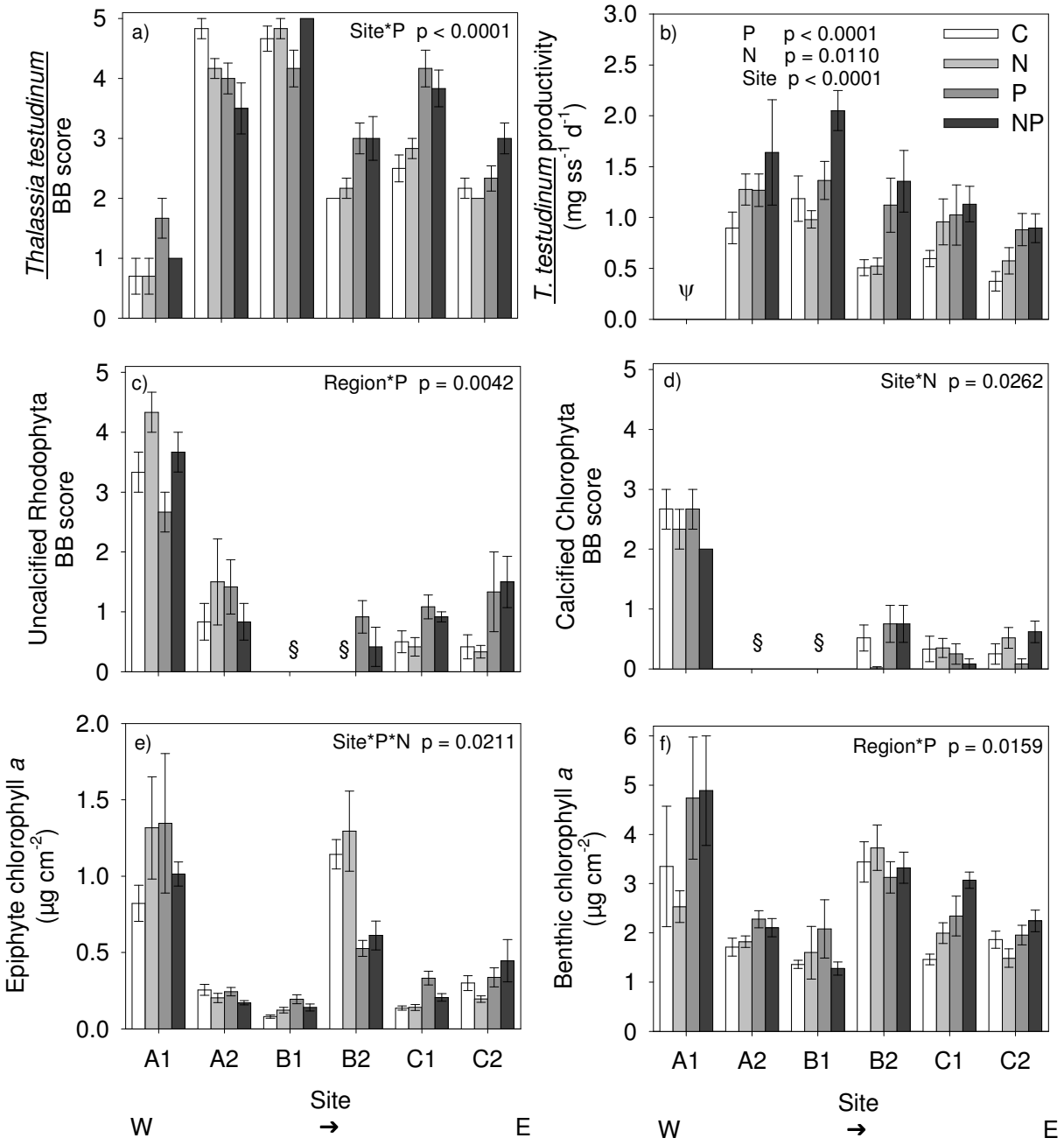

a) Site*P p = 0.0012
C
N
P
NP
Epiphyte %P
0.10
0.08
0.06
0.04
0.02
0.00
Ψ
b) Site p = 0.0050
Epiphyte %N
0.8
0.6
0.4
0.2
0.0
Ψ
c) Site*P p = 0.0046
Epiphyte N:P
80
60
40
20
0
Ψ
A1
A2
B1
B2
C1
C2
Site
W
➔
E

Figure 5


a)
Site*P p < 0.0001
Thalassia testudinum BB score
b)
P p < 0.0001
N p = 0.0110
Site p < 0.0001
C
N
P
NP
T. testudinum productivity (mg ss-1 d-1)
ψ
c)
Region*P p = 0.0042
Uncalcified Rhodophyta BB score
§
§
d)
Site*N p = 0.0262
Calcified Chlorophyta BB score
§
§
e)
Site*P*N p = 0.0211
Epiphyte chlorophyll a (µg cm-2)
f)
Region*P p = 0.0159
Benthic chlorophyll a (µg cm-2)
A1
A2
B1
B2
C1
C2
Site
W
➔
E

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University of
Montana

WATER QUALITY BENEFITS OF THE MISSOULA PHOSPHATE BAN IN THE CLARK FORK RIVER

By

Perry Berlind

Presented in partial fulfillment of the requirements

for the degree of

Master of Science

University of Montana

1992

Approved by

Vicki Watson

Director, Dr. Vicki Watson

Dean, Graduate School

October 5, 1992

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Berlind, Perry L., M.S., 1992 Environmental Studies

Water Quality Benefits of the Missoula Phosphate Ban in the Clark Fork River (72 pp.)

Director: Dr. Vicki Watson VW

Algal accumulation in the Clark Fork River in western Montana has reached levels of nuisance accumulation. Excessive nutrient loads in the river are believed to contribute to the nuisance algal accumulation. Studies on the Clark Fork River have shown that municipal wastewater treatment plants are responsible for much of the nutrient loading to the river. A phosphate detergent ban has been enacted by the City and County of Missoula to reduce nutrient loading to the river. This report analyzes the reductions in algal accumulation expected to result from decreased nutrient loading to the river because of the ban.

The most direct way to measure the benefits of the phosphate detergent ban on the algal accumulation in the river would be to simply compare pre- and post- ban levels of algal accumulation. Limited data exist for this comparison. However, the algal accumulation data collected can not be directly compared because of confounding environmental factors that influence algal accumulation. The pre-ban data were collected in a low flow year and the post-ban data were collected in an average flow year.

A model designed to simulate algal accumulation under differing environmental conditions has been developed to facilitate comparison of pre- and post- ban data. Nutrient concentrations in the Clark Fork River under differing nutrient load conditions have been determined by a nutrient model developed for this project.

Nuisance levels of algal accumulation in the Clark Fork River were shown to be substantially reduced as a result of the decreased loading achieved by the phosphate detergent ban. The largest reductions were found at sites farthest down the river from the Missoula wastewater treatment plant where nutrient levels were farther below saturation.

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CHAPTER 1. INTRODUCTION

The Clark Fork River Basin drains parts of Idaho, British Columbia, and most of western Montana. The water flows through the Clark Fork River into Lake Pend Oreille, and then through the Pend Oreille River into the great Columbia River. The water of the Clark Fork River Basin has been polluted by many industrial, rural, and urban activities. Mining, smelting, logging, paper production, agricultural, and urban wastes have created major water quality problems. The Section 525 amendments to the Clean Water Act in 1987 directed the State of Montana to comprehensively assess the impacts of these activities on the water quality of the basin. Many chemical and physical problems were identified by the Governor's Office Section 525 report (Johnson and Schmidt, 1988). Elevated levels of metals, nutrients, turbidity, and temperature, depleted levels of dissolved oxygen, dewatering, sedimentation, channelization, and hydroelectric dams were found to create major problems. The most significant water quality issues identified by the report result from the presence of heavy metals that have been mobilized in the system by mining activities. Mining and smelting wastes have introduced these metals into the water, sediments, and floodplains of the basin, adversely affecting the quality of the surface and ground water and causing acute and chronic problems for the local aquatic community.

The Governor's Office Section 525 report identified

nutrient pollution as the second most significant water quality issue facing the Clark Fork River Basin. Nutrients are put into the system from agricultural runoff, urban and industrial wastewater discharges, and groundwater infiltration. Excess bioavailable nutrients in the Clark Fork River contribute to nuisance levels of algal accumulation and to heavy nutrient loading and changes in the trophic status of Lake Pend Oreille. Nutrient concentrations in most reaches of the Clark Fork River are below levels that saturate maximum algal standing crop (Watson et al, 1990). Therefore, reductions in nutrient levels in the river may result in reductions of algal standing crop.

The City of Missoula has enacted a ban on the sale of phosphate detergents to help curb the amount of phosphorus discharged to the river from the Missoula wastewater treatment plant (WWTP). This report will analyze the realized reductions in algal accumulation achieved as a result of decreased nutrient loading due to the phosphorus detergent ban. In the classic impact study design, the changes in algal accumulation as a result of the ban are determined by measuring algal accumulation above and below the point of nutrient loading both before and after the ban went into effect. However, comparisons between these conditions are confounded by many uncontrolled environmental factors that affect algal growth, such as flow conditions and temperatures for a given year and flow and lighting differences from site-

to-site. These problems are only partially resolved by the classic impact study design.

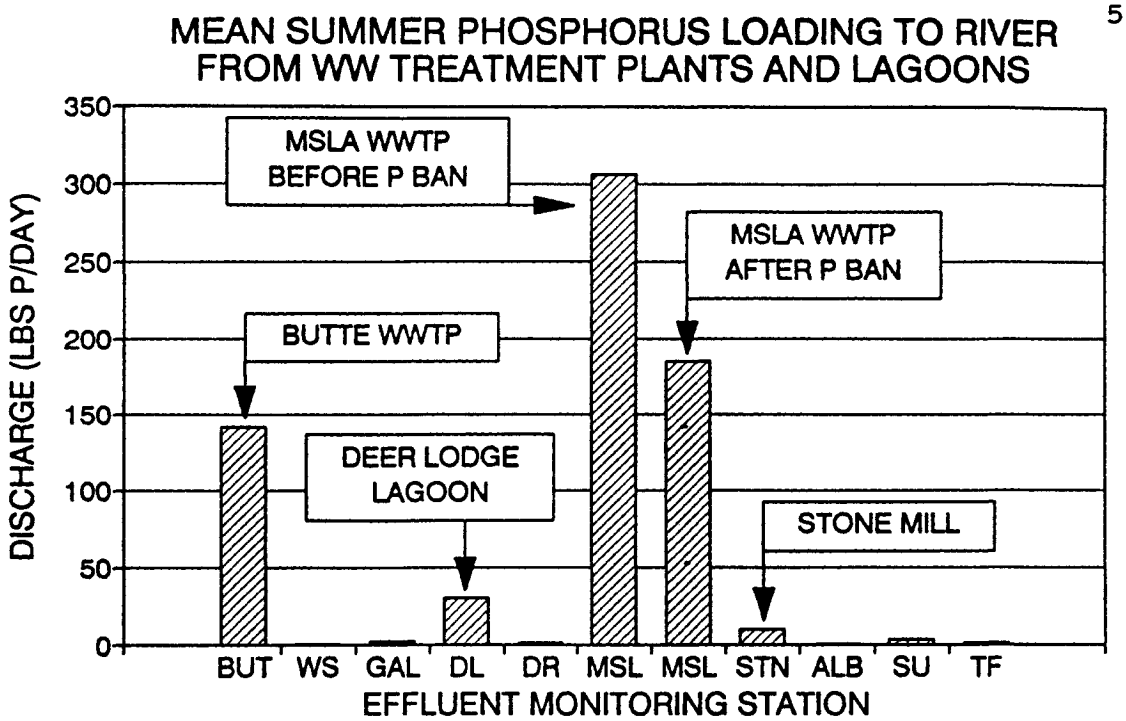
Another approach must be taken to account for these confounding factors and to allow an unbiased evaluation of the effects of the phosphate detergent ban on algal accumulation in the Clark Fork River. A numerical model has been developed to simulate levels of algal accumulation in the Clark Fork River under different nutrient, temperature, and light regimes. This model can be run to test the effects of changes in nutrient concentrations in the river as a result of the phosphate detergent ban. The confounding year-to-year factors are controlled by utilizing a nutrient concentration model developed for the Clark Fork River that estimates river nutrient concentrations from river flows and nutrient loads.

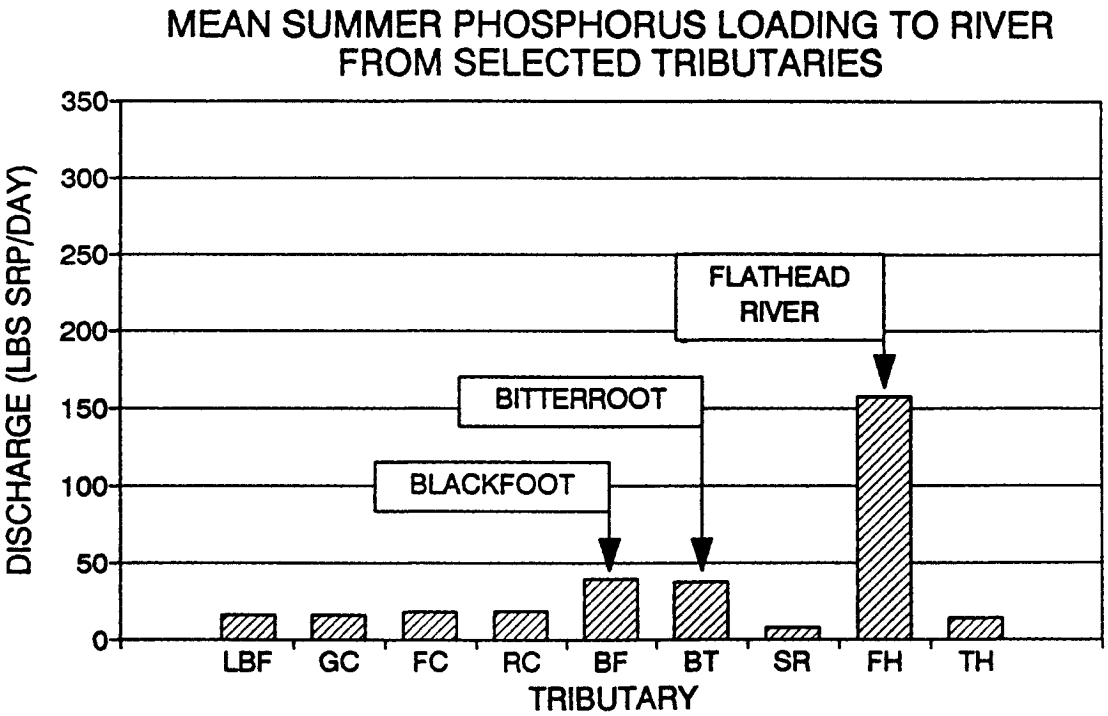
CHAPTER 2. NUTRIENTS IN THE CLARK FORK RIVER

The Section 525 water quality assessment of the Clark Fork River Basin has spawned additional studies designed to address the specific causes and effects of nutrient pollution. The bioavailable forms of the nutrients phosphorus and nitrogen are of concern for eutrophication control and algal management. Bioavailable nitrogen is in the form of nitrate (NO_3^-, nitrite (NO_2^-), and ammonia (NH_3^-), and the sum total of these forms is referred to as total soluble inorganic nitrogen (TSIN or SIN). The bioavailable form of phosphorus is soluble reactive phosphorus (SRP), chemically equivalent to inorganic orthophosphate (PO_4^{3-}).

NUTRIENT SOURCE ASSESSMENT

The Montana Water Quality Bureau has identified the major point and non-point sources of nutrients to the Clark Fork River Basin (Ingman, 1990). The point nutrient sources along the main stem and headwaters of the Clark Fork River include the municipal wastewater discharges from Butte, Warm Springs, Galen, Deer Lodge, Drummond, Missoula, Alberton, Superior, and Thompson Falls. The mean summer phosphorus loading to the river for each of these plants is shown in Figure 1. The Missoula and Butte wastewater treatment plants (WWTP) contribute the greatest loads of bioavailable soluble reactive phosphorus (SRP). The Stone Container pulp mill, which seasonally discharges wastewater pond effluent into the river, has achieved major reductions in phosphorus loading to the





Figures 1 (top) and 2. Mean summer phosphorus loading to the Clark Fork River from selected tributaries and wastewater treatment facilities.

river and has a low average discharge. The main tributary nutrient sources to the Clark Fork River include the Little Blackfoot River, Gold Creek, Flint Creek, Rock Creek, Blackfoot River, Bitterroot River, and the Flathead River. In the middle Clark Fork River, the tributary loadings contribute about one-third of the total nutrient load to the river. In the lower Clark Fork River, the SRP loading is dominated by the Flathead River (Figure 2).

NUTRIENT SAMPLING-METHODS

The Montana Water Quality Bureau began collecting and analyzing Clark Fork River water samples for total and dissolved nutrients in 1988. Grab samples were collected monthly at selected sites along the main stem and tributaries of the Clark Fork River. Main stem samples were collected from the headwaters of the Clark Fork River near Butte to a point nearly 375 river miles downstream, below Cabinet Gorge Reservoir in northeastern Idaho (Figure 3). The 18 station locations (see Table 1) are measured in river miles from Warm Springs. Samples for dissolved nutrients were filtered in the field through a standard 0.45 micron glass filter. Samples were packed on ice after collection and returned to the Montana Department of Health Chemistry Laboratory for analysis. The sample collection and analysis followed standard EPA approved methodology. To ensure the precision and accuracy of the data, strict quality assurance/quality control (QA/QC) protocols were implemented. All collection

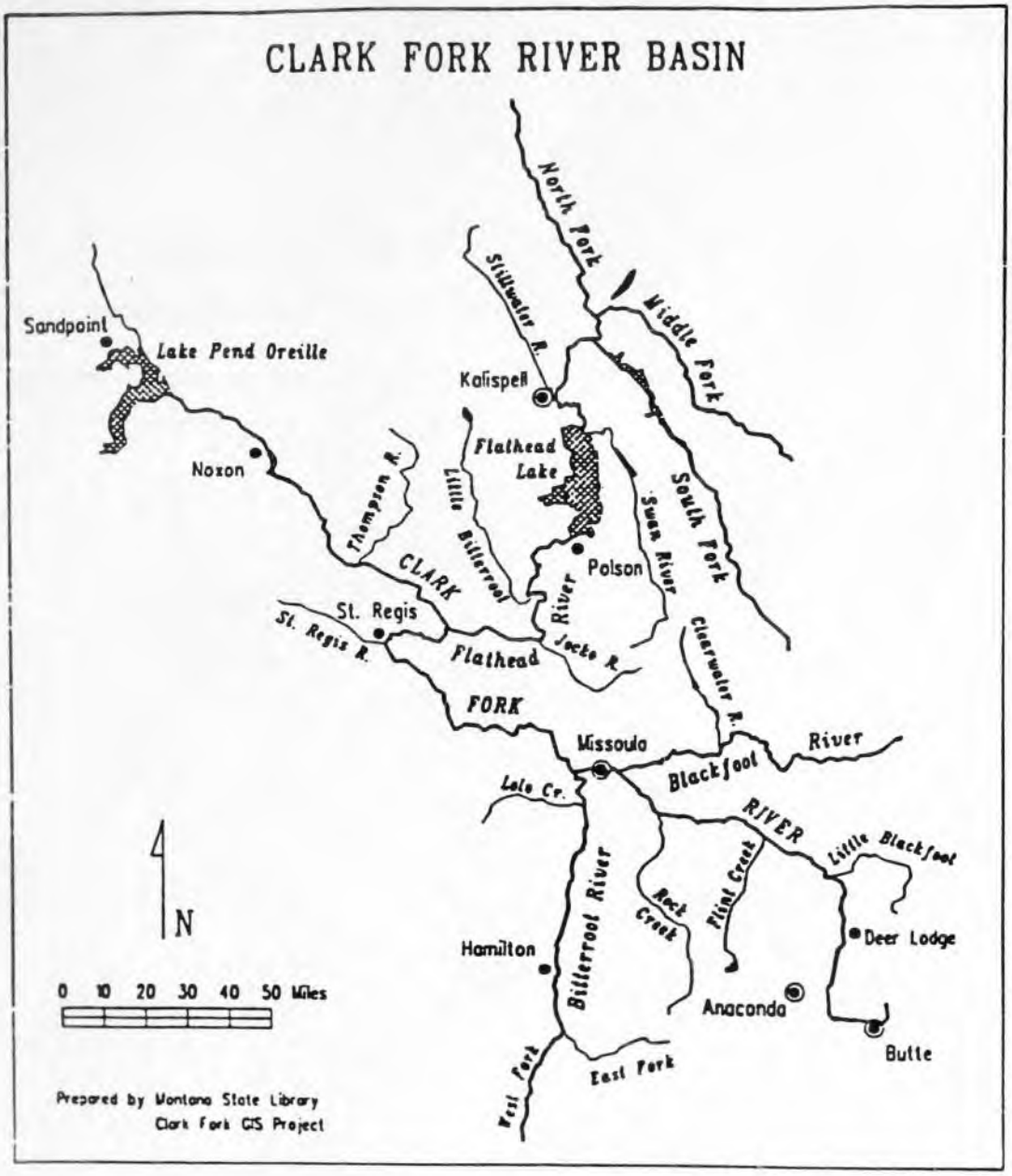


Figure 3. The Clark Fork River Basin.

Table 1. CLARK FORK RIVER MONITORING STATION LEGEND

STATION ID	RIVER MILES	STATION DESCRIPTION
WS	0	At Warm Springs, near Anaconda
DM	22	Near Dempsey
DL	33	As river enters Deer Lodge
ALB	48	Above the Little Blackfoot River
GC	61	Below Gold Creek, above Flint Creek
BO	112	Bonita, above Rock Creek
TU	130	Turah, below Rock Creek
AM	145	Above Missoula WWTP
BM	148	Below Missoula WWTP
HB	158	Harper's Bridge, below Bitterroot
HU	170	Huson, below Stone Container Mill
AL	181	Above Alberton
SU	219	At Superior
AF	258	Above Flathead River, near Plains
ATF	283	Above Thompson Falls
BTF	303	Below Thompson Falls
BN	340	Below Noxon Reservoir
BCG	362	Below Cabinet Gorge Reservoir

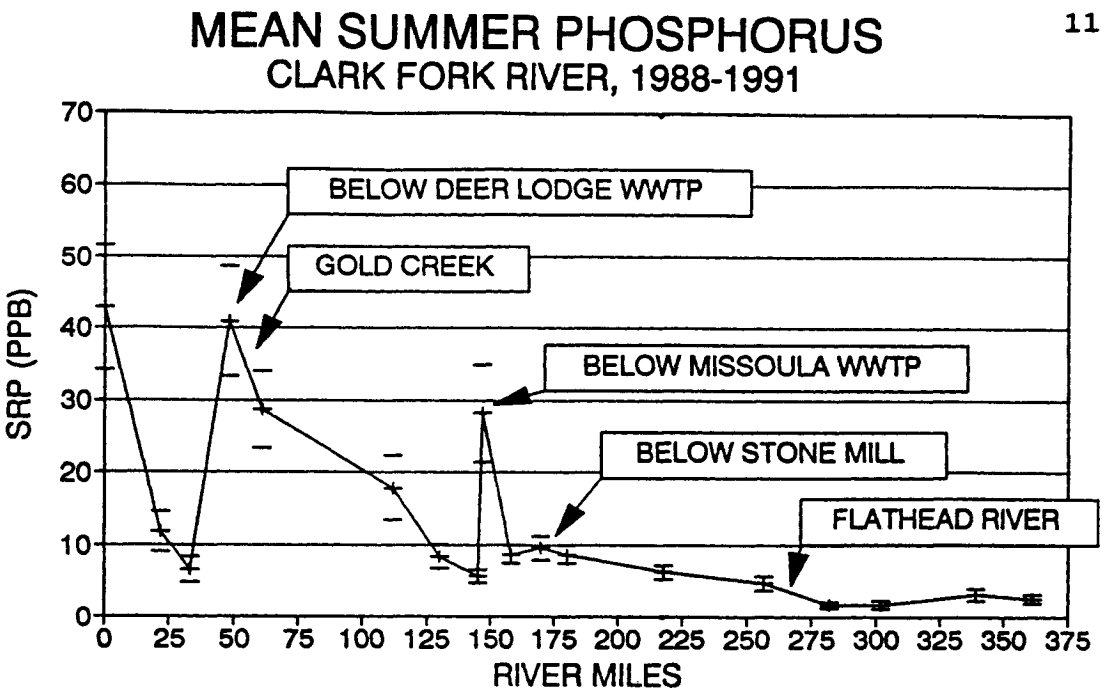
equipment was acid washed and rinsed to prevent sample contamination. Filtered blanks were also collected and analyzed. The state lab uses a QA/QC program of duplicates, spiked samples, known standards, and EPA audit samples to insure the integrity of the data (MDHES, 1974). To complement the state's monthly sampling, the University of Montana began collecting and analyzing biweekly samples during the summers of 1988, 1989, and 1990. Field collection and lab analysis were conducted under the same EPA approved techniques.

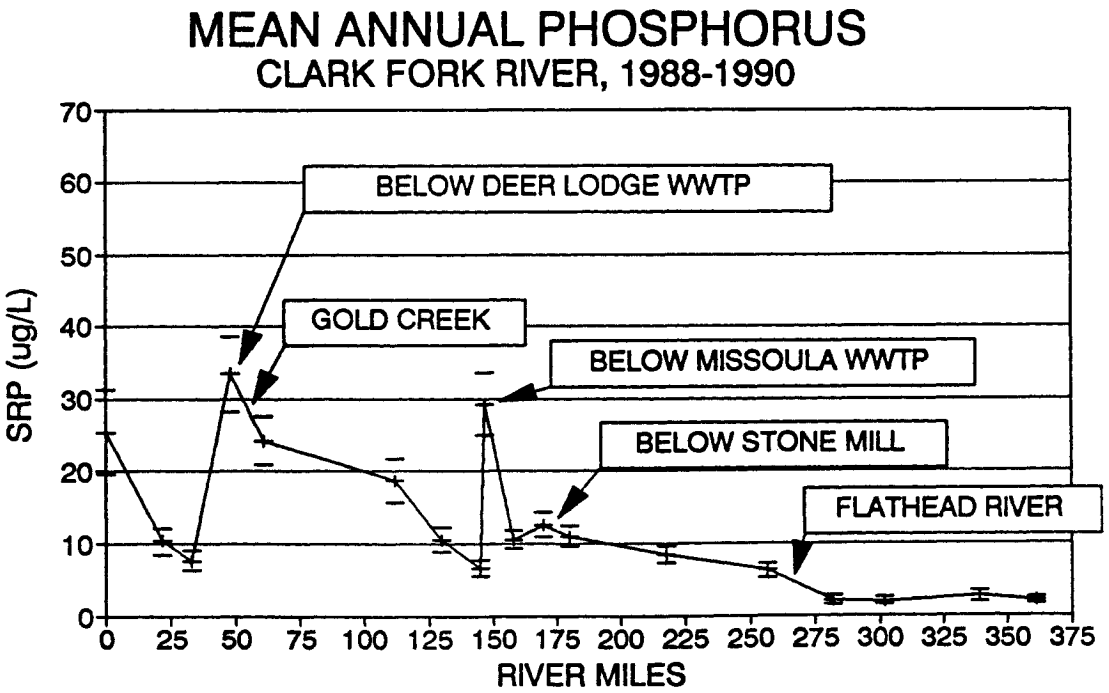
NUTRIENT SAMPLING-RESULTS

A plot of nutrient concentrations vs. distance down the river displays the dynamics of nutrient levels in the Clark Fork River. Figure 4 shows mean summer SRP levels along the river as averaged over the course of 4 years worth of data. The 95% confidence intervals are also presented to represent the variability of the data. The phosphorus level is near 40 ug/L (PPB) at the Warm Springs monitoring station near Anaconda. This value is high for the Clark Fork, but the actual phosphorus load at this point is low because of the low average flow (100 cfs) at this station. Along the reach of the river below Warm Springs, at the Dempsey and Deer Lodge stations, the SRP concentration has dropped to a value near 10 ug/L. The concentration has dropped because of dilution by nutrient poor water and by biological nutrient uptake by algae. The next station is below the Deer Lodge wastewater lagoon, and the SRP concentration consequently jumps back up

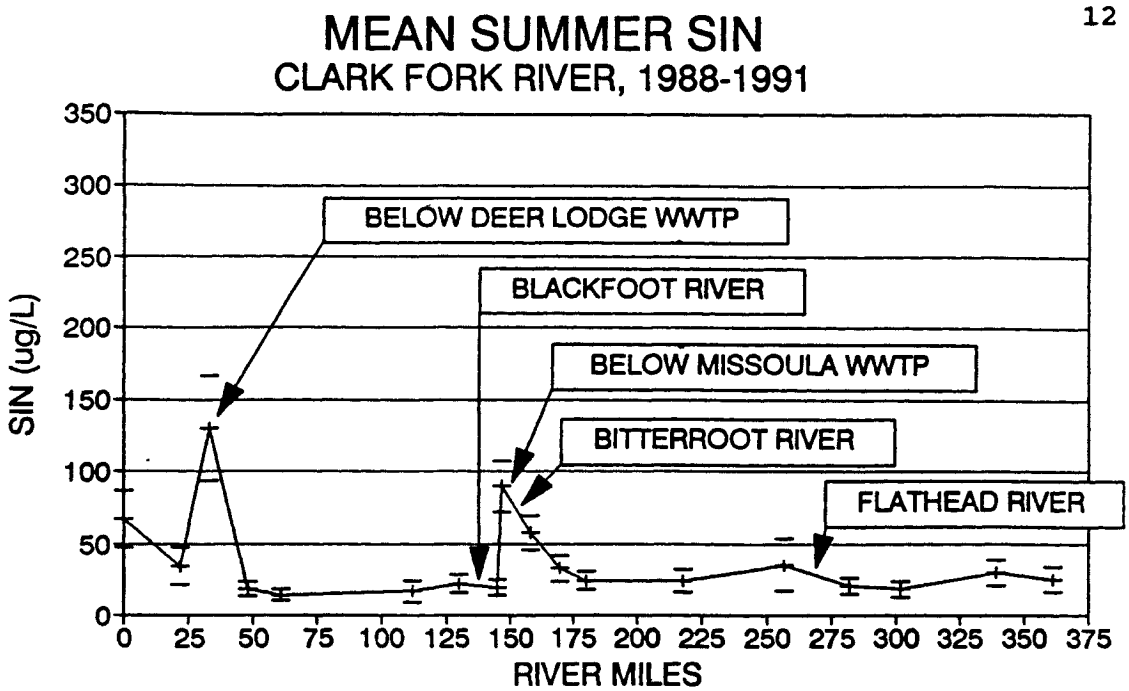
to 40 ug/L. The next station is below the confluence with phosphorus rich Gold Creek, and the SRP stays high near 30 ug/L. The SRP levels in the river gradually decrease downstream as the flows increase and more dilution and biological uptake occurs. After the Blackfoot River enters the Clark Fork almost 150 miles downstream from Warm Springs, the SRP concentration has dropped to 7 ug/L at the above Missoula station.

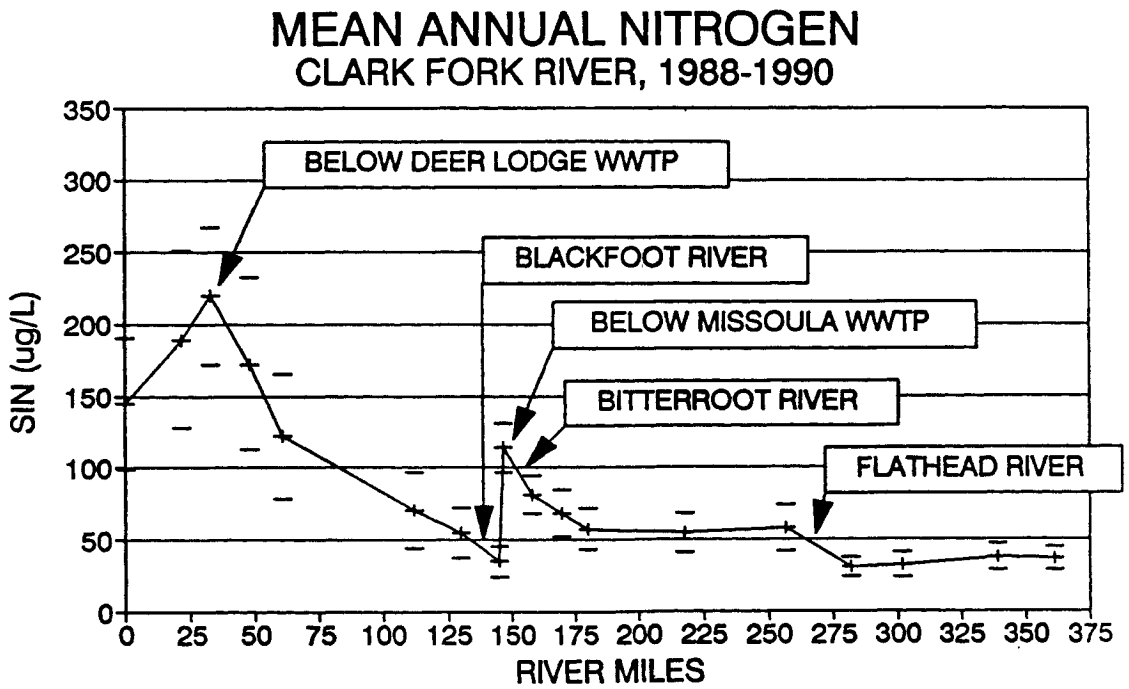
Nutrient levels would gradually decrease downstream from this point if there were no other significant point sources of nutrients. However, effluent from the Missoula wastewater treatment plant enters the river between the above and below Missoula stations, just past the 150 mile mark. At this point, the river's SRP concentration jumps to 30 ug/L. The large jump in concentration at this station represents the loading by the Missoula WWTP. The average annual daily load to the river from the Missoula WWTP is currently 185 lbs total P/day. The SRP concentration drops off downstream from the below Missoula station and stays near 10 ug/L at the Harper's Bridge, Huson, and Alberton stations because of dilution from the Bitterroot River and from algal uptake along this reach. The influence of the Stone mill discharges, located between the Harper's Bridge and Huson stations, is manifested as a small average increase in SRP levels at the Huson station. The lower Clark Fork River is characterized by low nutrient levels and large flows. There is a heavy nutrient load in the





Figures 4 (top) and 5. Mean summer and annual soluble reactive phosphorus (SRP) concentrations in the Clark Fork River.





Figures 6 (top) and 7. Mean summer and annual soluble inorganic nitrogen (SIN) concentrations in the Clark Fork River.

lower Clark Fork, defined by the large volume of water flowing towards Lake Pend Oreille.

The bioavailable nitrogen levels in the Clark Fork follow trends similar to that of phosphorus (Figure 6). Total soluble inorganic nitrogen levels peak downstream from wastewater discharges at Butte, Deer Lodge, and Missoula, and then the levels drop off. The nitrogen plots do not show the sustained high nutrient levels below Gold Creek, which is only a significant contributor to the phosphorus load. Nitrogen does not drop as dramatically as does phosphorus below the Bitterroot River because the Bitterroot is relatively high in nitrogen. Annual nutrient levels (Figures 5 and 7) are similar to the summer-only averages.

CHAPTER 3. ALGAL ACCUMULATION IN THE CLARK FORK RIVER

NUISANCE ALGAL ACCUMULATION

Algal accumulation in the Clark Fork River is a natural phenomenon that is beneficial to the local aquatic community. The algae serve as a food source and refuge for aquatic invertebrates, as well as acting as a critical keystone for nutrient cycles. However, like all good things, too much algae can be harmful. Algae levels can be considered a nuisance if the algae interferes with some aspect of recreational, commercial, or natural use of the river. This interference can be purely aesthetic or have some more tangible physical effect. Algae can block agricultural irrigation intakes, clog pumps, and stagnate irrigation channels. Algal mats can prevent recreational boat use and interfere with fishing. Algal accumulation also adversely affects river fisheries by clogging intergravel habitat, controlling the dynamics of local insect communities, and affecting dissolved oxygen (DO) levels.

Various attempts have been made to quantify algal accumulation and to define levels of nuisance algal accumulation. Algal biomass accumulation can be determined by the amount of chlorophyll present over a given area (measured as mg chlorophyll "a"/m^2), or by measuring the amount of ash free dry weight present per area. Nordin (1985) has found algal accumulation levels of less than 50 mg/m^2 to be acceptable aesthetically and for recreational uses. Welch

(1988) has found a biomass range of 100-150 mg chlorophyll/m^2 to represent a critical level for an aesthetic nuisance. This biomass level corresponds to filamentous coverage near 20%. Photographs of Clark Fork River algal research sites have been used to solicit comments on the aesthetic nuisance of attached algae. An informal survey of local citizens found unanimous agreement that biomass levels of 200 mg chlorophyll/m^2 is aesthetically unacceptable, and the majority felt that biomass levels of 100 mg chlorophyll/m^2 are unacceptable (Watson, pers. comm.). Algal accumulation of less than 100 mg chlorophyll/m^2 was not found to affect the local community of aquatic organisms adversely (Nordin, 1985). For this report, algal accumulation in the Clark Fork River that exceeds 100 mg chlorophyll/m^2 will be defined as nuisance accumulation.

ALGAL MONITORING-METHODS

In 1986, the Montana Water Quality Bureau surveyed attached algae levels along the entire length of the Clark Fork from the headwaters near Warm Springs to the Idaho border. This sampling program utilized algae grown on randomly selected natural rock substrates. Numerous algal communities exist in the Clark Fork River. The upper river is dominated by a filamentous green, *Cladophora*, while the middle and lower river is generally characterized by a mixed community of periphytic diatoms. Algal levels were found to exceed 100 mg chlorophyll/m^2 at many of the upper and middle river sites (Weber, 1988).

Algal accumulation on artificial substrates was monitored during the 1987, 1988, and 1990 summer growing seasons under a program developed by Dr. Vicki Watson at the University of Montana. These data were primarily collected to validate a model designed to simulate algal accumulation in the Clark Fork River. Algal samples were collected on both natural (rocks) and artificial (ceramic tiles or styrofoam) substrates. The substrates were kept at a constant depth of 20-30 cm and in flows near 0.3 m/s. Samples were collected and analyzed for chlorophyll and ash free dry weight in accordance with standard methods. Algal samples were scraped off the substrate, and the chlorophyll extracted with acetone. The samples were then centrifuged and spectrophotometrically analyzed. Ash free dry weight was determined by ashing and weighing the algae samples.

In 1987, unglazed ceramic tiles served as the artificial substrates that were placed in the river at sites above and below the Missoula WWTP, and at sites near Harper's Bridge and Huson, bracketing the Stone mill. Samples were collected and analyzed at the end of the growing season in September. Natural substrates were collected in 1988 at two sites, one further upstream from Missoula near Rock Creek, and the second at the below Missoula site. In the summer of 1990, artificial tile substrates were placed at the same sites as the 1987 substrates, and at four lower river sites, Alberton, Superior, St. Regis, and Plains. The Superior substrates disappeared

early in the experiment. Samples were collected weekly at the middle river sites and monthly at the lower river sites from the beginning of July to the end of September. Five to ten replicate samples were collected at each site and the samples were analyzed for chlorophyll and ash free dry weight.

ALGAL MONITORING-RESULTS

The results from the artificial substrate experiments confirm that the greatest levels of algal accumulation occur at sites downstream and closest to the Missoula wastewater treatment plant. Figure 8 shows the mean peak biomass attained at each of the sites as averaged over the course of the summer for each year. Algal accumulation found on the substrates at the above Missoula site are similar for all three years, with an average biomass near 25 mg chlorophyll/m^2. At the below Missoula site, algal accumulation is above the 100 mg chlorophyll/m^2 nuisance criterion all three years. Algal accumulation is highest in 1987 and 1988, low flow, pre- phosphate detergent ban years. 1990 was an average flow, post- ban year, and therefore has lower levels of attached algae. The lower river sites are characterized by low levels of algal accumulation. The variability of the replicate samples is highest at the sites with high levels of algal accumulation. Algal accumulation on the artificial substrates is most uniform at low (< 50 mg chlorophyll/m^2) levels. Algal accumulation data as measured over the course of each summer is presented in Figures 11

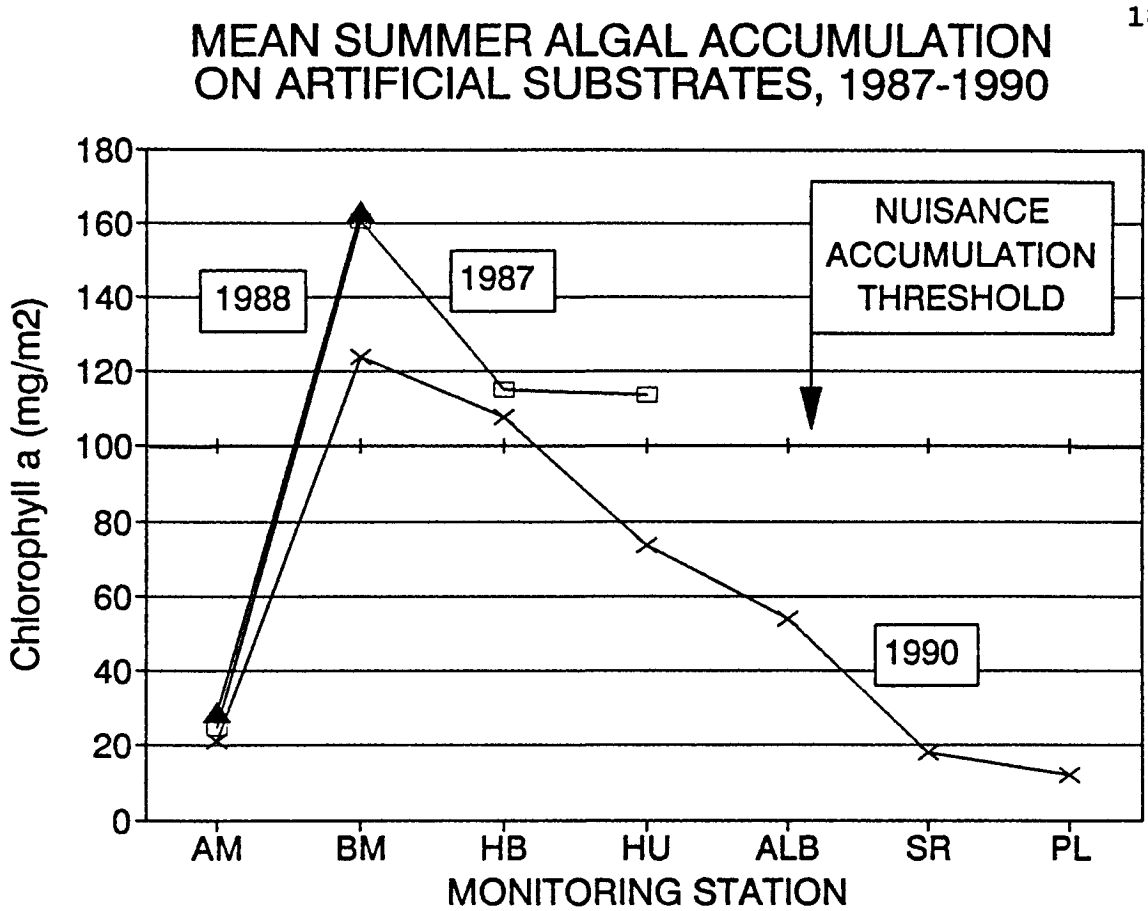


Figure 8. Mean summer algal accumulation as measured on artificial substrates in the Clark Fork River, 1987-1990.

through 18 as part of the algal accumulation model validation runs.

LIMITATIONS OF ALGAL GROWTH AND ACCUMULATION

The rate of algal growth and amount of accumulation in a given year is dependent upon many physical and chemical factors. Algal growth is primarily a function of temperature, light, and nutrient availability. In the Clark Fork River, the algal growing season is roughly limited to May through September, when temperatures and light levels are most favorable. Algal accumulation over time represents the balance between growth rates and algal die-offs and sloughing. Sloughing of the algal biomass occurs as filaments weaken and break away due to scouring high river flows and environmental stress. Environmental stress can occur from toxic discharge events, extreme temperature or flow fluctuations, and from nutrient limitation.

In all natural ecosystems, some combination of factors will limit growth. The most important limiting factor for algal growth and accumulation in the Clark Fork River is nutrient availability. Both nitrogen and phosphorus have been found to be low enough to limit growth and maximum standing crop at different seasons and different locations on the Clark Fork River (Watson et al, 1990).

CHAPTER 4. THE MISSOULA PHOSPHATE DETERGENT BAN

Many communities have adopted bans on phosphate detergents to reduce phosphorus discharges from local wastewater treatment plants. Phosphate detergents were identified as a problem in the Great Lakes states, Europe, and Australia in the late 1960's and early 1970's (DeJung, 1989, Hartwell, 1973, Hartig, 1990). Phosphorus discharges were strongly contributing to the eutrophication of receiving waters. In 1988, a phosphate detergent ban was proposed for Missoula to reduce phosphorus discharges from the Missoula wastewater treatment plant. A reduction in phosphorus discharges from the Missoula plant would prevent violations of the wastewater treatment plant's discharge permit, lower the bioavailable phosphorus load to the Clark Fork River, and allow the City of Missoula the opportunity to annex more homes and expand the city sewer system while remaining in permit compliance.

The County and City of Missoula jointly enacted the Missoula Phosphate Ordinance on November 21, 1988. The ordinance prohibits the sale of any laundry and dishwasher detergents, soaps, bleaches, and water conditioners that contain more than 0.5% phosphorus. This ban pertains to these cleaning products that are sold inside the city limits and within 3 miles of the city (Figure 9). The ban does not cover many outlying communities, including Bonner, Lolo, and Frenchtown, but these areas are covered by the ban de facto.

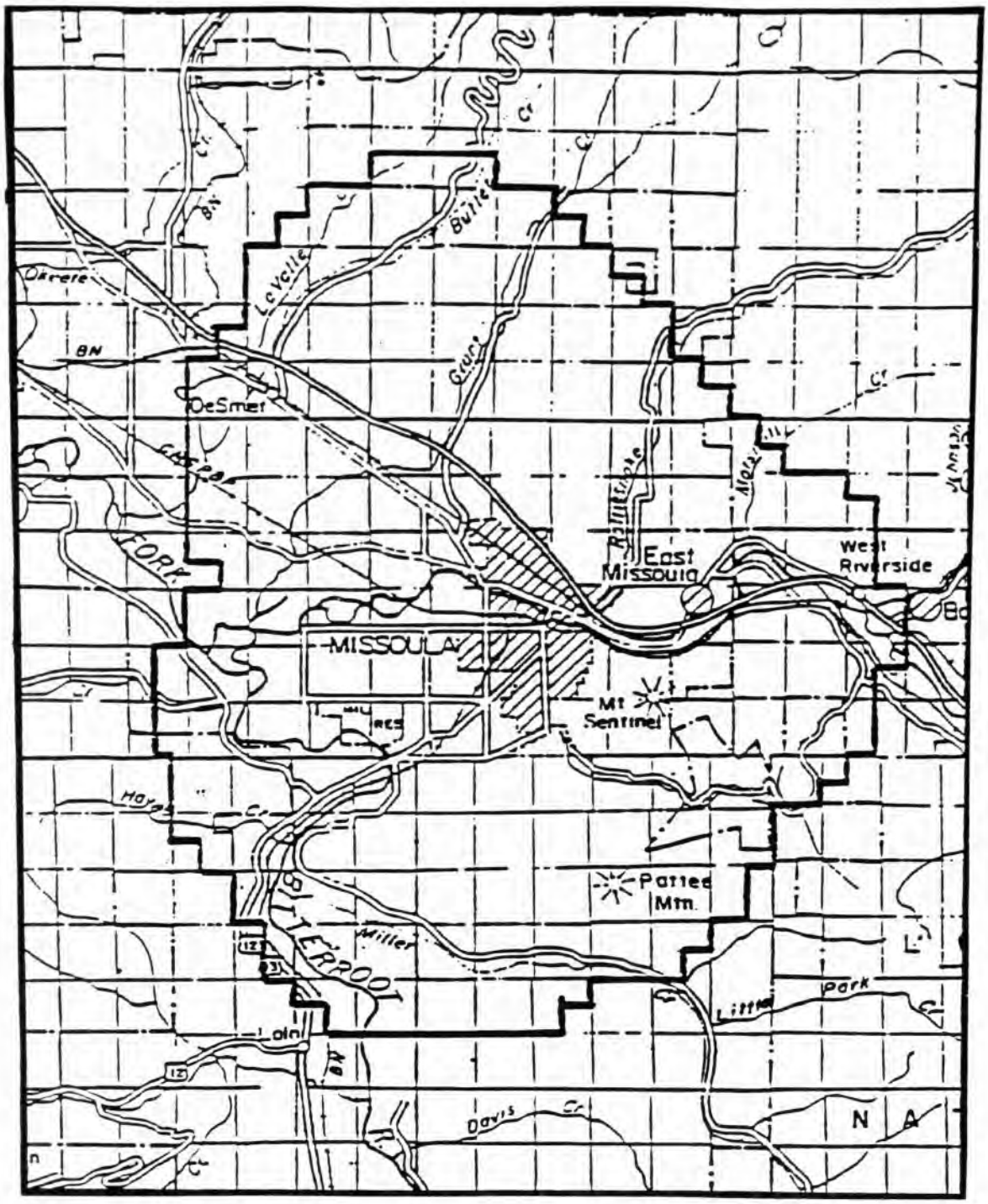


Figure 9. Area affected by the Missoula Phosphate Detergent Ban.

Many residents of these communities shop in Missoula, and Missoula detergent suppliers now supply these communities with phosphate free detergents.

PRE- AND POST BAN NUTRIENT LOADING FROM THE MISSOULA WWTP

The mean daily total phosphorus discharge to the Clark Fork River from the Missoula wastewater treatment plant over a six year period is shown in Figure 10. Before the phosphate detergent ban went in to effect in May, 1989, phosphorus discharges from the plant averaged 306 lbs/day and showed large monthly fluctuations. Monthly average discharges exceeded the plant's NPDES permit level of 375 lbs phosphorus/day on five months out of the six year period. The fluctuations represent changes in the plant's influent phosphorus levels and high phosphorus releases due to upset events occurring within the plant itself. Phosphorus discharges have decreased to an average of 185 lbs/day after the detergent ban was enforced, reflecting the decreased phosphorus load to the plant. This translates to a 40% reduction in annual phosphorus discharges to the river. Post-ban phosphorus discharges remain stable compared to the pre-ban discharge fluctuations. This is in part due to a conscious effort on behalf of the plant operators to avoid plant upsets and run the plant more consistently (Miller, 1991), and in part due to the closing of a Missoula dairy operation that had irregularly introduced phosphorus-rich wastes into the city wastewater system.

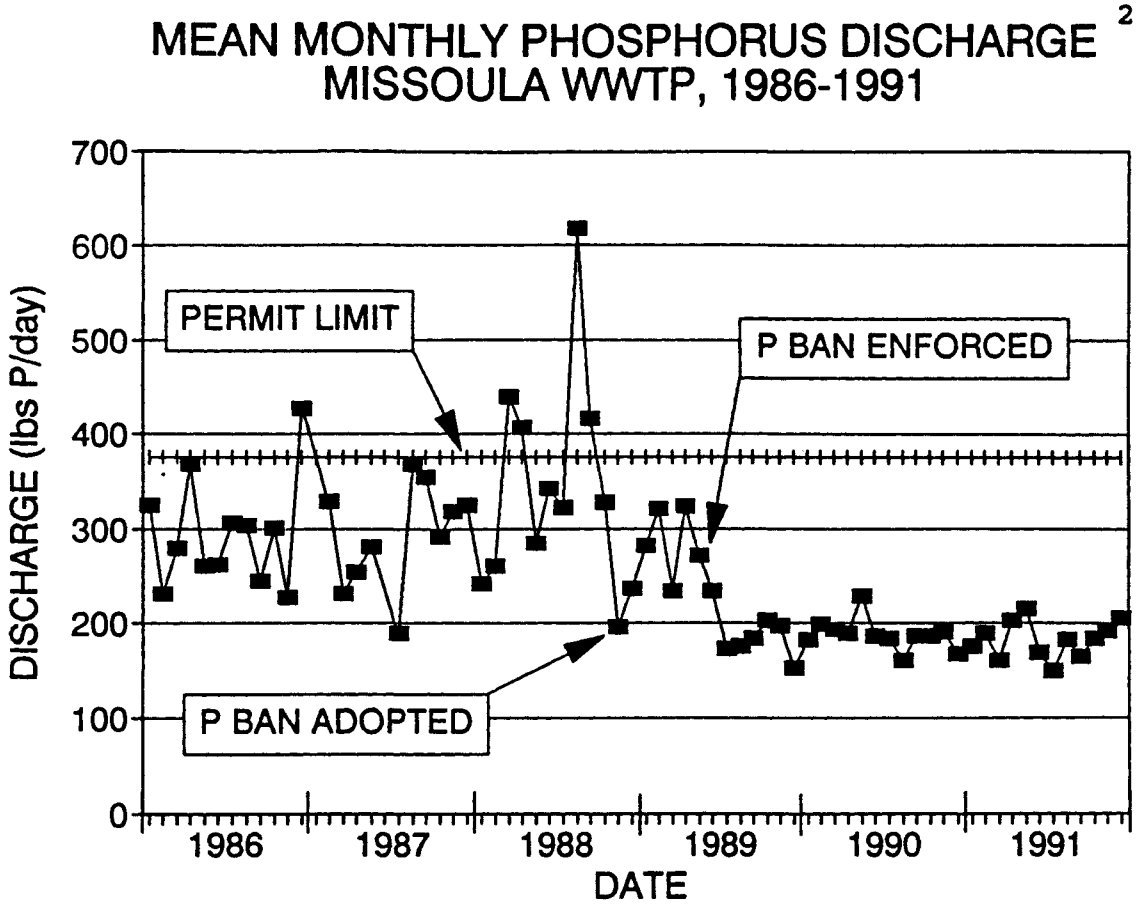


Figure 10. Mean monthly phosphorus discharges from the Missoula Wastewater Treatment Plant to the Clark Fork River, 1986-1991.

Phosphorus discharges from the Missoula wastewater plant could be reduced even further by more expensive and elaborate techniques. Metal ion coagulates such as alum (aluminum sulfate) or ferric chloride could be added to the secondary clarifier of the plant to complex and precipitate out phosphorus (Kerri, 1991). Effluent from the plant could also be land treated in the summertime, completely eliminating the nutrient load to the river.

CHAPTER 5. APPROACH TO ASSESSING THE BENEFITS OF THE BAN

The nutrient load reduction achieved as a result of the phosphate detergent ban has decreased algal accumulation in the Clark Fork River. Comparisons of measured algal accumulation before and after the ban data qualitatively show this to be true (see Figure 8). However, there are a number of confounding environmental factors besides nutrients that affect algal accumulation in the river. Yearly differences in cloud cover, temperatures, grazing, toxic events, and algal sloughing affect algal accumulation. The primary factor controlling algal accumulation is the amount of water discharged through the river channel. The flow controls water temperature, nutrient dilution, and substrate scouring. To normalize these confounding factors, a numerical model that simulates algal accumulation in the Clark Fork River has been developed. Algal accumulation in the river can be simulated under pre- and post- ban nutrient conditions with all other factors held constant. The realized benefits as a result of the phosphate detergent ban can then be quantified as reductions in accumulated algal biomass and as reductions of the frequency of occurrence of nuisance levels of algal accumulation.

The algal accumulation model simulates algal accumulation as a function of nutrient concentration. To normalize the confounding factors that affect nutrient concentrations in a given year, a numerical nutrient concentration model has been

developed. This nutrient model predicts what river nutrient concentrations are in the river as a function of nutrient loading and flow. The nutrient model predicts the changes in river nutrient levels as a result of decreased nutrient loading from the phosphate detergent ban by isolating the effects of the ban. The output of the nutrient model is used as the input to the algal accumulation model, and the algal model is then run to assess the benefits of the ban.

CHAPTER 6. THE ALGAL ACCUMULATION MODEL

NATURE OF THE ALGAL ACCUMULATION MODEL

A model designed to simulate algal growth and accumulation in the Clark Fork River was developed by Dr. Vicki Watson at the University of Montana. The model is given average daily light, temperature, and nutrient conditions as inputs, and then predicts total algal biomass accumulation for each day of the growing period. The model begins with a small initial biomass and then estimates the net growth, respiration, and sloughing rates on an hourly basis. The model then adds or subtracts this amount of biomass from the previous estimate of the biomass. This process then continues for each day of the growing period and algal accumulation increases or decreases as conditions demand.

The model is based on a number of simplifying assumptions. Growth limitation is based solely on phosphorus, temperature, and light limitation. Other nutrients are not considered. The model does not consider effects from insect grazing, scouring flows, turbidity, or cloudy weather. The model does a reasonably good job of predicting algal accumulation in the river and the simplifying assumptions do not seem to compromise the data. The kinetics of the model are described in further detail in Appendix A.

VALIDATION OF THE ALGAL ACCUMULATION MODEL

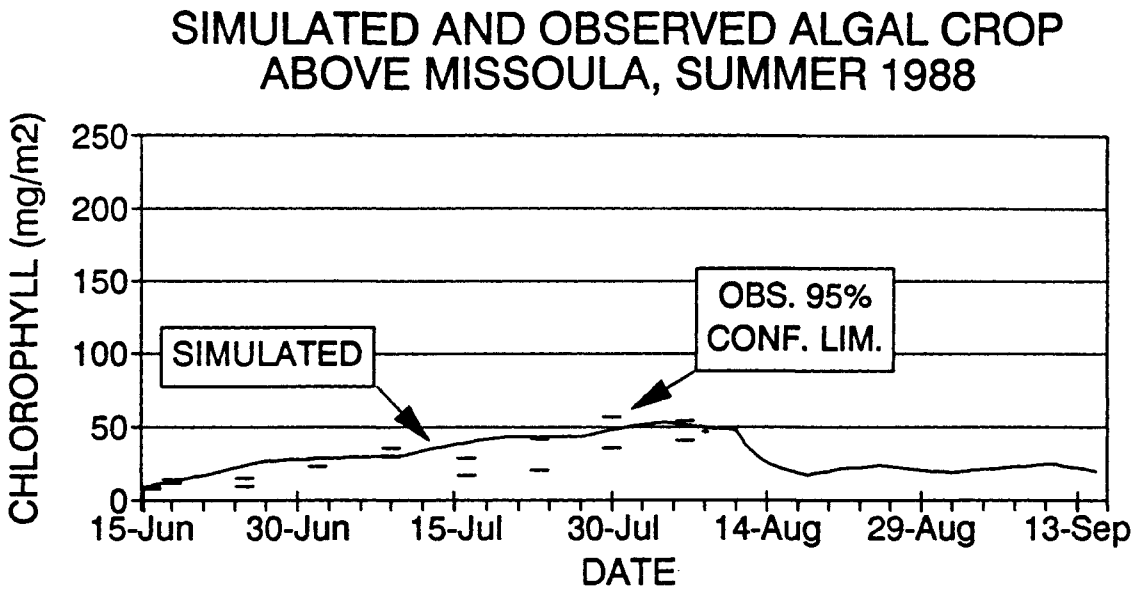
The model was validated by using field data (phosphorus concentration and water temperature) for the model inputs and

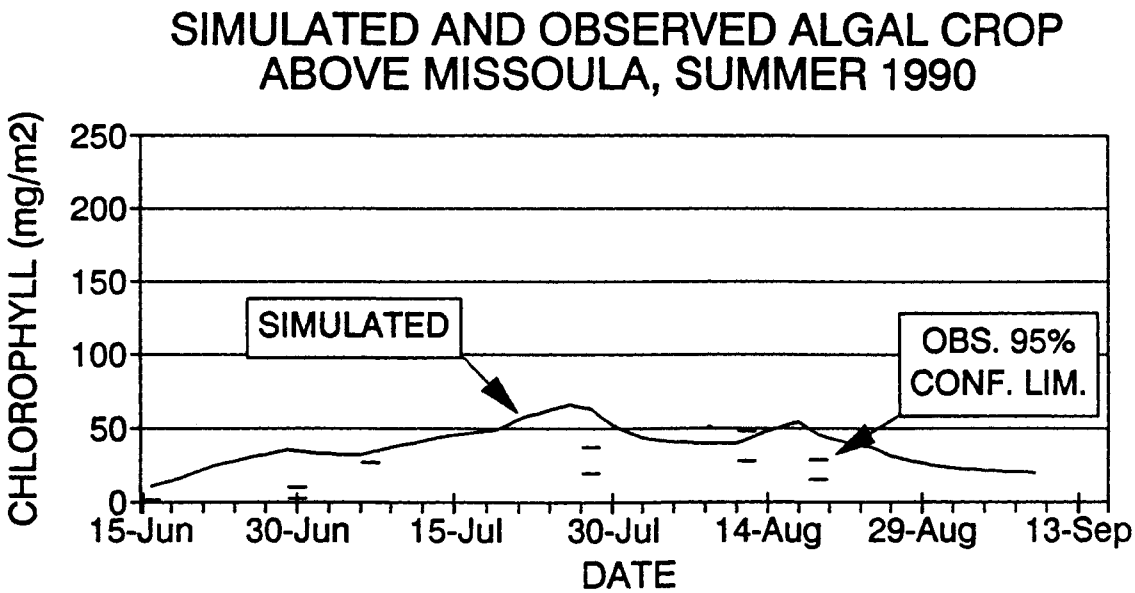
comparing the model's simulated levels of algal accumulation to actual measured algal accumulation at each site. Validation data were used from 1988 and 1990 at the above and below Missoula (AM and BM) sites and from 1990 at the Harper's Bridge (HB), Huson (HU), St. Regis (SR) and Plains (PL) sites. Model parameters, such as the growth kinetics and sloughing rates, were adjusted to achieve the best fit of the simulated data to the actual algal accumulation. The model was declared validated when the simulated algal accumulation reasonably matched the observed data on two independent parameters. Model simulations were validated against the peak biomass attained over the course of the growing period and the general growth and sloughing patterns. To facilitate the model validation, the observed and simulated algal accumulation levels were normalized to a 93 day summer growth period, from June 15 to September 15.

Results from the model validation runs are presented in Figures 11 through 18. The plots show the model simulation and observed algal accumulation for each site over the summer growing season. The observed data, from the artificial substrate experiments, are represented by the 95% confidence interval limits of the measured values. The simulated algal accumulation does a reasonably good job of representing the actual measured algal accumulation at each site, with the exception of the St. Regis site. The simulated and observed data generally both show similar growth and sloughing patterns

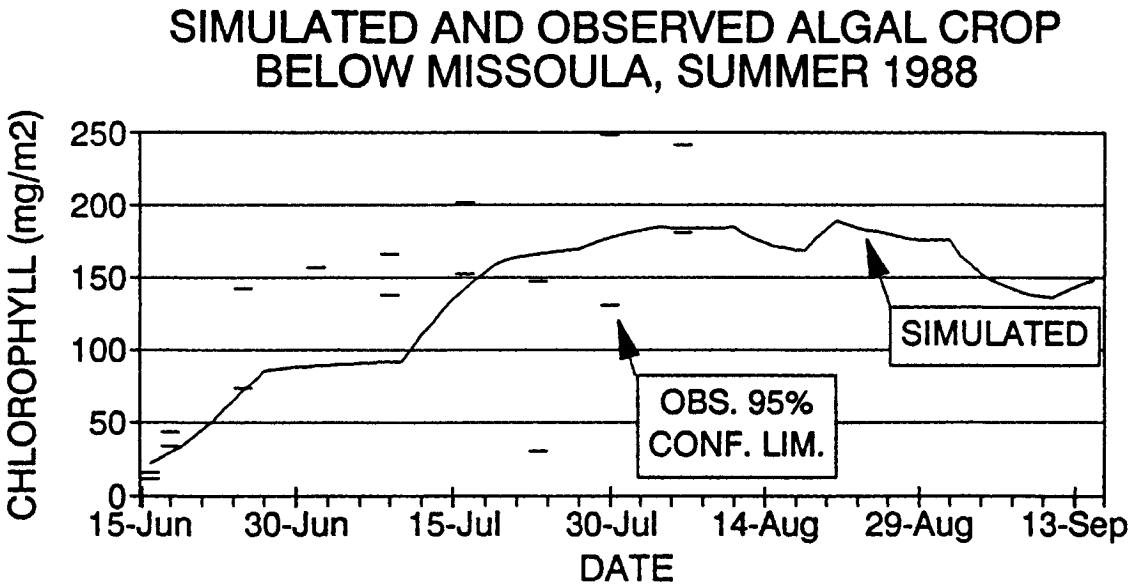
and the model accurately predicts the peak biomass level attained at the middle river sites. The simulated levels run high for the lower river sites because the observed algal accumulation does not accumulate as fast in the larger river channel as it does in the smaller reaches of the river. The observed data from the below Missoula site exhibit wide confidence intervals and large fluctuations between sampling dates. This is due to uneven algal accumulation on the artificial substrates and unexpected sloughing events. It is difficult to declare the model validated in these conditions. However, the simulated values do pass through many of the observed confidence intervals and appear to reasonably represent the real world.

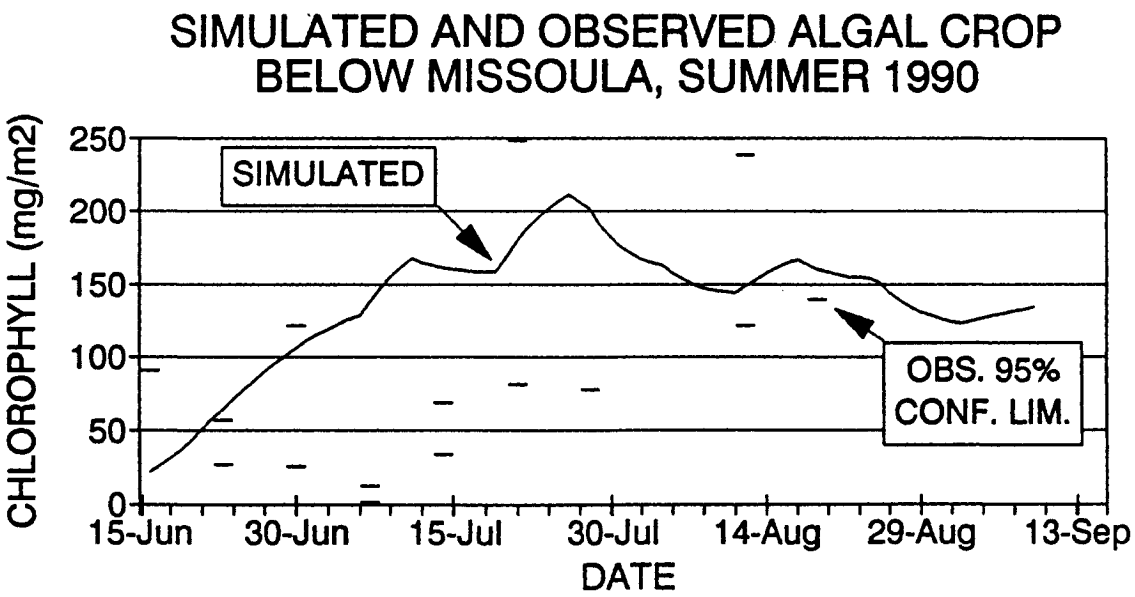
The observed nutrient and temperature data used as inputs to the model were based on biweekly intervals. The observed value was used to represent the temperature and nutrient conditions in the river for that entire period. Light levels were simulated based on typical light levels from this latitude. This simplification decreases model sensitivity by ignoring any small scale and diurnal fluctuations of these parameters. Model prediction could be improved by using more frequently measured input data that would more closely match the actual temperature and nutrient fluctuations seen in the river. Model sensitivity could also be improved by considering other physical parameters, most importantly nitrogen concentrations that are seen in the river.



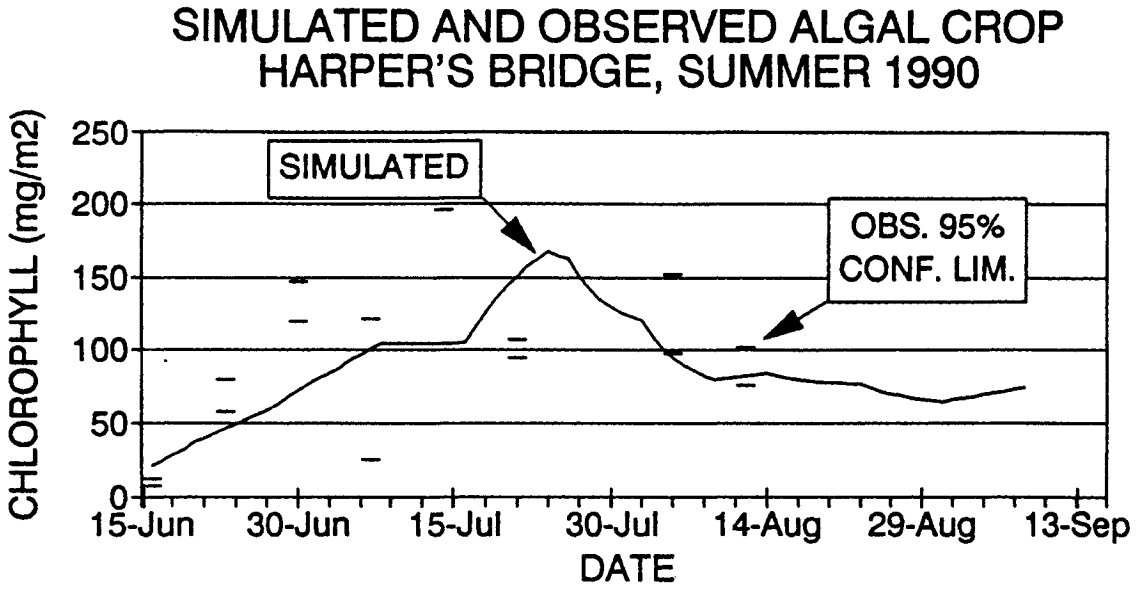


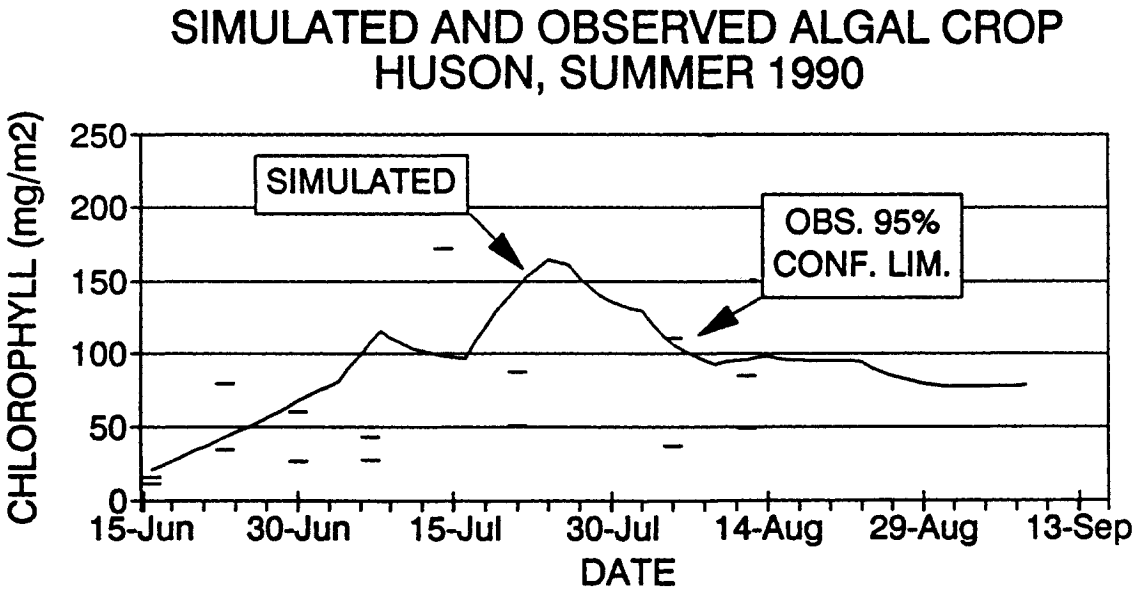
Figures 11 (top) and 12. Validation runs of the algal accumulation model. Simulated and observed algal accumulation in the Clark Fork River.



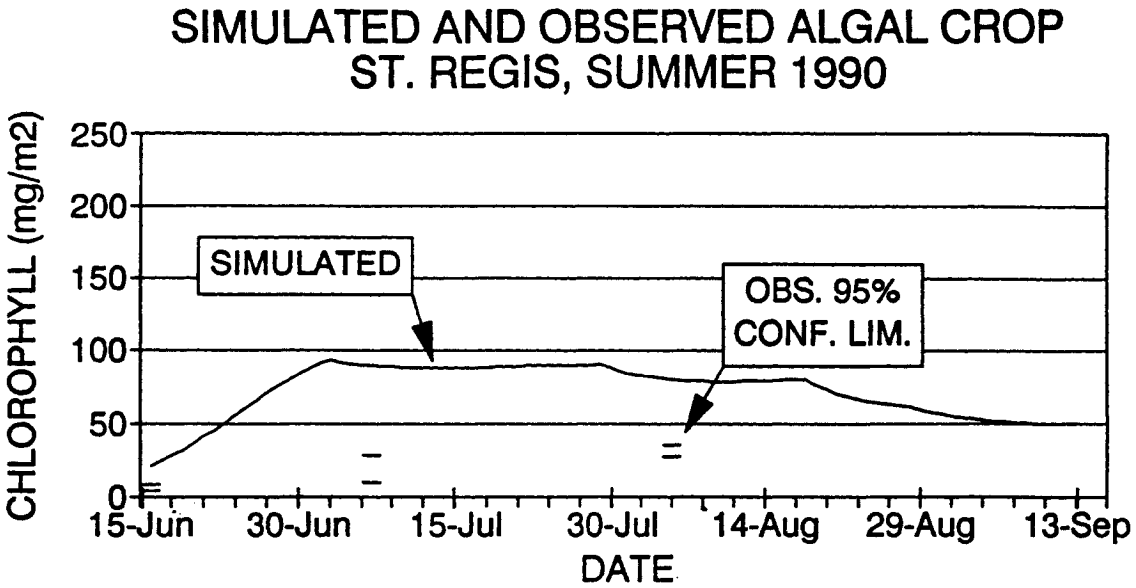


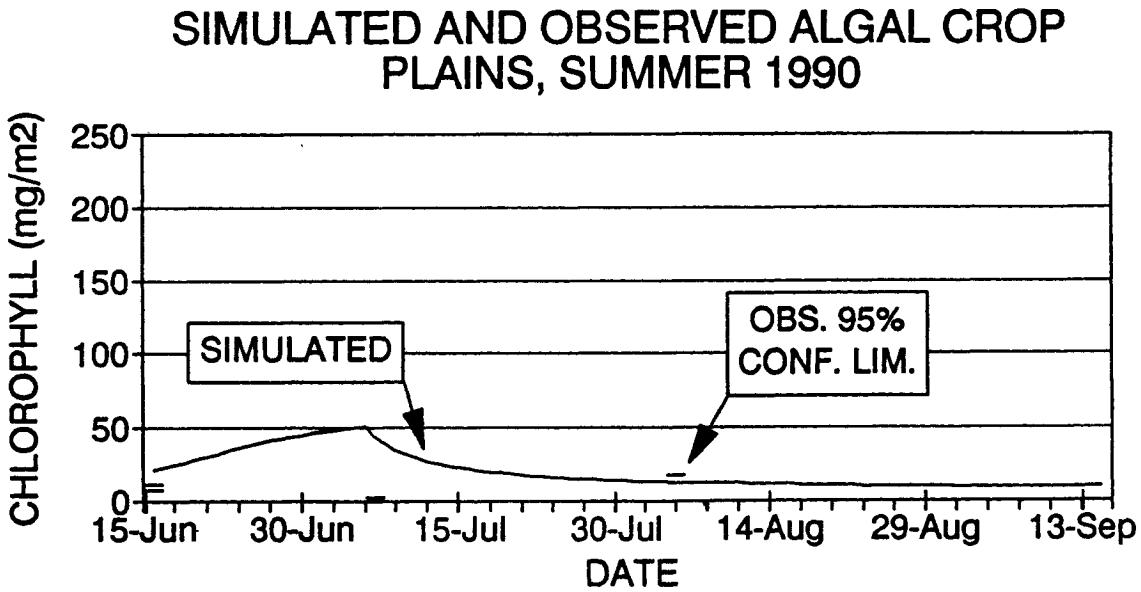
Figures 13 (top) and 14. Validation runs of the algal accumulation model. Simulated and observed algal accumulation in the Clark Fork River.





Figures 15 (top) and 16. Validation runs of the algal accumulation model. Simulated and observed algal accumulation in the Clark Fork River.





Figures 17 (top) and 18. Validation runs of the algal accumulation model. Simulated and observed algal accumulation in the Clark Fork River.

CHAPTER 7. THE CLARK FORK RIVER NUTRIENT MODEL

PURPOSE AND NATURE OF THE NUTRIENT MODEL

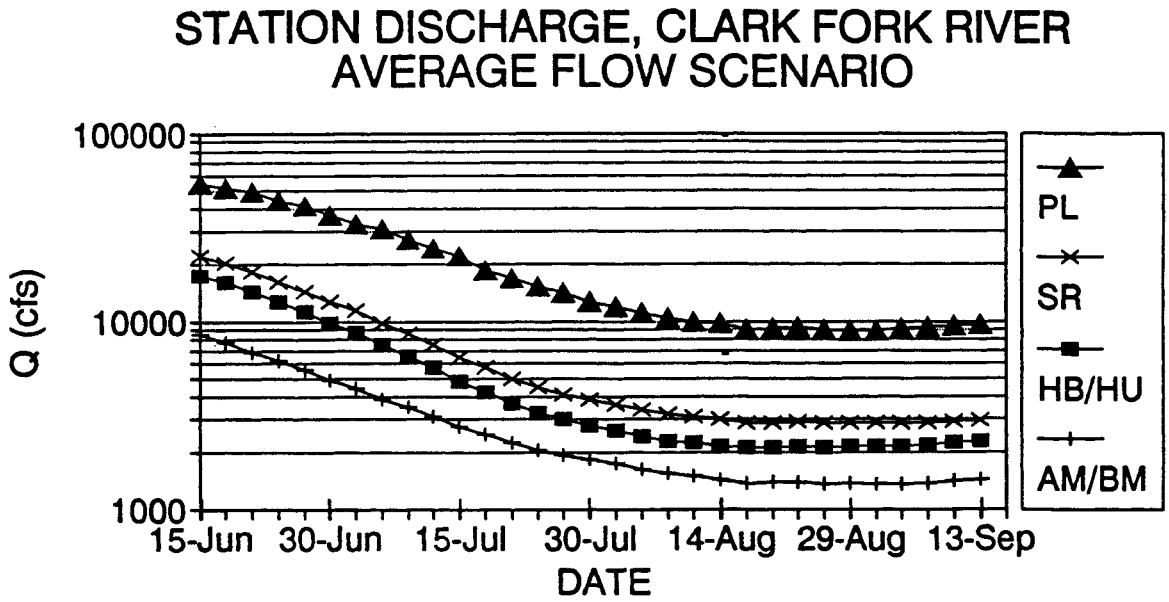
A Clark Fork River nutrient model was developed to provide nutrient level input data to the algal accumulation model that is normalized against confounding environmental factors. These inputs will allow valid comparisons of pre- and post- ban algal accumulation by isolating the effects of the phosphate detergent ban. The nutrient model predicts soluble reactive phosphorus concentrations at selected sites on the Clark Fork River for low and average flow conditions. High flow years are not modeled because of the high rates of sloughing and substrate scouring that would be expected to prevent accumulation of nuisance levels of attached algae.

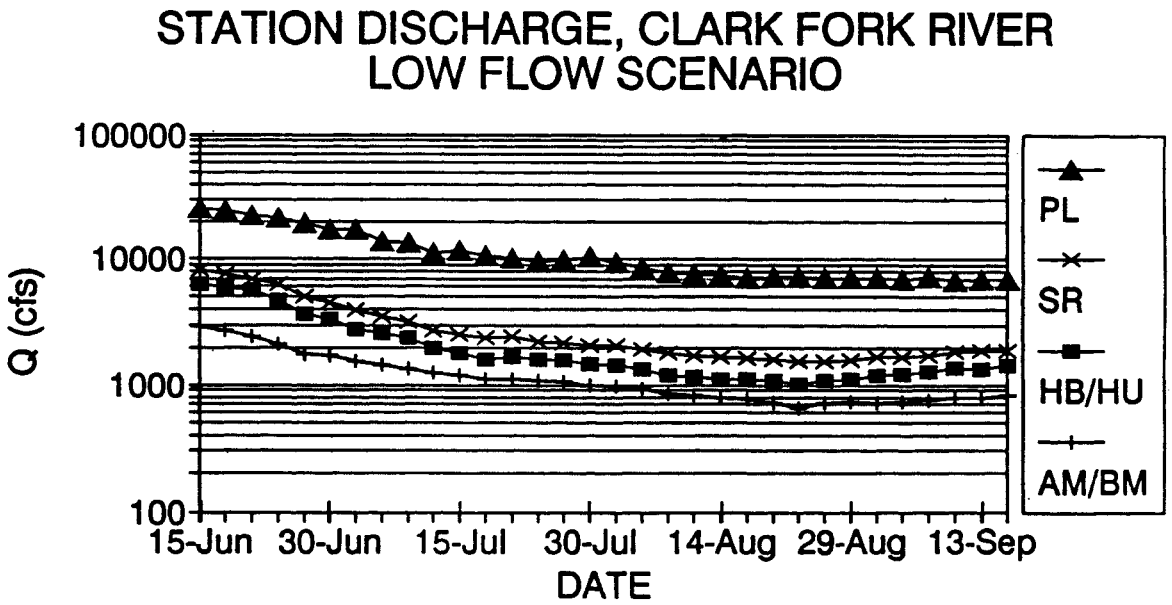
The nutrient model was developed by first defining three basic parameters that control the nutrient levels in the river. These parameters include flow, nutrient loading, and nutrient uptake and retention in the system. The model utilizes standardized flow conditions, a mass-balance approach to nutrients in the river, and empirically derived nutrient retention and uptake factors for each reach of the river. Nutrient levels are predicted for each of the sites where algal accumulation and nutrient data had been previously collected, namely the above and below Missoula sites, Harper's Bridge, Huson, St. Regis, and Plains. The nutrient model was validated with observed nutrient data collected by the Montana Water Quality Bureau and the University of Montana in 1988 and

1990.

The river's average and low flow conditions were defined from 62 years (1930-1991) of USGS gauging station records. The average flow scenario was defined by the average of the 61 year record available for each station. The 1990 water year was classified as an average flow year because the 1990 station discharge figures are within ten percent of this average. The low flow scenario was defined by the average of the lowest 10% of the flows, with the exclusion of one extremely low flow year. The 1987 and 1988 water years are within this low flow condition. The standardized flow scenarios so obtained are presented in Figures 19 and 20. The major tributaries between the gauging stations include the Bitterroot River between the below Missoula and Harper's Bridge sites, the St. Regis River between Huson and St. Regis, and the Flathead River between St. Regis and Plains.

The mass-balance approach to estimating phosphorus levels in the river begins by utilizing the nutrient concentrations at the above Missoula site from a low flow (1988) and an average flow (1990) year. The nutrient load at this location is known from the flow and nutrient concentration. Phosphorus loading from the Missoula wastewater treatment plant was calculated from the wastewater treatment plant's monthly discharge report (see Figure 10), and then this load was added into the river. The nutrient concentration at the below Missoula site was then calculated from the known nutrient load





Figures 19 (top) and 20. Clark Fork River station discharge, average and low flow scenarios.

and known river flow. Nutrient loading from tributaries was estimated by averaging nutrient and flow data from pre- and post- ban years, and added in at the appropriate locations. The nutrient load predicted by this conservative approach is greater than nutrient loads actually found in the river, suggesting that there is a net retention of phosphorus in each reach of the river. An algal uptake and retention term was introduced to reduce the excess nutrient load. The amount of phosphorus retained in each reach of the river was determined by empirical measurements of algal retention and uptake from each reach. A linearly increasing percent retention term was applied to each reach of the river over the entire summer growing season. The changing nutrient retention simulates the increasing retention due to increasing algal accumulation over the course of the summer.

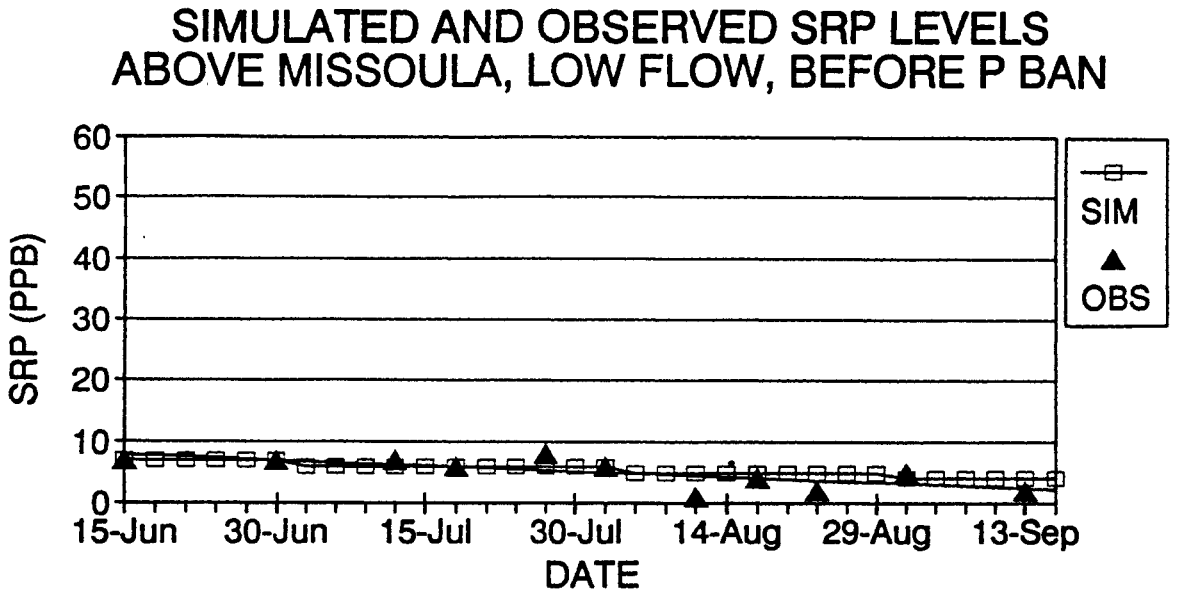
VALIDATION OF THE NUTRIENT MODEL

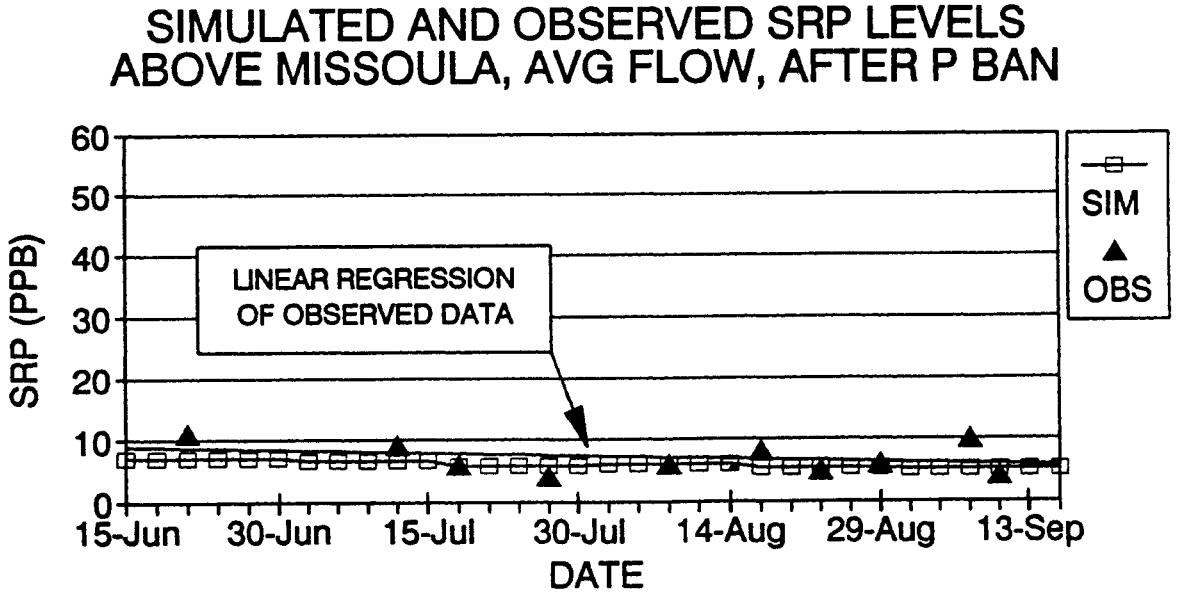
This nutrient concentration model was validated with available observed nutrient data. The model was run under low flow conditions with pre-ban loading and the output compared to observed data from 1988, a low flow, pre-ban year. The model was also run under average flow conditions with post-ban loadings and the results compared with observed data from 1990. The validation runs are presented in Figures 21 through 32. The simulated nutrient concentrations from the model are consistent with the observed data, and the model predicts peak concentrations reasonably well. To facilitate

the validation, a "best-fit" least-squares linear regression routine was applied to the observed data and is plotted along with the observed data. The below Missoula site (Figures 23 and 24) is the most difficult to model because of the large fluctuations in observed nutrient concentrations, and the therefore the simulated values are not as close a fit as the other sites. When observed data becomes available, the model should be validated for low flow, post-ban conditions.

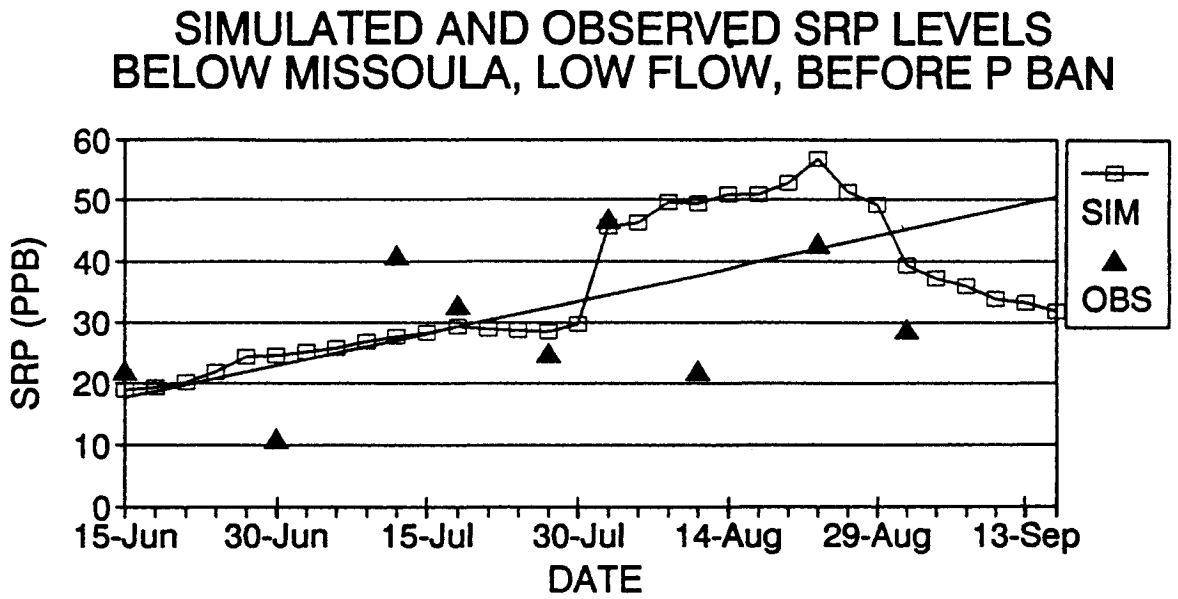
The validated nutrient model was then used to predict changes in instream nutrient concentrations solely as a result of the phosphate detergent ban. The nutrient model was first run for low flow, before the ban conditions. The nutrient loading from the Missoula wastewater treatment plant was then reduced to post-ban levels, and the model was run again. Figures 33 and 34 show the predicted nutrient reductions found in the river under low flow, before and after the ban conditions. The reductions in nutrient concentrations represent the effects of the phosphate detergent ban. The model was also run for average flow, before and after the ban conditions, and the results are presented in Figures 35 and 36. The nutrient model predicts an average 35% reduction in nutrient concentrations for the average flow scenario and an average 43% reduction for the low flow scenario at the below Missoula site. The other middle river sites, Harper's Bridge and Huson see similar reductions in nutrient levels. The lower river sites do not see quite as large a percent

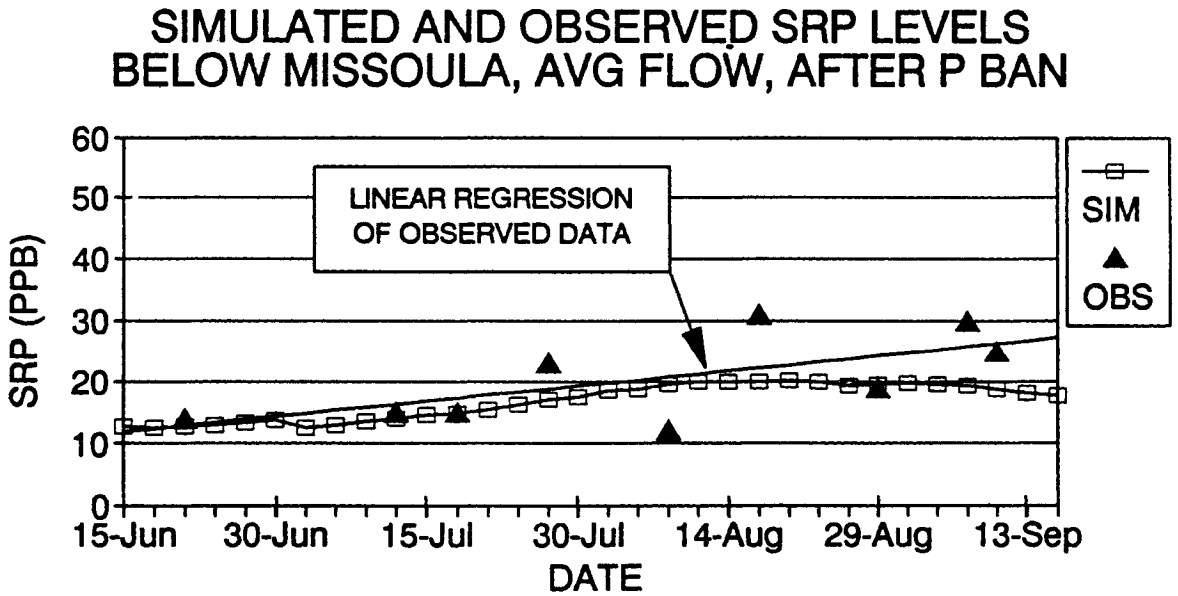
reduction in nutrient levels because of the smaller influence of the Missoula wastewater treatment plant nutrient loading at these sites.



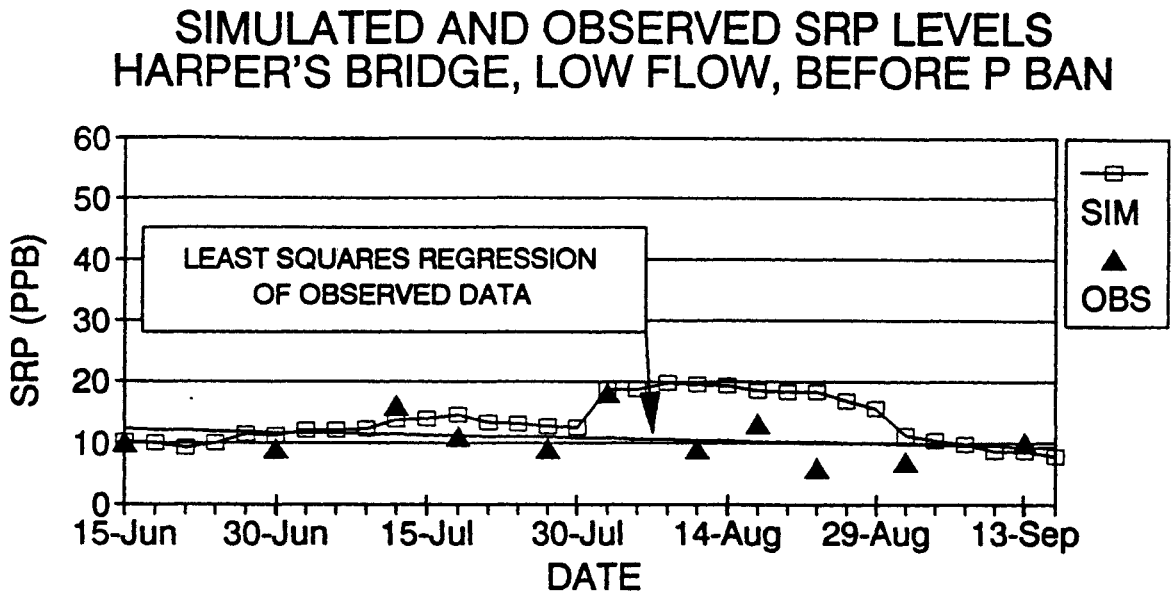


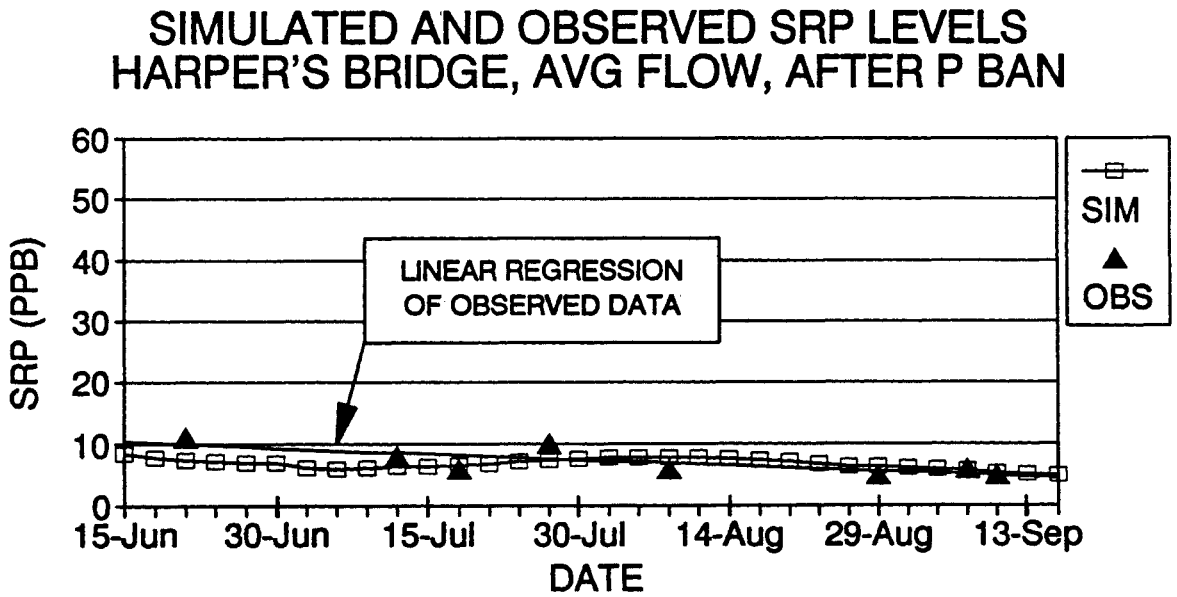
Figures 21 (top) and 22. Validation runs of the nutrient model. Simulated and observed nutrient concentrations in the Clark Fork River.



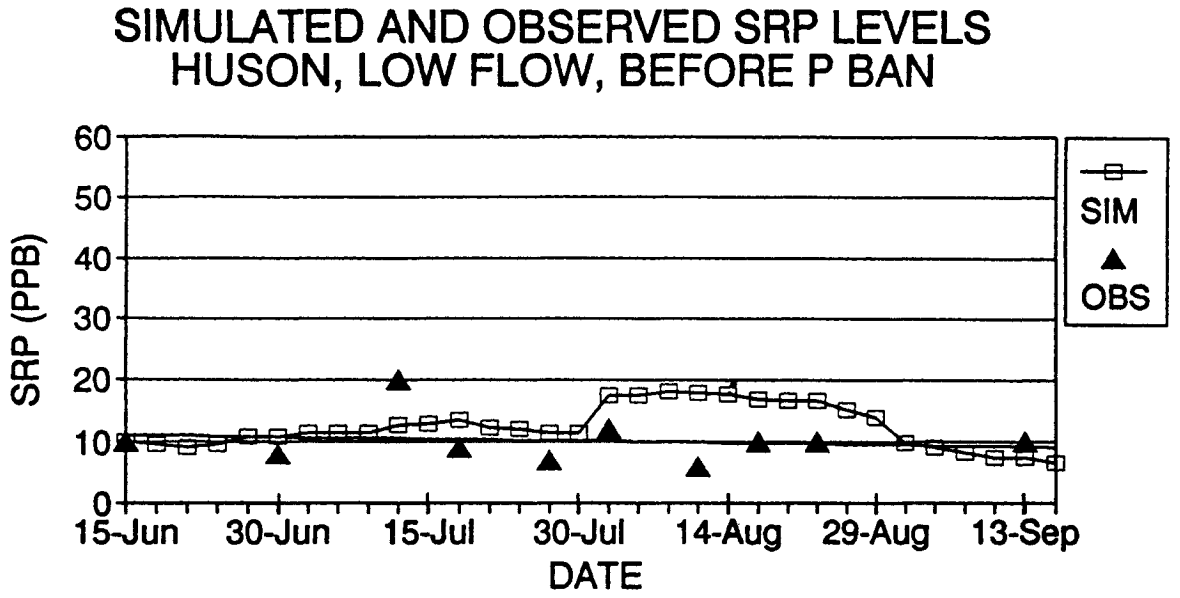


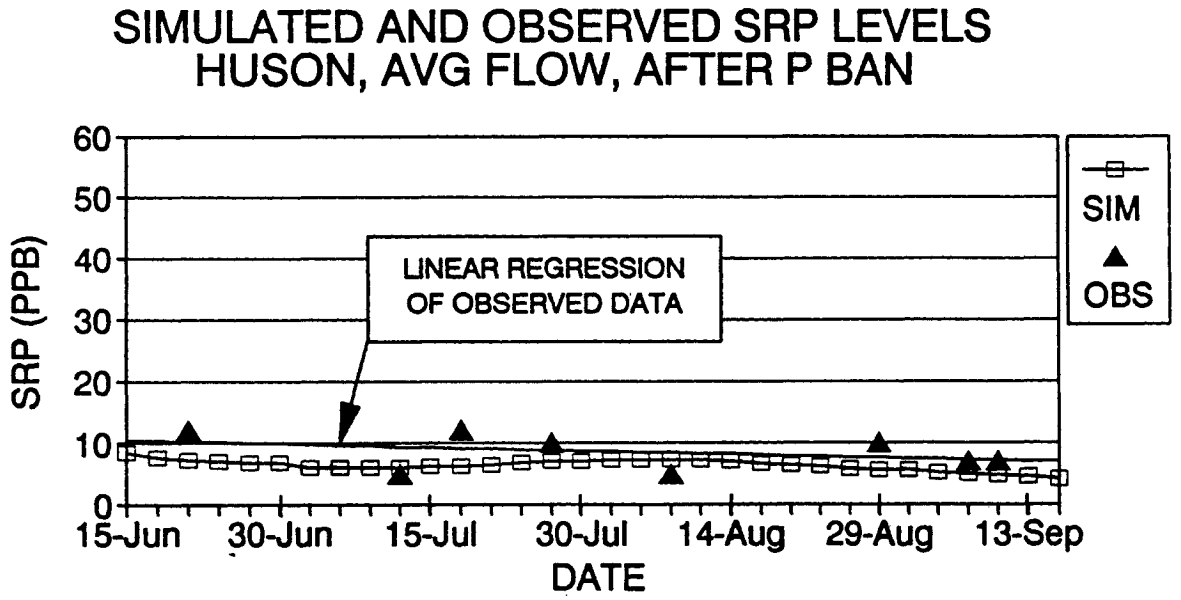
Figures 23 (top) and 24. Validation runs of the nutrient model. Simulated and observed nutrient concentrations in the Clark Fork River.



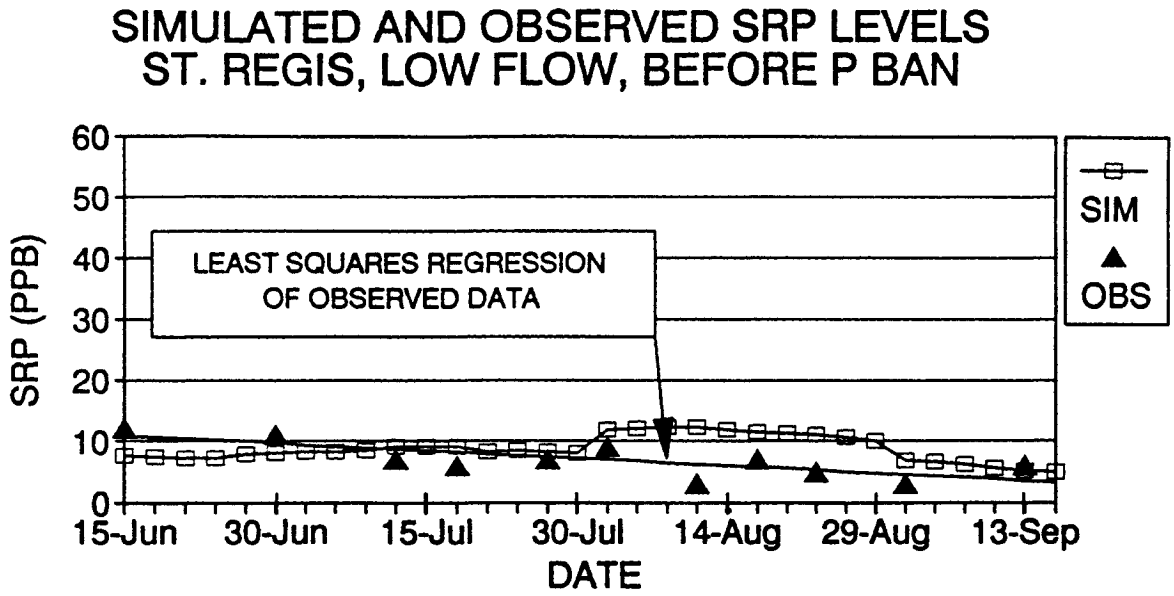


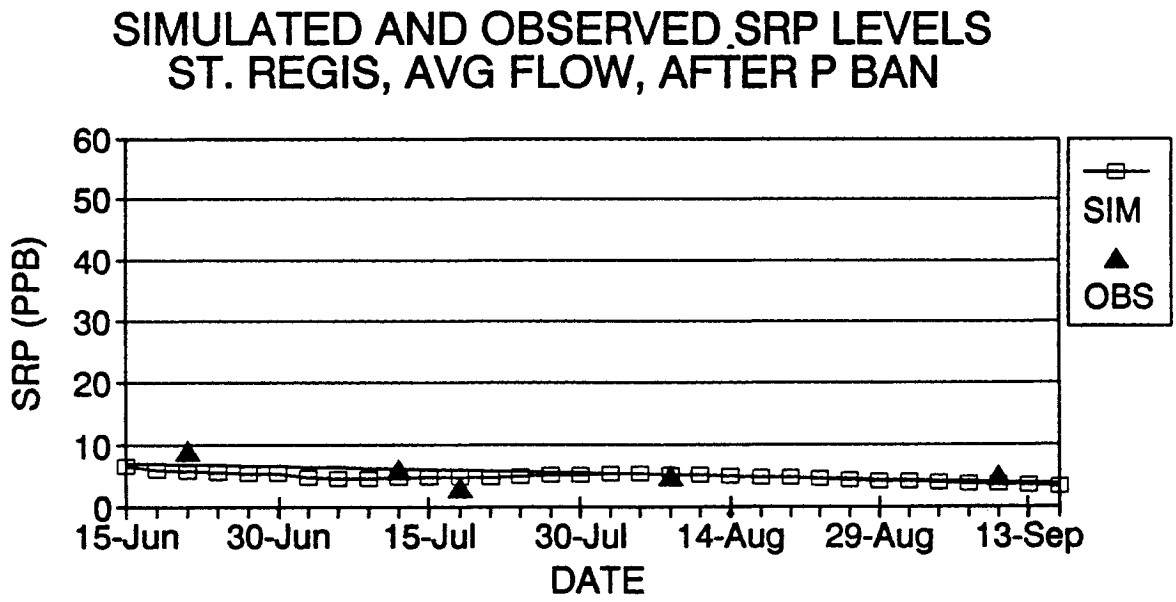
Figures 25 (top) and 26. Validation runs of the nutrient model. Simulated and observed nutrient concentrations in the Clark Fork River.



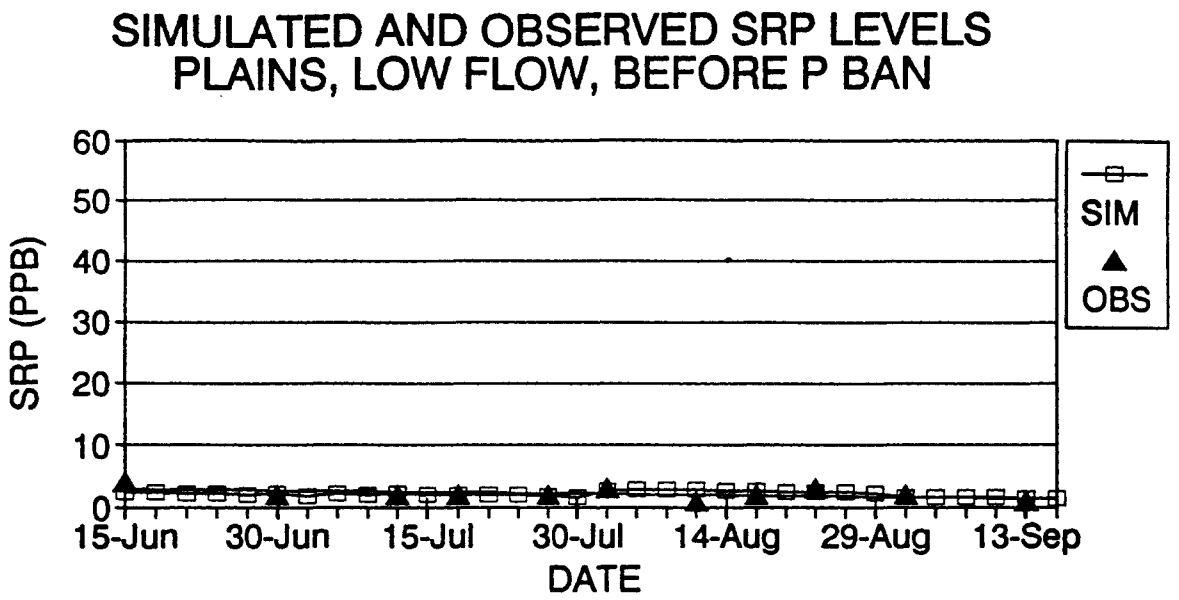


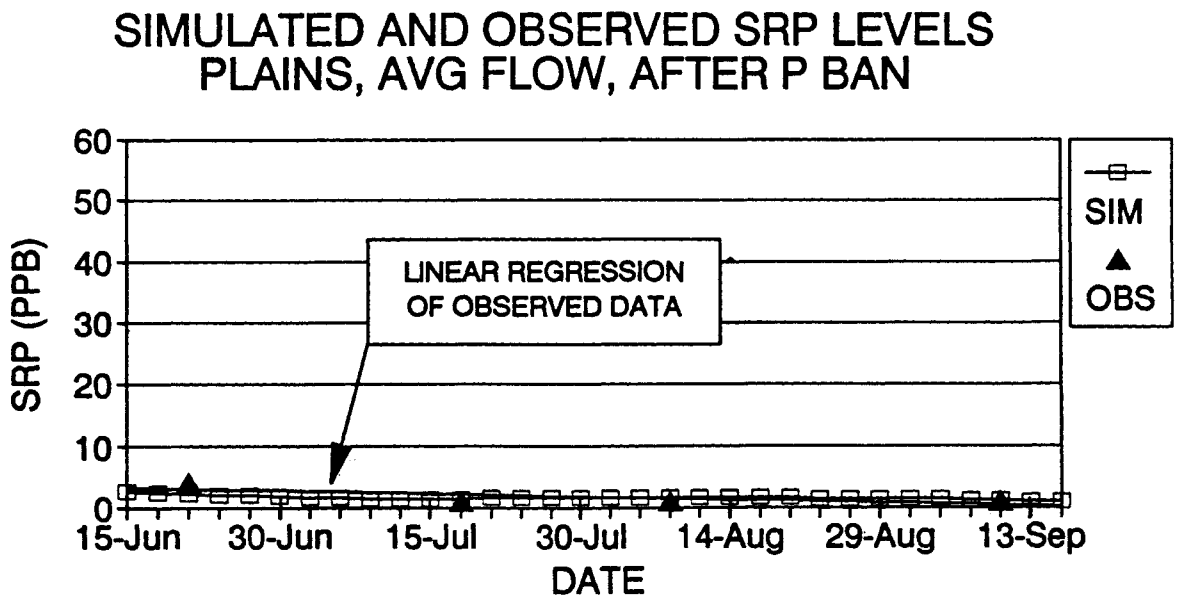
Figures 27 (top) and 28. Validation runs of the nutrient model. Simulated and observed nutrient concentrations in the Clark Fork River.



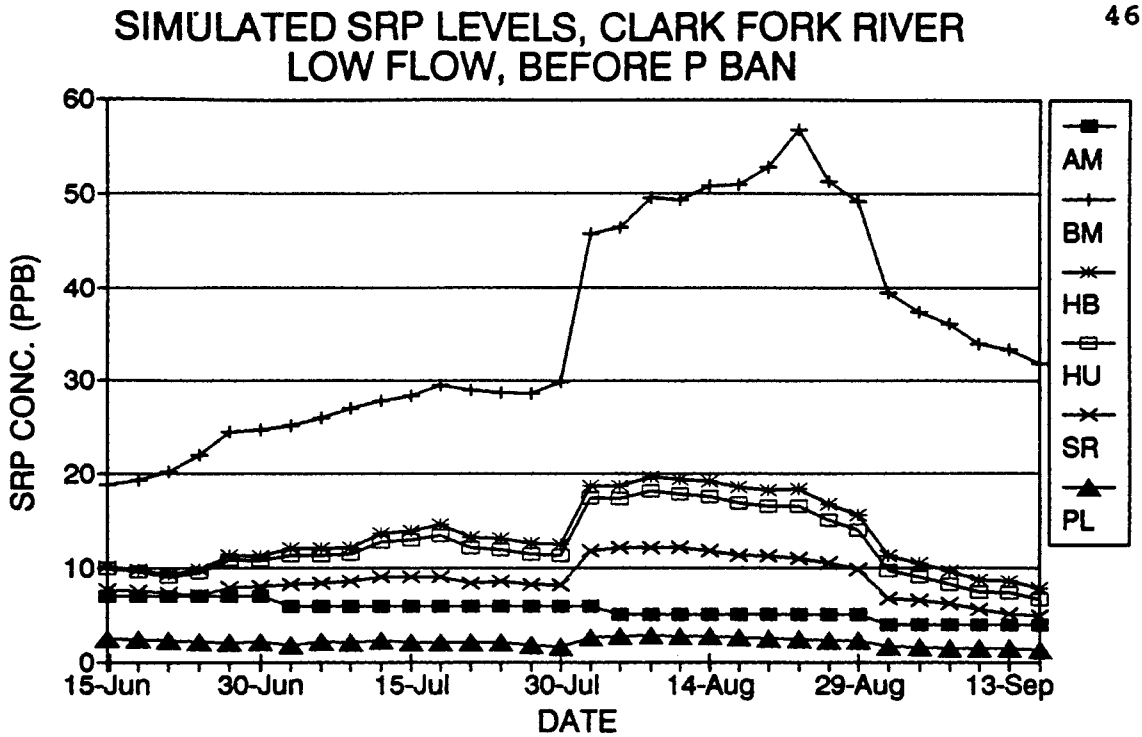


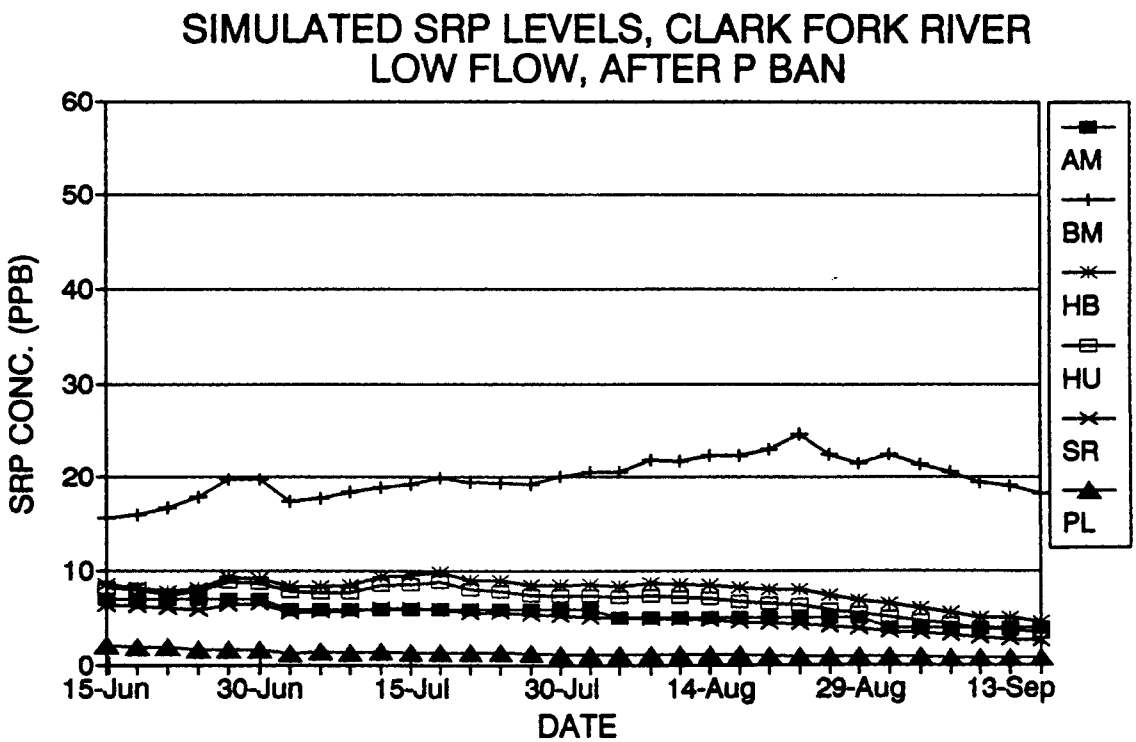
Figures 29 (top) and 30. Validation runs of the nutrient model. Simulated and observed nutrient concentrations in the Clark Fork River.



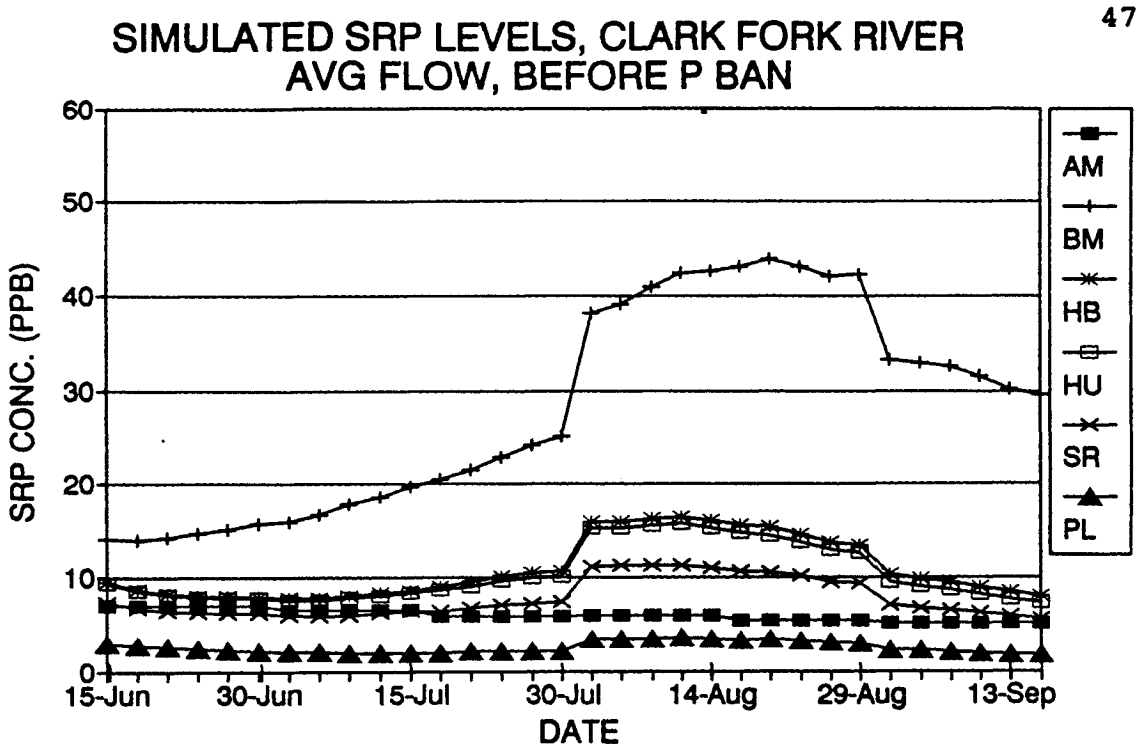


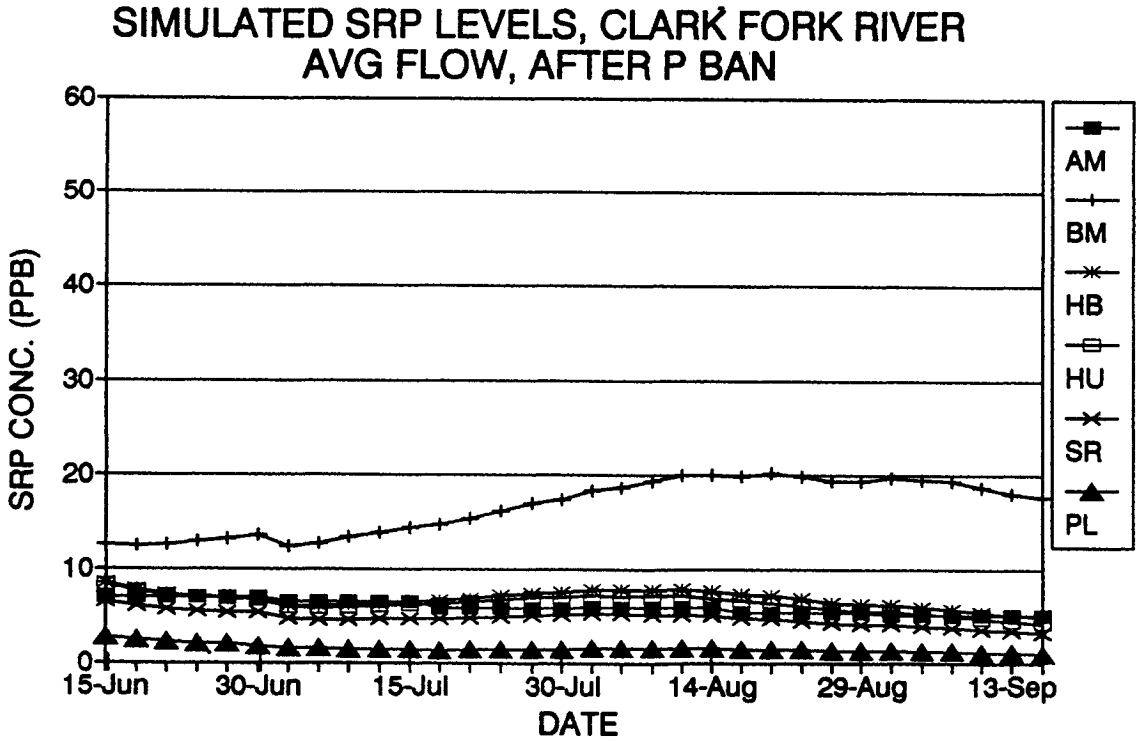
Figures 31 (top) and 32. Validation runs of the nutrient model. Simulated and observed nutrient concentrations in the Clark Fork River.





Figures 33 (top) and 34. Simulated soluble reactive phosphorus (SRP) levels for selected stations in the Clark Fork River, low flow scenario, before and after the Missoula phosphate detergent ban.





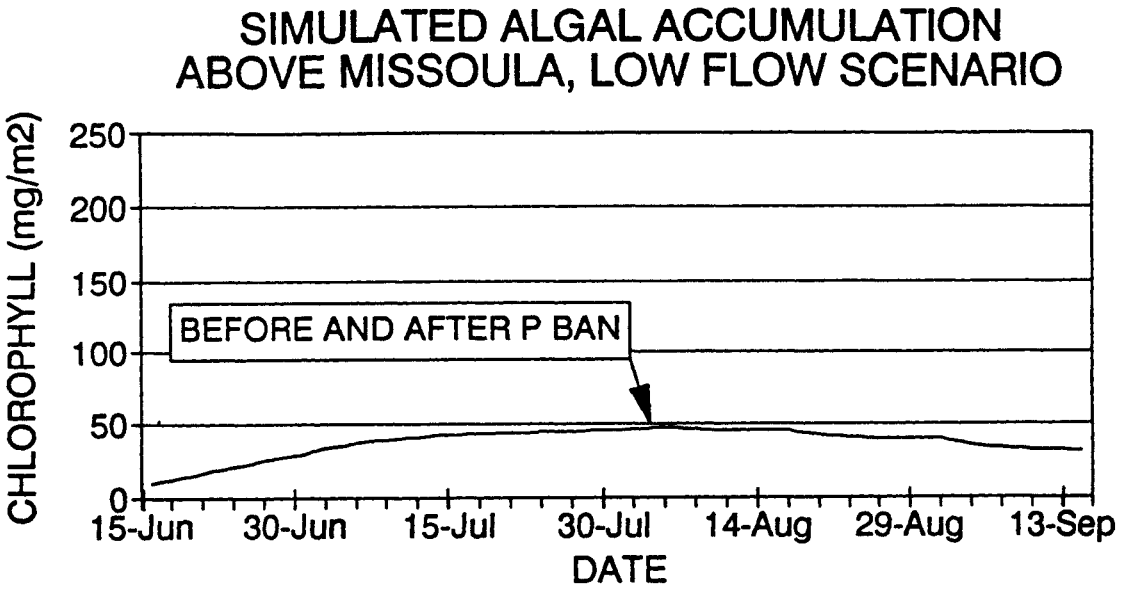
Figures 35 (top) and 36. Simulated soluble reactive phosphorus (SRP) levels for selected stations in the Clark Fork River, average flow scenario, before and after the Missoula phosphate detergent ban.

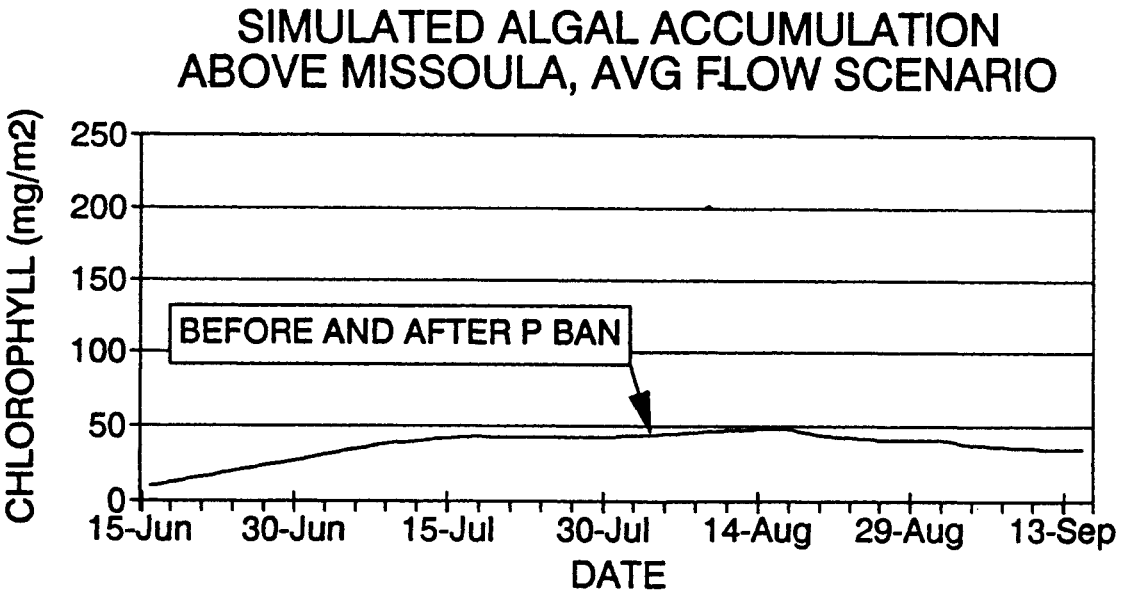
CHAPTER 8. ALGAL RESPONSE TO THE PHOSPHATE DETERGENT BAN

The nutrient model predicts how the nutrient levels in the Clark Fork River have changed since the inception of the Missoula phosphate detergent ban. This information can then be used in the algal accumulation model to predict changes in algal accumulation as a result of the ban. The algal accumulation model was run under the four scenarios of low and average flow conditions, before and after the phosphate detergent ban for each of the six study sites.

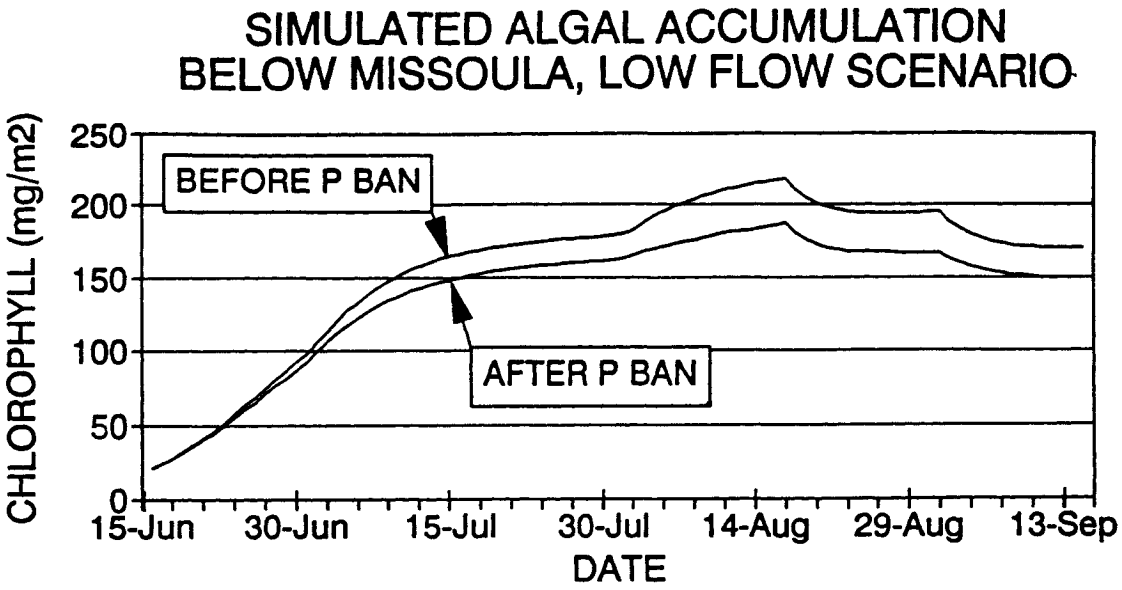
ALGAL RESPONSE-RESULTS

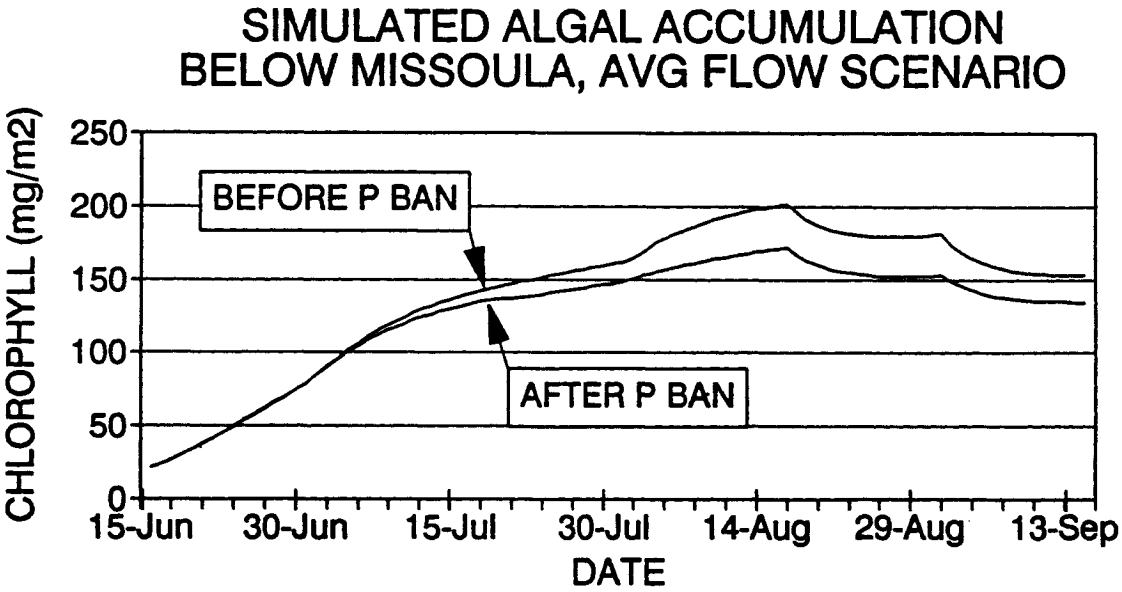
The predicted algal accumulation for each site is presented in Figures 37 through 48. Each figure shows algal accumulation before and after the phosphate detergent ban. The above Missoula site is not affected by phosphorus loading from the Missoula wastewater treatment plant and therefore shows no response to the phosphate detergent ban. The algal accumulation model predicts the highest algal accumulation at the below Missoula site and accumulation decreases downriver from that point. The below Missoula, Harper's Bridge, Huson, and St. Regis sites are characterized by a rapid increase in algal accumulation for the first 20 days of the simulation, followed by a gradual leveling off until mid-summer. Algal accumulation then increases to a peak biomass near mid-August. At this point, algal sloughing becomes greater than accumulation and levels of algal accumulation decline through the rest of the summer. The simulations for the Plains site



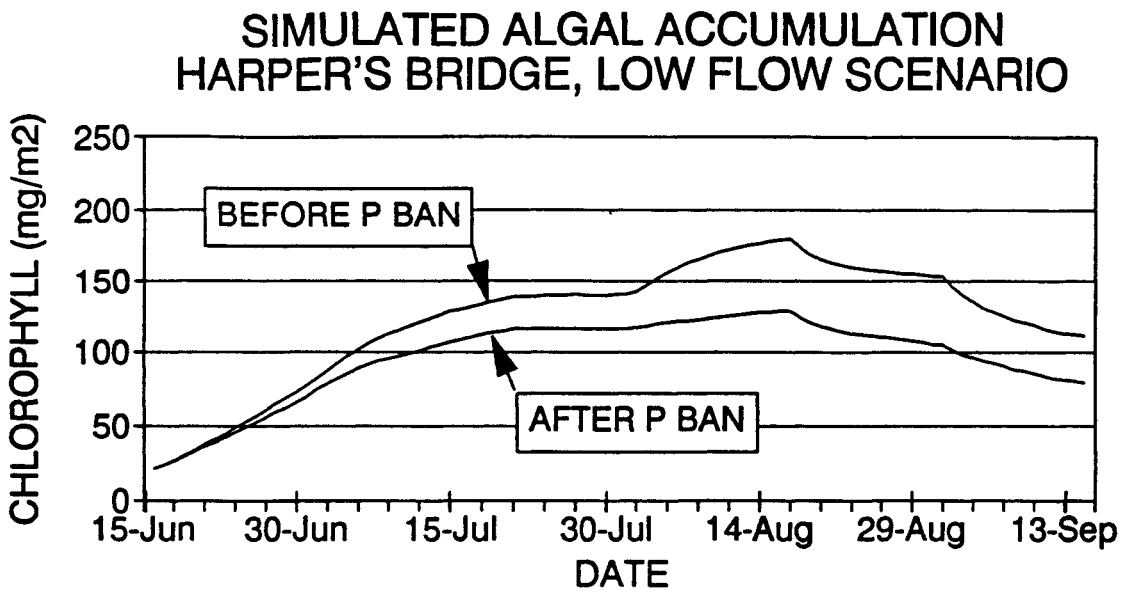


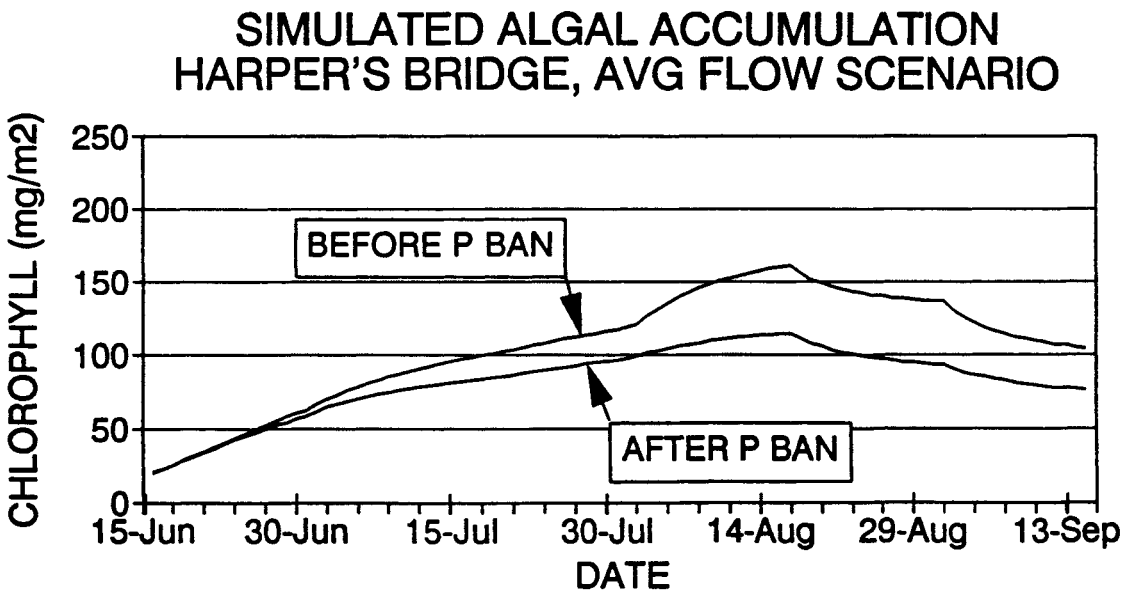
Figures 37 (top) and 38. Pre- and post- phosphate detergent ban algal accumulation, Clark Fork River.



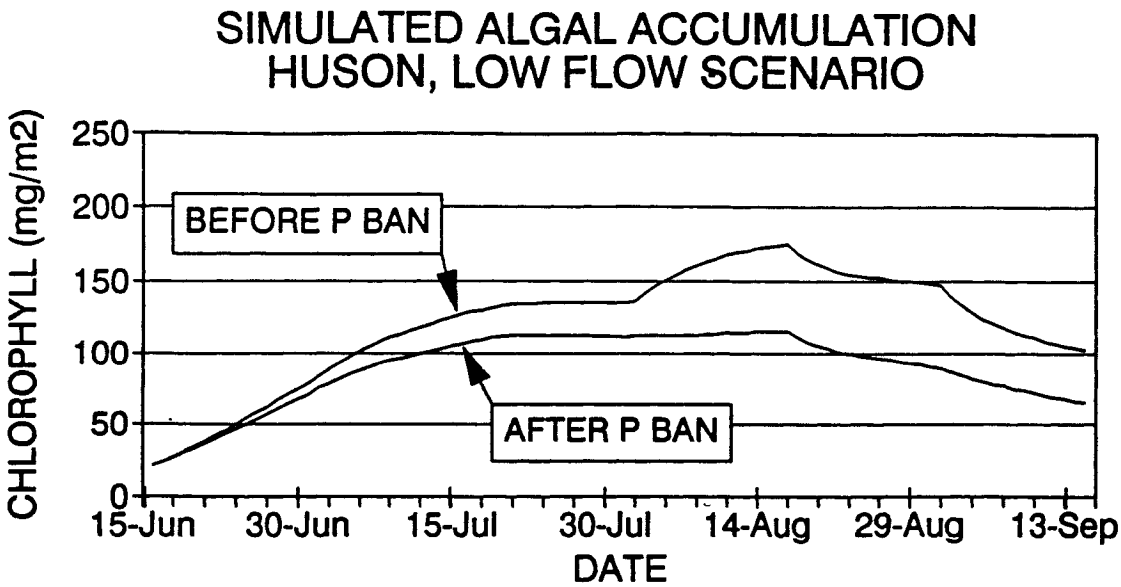


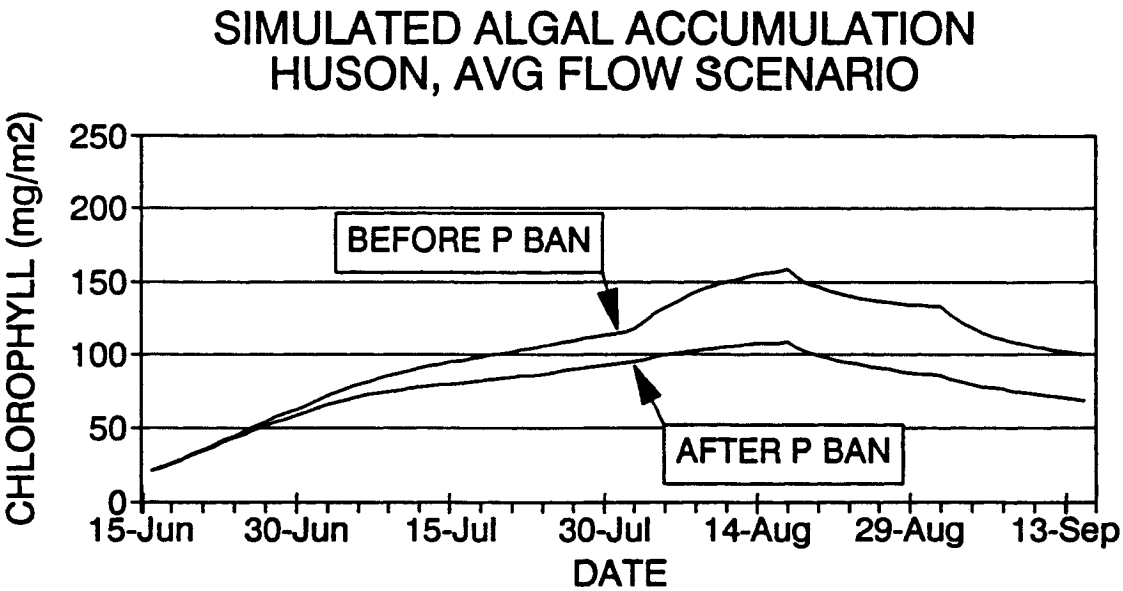
Figures 39 (top) and 40. Pre- and post- phosphate detergent ban algal accumulation, Clark Fork River.



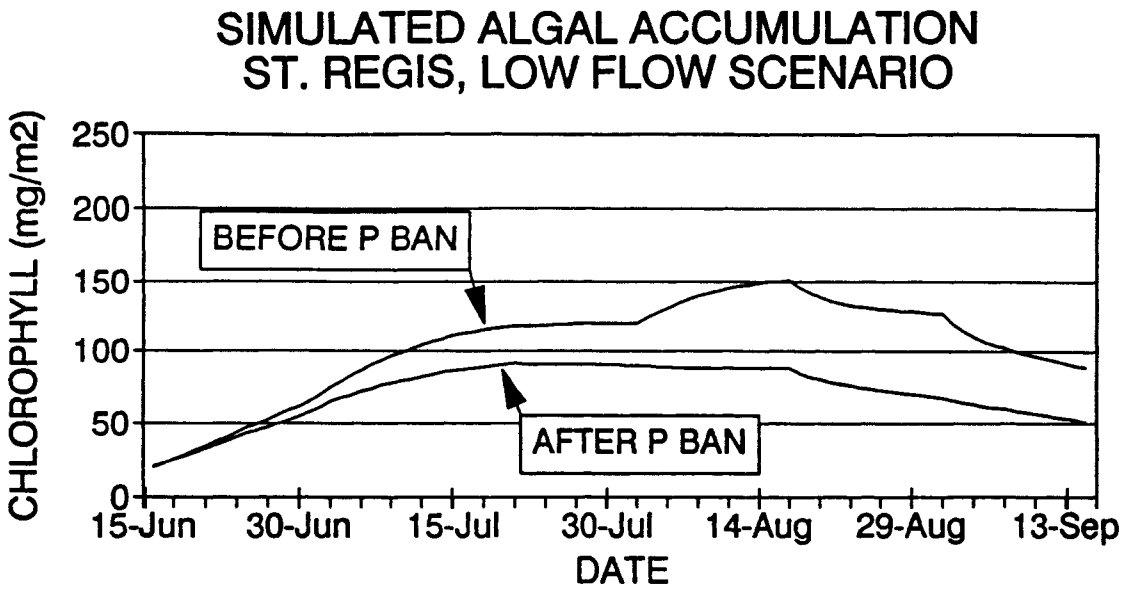


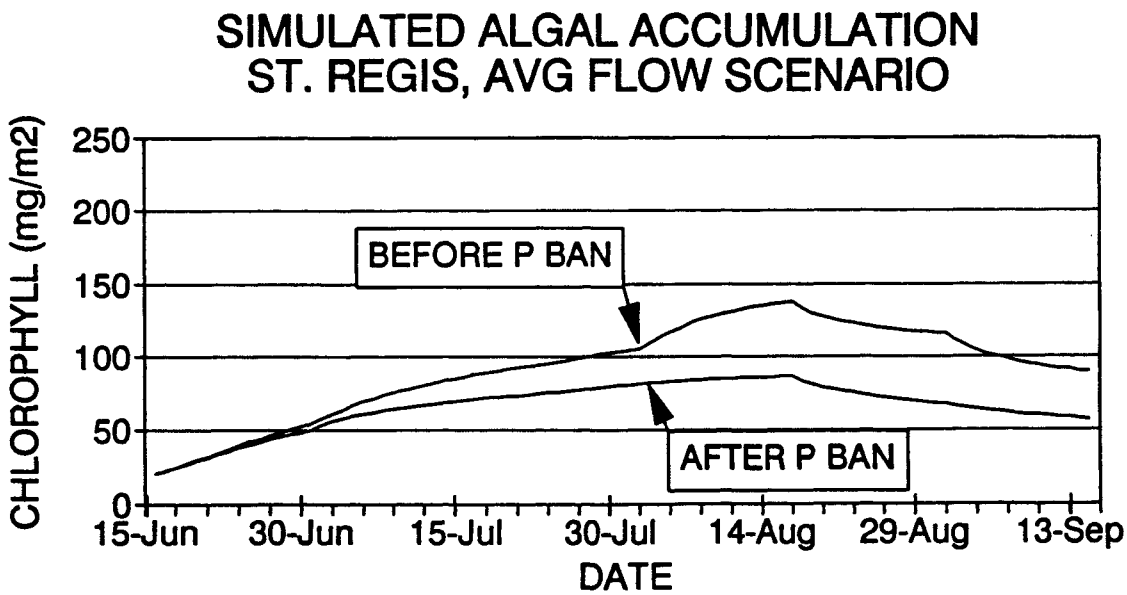
Figures 41 (top) and 42. Pre- and post- phosphate detergent ban algal accumulation, Clark Fork River.



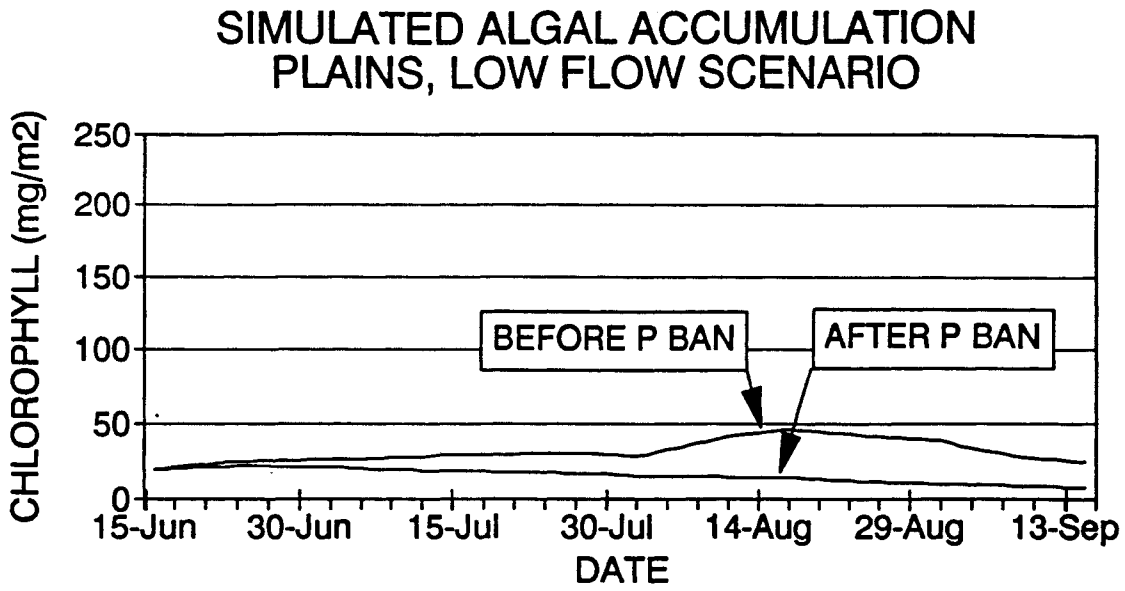


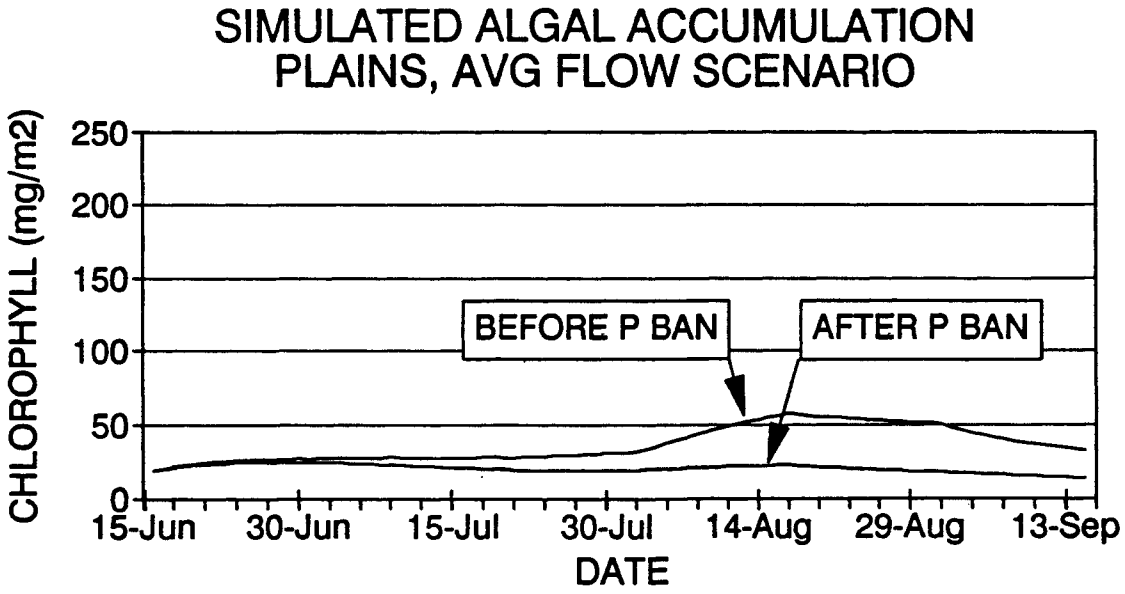
Figures 43 (top) and 44. Pre- and post- phosphate detergent ban algal accumulation, Clark Fork River.





Figures 45 (top) and 46. Pre- and post- phosphate detergent ban algal accumulation, Clark Fork River.





Figures 47 (top) and 48. Pre- and post- phosphate detergent ban algal accumulation, Clark Fork River.

are similar to the above Missoula site, with low levels of algal accumulation throughout the summer.

Two approaches were taken to summarize the differences between pre- and post- ban simulations. The first approach compares the peak biomass attained at each site for each pair of pre- and post- ban simulations. The second approach compares the average algal biomass accumulated over the summer before and after the ban for each site. The results from these calculations are presented as percent differences in Table 2.

TABLE 2. PREDICTED CHANGE IN ALGAL ACCUMULATION IN RESPONSE TO MISSOULA PHOSPHATE DETERGENT BAN

SITE	FLOW	% DIFFERENCE IN PEAK BIOMASS BEFORE AND AFTER P BAN	% DIFFERENCE IN TOTAL BIOMASS BEFORE AND AFTER P BAN
AM	AVG	0	0
BM	AVG	16	11
HB	AVG	33	25
HU	AVG	37	27
SR	AVG	46	33
PL	AVG	80	57
AM	LOW	0	0
BM	LOW	15	12
HB	LOW	33	25
HU	LOW	41	29
SR	LOW	49	38
PL	LOW	74	69

The results presented in Table 2 suggest that the greatest differences between pre- and post- ban algal

accumulation occur farthest down the river. Nutrient concentrations are farthest from saturation at the lower river sites and therefore exhibit the greatest response to a change in nutrient levels. Most of the reductions in peak and total biomass were slightly greater during low flow conditions. The model predicts a minimum change of 11% in total biomass at the below Missoula site. Variability in the replicate samples from artificial substrates at this site are at this magnitude, hence this 11% does not represent a significant change. The nutrient reductions achieved as a result of the ban do not translate into an equivalent reduction in algal accumulation at the below Missoula site. The nutrient levels are still high enough after the ban that they are not limiting algal growth or accumulation, and nuisance levels of attached algae can still be expected.

The percent difference in total biomass at the Harper's Bridge and Huson sites is near 25%. A predicted reduction of this magnitude should result in a noticeable difference in the river. The model suggests that total algal accumulation at the Plains site will be reduced by 60% to 70%. The nutrient levels at this site were below levels that saturate algal accumulation before the ban. The nutrient reductions achieved by the ban at this site therefore significantly reduce the expected algal accumulation.

The percent difference in peak biomass attained as a result of the phosphate ban at each site follow similar trends

as the percent difference in total biomass. The greatest differences occur with increasing distance down river, where nutrient levels are farthest from saturation.

The output of the algal accumulation model can also be used to predict the change in the frequency of occurrence of nuisance algal conditions as a result of the phosphate detergent ban. The percentage of days that algal accumulation exceeded the defined nuisance level of 100 mg chlorophyll/m^2 was calculated for each site. The percent reduction in the number of days of nuisance algal occurrence as a result of the ban was also calculated, and the results are presented in Table 3.

TABLE 3. PREDICTED CHANGE IN FREQUENCY OF OCCURRENCE OF NUISANCE ALGAL CONDITIONS IN RESPONSE TO MISSOULA PHOSPHATE DETERGENT BAN

SITE	FLOW	% OF DAYS ALGAL ACCUMULATION > 100 mg/m2 Chl BEFORE P BAN	% OF DAYS ALGAL ACCUMULATION > 100 mg/m2 Chl AFTER P BAN	% REDUCTION
AM	AVG	0	0	0
BM	AVG	79	79	0
HB	AVG	65	23	65
HU	AVG	63	16	74
SR	AVG	43	0	100
PL	AVG	0	0	0
AM	LOW	0	0	0
BM	LOW	83	81	1
HB	LOW	78	58	26
HU	LOW	78	47	40
SR	LOW	65	0	100
PL	LOW	0	0	0

The results from Table 3 suggest that nuisance levels of algal accumulation were attained before the ban at all sites except for the above Missoula and Plains sites. Nuisance algal conditions are more frequent in low flow years because of the higher nutrient concentrations and temperatures found in the river during low flow conditions. The below Missoula site shows a 79% frequency of days with nuisance algal levels both before and after the phosphate detergent ban, low and average flow conditions. Any slight reductions achieved at this site are not enough to pull algal levels below nuisance conditions.

As a result of the phosphate detergent ban, the frequency of nuisance algal conditions is substantially reduced at the Harper's Bridge, Huson, and St. Regis sites. Harper's Bridge and Huson show reductions of frequency of nuisance algal levels of 65% and 74%, respectively, for the average flow condition. These same sites show reductions of 26% and 40%, respectively, for the low flow condition. Greater reductions occur under the average flow conditions because nutrient levels are lower than the low flow levels, and therefore further from growth saturating levels. The frequency of nuisance levels at the St. Regis site varied from 43-65% of the summer before the ban, but no days of nuisance algal accumulation after the ban.

CHAPTER 9. CONCLUSIONS

Algal accumulation in the Clark Fork River has been predicted to be substantially reduced as a result of the Missoula phosphate detergent ban. The 40% reduction in nutrient loading to the river from the Missoula wastewater treatment plant as a result of the ban has likely resulted in substantial reductions in peak and total algal biomass accumulations. The frequency of occurrence of nuisance algal accumulation has likely been reduced by up to 100% at sites downriver from the Missoula wastewater treatment plant.

Nutrient concentrations in the river just below the Missoula wastewater treatment plant are still high enough after the ban that algal growth and accumulation are not nutrient limited. The greatest benefits were found at the greatest distances downriver from the Missoula wastewater treatment plant. Overall, the reduced nutrient loading as a result of the ban probably resulted in decreased levels of nuisance algal accumulation over a 100 mile stretch of the river, from Harper's Bridge to below Plains.

These conclusions are based on models validated with observed data from the Clark Fork River. These models should continue to be improved through validation of more data collected from post-ban years. Algal accumulation on artificial substrates and nutrient data should continue to be collected for this purpose. These models could also be improved by incorporating considerations for other physical

parameters, particularly nitrogen concentrations.

Greater reductions in algal accumulation could be achieved with greater reductions in nutrient loading to the river. Management actions should focus on reducing point source phosphorus discharges even further. The Missoula wastewater treatment plant nutrient discharge is still a contributor to nuisance levels of algal accumulation. The Butte and Deer Lodge nutrient loads have also been decreased as a result of recent phosphorus detergent bans passed in these communities. Management efforts should continue to be directed at reducing these nutrient sources by utilizing more advanced techniques for phosphorus removal in the wastewater treatment plants or by disposing of the effluent on land in the summertime.

APPENDIX A.

Kinetics of the Algal Accumulation Model by Vicki Watson

PERISIM is a program written in IBM BASICA which simulates the accumulation of attached algal biomass on river rocks over time. In its current form the model has been shown to simulate with reasonable accuracy the accumulation of biomass of the mixed diatom community that characterizes the middle and lower Clark Fork River during the summer growing season.

The model simulates attached algal biomass accumulation by estimating the production of new biomass (via photosynthesis) and the loss of biomass (to respiration and sloughing) every hour. Gains in biomass are added to the previous estimate of biomass and the losses subtracted. Thus the mass of algal material gradually increases or decreases depending on whether gains exceed losses or vice versa. That is, the change in biomass over time is simulated by the equation:

Bt = Bt-1 + Bt-1 * (rates of growth -respiration -sloughing)

where Bt = biomass at time t, Bt-1 = biomass at previous point in time. The time step used is one hour.

The ecological processes simulated are algal growth (via photosynthesis), respiration, and sloughing (or loss of biomass due to detaching of algae from the substratum and washing away). Each of these will be discussed separately.

Algal growth has been modeled with varying levels of complexity. While some of the more complex methods are considered to depict nutrient uptake and growth more realistically, some of the simpler methods often produce estimates that are as accurate (or more accurate). In PERISIM, algal growth is a function of available light, nutrients, and temperature. Under optimum conditions of light, nutrients, and temperature, algal biomass increases at a maximum exponential rate (that is, some fraction of the existing biomass is added each day). When any of these factors is less than optimum, the rate of increase is reduced by a specific formula. That is, the growth rate is estimated by:

u = umax * LD * ND * TD

where u is the rate of increase, umax is the maximum rate of increase of which that particular community is capable, and LD, ND, and TD are the light, nutrient, and temperature dependent functions that reduce the maximum rate of increase

to that expected under these suboptimum conditions (Lehman et al, 1975).

The light dependence formula is that used by Steele (1964, 1965) which recognizes that photosynthesis increases with light up to a point, then becomes saturated and finally inhibited at higher light levels. This relationship is depicted by the formula:

$$LD = (IZ/IOPT) * \exp(1-IZ/IOPT)$$

where LD is the light dependence of growth, IZ is the light at depth Z in calories/cm2/day, IOPT is the optimum light level. PERISIM estimates the surface light level for that time of year and time of day at the latitude being simulated. The light is then attenuated for the depth being simulated, using the formula:

$$IZ = Io * \exp(-nZ)$$

where Io is the surface light and n is the extinction coefficient.

Obviously, growth exhibits an increasing and decreasing response to temperature over a wide range of temperatures. Temperatures in the Clark Fork rarely exceed the optimum (around 25C for many species), hence growth may be represented as a simple increasing function of temperature by the formula:

$$TD \text{ or temperature dependence of growth} = 0.04 * T$$

where T is the ambient water temperature in centigrade.

The effect of nutrients on algal growth has been modeled in numerous ways. The formula used here is the simple Monod or Michaelis Menten formulation which assumes that algal growth rates can be estimated from ambient available nutrient levels. When estimating accumulation rates of a mixed community this method does as well or better than more complex methods (DiToro, 1981). Nutrient dependence is calculated as:

$$ND \text{ or nutrient dependence of growth} = P/(P+Kp)$$

where P is the concentration of soluble reactive phosphorus (SRP) in ppb in the ambient water and Kp is the half saturation constant or the concentration of P which produces half of the maximum growth rate. Note that when P = Kp then ND is 0.5 and the growth rate is 1/5 of the maximum rate. When P is much > Kp, this term approaches 1 and the growth rate approaches the maximum growth rate. When P is much < Kp, this term approaches zero. However, Kp is very low, 2ppb or less according to most research. Hence, when ambient SRP

is low relative to Kp, it is below detection.

Respiration is modeled as a simple function of temperature similar to the temperature dependence of growth:

R or respiration rate = 0.04 * T * Kr

where Kr is the maximum daily respiration rate (0.1/day). This equation produces respiration rates similar to those produced by the equation developed by Graham et al (1982) in which R = 0.151*(0.025T + 0.1).

Sloughing is a function of water velocity, turbulence, and the vigor of the algal community. Artificial stream studies show that following colonization, algal biomass increases at a rapid exponential rate then levels off as losses in biomass come to balance gains. As long as environmental conditions do not change greatly, a dynamic equilibrium biomass is established that seems to be a function of ambient nutrient levels. That is, under higher nutrient levels, algal biomass accumulates to a higher level before levelling off than it does under lower nutrient levels. Based on the work of Bothwell (1989) and Watson (1990), a formula was developed that described this relationship between ambient nutrient level and the maximum biomass sustained at this dynamic equilibrium:

Bmax = 10 + 60 * P/(P+Kb)

where 10 g/m2 is the biomass sustained at P levels below detection level, 60 is the maximum biomass sustained when P saturates standing crop and Kb is the SRP level that produces a standing crop that is about half of the maximum level.

As the biomass at a site approaches the maximum biomass that can be sustained given the nutrient levels there, sloughing increase. This is accomplished by the formula:

Sloughing rate = SLMAX * B/Bmax

where SLMAX is the maximum daily sloughing rate (set at 1/2 the standing crop per day). This approach is similar to that used by Auer and Canale (1980, 1982) except that their Bmax is fixed rather than a function of ambient nutrient levels. Actually Auer and Canale made two uses of Bmax to limit the standing crop of Cladophora. As B approaches Bmax, the growth rate slowed due to shading, nutrient limitation, and waste buildup and the sloughing rate increased. The formulations used were:

growth dependence = 1-(B/Bmax)
sloughing rate = max rate (B/Bmax).

Model Inputs

The model estimates the light levels for each day and hour. The environmental data that the model requires is water quality data, specifically water temperature (in degrees centigrade) and nutrient levels (SRP in ppb). These parameters were measured weekly during the summers of 1988 and 1990 on the Clark Fork. The model requires daily values, and another simple program (PERIFILE) was developed to produce daily water quality input files from the weekly measurements. An initial biomass value must be specified, typically between 1 and 3 g/m2 is used for simulations. For validation runs, the amount of biomass observed on artificial substrates after one week of colonization was used as the initial biomass and the simulation was started on that date. The model also requires the values of the rate constants and other constants in the simulation equations. These are summarized in the accompanying table.

Model Outputs

PERISIM estimates ash free dry weight of attached algae per square meter of river bed for each day of the simulation. Ash free dry weight is then converted to chlorophyll a by a conversion factor (CCF). This factor is 150 for high nutrient sites (below Missoula, Harper's Bridge), 300 for low nutrient sites (above Missoula, Plains), and 200 for moderate nutrient sites (all others).

RATE CONSTANTS AND OTHER PARAMETERS USED IN PERISIM

SYMBOL	VALUE USED	DEFINITION	SOURCE
M	378 cal/cm2/day	mean annual daily light intensity at 45 N latitude	Hutchinson 1975
VAR	249 cal/cm2/day	seasonal variation of light intensity either side of the mean	"
VARDL	4 hrs	seasonal variation of daylength either side of the mean	
N	0.5	extinction coefficient of water	"
MUMAX	1 per day (ie, doubles daily)	maximum growth rate of algae	Watson 1981, 1983 Whitton 1967
KI	10 cal/cm2/day	half saturation constant for light for photosynthesis	Watson 1981, 1983
IOPT	15 cal/cm2/day	optimum light level for photosynthe	"
KPMU	2 ppb	half saturation constant of phospho for algal growth	"
KPB	5 ppb	half saturation constant of phospho for algal standing crop	explained in text
SLMAX	0.5/day	maximum daily sloughing rate similar to 0.3/day used in	Auer & Canale 1980 Auer & Canale 1988
KR	0.1/day	respiration rate coefficient used in R = 0.04 * KR * T produces values similar to R = .151*(.025 T + .1) used by	Watson 1981, 1983 Graham et al. 1982

APPENDIX B.

The Missoula Phosphate Detergent Ordinance

ORDINANCE NO. 2643

AN ORDINANCE PROHIBITING THE SALE OF CERTAIN CLEANING PRODUCTS WITH MORE THAN A TRACE CONTENT OF PHOSPHORUS.

BE IT ORDAINED BY THE CITY COUNCIL OF THE CITY OF MISSOULA THAT TITLE 13 OF THE MISSOULA MUNICIPAL CODE BE AMENDED BY ADDING CHAPTER 13.10, SECTIONS 13.10.010 THROUGH 13.10.080, MISSOULA MUNICIPAL CODE.

Section 1. Chapter 13.10. Phosphorus Content of Wastewater Discharges, of Title 13, Sections 13.10.010 through 13.10.080, Missoula Municipal Code, Public Services, is hereby adopted as follows:

CHAPTER 13.10 PHOSPHORUS CONTENT OF WASTEWATER DISCHARGES.

13.10.010 Legislative Intent and Purpose

The intent and purpose of this chapter shall be to:

(a) Set forth regulations, prohibitions and requirements pertaining to phosphorus compounds for direct and indirect discharges into the City wastewater collection and treatment system enabling the City to better attempt to comply with the Montana Pollution Discharge Elimination System Permit.

(b) Generally protect the health, safety, and welfare of residents of the city and downstream users of the Clark Fork River with respect to quality of water available to them.

(c) Prescribe powers and duties of the City of Missoula and the City/County Health Department to be exercised within their jurisdictional area within the city limits and within three miles of the city limits.

13.10.020 Definitions

(a) "Chemical water conditioner" means a water softening chemical or other substance containing phosphorus which is intended to treat water for use in machines for washing laundry.

(b) "Commercial establishment" means any premises used for the purpose of carrying on or exercising any trade, business, profession, vocation, or commercial or charitable activity, including but not limited to laundries, hotels, motels, and food or restaurant establishments.

(c) "Household cleaning product" means any product including but not limited to soaps, detergents, laundry bleaches, and laundry additives used for domestic or commercial cleaning purposes, including but not limited to the cleaning of fabrics, dishes, food utensils, and household and commercial premises. Household cleaning product does not mean foods, drugs, cosmetics, or personal care items such as toothpaste,shampoo, or hand soap.

(d) "Person" means any individual, proprietor of a commercial establishment, corporation, municipality, the state or any department, agency, or subdivision of the state, and any partnership, unincorporated association, or other legal entity.

(e) "Phosphorus" means elemental phosphorus.

(f) "Trace quantity" means an incidental amount of phosphorus which is not part of the household cleaning product formulation, and is present only as a consequence of manufacturing, and does not exceed 0.5% of the content of the product by weight expressed as elemental phosphorus.

13.10.030 Application of Chapter

The provisions of this chapter shall be enforced by the City and the City/County Health Department and will apply to persons engaged in the sale or commercial distribution of products that have as their substantive content prohibited phosphorus compounds within the City of Missoula and within 3 miles of the Missoula City Limits.

13.10.040 Prohibited Phosphorus Compounds

(a) No household or commercial cleaning product shall be distributed, sold, offered, or exposed for sale within the City of Missoula or within 3 miles of the city limits if it contains phosphorus in concentrations in excess of a trace quantity, except as provided in this chapter, except that no dish washing detergent may be distributed, sold, offered, or exposed for sale if it contains phosphorus in excess of 8.7% by weight expressed as elemental phosphorus.

(b) No chemical water conditioner or softener which contains more that 20% phosphorus by weight may be distributed, sold, offered, or exposed for sale within the city limits or within 3 miles of the city limits.

13.10.050 Exceptions

The following cleaning agents and other products containing phosphorus are exempt from the provisions of this ordinance:

(a) Those used in food or beverage processing.

(b) Those used for industrial processes or for cleaning food and beverage processing equipment, medical or surgical equipment, or dairy equipment; and

(c) Those existing stocks of phosphorus cleaning products and water conditioners which are offered for sale within the City of Missoula and within 3 miles of the Missoula City limits, for a period of six months after adoption of this ordinance.

13.10.060 Labeling

None of the products listed below shall be offered for sale unless the item is clearly labeled with the percent elemental phosphorus content to the nearest one tenth of one percent accuracy, except that products which contain a trace quantity may be labeled "contains no phosphorus", "contains no phosphates", or similar labeling which makes a clear statement that phosphorus is not present in the product.

Products requiring labeling:

1. Powdered or liquid laundry detergents and soaps.
2. Powdered laundry bleaches.
3. Powdered laundry chemical water conditioners.
4. Powdered laundry pre-soak products.
5. Powdered and liquid automatic dishwasher detergents and soaps.

13.10.070 Penalty

A person involved in the sale or commercial distribution of any phosphorus compound prohibited by this chapter who is unaware of the provisions of this chapter, shall for a first offense of this chapter be notified of such noncompliance by either the City or by the Missoula City/County Health Department and shall be given 10 days from receipt of such notice to comply with the provisions of this chapter. Failure to comply with this chapter following this 10 day period shall be a misdemeanor. A minimum fine of $50.00 shall be imposed for each violation of this chapter. The maximum penalty that may be imposed shall be $500.00 and no imprisonment may be imposed. Each day a violation exists shall constitute a separate and independent violation of this chapter.

13.10.080 Annual Report

On or before the 1st day of August of each year the Public Works Director and the City/County Health Department shall prepare a summary of the reports transmitted to State and Federal agencies during the previous twelve month period as required by the Montana Pollution Discharge Elimination System permit issued to the City of Missoula under date of August 1, 1988. The said summary shall be submitted to the Mayor and the City Council on or before the 15th day of August of each year.

Section 2. Severability.

If any section, subsection, sentence, clause, phrase or word of this ordinance is for any reason held to be invalid, such decision shall not affect the validity of the remaining portions of this ordinance.

Codification Instructions

This ordinance shall be codified as 13.10.010 through 13.10.070 of Title 13.

Passed by a _____

Approved by the Mayor this _____ day of _____, 1988.

ATTEST: APPROVED:

_____ _____

City Clerk Mayor

(SEAL)

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Stream Periphyton Monitoring Manual

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Prepared for

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1 Introduction

1.1 Background

Water management in New Zealand is principally controlled by the Resource Management Act (1991) (RMA). The RMA is holistic in its approach and encourages the inclusion of a wide range of values in assessing the potential impacts of any water resources development. A fundamental part of river ecosystems, and the aesthetic, amenity, biodiversity and fishing values that they sustain, is the periphyton community. This community is the slimy coating found on rocks and other stable substrates in streams and rivers. It consists mainly of algae, but the term also includes fungal and bacterial matter, and varies greatly in appearance from a thin brownish or greenish film, to thick, dark-coloured "mats", to masses of streaming green or brown filaments. More specifically, periphyton:

- can provide much of the food, and therefore energy, to maintain higher levels of the food chain such as insects and the fish that feed on these insects;
- are an important agent to help purify waters. The periphyton absorb many impurities such as nitrogen and phosphorus (nutrients), heavy metals and are sites for the break-down of bacterial and other organic matter contamination;
- are very responsive to degradation of water quality, often changing in both taxonomic composition and biomass where even slight contamination occurs;
- can proliferate when high concentrations of nutrients occur in the water, velocities are low, and there hasn't been a flood for a while.

1.2 Purpose and scope

The *New Zealand Periphyton Guideline: Detecting, Monitoring and Managing Enrichment of Streams* (Biggs 2000a) reviews periphyton in the context of their importance to water resources management. In particular, a background overview is given of factors controlling periphyton growth in streams, communities normally found in different habitats, the use of periphyton as environmental indicators, and guidelines to prevent their proliferation. In this stream periphyton monitoring manual we build on this by describing a standard set of methods to enable Regional Councils, consultants and researchers to collect and analyse data on periphyton for resource surveys, impact assessments / monitoring, State-of-the-Environment (SOE) reporting and research studies. *We stress the need for comprehensive study planning and a clear definition of what the data are required to resolve before a study is commenced.*

Periphyton monitoring is suitable for use in streams and rivers with any type of substrate (silt through to boulders and bedrock). However, for practical and safety reasons, it is best to apply the sampling methods described in this manual in streams and rivers that can be waded, with water velocities of no more than about 1.0 m / s and a depth of < 0.75 m. Throughout this manual we use the term "*stream*" because it more closely reflects the type of environment where the methods are likely to be applied.

Internationally, many different methods are used for periphyton research and monitoring depending on the habitats and study objectives. It is not the intention to review all these methods in the present manual. Instead, the objective is to prescribe a set of protocols that will be applicable to most of the common stream habitats in New Zealand. We cover two general levels of data collection that are likely to be needed in resource management investigations: rapid assessments, and comprehensive (quantitative) assessments. The methods

have all been used extensively. We describe methods for:

- non-destructive sampling, surveying and data collection in the field;
- collection of periphyton samples from natural and artificial substrates for later analysis in the laboratory;
- in-stream nutrient-diffusing substrate assays for assessing the degree and type of nutrient limitation of periphyton growth;
- laboratory analyses for two measures of biomass (ash-free dry mass and chlorophyll *a*);
- laboratory analysis of taxonomic composition (including a guide to common taxa).

The manual also includes:

- guidance on design of sampling programmes;
- statistical definition of variability and numbers of sample replicates;
- procedures for sample identification and tracking;
- monitoring and cross-checking procedures;
- some approaches to data analysis.

We use "*sampling programme*" as a general term to mean a data collection exercise of no fixed frequency or duration. For example, this might be an experiment to define the nutrient that limits periphyton growth in a stream. Alternatively it might entail the detailed assessment of the effects of a discharge from a dairy shed using artificial substrate sampling above and below the discharge point on several occasions. A more specific sub-set of this is a "*monitoring programme*". This involves defining a set location, sampling interval and duration in relation to establishing some longer-term condition or potential effect on the resource. For example, you might sample a site monthly for a year to establish the average state of the community in relation to the degree of nutrient enrichment. Alternatively, a set of sites might be sampled quarterly over a long period (e.g., 10 years) to detect trends as part of SOE monitoring.

While this manual is geared toward agencies involved in water resources management issues, it should also be useful for researchers as a guide to some robust methods for use in New Zealand stream and river environments. It is hoped that the adoption of standardised approaches will greatly assist in data interchange throughout New Zealand.

2 Design of sampling programmes: “begin with the end in mind”

2.1 Introduction

In developing any sampling programme, whether it be for bio-monitoring of waste discharges, SOE monitoring or a research study, the quality of the information generated will largely hinge on good study planning. Good study planning, particularly the setting of clear objectives and hypotheses, can save time later in the study, and also greatly simplify data analysis and reporting. In other words, it really helps to “*begin with the end in mind*”. Time-consuming, and usually quite complicated data analysis is often needed where study objectives have not been explicitly developed and used to thoroughly plan a sampling programme. Such analyses can be very frustrating, and lead to imprecise results with weak conclusions. Indeed, your study is likely to be much more useful if you clearly set out what you want to define. We therefore discuss programme planning in some detail below. Two examples of planning and executing studies are used to illustrate the concepts and types of decisions that need to be made.

2.2 Types of sampling programmes

Three basic types of periphyton sampling programmes are likely to be needed in New Zealand.

1. *Resource surveys* establish general patterns of periphyton biomass and composition in time and / or space. Such data can then be used for desk-top assessments in discussions of possible changes to water resources / landuse management regimes, classification of waterways according to degree or type of human impact, etc.
2. *Impact assessments* define effects on periphyton of a change in management regime. These are best treated in terms of BACI designs (Before vs. After in time, Control vs. Impact sites in space) (e.g., Green 1979). However, this is rarely possible in practice so such assessments generally have to rely on Control vs. Impact site analysis.
3. *State-of-the-Environment monitoring* tests the effectiveness of regional water resources management policies. Sites are selected to represent some pre-defined condition within a region, and are monitored over an extended period so that any changes in these conditions that may relate to policy decisions will be unambiguously defined.

These study designs will be discussed in more detail below.

Before embarking on a sampling programme, it is essential that you clearly define the issues or questions being asked. As Green (1979, p.25) suggests: ”Be able to state concisely to someone else what question you are asking. Your results will be as coherent and as comprehensible as your initial conception of the problem”. It is particularly important that you study Figure 1 if embarking on resource surveys and impact assessments. This figure summarises a general approach for planning, setting consent criteria and verifying appropriateness of the chosen criteria for managing instream values in relation to periphyton. You should turn your questions into instream management objectives and then, if possible, develop testable hypotheses.

2.3 Defining your objectives

The most fundamental aspect of establishing a sampling programme and fulfilling the overall goals of your study is adequate definition of objectives. These objectives might be formal instream management objectives (ISMOs), as described in Biggs (2000a). However, if the

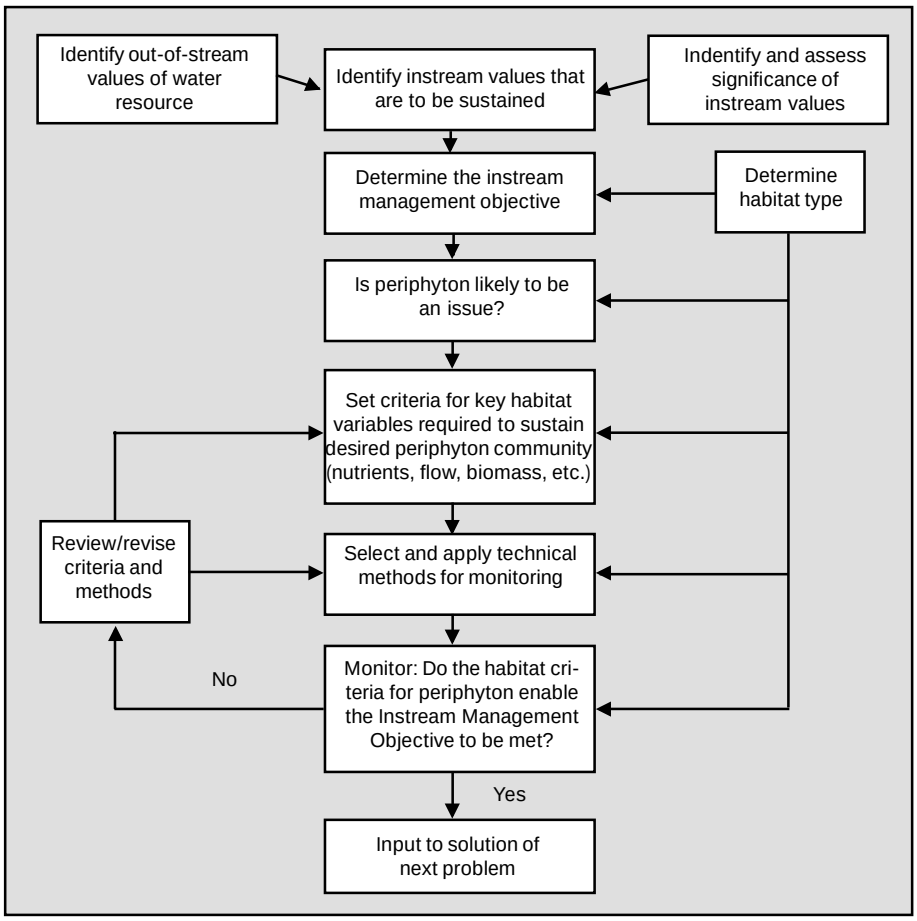


Figure 1: General procedures for planning, setting consent criteria and verifying appropriateness of consent criteria for managing instream values in relation to periphyton (from Biggs 2000a).

study relates more to general resource analysis, or testing for the effects of an existing water management practice, the objectives might be specific to your particular study. Well-defined objectives will help in resolving:

- where to sample;
- how often to sample;
- variables to consider;
- sampling methods and replication;
- study budget;
- approaches to data analysis;
- reporting milestones and formats.

Planning for many types of studies can be sharpened even further by translating the objective into a hypothesis testing framework. This might seem rather too scientific for many resource management studies or general resource surveys. However, the planning for even a simple study can benefit from hypothesis testing. One of the principal benefits of doing this is that it forces you to think about what important effects or differences you might need to be able to confidently detect and the processes that might be operating (i.e., "*start with the end in mind*"). For most questions you are confronted with, it should be possible to develop a reasonable study approach using your accumulated knowledge, literature, and some sensible reasoning (based on sound scientific principles) (see Section 2.5). A literature search is always a powerful entry point to a new study area or for solving a new problem. Electronic

searches should be possible through most libraries. NIWA holds databases on all material published in New Zealand. It might be possible to access overseas databases (e.g., "Aquatic Sciences and Fisheries Abstracts") through some University libraries.

The means of assessing sample numbers and performing calculations is given in Section 3. But first we need to consider some more general issues, such as scale. We then give some examples of planning and carrying out periphyton monitoring studies to illustrate the concepts.

2.4 Issues of scale

A component of clearly defining your objectives and setting hypotheses is developing a sense of the scale of your problem. This will enable you to better assess what data might be required to resolve your issue. Scale is a somewhat nebulous concept to most people, but underlies many discrepancies that arise in concepts and differences in data interpretation. It is also vital to understand scale when attempting to rationalise data collection to obtain the most cost-effective solution.

Set the right scale and you will establish the right reference point to analyse your problem. Scale is mainly about the size of the "window" that you use to see the biological and physical variability in nature. For example, our perception of the world as we walk down the street is different from that we obtain from an aircraft, which is different again from what we would obtain from a spacecraft. *In essence*, the patterns we perceive are strongly influenced by how big our window is in relation to spatial and temporal variability in our environment. So, a motile diatom that is <10 µm long sees the surface of a stone like we might see the Sahara Desert. The diatom might then perceive undulations on the surface of the stone as we would see a large sand dune. Conversely, a mayfly would see variability in terms of the shape of individual stones and their orientation to the flow and a trout would see variability in terms of the extent of pools, riffles and runs. Trout wouldn't be influenced by the surface texture of individual stones and a diatom wouldn't even know about pools, riffles and runs.

Humans have the ability, particularly through modern technology, to view things over a very broad range of scales. Often it is very tempting to become swamped by detail in an effort to understand variability in stream periphyton communities. At some point it becomes important to stand back and ask "what level of detail and quantification of variability do I really need to answer the question being posed?" Should I be looking at the spatial arrangement and type of community patches on individual stones (which might be controlled most by local variations in water velocity)? Should I be integrating everything over individual stones and looking at the variability within the reach (which might be controlled most by spatial variations in sediment type such as cobbles vs. sands)? Or, should I be looking at variability among reaches (which might be controlled most by variations in riparian vegetation)? Or perhaps it is more important for the study objectives to pool all the samples together within each stream, but sample many streams along an environmental gradient to determine the effects of, say, catchment development among streams?

In other words, clearly define the potential factor(s) whose effects you want to examine, and the scale of biotic response that could occur. While strongly influencing the nature and success of your study, these questions also have a major bearing on the cost of carrying out the investigation (see below).

Scale has important implications for environmental predictions (Levin 1994). In complex systems it is usually possible by carrying out time and/or space averaging, to reduce vari-

ability to such an extent that general trends or responses become clear and can be simulated. This might be the appropriate scale to base management on. Alternatively, so much detail may be lost that the predicted shifts in biological response lie within the error of the model. For example, periphyton biomass in streams is typically highly variable over time (e.g., Fig. 16, Biggs 2000a). Indeed, this variability has defied efforts to develop realistic time-series models of biomass as functions of controlling variables such as nutrients, floods, light, temperature and invertebrate grazing. However, considerable progress has been made in developing models by moving up to the next scale by averaging out the temporal variability over a year, and then comparing biomass from many streams that differ widely in their landuse and flow characteristics (e.g., Biggs 2000b).

Thus, while we cannot predict exact periphyton biomass in a given reach of a river at a particular time, we can get estimates of likely average and worst case biomass as functions of flood disturbance frequency and nutrient concentrations. The question then arises: are these estimates accurate enough to enable the required decision to be made, or are they within the range of error of the model? Such questions of scale need to be addressed at the planning stage of a programme. Obtaining sensible answers requires careful thought about the processes you wish to investigate and their effects.

2.5 Examples of setting objectives and carrying out a sampling programme

Two examples of setting objectives and how one might develop associated study strategies are summarised below. For completeness, we have also included a brief summary of the results. The first example is an investigation of the effects on periphyton of land use in the stream catchment. The second is an investigation of the effects of a change in point source pollution discharge from a meat works.

2.5.1 Example 1: Enrichment of the Kakanui River, North Otago

The Otago Regional Council (ORC) were confronted with a perceived issue of possible over-allocation of waters in the Kakanui River in North Otago. It was suggested by community groups that abstraction in summer was reducing flows to such an extent that it was compromising the "life-supporting capacity" of the river. In particular, there was a perception that trout fishing opportunities had decreased greatly in recent years in the lower river and this was possibly because low flows were causing periphyton proliferations. This provided a good research opportunity to develop a greater understanding of the interactions between flow regimes and stream enrichment. The study was carried out as a collaboration between NIWA and the Otago Regional Council and the results have been published in Biggs *et al.* (1998b). After considering the problem we established a monitoring objective within the context of an hypothesis testing framework as defined below. The primary questions were:

1. Is there a downstream increase in *mean* and *maximum* periphyton biomass associated with intensification of landuse and the accompanying reduced flows caused by abstractions?
2. If so, does biomass breach MfE guidelines for the maintenance of life supporting capacity in streams (i.e., are periphyton proliferations really to blame for a perceived degradation in the fishery)?

To sharpen the scientific and conceptual basis for the investigation we then formulated a simple hypothesis:

"We predict that the headwater reaches of the river will be less enriched, and have lower periphyton biomass, than the lowland reaches."

The testable null hypothesis then became:

"There will be no statistically significant difference in periphyton biomass among sites at the 95% confidence level".

The monitoring objective then became:

"To define the effects of downstream intensification of landuse on enrichment of the Kakanui River with particular concern for defining the timing and magnitude of possible periphyton proliferations."

From this monitoring objective we could start to address the specific issues of study planning, as noted above (Section 2.3):

- **Where do we sample?**

Sites were needed in reaches that were all morphologically similar (i.e., similar slope, substrate type, water velocity, degree of shading, etc.) in an undeveloped headwater reach, a moderately developed mid-catchment reach and a highly developed lowland reach. Ideally, to give robust and conclusive answers, it would be best to replicate sites within each of these reaches (this would allow assessment of within reach variability). However, because of budgetary constraints, this was not possible. In each reach we sampled in a riffle and a run in order to identify whether enrichment influenced these habitats differently.

- **How often should we sample?**

We were most interested in summer periods of low flows resulting from abstractions. However, many high country streams in the South Island have periphyton biomass maxima at other times of the year - particularly mid-autumn (Biggs and Close 1989, Biggs *et al.* 1999). Therefore we chose to sample monthly over 2 years. This would allow us to quantify temporal variability, the timing of maximum biomass, and the magnitude of these growths to see how well they correlated with periods of abstraction.

- **What variables should we consider?**

Enrichment effects will be manifested as high amounts of biomass. Samples could be analysed for either ash-free dry mass (AFDM – which measures total organic matter) or chlorophyll *a* (which measures the amount of live algae). Chlorophyll *a* is cheapest, and easiest to relate to existing guidelines. However, AFDM gives a better measure of overall mass and, with an extra step in the analysis, can generate data on siltation of the bed. We chose to carry out both analyses. We also chose to determine the type of periphyton dominating the mats. This is because the species (or taxa) dominating a community provides a good additional indicator of relative levels of enrichment and may become an important qualifier if, for example, invertebrate grazing is quite high (in which case biomass becomes a less useful indicator of enrichment). Habitat variables such as near-bed water velocities, dissolved nutrients in the water, and nutrient concentrations in the periphyton mat were also measured.

- **What sampling method and replication is needed?**

The objective was "To define the effects of downstream intensification of landuse ... on ... possible periphyton proliferations". If restrictions on land development and/or use of the water are to be contemplated, then we need to be sure that changes in biological response down the catchment really are significant and that biomass criteria for proliferations are being consistently broken. Therefore, we chose sampling method that allowed biomass to be

measured as weight per unit area (see Section 6.5). Further, it was also necessary to carry out a statistical comparison of biomass among the sites to test the null hypothesis (that there was no statistically significant difference in biomass among sites at the 95% level), so some degree of replication was required within the sites. The amount of replication needed to reflect the spatial variability of growths. After considering the degree of variability from a preliminary survey, the degree of difference in mean biomass that might be ecologically important among sites (> 50%), and the budget we ended up choosing five samples per transect for each sampling occasion (see Section 3.4 for more guidance on determining required numbers of replicates for different levels of precision).

- **How should we analyse the data?**

We needed to define:

1. changes in biomass as a function of position in the catchment;
2. the timing and magnitude of peak biomass events.

A graph summarising data from one of the sites is given in Fig. 16 of Biggs (2000a). Because the sampling programme had been planned around a testable hypothesis, it was possible to use analysis of variance (ANOVA) to infer whether biomass differed significantly (at the

Table 1: Summary of periphyton statistics from runs and riffles at three sites in the Kakanui River, Otago. Head = headwater reach, Mid = mid-catchment reach, Low = lowland reach. ANOVA - *P* is the probability statistic of a one-way ANOVA to determine whether the mean monthly chlorophyll *a* or AFDM at the sites is significantly different at the 95% confidence interval (*) or the 99% significance level (**). Samples collected at monthly intervals were considered to be independent of one another. N.S. indicates that the difference is not statistically significant at the 95% level. Statistics with the same superscript for a given variable are not significantly different from each other at the 95 % confidence level as determined from a post-hoc Tukey test. %C.V. is the percentage coefficient of variation (i.e., the standard deviation/mean x 100). The analysis was carried out after transforming the raw data to natural logarithms to correct for a non-normal distribution in the data as required by the ANOVA test. Periphyton mean values were calculated as geometric means (from Biggs *et al.* 1998a).

Variable	Head	Mid	Low	ANOVA - *P*
1. Runs				
Mean chlorophyll *a* (mg/m^2)	10.1	17.0	8.6	N.S.
% C.V.	88.7	48.3	83.0	
Maximum chlorophyll *a* (mg/m^2)	143 (May)	64 (Apr.)	104 (Sep.)	
Mean AFDM (g/m^2)	6.2	15.1	5.6	N.S.
% C. V.	84.9	50.5	56.8	
Maximum AFDM (g/m^2)	75 (May)	56 (Apr.)	33 (Sep.)	
2. Riffles				
Mean chlorophyll *a* (mg/m^2)	18.0^1	13.7^1	59^2	**
% C.V.	76.6	31.8	16.6	
Maximum chlorophyll *a* (mg/m^2)	220 (Aug.)	60 (Dec.)	159 (Jan.)	
Mean AFDM (g/m^2)	10.7	14.5	21.6	N.S.
% C. V.	84.9	27.6	17.9	
Maximum AFDM (g/m^2)	67 (Aug.)	33 (Mar.)	47 (Jan.)	

nominated confidence level) down the valley. If sites are replicated for a given landuse, then it is important to carry out a nested ANOVA to enable the among replicate site variability to be assessed against the variability among reaches (landuse treatments). However, this was not applicable in this example because only one site in each headwater, mid-catchment and lowland reach was sampled. A summary of the results is given in Table 1, together with the ANOVA results.

In brief, the results indicated the following.

- There was not a statistically significant difference in mean chlorophyll *a* nor AFDM biomass in the runs moving downstream, however there was a statistically significant difference in the riffles (for an explanation of this see below) which partially supported the hypothesis of increased levels of enrichment down the catchment;
- Maximum chlorophyll *a* concentrations in the runs did not show a clear difference down the catchment, while maximum AFDM did differ statistically down the catchment. Chlorophyll *a* was not greater than levels deemed to be a proliferation at any site and which could lead to the degradation of higher communities such as trout (= 200 mg/m^2 chlorophyll *a* – see guideline in Biggs 2000a). However, AFDM levels were greater than the recommended criteria (= 35 g/m^2 AFDM) at the headwater and mid-catchment sites suggesting a high accumulation of non-photosynthetic organic matter on the bed at these sites. These accumulations occurred in autumn (April and May) and not in summer, as expected.
- Maximum chlorophyll *a* concentrations in the riffles also did not increase downstream. Values at the headwater site and not the lowland site exceeded the proliferation criteria for chlorophyll *a*. This occurred in winter. AFDM in the riffles exceed the biomass criteria at both the headwater and lowland sites, in winter and summer respectively. The variation in timing of maximum chlorophyll *a* and AFDM was unexpected and did not support the contention that artificially reduced summer low flows were enhancing periphyton proliferations.

When interpreting the data for management decisions it is important to take into account the areal extent of the habitat that is being sampled. In the lowland section of the river, whilst high biomass of periphyton occurred in the riffles, they occupied only a small part (~ < 20 %) of the reach. The remaining area was dominated by runs which we found did not have a high biomass of periphyton. The reason for this was high grazing activity by snails. Experiments were carried out with nutrient-diffusing substrates to isolate nutrient and grazing effects (Biggs and Lowe 1994). These experiments clearly indicated that under the low water velocities of the runs the snails were able to build up high densities and graze most of the accumulating biomass production from the stones. However, higher velocities in the riffles prevented snails from accessing the stone surfaces so that they were unable to control periphyton biomass.

2.5.2 Example 2: Removal of a meatworks discharge to the South Branch of the Waimakariri: what were the benefits?

In 1985, Canterbury Frozen Meats introduced a new waste-water management policy in an effort to reduce the impact of their activities on the environment. This policy involved: commissioning an upgraded waste-water treatment facility to remove most of the solids from their effluent; removal of much of the oxidisable organic matter; and a reduction in phosphorus outputs. It was also planned to progressively reduce the amount of waste being discharged to the South Branch of the Waimakariri and eventually divert the entire, more highly treated, waste stream to the much larger main stem of the Waimakariri River.

The South Branch is a spring-fed stream with a mean flow of about 1 m^3/s, few floods, clear waters and a diverse, productive benthic community dominated by macrophytes and periphyton. In contrast the main Waimakariri is a flood prone gravel-bed river with a mean flow of ~50 m^3/s and a relatively depauperate benthic community. The waste was organic in nature and previous field observations had identified proliferations of sewage fungus in the reaches of the South Branch downstream of the discharge. The heterotrophic growths are undesirable because they suffocate the stream bed and can cause deoxygenation of the near-bed waters where the invertebrates live. A progressive reduction in waste volume started in May 1986 with complete diversion from the South Branch by September 1986. The question we had was "what benefits would an improvement in waste quality have on the ecology of the South Branch and what would be the benefits of complete diversion?". We carried this study out as a government-funded research exercise because it provided a good opportunity to test periphyton monitoring methods and the time required for communities to recover. As it turned out, no other studies were commissioned so it provided the only quantitative biological data to determine whether the very large expenditure on improved waste management had any benefits.

After viewing the discharge area, the following hypothesis was developed:

> *"The waste discharge is degrading the benthic communities in the South Branch by causing sewage fungus proliferations. As a result the periphyton communities are changing from being dominated by autotrophic to heterotrophic organisms".*

The monitoring objective then became:

> *"To determine the benefits of removing a treated meat works effluent discharge to the ecology of the South Branch of the Waimakariri River."*

The testable null hypothesis then became:

> *"There is no statistically significant difference at the 95% confidence level in the proportions of autotrophs and heterotrophs in periphyton between sites upstream and downstream of the discharge."*

Because we were forewarned, we had the opportunity to collect data prior to a change in discharge. Thus, we were able to develop a BACI type study design (i.e., sample before any change in management, after the changes, and with upstream control sites and downstream impact sites; see Section 4.2 for more information on this type of monitoring design).

- **Where do we sample?**

Sites needed to be carefully selected so that the physical constraints were identical at both the control and impact sites (i.e., any observed differences in periphyton would be due to water quality, not physical differences between sites). Also, it was essential that the impact site be "after full mixing" of the effluent since most consent conditions will specify this. It would have been preferable to replicate sites but this was not possible because of a major increase in encroachment of riparian willows on the channel immediately upstream of the control reach. Sampling locations were chosen to avoid effects of changes in local hydraulic conditions (velocity and turbulence) and of the effects of patches of macrophytes.

- **How often should we sample?**

At least two samplings before, and two samplings after, the change in management were nominated. Greater numbers of samplings would have been desirable but were not possible because of the timing of the planned waste reduction programme. We were also able to carry out four samplings during the period when the effluent quantity was being reduced.

- **What variables should we consider?**

We needed fully quantitative variables to assess the effects on heterotrophic (sewage fungus) growths vs autotrophic (algal) growths. We also needed quantitative analysis of the relative abundance of different algal taxa and sewage fungus. Therefore, we chose to analyse samples for ash-free dry mass, chlorophyll *a* and the density of cells in different taxonomic groups. We also calculated the autotrophic index (AI). This is the ratio of ash-free dry mass to chlorophyll *a* where high values indicate large amounts of non-photosynthetic organic material compared to live plant material (see Section 7.1).

- **What sampling method and replication is needed?**

Sampling needed to be quantitative. However, inspection of the reaches above and below the discharge point revealed that the local substrate conditions differed between the two reaches. It was therefore necessary to employ artificial substrate samplers in order to remove the influence of differences in bed habitat (see Section 6.6). Three sets of samplers, each with five

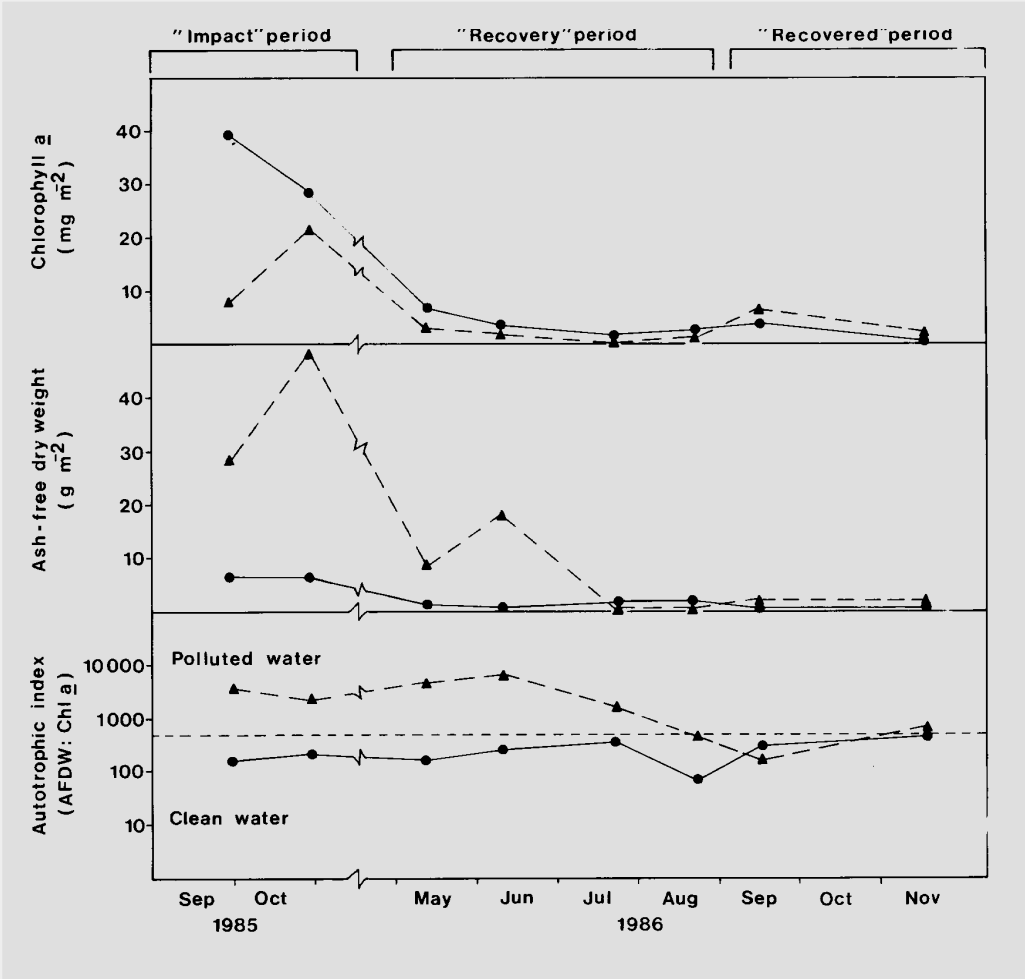


Figure 2: Changes in chlorophyll *a*, AFDM, and the autotrophic index (AI) over time in the South Branch of the Waimakariri River. Periods of full effluent discharge ("Impact" period), reducing discharge ('Recovery period'), and no effluent discharge ("Recovered period") are shown. The fine dashed line on the AI graph denotes a value of 400 which has been suggested by Collins and Weber (1978) as the upper limit for clean waters (•, control site; ▲, impact site) (from Biggs 1989, reproduced with permission of the *New Zealand Journal of Marine and Freshwater Research*).

replicate substrates, were used in each of the reaches above and below the discharge point. The sampling plates were loaded vertically in the sampler rack to avoid siltation. Previous analysis (Biggs 1988a) had identified that 28 replicates per sampler would be needed to estimate mean biomass to within ±20%. However, this was not possible because of budgetary constraints. So five replicates were collected from each of three samplers, giving quite a low level of precision per sampler, but improving to around 25 % with a pooling of the results within a site once the blocking effect of the different samplers was accounted for in the ANOVA (see Section 3.4 for more guidance on determining required numbers of replicates for different levels of precision in impact testing).

- **How should we analyse the data?**

Two main approaches were used:

1. ANOVA of differences between sites;
2. analysis of trends over time.

Prior to the improved waste treatment, the most abundant taxa at the control site were unicellular diatoms that indicated reasonably clean or slightly enriched waters whereas at the downstream impact site the communities were dominated with “sewage fungus”. This resulted in statistically significantly different AI between the control and impact sites. These differences can be clearly seen in Figure 2.

With a reduction in effluent volume over a four-month period there was a decrease in quantity of sewage fungus in the mat at the impact site and an associated reduction in the AI. Within a month of complete diversion of the effluent, ANOVA indicated that there were no statistically significant differences in the AI between control and impact sites.

2.6 Reference sites

Reference sites are sites located in areas that are not clearly impacted by human activity. Possible locations for these can be identified through the process of physical habitat classification or local knowledge. Ideally, reference sites as well as impacted sites should be included in all long-term monitoring programmes (e.g., State of the Environment). Reference sites are essential if the objective is to determine the effects of environmental changes brought about by a specific activity that could introduce non-point source contaminants, such as forestry or horticultural developments. This is because broad-scale changes may also be occurring as a result of, say, climate change. It is then necessary to separate the effects of changes in management from such broad-scale influences.

Reference sites should not be confused with “control” sites. Control sites are sites upstream of a specific perturbation that are paired with a downstream “impact” site and are used to define the effects of that perturbation regardless of what the upstream conditions are. While reference sites might serve the needs of a control site (depending on where in the catchment the analysis is located), the reverse need not be true.

In choosing reference sites, it is important to define the habitat type being represented. This will usually only need to be done at quite a high level of the hierarchy of controlling variables in a region, and for controllers that are largely unaffected by human activity, plus the local native vegetation type. So for example, in Hawke's Bay we might define a reference site for hill country streams with predominantly greywacke geology at a location near the head of the catchment where the land cover is predominantly beech and broadleaf forest. We may identify another reference site in a hill country stream with predominantly soft, Tertiary mudstone and a native bush upper catchment to represent the natural background setting/

conditions in Tertiary hill-country streams of the region. These sites are then used as the reference points against which data from downstream, impacted, reaches are compared. These sites may also serve as reference points for other streams / catchments in a region that share the same base classification of source of flow and geology. Ideally, one should attempt to sample at least three reference sites on several occasions for a given habitat class to gauge the variability among them before settling on using one for long-term monitoring.

It should be recognised that it may not be possible to obtain reference sites for some major habitat types in many regions. For example, finding a lowland stream with a predominantly forest catchment upstream of a given point is now very difficult. A regional river environment classification will assist in defining the availability / rarity of such habitats (see Snelder *et al.* 1998).

2.7 Maximising information for the available budget

In developing a sampling programme, there will always be a trade-off between the detail required and the resources available. This problem arises because of the amount of variability in nature and the scale of the changes or impacts that you usually need to define for management of streams. These considerations impinge heavily on replication and the degree of statistical precision (i.e., degrees of freedom) required for the analysis (this is discussed more in Section 3). There are always budget limits, and there will always be compromises. The compromises should be based on prior knowledge and the requirements of each study. The limits to compromise also need to be set from this prior knowledge. The limit is the point at which the data collected are unable to satisfactorily resolve the issue(s) under consideration.

In the past, we have often compromised detail in our investigations in the following order (i.e., first to last):

1. analytical detail such as the number and type of parameters (this should not be confused with analytical precision which relates to how well an analysis is performed);
2. analytical replication;
3. sampling replication in space;
4. sampling replication in time.

These reflect a hierarchy of increasing sources of variability in periphyton data. In other words, periphyton communities are most variable in time (periods of weeks to months), followed by being quite variable across the bed of a stream within a given reach or even valley segment, followed by being less variable among analytical replicates. Finally, the type and detail of the analysis can add a huge amount to the cost of a study (perhaps doubling it), but for only a marginal increase in quality of information. Unless that increase in quality of information could become vital at a later stage, then save your resources and only analyse for the variables that are absolutely necessary. There are a number of ways to optimise the quality of the data, whilst reducing the cost of a programme. A real example of such a series of compromises to fit a relatively small budget is as follows. This was to enable the study in Example 1 (Section 2.5.1) to be carried out (from Biggs *et al.* 1998a):

Study objective:

To define the effects of downstream intensification of landuse on periphyton communities in the Kakanui River catchment, North Otago, with particular concern for defining the timing and magnitude of possible proliferations.

What we most needed to know (arranged in decreasing order of priority):

- average monthly periphyton biomass over a year;
- maximum biomass (to see if it breached proliferation criteria);
- what taxa dominated peak biomass;
- variability / gradients in community biomass / composition within the catchment;
- overriding effects of other perturbations / disturbances (e.g., invertebrate grazing and floods).

Sampling regime:

In order to satisfy the above priorities, while staying within the budget, we decided on a periphyton sampling and analysis programme as follows.

- Monthly sampling over two years at three sites representing the three main valley segments in the catchment (headwaters segment, mid-catchment confined segment, lowland segment) with sites located near water-level recorder sites;
- Five replicate samples scraped from a set area on the tops of stones along a transect across a riffle (see Section 6.5.5). These replicates were then pooled into one sample per sampling date rather than being kept separate for individual analysis. Another five replicates were then collected in the same way from runs. This protocol was repeated at each site on every visit.
- In the laboratory, each pooled sample was thoroughly blended and then three aliquots (sub-samples) of suspension were removed and pooled for analysis of one sub-sample for biomass. A further pooled sub-sample was retained and frozen. Once the sampling programme and all biomass analyses were complete, then sampling occasions when peak biomass occurred were analysed for taxonomic composition using a rapid assessment method to define relative abundance (see Section 8.3).

Sampling regime and budgetary compromises and efficiencies:

- We sampled for two years because the catchment was known to be prone to major inter-annual variations in flow regime. A single-year sampling programme would not enable the average conditions of the river to be characterised, nor the effects of meso-scale variations in climatic regimes. A longer time period would have been preferred to establish year-to-year variability; two years is the minimum time required to start to define inter-annual variability.
- Monthly sampling was carried out as part of the standard hydrological / water-level recorder site visits to the river by the Otago Regional Council. This meant that specific field visits by our team (including the down-time of travel from Christchurch to the Kakanui River, 4 hours each way) was not required. Minimal new resources were therefore necessary to carry out the sampling. It would have been good to have extra sites in order to determine variability within each section of the river, but these could not be accommodated within the budget. A once-off, more detailed synoptic survey of 8 sites down the catchment was carried out during summer low flows to help define downstream variations in biomass and composition.
- Only five samples were collected which would have give means with quite high variability, but we expected to be able to confidently detect differences of > 50% with 95% confidence (using formulae as defined in Section 3.4). A much lower error

would have been achieved had we collected 10 samples per habitat (a standard error of ~ 10–20% of the mean, depending on how patchy the distribution of the periphyton was). However, we needed to sample both riffles and runs (as opposed to just runs, which is our normal sampling habitat) because proliferations had been observed in the runs in headwater reaches and riffles in downstream reaches. The time required to collect 20 samples per site (10 from the riffle and 10 from the run) would have meant that the field team could not complete the sampling and all their other tasks within daylight hours in winter. Another consideration in the decision was that the time-scale of the programme was long (2 years) and so we expected that the size of the temporal fluctuations in biomass would have been much greater than the errors associated with individual measurements (2–20 times greater).

- The decision to pool the five replicate samples was to enable analytical costs to be greatly reduced (to about 30% of what they would have been). We lost the ability to test for significance of difference between habitats and sites on any given sampling occasion, or to test the effects of specific perturbations (e.g., the effect of a given flood event). However, the primary objective was to identify differences among sites over at least a year and the magnitude and timing of biomass peaks. This was the primary scale of our objective and thus we didn't believe that pooling of replicates would compromise this objective greatly. For example, the single values for each month resulted in 12 values for a given habitat over a year. This provided the replication required to test for differences among sites in mean monthly biomass (i.e., a comparison of the average of the monthly measurements at each site). These data were also suitable for testing differences among years for a given site.
- Analysis of individual aliquots would have enabled the error in sub-sampling + analysis to be assessed. However, previous studies have shown that the standard deviation was in the order of only 12–15% of the mean (Biggs 1987), so pooling sub-samples reduced analytical costs greatly. It was not anticipated that the data from analytical replication would be used. The samples were thoroughly blended before sub-sampling because periphyton tend to aggregate into clumps, which can result in very large sub-sampling error and the necessity for analysing many more replicates (see Section 7.1). Every taxonomic analysis is quite time consuming. By only analysing samples from specific times of interest (i.e., times of high biomass), it was possible to also greatly reduce the expense of this part of the study.

3 Determining data precision and number of sampling replicates required

3.1 Background

In any quantification of the environment, we are attempting to take a representative "snap-shot" of the conditions present. In other words, we analyse a sub-set of the overall conditions or populations and make inferences about the way the whole population or community works from this sample. The degree to which our "sample" represents the "whole" of what we are interested in is a function of the number of samples we take in relation to the degree of variability (or patchiness) of communities or populations.

To illustrate ways to deal with variability we can use a simple hypothetical example based on a well-known terrestrial "habitat": a golf course.

Suppose we are presented with the question: "what is the average biomass of plant communities on the putting green around the 7th hole?" To determine this we might retrieve three replicate samples from different parts of the putting green (e.g., as a core down through the grass to a depth of ~5 cm). Because putting greens are generally created and maintained to be very uniform, the three samples might give us data on the mean biomass of vegetation (grass) that is very close to the true average for the whole putting green (e.g., the sample mean might be within, say, 10% of the true mean).

However, the question might become: "what is the average biomass of vegetation on all 18 putting greens of the golf course?" In this situation, the results from the 7th green would probably be a poor representation of all the greens. It would then be necessary to randomly select (i.e., using a set of random number tables) several of the greens to sample. If the greens were all being maintained in a similar way and had very similar vegetation cover, then a set of three samples from each of three greens might represent the full population of greens very well. However, if for some reason (e.g., soil type, irrigation regime etc) there was considerable variability in grass growth among greens then a sample of three greens would give a poor representation of average green vegetation biomass.

At an even higher spatial scale, the question might become: "what is the average biomass of vegetation for the golf course?" Clearly, if we were to take three randomly located samples of vegetation from the whole golf course, we would obtain a mean that is likely to be very different from the true mean because of an increase in the degree of variability scaling up from the case of a single green to the whole course. One sample might be located in a bunker, another on a green and yet another in the rough. What about the stands of trees that line some areas and the extensive areas in fairways? In essence, how many random samples would we need to represent the mean biomass of vegetation for a high level of precision in such a heterogeneous habitat? This is the sort of problem we are continually confronted with when sampling stream periphyton.

In the following sections we discuss several measures of variability, illustrate some important concepts with regard to variability and the effects of sample number, and give guidance on how to estimate the number of samples needed for determining the mean of a variable for any pre-determined level of precision. Different approaches are required for estimating sample numbers in different types of studies and tests. Here we cover estimating sample numbers for: (1) general resource surveys; (2) testing for differences in the means of variables between two sites / populations; and (3) testing for differences between the mean level of a variable and a guideline or standard. Finally, we describe how to stratify sampling according to

physically distinct habitat units (e.g., greens vs. bunkers vs. patches of trees, etc.) in order to make more effective use of resources and increase information content about the habitats.

3.2 Measures of variability

The standard error of the mean is generally used as a measure of how reliable the sample mean may be when it is used to estimate the true population mean (note that the standard error is not a measure of variability – the standard deviation does this). Indeed, when presenting summary plots of the data, it is standard practice to include standard error bars on mean values to show the precision of our estimate of that mean (i.e., an indication of how precisely we have quantified the population mean). The standard error of the mean (*SEMx*) is calculated as the standard deviation of the data (*s*), divided by square root of the number of samples taken (*n*). In symbols, this is:

$$SEM_{\bar{x}} = \frac{s}{\sqrt{n}} \qquad (1)$$

For most periphyton assessments we wish to be able to state that:

"the mean abundance or biomass is $\bar{x} \pm y$ *numbers of cells or biomass/m*^{2}*".*

In practice, we have to accept that the data we use to estimate the mean are "normally" distributed about the true (and unknown) value so that no matter how large *y* is, there is a chance that our estimates could be further away from the true mean than we have stated. It is common to accept a 5% chance of being wrong and this is achieved if *y* is approximately twice the standard error of our estimate of the mean. In other words, there is a 95% chance that the true mean will be within two standard errors of our sample mean. The standard error of our estimate of the true mean can be made as small as we like by taking more replicate samples. That is, the more we sample from an area, the better we average out the variability. We discuss this more below.

It is the 95% confidence intervals (C.I.) around the sample mean that are the most useful statistics for interpreting whether sets of samples from different populations are statistically significantly different. These are easily calculated from the standard error of the mean using Student's *t*-statistics as follows:

$$\bar{x} \pm (t_{\alpha(2),\, n\text{-}1}) \times SEM_{\bar{x}} \qquad (2)$$

where the *t*-statistic is obtained from tables of Student's *t*-values by selecting the required % chance of being wrong (α = 0.05, in this case), whether the test is one-tailed or two-tailed (indicated by the subscript (1) or (2) – see page 23), and also the number of degrees of freedom (indicated in the tables by ν, where $\nu = n - 1$ degrees of freedom). (See, for example, Appendix Table B.3 of Zar 1996). If the 95% C.I. for two sets of samples do not overlap (e.g., when the means and C.I.s of the two samples are represented together on a bar graph), then the samples are considered to be statistically significantly different with a 95% probability that this conclusion is correct (i.e., $P < 0.05$ for the test).

3.3 Estimating the number of replicate samples needed for general resource surveys

For general surveys, we can use the standard deviation of the data to tell us how many samples (*n*) we might need to collect to achieve a pre-selected precision (after Zar 1996, p. 107). We would use preliminary survey data to calculate the initial standard deviation (as a measure of the variability), or data from a similar site collected previously. We then enter this data into the following equation:

$$n = \frac{s^2 \; t^2}{d^2} \qquad (3)$$

where s is the standard deviation of the preliminary data, $t = t_{a(2)}, n_{-1}$ the two-tailed critical value of the Student's t distribution with $v = n - 1$ degrees of freedom and $\alpha = 0.05$ (found in Appendix Table B.3 of Zar 1996), and d is a pre-selected half-width of the desired confidence interval of the sample mean. [Note: this formula corrects that given in Zar (1984) which is incorrect (G.B. McBride, NIWA pers. comm.)].

The degrees of freedom for t are not known because n is unknown, so it is necessary to iterate Equation (3) to obtain a solution. The half-width of the pre-selected precision, d, is set based on the objective of the study and practical or resource limitations (e.g., we may wish the confidence interval around a mean to be ±30% of the mean so $d = 0.3/2$ x the mean = 0.15 x the mean). As we discuss further below, as the level of precision increases more samples need to be collected and processed. This is because with a doubling of precision, the required number of samples increases not 2-fold, but 4-fold (i.e., to the power of two). The balance that needs to be struck between being over-precise and under-precise, will be discussed further below.

The above assessment assumes that the preliminary data for assessing variance are drawn from a population with a statistical distribution that is approximately "normal". If the data are very skewed to the right (as is often the case for periphyton measurements) then a logarithmic transformation can be used to normalise the data and the calculations done using the standard deviation of the logs. However, for very skewed data, the mean is a very poor representation of central tendency and it may, therefore, be better to use the median. Procedures for assessing the number of samples required for calculating medians with a given level of precision are given in texts such as Gilbert (1987, p. 174, eq. 13.23).

Let us briefly work through an example. Suppose we want to know the mean chlorophyll *a* concentration of periphyton in a run of a foothills stream (we could be interested in the extent of enrichment from agricultural activities in a catchment). We might want to estimate this mean biomass with a precision that enables the sample mean to lie within a 95% C.I. that is ±20% of the mean (i.e., the sample mean is to be within ± 20% of the population mean at $p \oplus 0.05$). How many samples would be required? Preliminary survey data of chlorophyll *a* from one reach were tested and found to be approximately normally distributed. The mean of these data was 267.5 mg / m^2 chlorophyll *a* (n = 10), with a standard deviation of 86.4, and d = 0.2 x 267.5 = 53.5. We then need to start the process of iteration by guessing the number of samples that might be required and then using this as a basis to select a critical value for the t distribution. We start by guessing that 15 samples would be required (for the iterations it is better to initially overestimate the number required). Therefore, the critical value for the t distribution is $t_{0.05,\,15-1} = 2.145$. Inserting our values for s, t and d in equation (3) and completing the calculation we therefore have:

$$n = s^2 t^2 \,/\, d^2 = 86.4^2 \times 2.145^2 / 53.5^2 = 12.00 \text{ samples}$$

We then iterate the equation again to see if we can get close to 12 by using a smaller starting value than 15. If we use 12 samples as the starting point we insert a critical value for the t distribution for n = 12 – 1 degrees of freedom (= 2.201). This iteration then gives an estimate of 12.6 samples required. This is close enough to the first estimate of 12 to conclude that we probably need 12–13 samples to enable us to be 95% confident that our sample mean will be within ± 20% of the population mean.

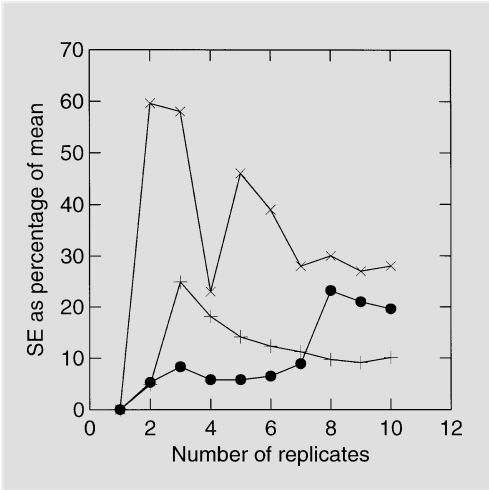


Figure 3: Effect of increasing number of sample replicates on the standard error of the mean (*SEM*) (normalised to its respective means) for chlorophyll *a* of diatom dominated communities on cobbles at three sites. +, mean chlorophyll *a* with 10 samples = 268 mg/m^2; X, mean chlorophyll *a* = 64 mg/m^2; •, mean chlorophyll *a* = 4 mg/m^2.

Such assessments can be easily carried out for any parameters. However, in pre-selecting precision there is a trade-off between the precision of estimating the population mean (which influences the chance of incorrect conclusions) and resource availability for the study.

Let's take a closer look at the effect of the number of replicate samples on how precisely we can estimate the mean. We will consider biomass and then species richness (i.e., total number of species encountered) because analysis of variability for these requires different approaches.

In Figure 3 we plot the *SEM*s (as a percentage of each mean) for chlorophyll *a* as a function of increasing replication. The samples are from low, medium, and high biomass diatom dominated communities. These data show several important patterns. First, with few samples for the medium and high biomass communities the variability in the data tends to be high, and thus the standard error of our estimate of the mean is high relative to the mean. However, with increasing numbers of replicates the error decreases, then stabilises (at ~ 7–8 replicates).

Second, precision is lowest (i.e., the *SEM* is highest relative to the mean), and more variable, for the medium biomass community than for both high and low biomass. With medium biomass, periphyton cover is usually more patchy and we often see stones with large tufts of green filaments in reaches that otherwise only have mats of diatoms (see Figure 6 and Figure 28 in Biggs 2000a). Once a tuft of filamentous green algae is encountered, variability suddenly increases (e.g., at 5 replicates for the medium biomass community, Figure 3). This variability in the *SEM* does not tend to occur as much with high biomass communities (particularly of diatoms), because the distribution of organic matter at such times has generally become more uniform across the stream bed. Thus, variability peaks at 3 replicates and then decays with increasing sample replication.

Third, with low biomass communities, variability tends to be very low because cover is also usually quite uniform. However, as with the medium biomass community, there is an increased chance of encountering higher biomass tufts with more sampling such as occurred for replicate 8. Such tufts may have developed from relict communities that survived the last flood disturbance, or they may be near some localised enrichment (e.g., from groundwater sources). The analysis of changes in standard errors as a function of numbers of replicates can also be carried out on cell count data.

A different approach is normally used for determining “species richness” in a population (NB: we use the term “species” liberally here because many of the identifications can only be made to the generic level with periphyton, but “species richness” is the accepted terminology in other fields of ecology). When species richness is an important consideration, we are

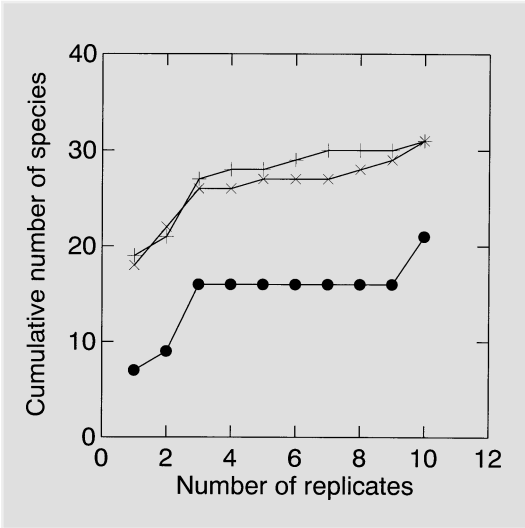


Figure 4: Effect of increasing number of samples on total species richness of periphyton samples at three sites in a cobble bed river. +, mean chlorophyll *a* with 10 samples = 69.3 mg/m^2; x, mean chlorophyll *a* = 45.0 mg/m^2; •, mean chlorophyll *a* = 2.1 mg/m^2.

generally interested in knowing what the total species pool might be (e.g., for assessing biodiversity values or the effect of, say, landuse on community composition). We therefore assess cumulative richness as a function of increasing number of replicate samples in our power analysis. As an example, we have summarised the cumulative number of species as a function of cumulative replicates for medium and low biomass communities in Figure 4. These illustrate two common features. First, low biomass communities tend to have fewer species because succession has not usually proceeded far enough for a more complex community to develop. Second, low biomass communities tend to be more stable in their species richness unless, as occurred in the example for replicate 10, a much higher biomass "patch" is encountered. With medium (and high) biomass communities, there is a rapid increase in recorded richness to 3 replicate samples, after which the increase slows. However, it does still trend upward and generally levels off at about 7–10 replicates.

It is often worthwhile to check the variability of sample values for resource surveys using the approaches described above. Consistent redundancy in the information collected can justify reducing sampling effort. Conversely, with high variability, more sampling effort may be required.

3.4 Estimating the number of replicate samples required for testing hypotheses of differences in two population means for periphyton biomonitoring and consent evaluation

There are situations where we may be required to test differences between two population means. An example is looking for the relative differences in some variable between two sites (e.g., impact vs. control or reference sites) as part of a biological monitoring programme. For this, we might need to set a level of difference between the two sites that should not be breached (e.g., as part of a consent condition). The study will then set out to establish whether a breach of the specific condition is occurring. In this case we need to determine how many samples are required from both sites at the nominated level of difference to ensure a low probability of being wrong. Assessing differences where there are gross disturbances or differences is a trivial procedure. However, if we are to distinguish more subtle differences then we need to be sure that we have collected enough samples so that our error does not swamp the differences we are trying to detect. For example, it might be that the biomass of the periphyton mat needs to change by >30% (measured as chlorophyll *a*) compared with the

control site before the consent has been breached (i.e., any change of less than 30% might be considered ecologically unimportant because of natural spatial or temporal variability). The formula for such estimates (from Zar 1996, p.133) is:

$$n \geq \frac{2s_p^2}{\delta^2}(t_{\alpha(2),\nu} + t_{\beta(1),\nu})^2 \qquad (4)$$

where n is the size of the sample from each site (or at one site over time), δ is the minimum difference in populations that we wish to detect ($\delta = \mu_1 - \mu_2$ for the two-sample t-test of the hypothesis, where μ indicates a population mean), sp is the pooled within-population standard deviation determined from previous data, $t_{\alpha(2),\nu}$ is the critical value for the t-distribution for $\nu = 2(n-1)$ degrees of freedom and with α, the nominated significance level, usually set at 0.05 (or perhaps 0.01 or 0.001 for really stringent tests). We can set the significance level of the test, α, to anything we want. However, $p = 0.05$ is a generally accepted limit and tells us that we have less than a 5% chance of making a Type I error (i.e., falsely rejecting the null hypothesis that there is no difference between the two population means). β is the probability of committing a Type II error (i.e., erroneously concluding that there is no difference between our populations) and $1 - \beta$ is the power of the test to detect a difference between population means. Values for $1 - \beta$ may be set at 0.8, 0.9, 0.95, etc. depending on what are considered to be ecologically important differences in population means, and budgetary constraints. High values require larger sample sizes. The value of $t_{\beta(1),\nu}$ is related to the probability that the desired exceedence will be detected (see Table B.3, Appendix B of Zar 1996 for the t values).

Because n is on both sides of the equation (the degrees of freedom depend on n), the solution can be found only by trial and error. However, this is usually done in only 2 or 3 iterations. Note that this is a "two-tailed" statistical analysis. In other words no assumption is being made of whether the change is up or down, or that a specific criterion is going to be exceeded. If the latter circumstances prevail, then a "one-tailed" test is required (see below). Also, the analysis assumes that the samples are from two populations with approximately normal distributions. If the data are strongly skewed to the right, transformation of the preliminary data (e.g., to logarithms) will be required before calculating the variance for the analysis .

Let us work through an example. The data are from the patchy "intermediate" biomass community used in Figure 3. Suppose that these data are representative of an upstream control site and there is a discharge of treated dairy shed effluent that is moderately enriched with phosphorus just downstream of the site. We set up a physically identical "impact" site in a reach after full mixing of the effluent (say 0.5 km downstream of the discharge). We need to assess, using a once-off survey during worst-case conditions during summer low flows, whether the conditions for the discharge developed as part of the consent procedures under the Resource Management Act have been broken. The consent is defined as:

> "there shall not be more than a 30% change in chlorophyll *a* between upstream and downstream sites (after full mixing) at any time over the summer months as a result of the discharge".

While we would expect that a discharge of treated dairy shed effluent would result in nutrient enrichment, and therefore an increase in periphyton biomass, we can never be sure of this. For example, in some situations higher levels of suspended solids may restrict periphyton production. We therefore want to test for *any* change in chlorophyll *a* (a "two-tailed" test) rather than testing for a specific level of increase in biomass (a "one-tailed" test). To do this we need to sample the impact and control sites with sufficient precision that we could detect a difference of ±30%. We would normally nominate a level of significance (α) of 0.05 for the

test, with a power $(1 - \beta)$ of say 0.90 (i.e., $\beta = 0.1$). Our preliminary study determined that the *arithmetic* mean biomass at the control site was 63.9 and the within-population variance (i.e., s^2) was 2865. However, the test assumes that the samples are drawn from two approximately normally distributed populations and a quick plot of the density distribution of the raw data identified that they were right skewed. We therefore require a logarithmic transformation of the data before determining sample size (indeed such a transformation would also be required before carrying out any parametric statistical tests such as *t*-tests or analysis of variance (ANOVA)). The mean of the logged data (using natural logs) was then 3.8898 and s^2 of the logged data was 1.7122. The antilog of the mean of the logarithms is the *geometric* mean, and this is exp(3.8898) = 48.90. (Note that the geometric mean is close to the median of the untransformed data.) Thus, the change to be detected (δ, best called the "minimum detectable difference") with the desired power is $\delta = 0.3 \times 48.9 = 14.67$, and in terms of logarithms, the difference is $\ln(\delta) = 2.686$. We need to guess what the sample size, *n*, might be so we will start with $n = 10$. Therefore, the critical value for significance level of 0.05 on the *t* distribution is calculated based on $2(10 - 1) = 18$ degrees of freedom, so for a two-sided test $t_{0.05(2),18} = 2.101$. The critical value of β, the probability of committing a Type II error, is determined for a one-sided test as $t_{0.1(1),\,18} = 1.330$. We then insert these numbers in equation (4) above:

$$n \geq \frac{2\,(1.7122)}{(2.686)^2}\,(2.101 + 1.330)^2 = 5.60$$

We now iterate the calculation again using a number near the result of the first iteration to see how close the next result is to the first. Say we try with $n = 6$:

$$n \geq \frac{2\,(1.7122)}{(2.686)^2}\,(2.225 + 1.372)^2 = 6.14$$

We therefore conclude that we need at least 6 samples per site to be able to detect a 30% change in chlorophyll *a* between the two sites with a power of 90%.

A few general, perhaps obvious, comments are warranted here. First, as noted in Section 3.3, the smaller the difference that we want to detect, the larger the number of samples we will need. Thus, in the above example, if we wanted to detect a difference of just 10% instead of 30% then we would need 16 samples per site. Second, the number of samples also increases greatly with increased variability, or patchiness, of the community. In the example there was a relatively low variability once the data had been log transformed. Many sites will have communities with considerably more variation. Third, if we desire a high probability of detecting a difference between population means (i.e., having a low probability of committing a Type II error), the power of the test, $1 - \beta$, needs to be relatively large which will, in turn, result in a larger sample size.

3.5 Estimating number of replicate samples required for testing hypotheses regarding compliance with periphyton cover or biomass guidelines

Another common situation to arise is monitoring to determine whether a breach of a specific guideline or standard on a consent might have occurred (e.g., for cover of filamentous algae or biomass). In such situations it is advisable to try to estimate the mean with as much precision as possible because the consequences of an incorrect assessment could be major for the Resource Consent holder or the environment. In other words, one could easily accept that there is a difference between the sample mean and the guideline value when there really isn't

(a Type I error in statistical terms), or that there isn't a difference between the sample mean and the guideline value when there really is (a Type II error). Both these scenarios can be problematical, the first might result in unnecessary restrictions for a water user (or prosecution) and the second might result in under-protection for the environment. This analysis is very similar to that described in the preceding section. However, the equation is slightly different because our interest lies in whether a given level for a variable is exceeded and not the simpler case of whether a significant change in any direction had occurred. Thus, we must use a "one-tailed" or ("one-sided") test. Zar (1996, p. 108) defines the following equation:

$$n \geq \frac{sp^2}{\delta^2} (t_{\alpha(1),\nu} + t_{\beta(1),\nu})^2 \qquad (5)$$

where n is the size of the sample for the site, δ is the minimum difference in population that we wish to detect, s is the within-population standard deviation determined from previous data, $t_{\alpha(1),\nu}$ is the critical t-value at the nominated significance level for the test (α) at $\nu = n - 1$ degrees of freedom (the significance level is usually set at 0.05, but could be 0.01 or 0.001 for really stringent tests). β is the probability of committing a Type II error (i.e., erroneously concluding that there is no difference between our populations) and $1 - \beta$ is the power of the test to detect a difference between population means. Values for $1 - \beta$ may be set at 0.8, 0.9, 0.95, etc. depending on what is considered to be an ecologically important difference in population from the guideline value and budgetary constraints. High values require larger sample sizes. The value of $t_{\beta(1),\nu}$ is related to the probability that the desired exceedence will be detected (see Table B.3, Appendix B of Zar 1996 for the t values).

As with Equation (4), n is on both sides of the equation (the degrees of freedom depend on n), so the solution can be found only by trial and error. However, this is usually done in only 2 or 3 iterations. As noted above, this is a "one-tailed" statistical analysis. In other words we are testing whether our population is *greater* than a hypothesised (or guideline) value. Also, the analysis assumes that the samples are from a population with approximately normal distributions. If the data are strongly skewed to the right a transformation of the preliminary data (e.g., to logarithms) will be required before calculating the variance for the analysis.

We will now work through an example, again using the data from Figure 3. Suppose we want to detect an exceedance of the 50 mg / m^2 chlorophyll *a* biomass guideline for the protection of natural biodiversity values in streams (Biggs 2000a) and the minimum ecologically important exceedance of this value that we are interested in is >30%. The mean of the logged data was then 3.8898 and s^2 of the logged data was 1.7122. Thus, a change in biomass of ±~30% of the mean at the control site would equate to needing to detect a difference in the geometric mean of the data of δ = 48.9 x 0.3 = 14.67, and ln(δ) = 2.686, which replaces δ in Equation (5). We need to guess what the sample size, n, might be so we will start with n = 10. The critical value for significance level of 0.05 on the t distribution is calculated based on n = 10 – 1 = 9 degrees of freedom, so for a one-sided test $t_{0.05(1),9}$ = 1.833. Also, with β = 0.1 and ν = 9, the critical value of $t_{\beta(1),\nu}$ = 1.383. We then insert these numbers in Equation (5) above:

$$n \geq \frac{(1.7122)}{(2.686)^2} (1.833 + 1.383)^2 = 2.45$$

We then iterate the equation again by starting with n = 3 and determining new critical values on the t distribution for α and β based on 3 – 1 degrees of freedom for the one-sided test. This gave an estimated number of samples of 5.5, and repetitions lead to alternative values of 3

and 6. We would therefore settle on a sample size of 6 as the minimum required to fulfil the design criterion of being able to detect a 15 mg / m^2 (i.e., 30%) exceedance of the 50 mg / m^2 chlorophyll *a* guideline at the given site.

3.6 Stratifying sampling effort to increase precision and reduce effort

In highly heterogeneous environments, or with very patchy communities, there are simple ways to focus sampling effort to give more cost-effective results (i.e., maximising precision for a given cost). This is carried out by dividing an area or reach into several more-or-less homogeneous strata. This is also useful because the assumption that is the basis of random sampling – that features vary randomly in time and / or space – is not always true. If we partition our sampling effort by strata, it allows us to increase the precision of population estimates and to "partition variance". Let us return to the hypothetical example of assessing the mean biomass of the golf course, introduced in Section 3.1 above. Rather than take many samples randomly over the golf course, we would be better off to identify physically distinct sub-units (or strata), characterise these, and then determine their areal extent. These sub-units might comprise the greens, fairways, bunkers and "the rough". The parallel for stream periphyton sampling might be to divide a reach of interest into areas of pools, riffles and runs or based on substrate type such as bedrock vs. sands and silts vs cobbles. At a larger scale, dividing streams up according to source of flow and then local geology and landuse, as done using stream classification, is another form of stratifying stream systems. This type of stratified sampling is usually done when carrying out general resource assessments rather than testing specific hypotheses.

First, we need to define the areal extent of the habitats and decide how many samples are required meet our monitoring requirements. We then allocate the sampling effort in proportion to the contribution of each area to the overall study area or catchment. The actual areas to be sampled within each stratum are then located randomly. However, if the variance of the variable is known within each stratum, then the optimum allocation of sampling effort is to allocate samples in relation to variance within strata (Snedecor and Cochran 1980):

$$n_i = \frac{N_i s_i}{\sum_{i=1}^{k} N_i s_i} \qquad (6)$$

where n_i is the number of samples in stratum *i*, N_i is the total number of sampling units available in the *i*th stratum, s_i is the standard deviation of the *i*th stratum.

Stratified samples have an overall mean calculated as:

$$\bar{x} = \frac{\sum_{i=1}^{k} n_i \bar{x}_i}{\sum_{i=1}^{k} n_i} = \frac{n_i\bar{x}_i + n_2\bar{x}_2 \ldots + n_k\bar{x}_k}{n} \qquad (7)$$

where $\bar{x}$ is the overall sample mean, *n* is the total number of samples and the other samples are as defined above.

The sample variance of the overall mean is calculated as follows:

$$s^2_{\bar{x}} = \sum_{i=1}^{k} \left[\frac{N_i}{N}\right]^2 \left[\frac{s_i{}^2}{n_i^2}\right]\left[1 - \frac{n_i}{N_i}\right] \qquad (8)$$

where s_i^2 is the sample variance for *i*th stratum for a random sample, N_i, n_i and *N* are the total number of sampling units available in the ith stratum, the number of sampling units

sampled in the *i*th stratum, and the total number of sampling units in all strata. The standard deviation of the overall mean is simply calculated as the square root of overall sample variance.

3.7 Estimating the number of replicate samples required and level of differences in testing effects: a cautionary note

There are several issues relating to application of the above equations for estimating sample size and required precision that require some cautions. First, the equations give only a rough approximation of the number of samples needed to achieve prescribed levels of precision. Periphyton have high spatial and temporal variability, so a preliminary estimate of the population variance at one point in time may not result in a very accurate estimate of number of samples in other locations or at other points in time. In particular, if there has been flood disturbance since a preliminary sample was obtained, then the degree of variability is likely to be quite different. In many situations, it may not be possible to collect preliminary samples or there may be doubt about the transferability of data from another site. In such situations, our recommendation is to collect at least 10 replicate samples which will normally give quite a high level of precision. Indeed, this has become our standard number of replicates per site because we are rarely afforded the luxury of preliminary sampling.

Second, as shown in the preceding section and Figure 3, it is possible to narrow down the error of estimates by taking more replicate samples. In doing this, it might then be possible to discriminate statistically significant, but very small (and possibly trivial) differences in periphyton communities among sites (e.g., control vs. possible impact sites). Very small differences if placed in the context of natural variability of the communities in both space and time may have little meaning for the ecosystem as a whole. Thus, we need to ensure that we give adequate thought to the fundamental question: "what differences are likely to be ecologically important?" There is further discussion on this by G.B. McBride in Appendix 1.

There are no set rules for determining what the most ecologically important differences in space or time might be for periphyton communities, which may then be used as a basis for assessing impacts or breaches of guidelines. It will vary with variables and habitats in relation to how sensitive a particular set of wider ecosystem processes might be to the variable in question.

For example, a 20% breach of the 200 mg/m^2 chlorophyll *a* guideline for the protection of trout and fishing values could be unacceptable during summer low flows because of the low capacity of the waters at high temperature to hold oxygen, periphyton respiration rates are high (respiration rates tends to double with every 10^oC increase in temperature), and lower volumes of water to buffer the system from periphyton mediated diurnal fluctuations in dissolved oxygen. However, such a breach may be of little or no consequence in winter when water temperatures are much lower, periphyton respiration activity is lower, and river flows are higher (unless the area of concern is a prime spawning habitat as well as fish rearing location).

Thus, determining whether a difference in a particular variable is of much management consequence must be done with some common sense, consultation with river managers and other end-users, knowledge of the interdependence of different communities or levels of the food chain, and knowledge of the natural variability of communities.

4 Specific considerations in planning studies

4.1 Resource surveys

4.1.1 General

Resource surveys are probably the least complicated sampling programme. However, they still require good planning to generate useful data. The aim of a resource survey is to describe habitats, and associated communities, and are generally only carried out on a "once-off" basis, or very irregularly. They might be required in the following circumstances:

- classification of the enrichment status of different valley segments in a catchment (along with some water quality indicators);
- definition of the location, extent and timing of periphyton proliferations;
- identification of unique, rare or endangered populations or communities;
- a baseline to enable assessments of the effects of a planned developments (e.g., a dam or discharge of pollutants);
- to characterise habitats that might be important for specific values (e.g., conservation status, salmon spawning, aesthetics, etc.);
- testing or validating the biological significance of catchment / valley segment habitat classifications based on physical variables.

It is also possible, with care, to use resource survey data as a basis for predicting possible communities and biomass in similar habitats elsewhere in the region. A table of commonly found community types is given in Appendix 2, together with a description of the habitats in which they are normally found. Resources surveys could also be used to test these community / habitat classifications and then modify or add to them as necessary for a particular region.

When using data from such resource surveys that have been collected more than, say, a year previously it is important to consider whether the data are still valid. Factors to review might include:

- whether there have been significant changes in landuse upstream of the site since the original survey;
- how representative weather conditions were during the year of the survey (e.g., was it a drought year?);
- whether the extent of abstraction and / or baseflows has changed markedly since the original survey,
- whether the bed sediment composition or channel configuration has changed.

All these factors have an important bearing on transferability of data from the past to the present.

After setting your objectives, the following main steps are required for a once-off resource survey.

- Carry out a river environment classification of the catchment to define the main combinations of climate, sources of flow, geology, landuse and frequency of flood events (Snelder *et al.* 1998). If there are major differences in valley morphology, you may also need to carry out valley segment classifications.
- Select a sample of the most common habitat types where access is good and the data

are likely to be most relevant to management issues. If possible, make sure that there is at least one "reference" site. This will be a site with minimal human impacts, which will provide a reference against which to compare conditions elsewhere in the catchment (see Section 2.6).

- Try to sample at a time of year that is most relevant to the questions being asked and that represents the usual state of periphyton growth for the types of habitats being sampled. For example, a common objective might be to define when and where in a catchment proliferations might occur. In hill-country stream (where highest biomass often occurs in spring and autumn), surveys in spring and autumn would be appropriate. However, for surveys on lowland-fed streams, or low altitude parts of hill-country fed rivers, then it might be best to sample in mid to late summer.
- Regardless of season, always try to sample at least 4 weeks after last flood event that moved the coarse parts of the stream bed, so that communities have developed to a reasonably mature state which will reflect their local environment. If you are in a catchment that is prone to frequent spates or floods, then sample as long after the last flood as is sensibly possible.
- Select sampling and analytical methods. These should reflect the detail with which you need to characterise the communities. In many circumstances, a rapid assessment survey using the SHMAK protocol (Section 6.4.3) will be sufficient. However, if it is likely that the data might be used to assess values for a contentious development, then quantitative sampling with semi- or fully quantitative analyses will be needed.
- Carry out the survey. Attempt to complete the sampling in the shortest time possible so that there are no major changes in flow or weather conditions which may influence the periphyton while carrying out the survey.
- Analyse your samples in the laboratory (if a quantitative survey).
- Analyse and report your data. In particular plot up your results in relation to, say, habitat type or variations in some environmental gradient such as percentage of the catchment developed for agriculture. *Use your original objectives to focus the analysis on specific issues.*
- Store your data in a form that is safe and accessible to others. Historical data collections are becoming increasing valuable as benchmarks against which longer-term changes in the environment can be assessed. At present there is no national database for stream benthic communities such as exists for hydrological and freshwater fish data.

4.1.2 How many sites should we sample?

This is a perennial question that defies a simple recommendation. If we were to be completely objective, the number of sites should reflect the heterogeneity of the environment and the scale at which it was necessary to characterise habitats and communities. The more variable the environment the more sites we generally need. If possible, we recommend statistically based approaches to assess the number of sites, based on variability in data from preliminary surveys such as described in Section 3.3 for sample replication, but treating sites as sampling replicates for the statistical analysis. However, in many situations the resources don't permit such an approach and some reasoned pragmatism is called for. If you are in this situation use your habitat classifications to guide you. This process partitions much of the broadscale variance and allows you to focus on habitats of greatest relevance. A general rule is:

Aim for at least three sites in the main habitat types of interest so that within-habitat variability can be quantified.

Formal habitat classification (Snelder *et al.* 1998) allows division of the environment and allocation of the location and number of sampling units to be carried out objectively / reproducibly. Basing sample sites and locations on habitat classifications generally results in fewer sites being required than if you were to sample a region randomly. Such sampling is known as "stratified random sampling" (see Section 3.6).

4.1.3 Where should sites be located: riffles, runs, pools or backwaters?

Once again, the answer to this question depends on the objectives of the survey and the habitats being considered. Runs are by far the dominant meso-scale habitat feature in most New Zealand streams and should be the first priority for sampling to gain the best representation of the general nature of the resource. This is the area where periphyton proliferations will usually develop because lower velocities allow higher biomass to accumulate under enriched conditions. In streams with moderate to low nutrient levels, riffles will usually have the highest biomass because of greater nutrient mass transfer (e.g., see Biggs and Hickey 1994, Biggs *et al.* 1998b). However, while biomass in riffles can be high, it usually does not exceed proliferation guidelines because higher velocities in this habitat slough accumulations from the bed. If a comprehensive study is required on a large river system, such as to assess the effects of future river diversion or damming, then stratify your sampling by mapping the extent of meso-habitats (as noted above and described in general terms in Section 3.6). Among other things, this will help identify unusual or rare communities and habitats. An example of such an approach is given in Biggs and Shand (1987).

4.1.4 How many replicate samples should be taken at each site?

For valid statistical comparisons of stream communities in different habitats, resource surveys must include replicate samples at each site. Each replicate is collected independently of the others, but must be taken in exactly the same way from each sampling point. The more replicates taken at a site, the more statisically robust the analysis will be (see Section 3). However, as noted earlier, there is a trade-off between increasing the accuracy of the survey and the extra cost of more sampling replicates. The level of discrimination is very much dependent on local variability or "patchiness": the more patchy, the less precision there will be for a given number of samples (e.g., see Section 3.3).

4.2 Impact assessments

4.2.1 General

The aim of periphyton biomonitoring for impact assessment is often to compare biomass and taxonomic composition of communities:

- that have been impacted by a disturbance plus at least one paired control site; *or*
- at a site before and after a disturbance; *or*
- at impacted and paired control sites, both before and after the disturbance.

Impact assessments are probably the most complicated sampling programmes because the effects of a particular pollutant need to be isolated from the effects of the local physical habitat. They require good planning to generate data that will be defensible. Unlike resource surveys, impact assessments do not usually enable a full characterisation of the aquatic habitats and associated communities. They normally focus on testing for a specific effect / impact. Com-

monly they might be used in the following situations:

- definition of the extent and cause of enrichment from a point source discharge such as an oxidation pond or a zone of possible diffuse source enrichment along a stream;
- monitoring for compliance of conditions on a consent;
- identifying possible impacts from an existing discharge where there is little historical information or if there has been a change in effluent quantity / quality;
- testing or validating conditions on a resource consent.

After setting your objectives, the following main steps are required for implementing an impact assessment.

- Carry out a reach-scale river environment classification in the vicinity of the impact point to help establish where control and impact sites might be located.
- Select at least two sites above (control sites) and two sites below (impact sites) the point / zone under investigation. Attempt to have all physical habitat variables the same among the sites (e.g., shading, depth, velocities, temperature, etc.) so that the periphyton results reflect only the effects of the impact being investigated.
- Attempt to carry out sampling at a time of year that is most relevant to the questions being asked and the usual state of periphyton growth for the types of habitats being sampled.
- Regardless of season, always try to have about >4 weeks of stable flow prior to sampling, and during the incubation period (if artificial substrate samplers are being used). This is to ensure that communities have developed to a reasonably mature state that will reflect their local environment and any effects of the potential problem being investigated. If you are in a catchment that is prone to frequent spates or floods, then sample as long after the last flood as is sensibly possible.
- Select sampling and analytical methods. These will need to reflect the detail with which you need to characterise the communities. In most circumstances, a quantitative approach will be required.
- Carry out the assessment. Attempt to complete the sampling in the shortest time possible so that there are no major changes in flow or weather conditions which may influence the periphyton during sampling.
- Analyse your samples.
- Analyse and report your data. In particular, plot up your results in relation to the disturbance being investigated. Commonly, histograms are used for such analyses followed by analysis of variance. *Use your original objectives to focus the analysis on specific issues.*
- Store your data in a form that is safe and accessible to others.

4.2.2 How many sites should be sampled?

Ideally there should be multiple impact and control sites. With two or more control sites we can better assess any longitudinal variation in periphyton along the stream between apparently similar sites which cannot be attributed to the disturbance. With two or more impact sites it might be possible to identify downstream recovery. We are then looking for differences between the impact and control sites which are greater than any which exist within each set of sites. An example of this is shown in Figure 5.

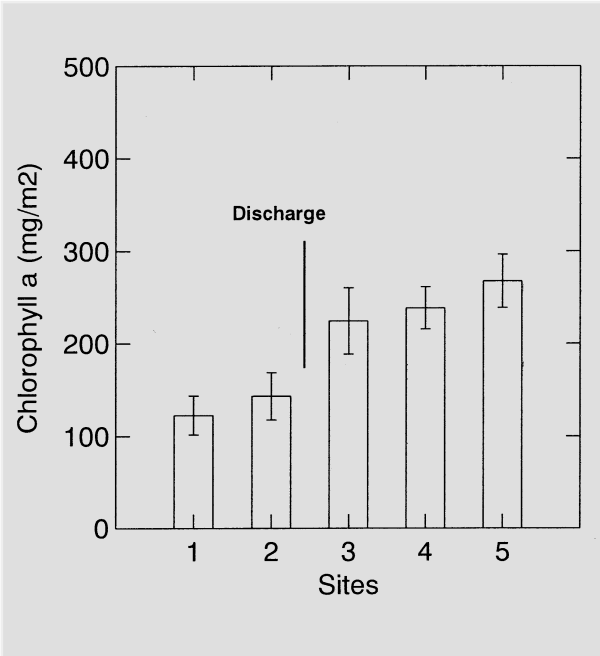


Figure 5: Chlorophyll *a* concentrations from 10 replicate samples across the bed at two sites above and three sites below a nutrient discharge. All sites were in runs. The data show a minor increase in periphyton chlorophyll *a* moving downstream before the discharge point (Sites 1 and 2). This increase continued downstream of the discharge (Sites 3–5). A nested analysis of variance showed that the chlorophyll difference was not statistically different among the upstream sites and among the downstream sites. However, the difference between upstream and downstream was highly significant.

Again, it is important to ensure that all impact and control sites are matched in all conditions except those resulting from the disturbance. Where monitoring is requested in an area which has highly variable physical characteristics, then it may be difficult to isolate the effects of the human disturbance on the periphyton from other habitat influences. The number of replicate sites may also be limited by the size of the stream. A further limit may be set by the available budget, as mentioned previously. Single impact and control sites will provide some information, but the statistical comparison is not nearly as robust as that provided by multiple sites. In cases where there have been limitations on placement of sites, then be sure to make other data users aware of the problems.

4.2.3 Where should sites be located?

The locations of impact and control sites must be selected on a case-by-case basis. It may be possible to select general locations for sites from a 1:50,000 map of the area. The exact location is determined in the field after a reach-scale habitat assessment.

For impact assessments, choose sites in river runs wherever possible. It is understood that in some streams it may be difficult to find areas of runs large enough for sampling. As noted earlier, the most important consideration in site selection is to ensure that impact and control sites are similar in all respects *except* in location relative to the disturbance and in relation to habitat / water quality features being influenced by the disturbance. The main local habitat features to consider in site selection are:

- bed slope
- water velocity
- water depth
- bed sediment composition
- shade
- other disturbances (e.g., stock crossings).

If depths and velocities are highly variable and difficult to standardise, or if the bed is silty/sandy, then consider using artificial substrates (see Section 6.6). These enable a much more accurate isolation of effects of specific water quality disturbances.

4.2.4 How many replicate samples should be taken at each site?

As discussed in earlier sections (e.g., see Section 3.5), it is necessary to collect replicate samples at each site for valid statistical comparisons of stream communities at different locations on a stream or river to accurately determine the effects of some perturbation. Each replicate is collected independently of the others and must be taken in exactly the same way from a spot within the site which is similar to that for the other replicates. Replicates are not sub-samples from a single sample obtained from a site. The more replicates taken at a site, the more statistically precise the analysis will be and the smaller the differences that can be detected among sites. A guide to calculating the likely numbers of replicates for a given level of precision (which needs to reflect the degree of difference among sites that you think is ecologically important; see Section 3.7) is given in Section 3.5.

4.3 State of the Environment monitoring

State of the Environment (SOE) monitoring is a relatively new concept that was initiated in New Zealand following a Cabinet directive in April 1993 with the overall purpose of measuring and reporting how well we are looking after the environment (MfE 1997). The specific objectives are to:

- systematically measure the performance of environmental policies and legislation;
- better prioritise policy and improve decision making;
- systematically report on the State of New Zealand's environmental assets.

Section 35 of the Resource Management Act requires Regional Councils to monitor the effectiveness of policies at the regional level.

At present there are no frameworks or guidelines for setting up and reporting SOE monitoring. These will be needed to ensure that data will be usable for assessing the effects of policy, and making connections between the indicator data and environmental issues. The indicators used will need to be sensitive to the types of pressures expected to influence the environment and have a clear link back to mechanisms causing changes in these variables (e.g., percentage silt in the periphyton matrix in rivers used for gravel extraction). Any framework will also need to incorporate an environmental classification system for identifying reasonably homogeneous parts to the environment and to allow stratifying of monitoring effort. Such classification also allows the identification of “reference” sites, enables physically similar environments to be compared over time, and enables data and conclusions from individual sampling sites to be extrapolated objectively to larger areas or a whole region.

Percentage cover of the stream bed by different types of periphyton has been included as part of a proposed suite of environmental indicators for SOE monitoring of New Zealand's freshwaters (MfE 1997). This inclusion recognises the importance of periphyton as an indicator of environmental quality.

To date, regional and national SOE reports have generally just collated monitoring data. These data are either summarised as means for different sites/geographic areas (to define “snap-shots”) of current conditions and/or are summarised as time series for indicators in an attempt to define trends over time (e.g., Waikato State of the Environment Report 1998). However, a few simple steps can be included in these reports that would greatly add to their

value and information content. In the following we suggest some general steps to be considered if using periphyton for SOE reporting. A number of these principles are common to those required for resource surveys and impact assessments.

- Set clear objectives that explicitly focus on measuring the success of Regional Policy Statements and Regional Plans so that changes that might ensue (to the most affected habitats) can be measured with the pre-selected level of precision.
- Carry out a habitat classification of the catchment to define the main combinations of climate, sources of flow, geology, landuse and frequency of flood events. If there are major differences in valley morphology, you may also need to carry out valley segment classifications.
- Select a sample of the most common habitat types where access is good and the data are likely to be most relevant to management issues. If possible, make sure there are at least three "reference" sites (i.e., where no human induced changes occur; see Section 2.6) for each of the main habitat types. Data from reference sites are essential if you want to effectively determine the long-term benefits of policies designed to improve stream health (such as planting riparian buffer strips in pastoral lands).
- Select indicator variables that are most relevant to the questions being asked and that will be sensitive to expected changes in environment. Also, ensure that there is a clear link back to mechanisms of change and management policies.
- Set a sampling frequency that targets your information needs while taking into account the natural temporal variability of the variable. For example, you may wish to know if a new policy of increasing the extent of riparian buffer strips in pastoral lands has resulted in a reduction in frequency of periphyton proliferations. Thus, we might nominate to carry out monthly surveys of percentage cover of filamentous algae and after 10 years of monitoring assess the trends in terms of the number of months per year when long filamentous algae exceeds 40% cover.
- Unlike resource assessment surveys, which can be carried out a certain times of the year to target specific conditions, SOE monitoring involving periphyton will generally need to be carried out on a routine basis with frequent sampling during the year to establish "average" conditions. See Section 3 for statistical methods for assessing number of replicate samples that may be required for testing differences in space or time at different levels of precision.
- Plot up your results according to broad habitat types to obtain an overall "snap-shot" characterisation. Then carry-out any statistical tests required to compare results from sites where you expect a change because of a particular policy decision with results from your reference site. Trends in indicator variables over time can also be plotted with test- and reference- site data overlayed on single graphs so that departures in trends over time can be easily detected. *Use your original objectives to focus the analysis on specific issues.*
- As for resource surveys, ensure that you store your data in a form that is safe, and accessible to others.

Discussion of other details applicable to SOE monitoring including number of sampling sites, where sites should be located, and how many replicates to collect is contained in Sections 4.1.2, 4.1.3, and 4.1.4. Discussion on sampling statistics is in Section 3.

4.4 Some practical considerations before you start your study

Before starting a monitoring programme, do some homework on the area and sites for which periphyton monitoring is proposed. This preliminary work should be done before designing the specifics of the programme. Consider the following.

- Examine maps of the area. Identify exactly where human disturbances might occur or are proposed. Identify other possible disturbances and select catchments in which control sites can be located.
- If possible, obtain a flow record for the stream in question, or for the nearest stream with a similar type of catchment in the area. This, combined with the GIS habitat classification information will give you a good idea of the type of stream that you are dealing with.
- Obtain any previous data that might be available (e.g., from previous monitoring programmes). Also, obtain information about any resource consents issued on the stream. This information might be a good lead on what communities to expect, how variable communities might be in space and / or time, the possible confounding effects of disturbances, etc.
- Visit the area and familiarise yourself with the different stream / river reaches before developing any specific monitoring plans.

5 Common New Zealand stream habitats and their periphyton communities

In this section we summarise the types of periphyton communities that might be found in different habitats around New Zealand. These habitats are defined in the first instance by differences in source of flow, because this dictates the general hydrological and sediment transport regime of streams and rivers. Sources of flow have an overriding influence on local habitat conditions and, through the associated disturbance regimes (which includes flood events), constrain the potential effects of the lower-order habitat variables of geology and landuse, which most commonly control the nutrient supply. These habitat features form the basis of the hierarchical river environment classification that is currently being developed for New Zealand (Snelder *et al.* 1998) and referred to earlier. General periphyton community types have been discussed in Biggs (2000a) and are further summarised in Appendix 2. The following sections discuss habitat characteristics of the main sources of flow of most New Zealand streams and rivers, and the periphyton communities most commonly found there.

5.1 Spring-fed streams

The overriding habitat characteristic of spring-fed streams is the high degree of flow stability, followed by a generally low bed gradient, fine bed sediments (usually silts, sands and fine gravels) and stable water temperatures. Thus, these habitats are often colonised by aquatic macrophytes. Indeed, gravel / cobble substrata for periphyton to adhere to may be rare. If the bed gradient and associated water velocities are sufficiently high, then areas of coarse gravels and cobbles may become more prevalent. Spring-fed streams are often quite enriched, depending on the geology of the catchment or whether the seepage waters are derived from gravels in an unconfined aquifer. Thus, a broad range of community types can be expected in such habitats. *Vaucheria* can often be found in cold, spring-fed streams forming very large mats over the silts and sands.

Of all the different stream types, spring-fed streams are most likely to have seasonal variations in biomass because spates don't occur regularly or often enough to “re-set” community development to early successional stages (Biggs 1996). For example, in spring-fed streams of Canterbury, the highest biomass (usually caused by blooms of *Ulothrix zonata* or *Oedogonium* depending on the degree of enrichment) occurs in late summer / autumn and late winter. Between these times, a moderate to low biomass of periphyton usually persists on the rocks. Intense invertebrate grazing during these periods probably keeps biomass from accruing. The reason for the two seasonal peaks in biomass of filamentous green algae is unclear. An intense period of sporulation may be triggered in these taxa by a change in light intensity or photoperiod. Water temperatures do not change much between winter and summer in spring-fed streams (except in downstream reaches, far from source) and always appear sufficient for high invertebrate activity.

5.2 Lake-fed streams

Lake-fed streams have some physical elements in common with spring-fed streams, in particular they generally have low flow variability. Both stream types usually have high densities of benthic invertebrate grazers. However, there is a great range in bed gradients and flows among lake-fed streams and, therefore, in water velocities and substrate particle size.

There is also a considerable range in the trophic state of lake outlet streams, reflecting the varying trophic states of the feeder lakes.

A wide range of periphyton communities is expected to dominate such lake-fed streams, however, few data have been collected in such habitats. Most sampling has been carried out in regulated lake-fed rivers in relation to hydroelectric power development, and it is unlikely that this information is totally applicable to unregulated lake-fed systems. Some lake-fed streams near their outlet are dominated by diatoms such as *Synedra*, *Gomphoneis* and *Diatoma*, whereas others are dominated by green algae such as *Ulothrix*, *Oedogonium* and *Bulbochaete* (S. Moore unpublished data; B.J.F. Biggs unpublished data).

5.3 Wetland-fed streams

Wetland-fed streams are distinctive in generally being low gradient with associated moderate or low water velocities and fine bed sediment particles. These streams are also often low in nutrients, and the waters often have a low pH and are dark through tannin staining. This represents a distinctive environment for periphyton. Much of the periphyton community is expected to be episammic (associated with sands) and epiphytic with abundant desmids and Zygnemataceae (*Spirogyra*, *Zygnema*, *Mougeotia*) and *Microspora* and filamentous diatoms such as *Eunotia*, *Fragilaria* and *Tabellaria*.

Few wetland-fed streams have been surveyed in New Zealand, so little definitive information exists to confirm these predictions. Considerably more basic research is needed on these habitat types, particularly given that such ecosystems have greatly diminished over the last 100 years, and are still under considerable threat. It is quite possible that they could harbour some unique communities and even new taxa. Several new diatom species have been discovered recently in brown water streams on the West Coast and on Stewart Island (R. L. Lowe and C. Kilroy, pers. comm.).

5.4 Low-altitude streams

With an average catchment elevation below about 400 metres, most of these streams are low gradient (with associated long reaches with low velocities and fine silty / sand bed sediments) located near the coast. Most of these streams are at least partially spring-fed and thus tend to have a low variability in flow (although some may be ephemeral) and tend to have prolonged periods of low flow in summer. They also tend to be very enriched, being at the most downhill end of farming activities. Few of these streams have natural, intact, riparian and / or catchment vegetation and we have no knowledge of what periphyton communities would naturally exist in such habitats. Some basic research is required on the communities inhabiting the remaining, natural lowland streams around New Zealand (mainly Fiordland, parts of Southland and the West Coast).

In the agriculturally developed areas such streams often don't have significant periphyton communities because of a lack of suitable substrate. Where there are extensive beds of macrophytes, filaments may grow as entangled masses over the larger plants. Where bed gradients are steeper and cobbles and gravels predominate, mesotrophic and eutrophic communities tend to occur, often forming a very high biomass. In particular, *Oedogonium* and *Microspora* can form very extensive cover in enriched South Island lowland streams, and *Cladophora glomerata* in North Island lowland streams.

5.5 Hill country-fed streams

This is by far the most common stream type in New Zealand and has the greatest range in physical conditions. In particular, most of these streams are flood prone with a frequency commonly being between 10 and 30 events per year. These streams are generally relatively steep so often have strongly developed reach-scale morphological features (e.g., pools, runs and riffles), the bed sediments are generally gravels and cobbles (except in mudstone catchments), and there is a very wide range of water velocities. They also encompass the full range of enrichment regimes expected for streams as a result of differences in land use and catchment geology. On stable substrates during summer low flows, the specific dominant community is usually determined by (in decreasing order of importance): shading, enrichment, water velocity and invertebrate grazing. Since most New Zealand streams and rivers do not have riparian shade except in their very headwater reaches, the most important controllers during low flows are usually enrichment, velocity and invertebrate grazing.

Diatom communities (e.g., *Fragilaria* and *Gomphoneis* groups) that rapidly colonise the stream bed most commonly dominate such streams over the year. However, if sediments are reasonably stable (armoured or imbricated) then a range of different communities will develop during low flows reflecting the local conditions of nutrient and light supply, and reach velocities. In oligotrophic streams draining bush or tussock catchments during such summer low flows, cyanobacterial communities dominated by *Schizothrix* / *Calothrix* / *Lyngbya* are often abundant as small black patches on the stones. *Coleodesmium* can be abundant in hill-country streams of Otago (S. Moore, Otago Regional Council pers. comm.). The communities appear to be very resistant to grazing by invertebrates. The cold water streams in the upper catchments of South Island hill country streams are often dominated by the green filamentous alga *Ulothrix zonata*. Communities dominated bythe red alga *Audouinella* tend to be restricted to stream reaches with very stable boulder or bed rock substrata. Mats dominated by this taxon are often light purple in colour.

Where low–moderate enrichment occurs, the low-flow communities in swift flowing reaches and riffles of such New Zealand streams are most often dominated by thick mucilaginous

Figure 6: Distinctive, bright green, tufts of the branched filamentous alga *Stigeoclonium lubricum* growing through an understorey of *Gomphoneis minuta* var. *cassieae* in a moderately enriched foothills-fed river.

mats of *Gomphoneis / Cymbella*. These can be overgrown by dark filamentous cyanobacterial (*Phormidium*) growths late in the summer (or late in the successional cycle if prolonged low flows occur at other times of the year). Large tufts of the branched filamentous alga *Stigeoclonium lubricum* often develop among the diatom community in moderately enriched streams and are a very conspicuous bright green colour (Figure 6). It is rare for a whole stream reach to be dominated by *Stigeoclonium*.

Similarly, *Spirogyra* is probably the most common filamentous alga throughout New Zealand. However, it is rarely the dominant community when a whole stream reach, or a transect across a stream, is surveyed. This taxon is often common among communities dominated by *Oedogonium* and diatoms, or as tufts of dark green filaments on stable stones in mesotrophic streams. *Spirogyra* can also form clouds of green filaments in pools during summer low flows.

With increasing enrichment, it is common to find extensive areas of hill country-fed gravel-bed streams dominated by *Oedogonium / Microspora / Zygnema*. These taxa can form very high biomass in the lower velocity runs of such streams (so long as there are adequate stable substrata).

5.6 Non-glacial mountain streams

Non-glacial mountain streams and rivers are usually characterised by harsh hydrological conditions of frequent flood events, steep, high-energy channels with generally unstable bed sediments, are rarely shaded and are most commonly unenriched. The windows of stable conditions that enable communities to proceed through to maturity are rare.

The overall species pool in the upper reaches of such rivers is probably limited by the frequency of flood disturbance events and thus even during infrequent periods of low flows the periphyton communities will usually be dominated by rapidly colonising diatoms (e.g., *Gomphoneis / Cymbella* communities and communities of *Fragilaria / Diatoma* and associated taxa) with *Ulothrix zonata* being the principal filamentous green alga (because this taxon is particularly disturbance resistant). Steep mountain headwater streams are often dominated by the filamentous diatom *Diatoma hiemale*. Grazing mayflies probably exert considerable "top-down" control on such communities because these invertebrates can be very common and the rate of periphyton production is usually quite slow because of low levels of nutrients. As a result of these joint processes, mean and peak biomass are usually relatively low.

5.7 Glacial mountain streams

These habitats are strongly dominated by high levels of sediment supply and associated bed sediment movement, and also have low levels of nutrient supply. Flood disturbance events are usually more seasonal than in non-glacial mountain and foothills-fed rivers, with the lowest flows usually occurring in late summer / early autumn when lower precipitation falls and then in winter when most precipitation falls as snow. Because of the high suspended and bedload sediment supply in these systems only minor increases in flow can mobilise sediments creating harsh abrasive conditions for benthic biota. These sediment supply processes are so prevalent that river communities may be strongly controlled by the glacial origin of flows even though glaciers may only constitute a very small proportion of the catchment. For example, at its mouth to the Pacific Ocean, only 2% of the catchment of the Rakaia River is of glacial origin, but the physical regime (flow variability and sediment movement) and benthic communities are strongly influenced by this source of flow.

It is important to recognise different valley segments because physical conditions change strongly down the river continuum in these glacial rivers. For example, conditions are very harsh in the single-thread, down cutting phase of these rivers, but where they braid in the less confined outwash plains, many sections of the braids usually have considerably less hydraulic power with very stable (often armoured) bed sediments. Periphyton are almost always very sparse in the main channels with the only cells being diatoms washed in from the more stable braids. However, extensive mats of diatoms (particularly *Gomphoneis* / *Cymbella* dominated) and mats of the green filamentous alga *Ulothrix zonata* can occur during periods of stable flow in the braids with stable sediment. Mats dominated by the red alga *Audouinella hermanii* can also occur in these stable braids.

In Canterbury, many braided reaches of such rivers receive groundwater inflows enriched with nitrate. These can result in locally high biomass (and sometimes proliferations) of taxa such as *Ulothrix* along the edges. High quantities of silt usually accumulate in such mats.

5.8 Streams regulated by dams

As already discussed in Biggs (2000a), rivers regulated by mainstream dams have unusual hydrological disturbance regimes which tend to encourage the development of high biomass communities of diatoms and filamentous green algae. These conditions include a reduction in bed sediment supply, which results in armouring, of the river bed (see Figure 19 of Biggs 2000a); a reduction in the frequency of small and medium sized flood disturbance events; and enrichment of waters from groundwater upwelling during periods of very low flows (Figure 7). The dominant periphyton cover during low flows in such systems is often filamentous and, depending on the degree of enrichment, can be either indicative of oligotrophic (e.g., *Ulothrix zonata*) or mesotrophic habitats (e.g., *Oedogonium*, *Phormidium*). Thick mucilaginous diatom slimes composed of *Gomphoneis*, *Cymbella* and *Synedra* populations occur in some South Island regulated rivers during summer low flows (Biggs and Hickey, 1994).

Figure 7: Boulder bed, typical of a regulated river downstream of dams, with luxuriant growths of periphyton dominated by the mucilage forming taxa *Cymbella kappii*, with *Gomphoneis minuta* var. *cassiae* as an understory and overgrowth of patches of the green filamentous alga *Spirogyra*.

6 Field procedures

6.1 General

Floods, particularly those which cause the bed material to move, can scour away much of the periphyton from the stream bed. It may take much more than four weeks of stable flows for periphyton communities to regain the biomass and species diversity present prior to such floods (see Biggs 2000a). Therefore, to obtain a representative sample of periphyton, at least four weeks should elapse between floods that have caused major scouring of the coarse bed sediments and sample collection. Such floods can generally be taken as at least 5-6 times the average flow for the previous week (if the flow was constant). Note that for oligotrophic streams such a change may not re-set the periphyton communities significantly because they are generally lower in growth form and tightly attached. In these cases a scouring flood can be taken as one capable of moving at least the 84th percentile size fraction of the bed, which usually takes a much higher increase in flow. If you are in a catchment that is prone to frequent spates or floods, then sample as long after the last flood as is sensibly possible. Conversely, long periods of low flow may lead to the natural growth of large accumulations of algae, particularly filamentous types. However, after peak biomass for the accrual cycle has been reached, extensive natural sloughing often occurs. If sampling takes place while sloughing is in progress you are likely to get highly variable biomass amongst replicate samples.

In the following sections, we describe methods for a rapid, semi-quantitative, assessment of periphyton as a function of cover of the streambed by different groups of algae. For this method, all information is collected in the field. The rapid assessment method does not provide information on community composition, nor biomass. However, the thickness of the different types of mat is assessed. It is also possible with some experience to identify many of the macro-algal taxa in the field and express the results in terms of percentage cover of a reach by certain community dominants. Such methods are very useful for broadscale surveys, for example, to understand the effects of variations in landuse on low flow periphyton growth, or monitoring adherence with consent conditions for the prevention of proliferations. We then describe fully quantitative methods of sampling, where the samples then need to be returned to the laboratory for later analysis. These methods include natural and artificial substrate sampling. Natural substrate sampling is recommended where ever possible, but artificial substrates are valuable to isolate most clearly the effects of water quality / pollution from other habitat constraints which might complicate interpretation of results. Quantitative methods need to be used if attempting to provide clear and comprehensive information on the possible effects of any perturbation to water quality or physical habitat.

6.2 Location of sites

Always select sites that are physically representative (i.e., depths, velocities, bed sediments, shading, etc.) of the reach in the stream that you need to characterise or monitor. As noted in Section 4, this will most commonly be in a “run”. These are places (or habitat units) where the water velocities are uniformly moderate–low (e.g., 0.2–0.4 m / s), the water depths are 0.2–0.6 m, and the bed sediments are uniformly similar in size and without significant variations in bed level. The water surface is also unbroken, or rarely covered by ripples.

For more details on requirements for study planning, site selection and determining the number of replicate samples required, refer to Sections 2, 3, and 4.

6.3 Site records

It is important to record all background information at a site. This might include:

- site name (the same as you put on sampling containers);
- map reference;
- vegetation on left bank; on right bank;
- degree of shade;
- water surface slope;
- catchment vegetation;
- water depth & velocity (measured at each sampling point on each sampling occasion);
- substrate composition (by visual assessment or Wolman particle size count (Wolman 1959)).

Also, take a photograph of the site on each monitoring occasion from a fixed location. A polarising filter improves the view through the water to the bed of the stream.

This record of the site is used both to confirm the exact location of the site and to establish the degree of similarity of conditions amongst sites, or at a site over time, during a monitoring programme. Site information is usually important in the interpretation of the results.

6.4 Rapid assessment protocols

6.4.1 Introduction

Many studies will require only rapid assessment of the cover of the stream bed by different general types of periphyton. We define two line transect methods below. The first is the simplest and focuses on measuring the cover of a site by filamentous algae using quadrats. This is specifically designed for assessing compliance with the periphyton guidelines for cover to protect aesthetic, recreational and fishing values (see Table 14; Biggs 2000a). The second method, while still a rapid assessment protocol, is more detailed focussing on the cover of a sample of individual rocks and enables the general evaluation of the degree of enrichment and water quality at a site. This method was developed for the Stream Health Monitoring and Assessment Kit (SHMAK) (Biggs *et al.* 1998c) and recognises 12 main types of periphyton based on colour and thickness. Repeated surveys at regular intervals over a year (e.g., monthly) using this method will provide a comprehensive picture of the temporal dynamics of communities and state of the environment. Necchi *et al.* (1995) compared line transect, point intercept and quadrat methods for rapid assessment of cover of macroscopic growth forms of periphyton and found little difference in overall results among the methods, but did show some differences in how depiction of spatial distributions. See Necchi *et al.* (1995) for more details.

Recordings from both rapid assessment methods can be supplemented with more detailed analyses of, say, biomass and taxonomic composition at certain times of the year. This might be during summer low flows when maximum biomass for the year has developed. The data that these methods generate are quantitative and thus can be analysed statistically. As mentioned above, an experienced person can also usually identify the predominant taxa forming the most conspicuous catagories of periphyton rather than just relying on the colour descriptors (a field microscope really helps such evaluations). There is some potential for inter-operator differences in assessments through different perceptions of colour, estimates of thickness and estimates of percent cover of the substrates. However, during testing of the methods, we have found that such variation is usually much smaller than differences in the community

over time or among sites covering a broad range of conditions. Nevertheless, there are advantages in standardising the person who carries out the assessment at a given site for routine surveys, or for multiple sites across a region where variations among sites / streams are to be assessed for a given time of the year.

Prior to using the following methods, ensure that your study objectives are clearly defined (preferably in writing) and that you have chosen a suitable site in the stream reach of interest. Refer to Sections 2, 3 and 4 for more details on design of sampling programmes sampling replication.

6.4.2 Rapid Assessment Method 1 (RAM-1): Quadrat method for percentage cover of a site by filamentous green/brown algae for assessing compliance with aesthetic/recreational guidelines for proliferations

This method involves setting up transects across a site and recording the percentage cover of filamentous algae > 3 cm in length for a given number of quadrat points. Percentage cover values for the individual points are then averaged to obtain an estimate of the average cover of the site by filamentous green / brown algae. These individual records can also be used to later construct a map of the distribution of filamentous algae and, if repeated sampling is performed, then changes in the distribution of mats or patches of these algae can be traced over time. Such analyses, if combined with some physical measurements (e.g., shading, water velocities, depths and / or substrate composition), can provide useful insights into the primary factors controlling the local development of proliferations.

Equipment:

1. 2 tape measures (50 m and 20 m long).
2. 4 pegs (> 20 cm long) and mallet.
3. Sampling quadrat consisting of an open steel / alloy ring or square 15 - 20 cm in diameter (size is not critical, but try to use the same size consistently for a given stream / site).
4. Glass or clear plastic bowl ~ 20 cm in diameter, with a flat bottom for looking into the water.
5. Field data sheet (preferably of waterproof paper).

Procedure:

- Select a reference point at the downstream end of your site and on one bank drive a peg into the ground.
- Attach the 50 m tape measure to the peg and lay it out taut for its full distance (or 5 x the stream width, whichever is the smaller). Attach the upstream end of the tape to a second peg.
- Calculate 10 equally spaced intervals along the tape measure (e.g., 5-m intervals for a 50 m distance).
- Attach the end of the 20 m tape measure to a third peg at the location of the reference peg and unwind the tape at right angles to the main tape. Anchor the far end with the fourth peg on the far bank.
- Divide the width of the stream (water's edge to water's edge) into 10 equally spaced points.
- Place the sampling quadrat on the stream bed centred on the first point.
- Hold the glass bowl on the surface of the water to obtain a clear view of the stream bed

(this may not be necessary in shallow, clear streams).

- Estimate the percentage cover within the quadrat of filamentous green / brown algae which have filaments > 3 cm long. Record these on your field sheet then move across to the next point.
- Complete the transect then move the transect upstream for the pre-selected interval and repeat the recordings.
- When complete, enter the data onto a spreadsheet and calculate the mean percentage cover of the site by filamentous algae > 3 cm long.

Notes:

- If the cover of the site is clearly very homogeneous (e.g., broad mats just along the periphery of the channel) then it is acceptable to reduce the number of points across each transect from 10 to 5.
- If the stream is so narrow that the area of the quadrat from one sampling interval overlaps with adjacent points then it is also acceptable to reduce the number of points across each transect or to employ a small quadrat.
- If the stream is > 20 m in width or too deep to wade safely, then sub-divide your site in a stream-wise fashion. This involves setting the limits of your transects out into the river based on a fixed depth or distance from shore. Using depth contours is most preferred. You then need to express your results in terms of percentage cover of filamentous algae for, say, the < 0.6 m deep section of the reach.
- There may be some difficulty in determining what constitutes a green / brown filamentous algal community with filaments > 3 cm long. In such cases, familiarise yourself with the communities at the site before commencing the survey by picking up stones from the stream bed and examining them carefully just below the surface of the water (so that the filaments stream in the current), then lift them above the water for closer examination. Note that the mats can look quite different under the water vs. out of the water.
- Taxa which form such growths include: *Spirogyra*, *Oedogonium*, *Stigeoclonium*, *Microspora*, *Mougeotia*, *Cladophora*, *Rhizoclonium* and *Zygnema*.

6.4.3 Rapid Assessment Method 2 (RAM-2): Line transect – point method for percentage cover of substrates by different categories of periphyton for general resource surveys and assessing broadscale effects of perturbations

As with RAM-1, this method involves setting up transects across the site of interest and recording the percentage cover of algae at fixed points. However, much more information is obtained on the types of algae present and many fewer points are examined. Four transects are used with five points across each transect. The number of points and / or number of transects can be increased for greater levels of precision. This method does not allow an estimate to be obtained of the percentage cover of the site by different algal groups. However, the percentage cover values for various types of algae are estimated, then multiplied (i.e., weighted) by a pollution score to give a general assessment of water quality conditions. Such analyses, if combined with some physical measurements (e.g., shading, water velocities, depths and / or substrate composition), can provide useful insights into the primary factors controlling the local development of different periphyton communities and assessment of causes of stream habitat degradation.

Equipment:

1. 2 tape measures (10 m and 20 m long).
2. 4 pegs (> 20 cm long) and mallet.
3. Small tea strainer (approximately 8 cm in diameter).
4. Field data sheet (preferably of waterproof paper).
5. Periphyton field identification chart (Appendix 3).

Procedure:

- Select a reference point at the downstream end of your site and on one bank drive a peg into the ground.
- Attach the tape measure to the peg and lay it out taut for a distance of 10 metres (or 5 x the stream width, whichever is the smaller). Attach the upstream end of the tape to a second peg.
- Divide the distance along the tape into thirds and mark the tape (i.e., 3.3-m intervals for a 10 m site distance).
- Attach the 20-m tape measure to a third peg at the location of the reference peg and unwind the tape across the stream at right angles to the main tape. Anchor the tape on the far bank with the fourth peg.
- Divide the width of the stream (water's edge to water's edge) into 5 equally spaced points.
- Working from the downstream end of the site, move out to the first point across the first transect to be sampled (this will be near the water's edge on one side of the stream). Bend down to lightly touch the bed sediments without looking at what is there. Ideally, pick up the first stone that you touch. Because the stones need to be a reasonable size to provide useful information sometimes you will have to disregard the initial piece of sediment that you touch if it is very small (e.g., gravel or sand) and pick up a stone no more than 10 cm away which is bigger than about 4 cm across.
- If the stream bottom is gravel, sand or silt, take a scooped sample at the sampling point with the tea strainer.
- Examine each stone carefully and identify the categories of periphyton present according to their colour and thickness using the periphyton field identification chart in Appendix 3. Estimate the percentage cover of the stone in each category (+ 5%) and enter this on the field data sheet reproduced on the next page.
- Complete the transect then move the tape upstream for the second transect at one-third interval and repeat the recordings.
- When complete, calculate the mean percentage cover of sampling points for each category of periphyton. You can also use the table shown below the sampling form to calculate an overall score for the sampling.

Periphyton (on exposed surfaces)			**Stone number:**									
		Peri.	**Transect 1**					**Transect 2**				
		score	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Thin mat/film:	green	**7**										
(under 0.5 mm thick)	light brown	**10**										
	black/dark brown	**10**										
Medium mat:	green	**5**										
(0.5-3 mm thick)	light brown	**7**										
	black/dark brown	**9**										
Thick mat:	green/ light brown	**4**										
(over 3 mm thick)	black/dark brown	**7**										
Filaments, short	green	**5**										
(under 2 cm long)	brown/reddish	**5**										
Filaments, long	green	**1**										
(over 2 cm long)	brown/reddish	**4**										
		Peri.	**Transect 3**					**Transect 4**				
		score	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Thin mat/film:	green	**7**										
(under 0.5 mm thick)	light brown	**10**										
	black/dark brown	**10**										
Medium mat:	green	**5**										
(0.5-3 mm thick)	light brown	**7**										
	black/dark brown	**9**										
Thick mat:	green/ light brown	**4**										
(over 3 mm thick)	black/dark brown	**7**										
Filaments, short	green	**5**										
(under 2 cm long)	brown/reddish	**5**										
Filaments, long	green	**1**										
(over 2 cm long)	brown/reddish	**4**										

		Transect 1					**Transect 2**				
Stone/sample no.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
(a)	Enter total % of stone surface covered by all types of periphyton										
(b)	Enter list of: percentage cover x score for that type of periphyton										
(c)	Sum of all multiplied % scores										
(d)	Average score per stone/ sample [(c) / (a)]										
(e)	Total of all average scores in line (d)										
(f)	Total average periphyton scores (d) /no. samples with periphyton (= overall avge.)										

Notes:

- In the present procedure we are recommending the use of 4 transects per site with 5 points / stones being examined per transect giving a total of 20 assessments / replicates per site. This is equivalent to the most detailed level of the SHMAK protocols (Level 2+) and will allow a good level of precision for activities such as State of the Environment reporting and detailed regional water quality assessments.
- Table 2 summarises the main taxa that typically dominate the different periphyton categories. Note that some taxa are found in several categories and it is not just their presence, but the thickness of the mat (i.e., degree of biomass development) which is important for the evaluation of water quality
- The indicator scores in Table 2 are preliminary at this stage and are derived from Biggs *et al.* (1998c) and unpublished data of the authors. These will be refined in future years with greater experience in their application in various habitats.

Table 2: Summary of periphyton categories for field assessment of community type in the SHMAK rapid assessment protocol. Also given are the enrichment indicator scores and taxa which could be expected to be dominating the biomass of the samples (*, diatom epiphytes give the green filaments a brown colouring).

Periphyton category		Indicator score	Typical taxa
Thin mat/film: (<0.5 mm thick)	Green	7	*Cymbella, Achnanthidium, Cocconeis, Ulothrix, Stigeoclonium* (basal cells), young *Spirogyra*
	Light brown	10	Assorted diatoms and cyanobacteria (e.g., *Cocconeis, Fragilaria, Synedra, Cymbella, Lyngbya, Amphithrix*
	Black/dark brown	10	Assorted cyanobacteria (*Schizothrix*, *Calothrix*, *Lyngbya*)
Medium mat: (0.5-3 mm thick)	Green	5	*Stigeoclonium, Bulbochaete, Chaetophora, Oedogonium, Spirogyra, Ulothrix*
	Light brown ±dark green / black bobbles	7	*Gomphonema, Gomphoneis, Synedra, Cymbella, Diatoma, Fragilaria, Navicula, Nostoc*
	Black/dark brown	9	*Tolypothrix, Schizothrix, Phormidium, Lyngbya, Rivularia*
Thick mat: (< 3mm thick)	Green/light bown	4	*Navicula*, *Gomphoneis*, *Synedra, Rhoicosphenia, Ulothrix, Oedogonium, Microspora, Spirogyra, Vaucheria*
	Black/dark brown	7	*Phormidium, Schizothrix, Audouinella, Batrachospermum, Nostoc*
Filaments, short (<2 cm long)	Green	5	*Ulothrix, Oedogonium, Microspora, Spirogyra, Cladophora*
	Brown/reddish	5	*Cladophora*, Oedogonium*, Rhoicosphenia, Navicula, Batrachospermum, Diatoma*
Filaments, long (>2 cm long)	Green	1	*Ulothrix, Oedogonium, Microspora, Zygnema, Spirogyra, Cladophora, Rhizoclonium*
	Brown/reddiish	4	*Melosira, Cladophora*, Rhizoclonium**

6.5 Quantitative protocols

6.5.1 Introduction

For many resource surveys and most impact assessments, sampling and analyses must be quantitative to enable testing for statistical significance of differences amongst sites and for diagnosis of impacts. There are two main parts to such sampling:

- locating the sampling points along a transect, and
- collecting the periphyton from the substrata.

Sampling points are located using transects as described for the Rapid Assessment Method 1 (Section 6.4.2) except that only one transect with 10 equally spaced points is used instead of 10 transects. Each point is defined by dropping an imaginary vertical line from the transect at a set interval; then picking up the nearest stone.

Transects have an important drawback for quantitative sampling in impact assessments. This is that it may not be possible to match physical characteristics of the sites at both control and impact sites. This may result in differences in periphyton community composition and biomass that are unrelated to the effects of the effect being monitored. In such situations it may be possible to just sample a sub-set of conditions that are common to each site (e.g., depths of 0.2–0.4 m, velocities of 0.3–0.5 m / s, only sample cobble sized substrates, etc).

When collecting the periphyton sample, the aim is to take samples in a consistent way from a known area. Different methods are used depending on whether the stream bed is:

- Gravel / cobble (2 methods, Method 1a, 1b),
- Bedrock / boulders (Method 2), or
- Sand / silt (Method 3).

The two most common methods for collection of periphyton samples from gravels and cobbles are whole stone sampling (Method 1a) and scraping and / or brushing a sample from a defined area on the top of the stones (Method 1b). With Method 1a, the samples provide representative data on the whole community inhabiting the stone, whether it is located on the tops or sides of the stone. There will be little difference in communities among such micro-habitats in fine cobble and gravel dominated habitats. However, high heterogeneity in biomass and taxonomic composition can occur over the surface of individual substrates where they are moderate sized cobbles and larger. This method is preferred for overall resource assessments and studies of instream ecological processes where it might be important to assess the total biomass and composition of the community across micro-habitats. Data generated from this method of sampling are usually expressed in terms of surface area of exposed sediments.

If you are only interested in the chlorophyll *a* concentration, then it is also possible to collect the rocks and return them with their communities intact to be directly extracted for chlorophyll in the laboratory.

With Method 1b, samples are only collected from the tops of the stones (i.e., the area of the substrata exposed to the direct flow of the water). This helps to remove the effects of spatial differences in water velocity, erosion of communities along the edge of the substrata, and effects of grazing invertebrates that usually spend most of their time under or along the edges of the substrata. Thus, this method is preferred for assessments of pollution effects, enrichment, etc. It is also the best method to use if large mats of long filamentous algae are expected because such mats can be “cored” with this method for an aerial estimate of biomass. Data generated from this method are expressed in terms of plane surface area of the stream

bed (which is, of course, considerably less than the total surface area of substrates exposed to the water).

With any quantitative method it is important to have an appreciation of the degree of error associated with your sampling. This reflects the natural heterogeneity of the periphyton, size of your sampling unit and the number of replicates. It is generally most easy to vary the number of replicates. Methods for calculating error are described in Section 3. In general we recommend at least 10 replicate samples per site, because periphyton are particularly heterogeneous. However, more may be required in some situations.

Before describing the sampling methods in more detail, we will outline requirements for sampling containers and labelling.

6.5.2 Sample containers

Different kinds of sample collection containers will be needed for different projects according to the volume of the samples being collected. For many purposes, Elkay-type containers are ideal. These are stackable flexible polyethylene specimen cups with screw lids and a volume of about 125 ml. Smaller (60 ml) plastic containers are also suitable for small samples.

For whole stone sampling, strong plastic bags (especially the snap-lock type) make the most convenient collection containers. Alternatively, 500 ml plastic bottles are also suitable.

6.5.3 Sample labelling

Prepare suitable containers for samples prior to field collection. Ensure that all containers are labelled with the following information:

- Date of sample collection.
- Name of collector (with organisation name if applicable).
- Job number (or job name).
- Site name or number.
- Sample number.
- Analyses to be done.

An example of label follows:

Date:	16 Feb 2000
Collected by:	A. Helper (ORC)
Job:	Kakanui monitoring
Site:	A
Sample:	1
Analyses:	Chla, DM, AFDM, Taxon.

Use pre-prepared paper labels, or write directly on the containers using a permanent marker pen. Remember that permanent marker ink is soluble in ethanol and do not label the lid (these can get mixed among samples very easily).

Correct labelling of sample collection containers at the time of sampling is crucial for the validity of the entire sampling programme.

6.5.4 Quantitative method 1a (QM-1a): Whole cobble/gravel sampling

Equipment:

1. 20–30 m tape measure.
2. 2 pegs (> 20 cm long) and mallet.

3. Deep sided laboratory tray or container (e.g., an ice-cream container).
4. A range of scrubbing brushes (from toothbrush size to nail brush – the size depends on the size of stone).
5. Scalpel.
6. Squirt bottle filled with stream water.
7. Labelled sample containers (500 ml bottles or large zip-lock plastic bags).
8. Plastic calipers.

Procedure:

- Select a reference point in the middle of your site and on one bank drive a peg into the ground.
- Attach the tape measure to the peg and lay it out taut across the stream. Anchor the far end with the second peg.
- Divide the width of the stream (water's edge to water's edge) into 10 equally spaced intervals.
- Move out to the first point across the transect (this will be near the water's edge on one side of the stream). Bend down to lightly touch the bed sediments without looking at what is there. Ideally, pick up the first stone that you touch. If it is too big to retrieve, then take the nearest one that can be picked up. If you touch a small silty or sandy patch among the cobbles, then also take the nearest stone that can be picked up.
- Place the stone on the white tray with a small amount of stream water and return it to the stream bank.
- Use the scalpel to scrape off any filamentous algae and thick growths of brown algae from the stone. Wash onto the tray using minimal water from the squirt bottle.
- Then use the brush(es) to scrub the stone thoroughly. Periodically rinse off the stone and brush into the tray. Scrub all sides of the stone to remove as much periphyton as possible. A standard scrubbing time of 2 minutes is suggested for cobble sized material.
- Transfer the contents of the white tray into your sample container (you may need to use a funnel if you have a narrow necked bottle).
- Finally, rinse the tray into the sample container until no trace of periphyton remains.
- Store the labelled container on-ice in a chilli-bin (cooler) for transport to the laboratory.
- Measure the *x*, *y* and *z* dimensions of the stone with the plastic calipers.
- Proceed to the next sampling point and repeat the above procedures.

6.5.5 Quantitative method 1b (QM-1b): Scraping or brushing a sample from a defined area on the top of a stone

Equipment:

1. 20–30 m tape measure.
2. 2 pegs (> 20 cm long) and mallet.
3. Deep sided laboratory tray or container (e.g., an ice-cream container).
4. Scalpel.
5. Small scrubbing brushes.
6. Labelled containers (e.g., 120 ml specimen cups; 60 ml rigid clear plastic pottles).
7. Pipettes (small “eye-dropper” is sufficient).

8. Squirt bottle containing stream water.
9. A ring of an appropriate size which can be used to define a sampling circle on each stone (e.g., the top of the sample collection container can be used).

Procedure:

- Select a reference point in the middle of your site and on one bank drive a peg into the ground.
- Attach the tape measure to the peg and lay it out taut across the stream. Anchor the far end with the second peg.
- Divide the width of the stream (water's edge to water's edge) into 10 equally spaced intervals.
- Move out to the first point across the transect (this will be near the water's edge on one side of the stream). Bend down to lightly touch the bed sediments without looking at what is there. Ideally, pick up the first stone that you touch. If it is too big to retrieve, then take the nearest one that can be picked up. If you touch a small silty or sandy patch among the cobbles, then also take the nearest stone that can be picked up.
- Place the stone on the white tray with a small amount of stream water and return it to the stream bank.
- Place the ring on top of the stone to define a circle in the centre, or as near as possible to the centre, of the stone.
- Scratch a mark on the stone around the outside of the ring with the tip of a scalpel blade. Then, scrape away from the outside of the ring all the surrounding periphyton.
- Remove the ring and then scrape off as much periphyton growth as possible from within the circle and rinse it off the scalpel into an appropriately labelled container.
- Scrub the defined area for ~30 seconds with a toothbrush then remove the slurry from within the circle using the small pipette (Figure 8).

Figure 8: Sampling periphyton from a defined area on the surface of a stone. *Left:* scraping the diatom film from within circumscribed area to show the difference in colour of the sampled area within the circle and the diatom film (additional scrubbing with a coarse toothbrush will complete the sampling). *Right:* a defined circle of periphyton mat which will be scraped/brushed off into a container as the sample.

- Rinse the area with a minimal amount of water. Remove any surplus water using a pipette and transfer into the sample container. Thoroughly rinse the brush into the container.
- Finally rinse the tray on which the stone was resting into the sample container until no trace of periphyton remains (Note: only use small amounts of wash water because you will quickly run out of space in the containers).
- Store the labelled container of periphyton sample on-ice in a chilli-bin for transport to the laboratory.
- If the sampling point falls over a mat of filaments streaming in the current then a slightly different approach is required for sample collection. Slide your hand underneath the filaments and gently raise them to the surface taking care to not disturb their alignment. Take the ring used for defining a set area and press it down firmly on top of the filaments and into the palm of your flat hand. This action will cut a core out of the mat which then becomes your sample. If necessary, use fine nail scissors to cut the filaments from around the edge of the ring.

6.5.6 Quantitative method 2 (QM2): Underwater bedrock/boulder sampling

It will be possible to use QM1a,b for most sites in New Zealand streams. However, sites with bed sediments of predominantly large cobbles, boulders or bedrock that are too large to retrieve for sampling (e.g., Figure 7), the samples must be collected in-situ with an underwater sampler. This sampler is made up from 2 x 60 ml wide-bore syringes with one of the syringes having a soft, thick rubber flange on the bottom to seal against the rock. The head of a flat toothbrush is attached to the plunger and this is used to brush the periphyton off the rock within an area defined by the base of the syringe (Figure 9). A second syringe is used to suck the slurry containing the abraded periphyton from within the base of the first syringe. This slurry is retained as the periphyton sample. The method was developed by Loeb (1981).

Figure 9: Double syringe periphyton sampler. *Top:* the apparatus, showing the components, and an assembled set. *Bottom:* the device in position in a stream ready for use.

Equipment:

1. 20–30 m tape measure.
2. 2 pegs (> 20 cm long) and mallet.
3. Double-syringe underwater sampler.
4. Squirt bottle filled with stream water.
5. Labelled containers (e.g., 120 ml specimen cups; 60 ml rigid clear plastic pottles).

Procedure:

- Select a reference point in the middle of your site and on one bank drive a peg into the ground.
- Attach the tape measure to the peg and lay it out taut across the stream. Anchor the far end with the second peg.
- Divide the width of the stream (water's edge to water's edge) into 10 equally spaced intervals.
- Move out to the first point across the transect (this will be near the water's edge on one side of the stream). Press the sampler firmly against the boulder / bed rock at this selected point, ensuring that there is a good seal around the base. The horizontal syringe should be inserted in the base of the main syringe as illustrated in Figure 9.
- Push the plunger down in the first syringe so that the brush is in contact with the substrate. Turn the plunger three full rotations to the right, then three full rotations to the left
- Draw some water into the vertical syringe by raising the plunger about 1 cm. Then draw this water / periphyton mixture into the horizontal syringe (this takes some dexterity, but is reasonably easy with practice).
- Carefully remove the apparatus from the water and discharge the sample into its appropriate container. Rinse off the brush into the container.
- Store the labeled container of periphyton sample on-ice in a chilli-bin for transport to the laboratory.
- The recommended number of rotations of the plunger may be insufficient to remove tightly attached periphyton. If possible test the method in a shallow area, or on a stone that can be retrieved at your site, so that you can check periphyton removal efficiency before using it for proper sample collection. If necessary increase the number of rotations, but then ensure that you use this number consistently for all samples.

  Note: Cattaneo and Roberge (1991) have tested the efficiency of this sampler for removal of periphyton in streams and lakes. They found that it depends largely on the relative proportions of loosely and tightly attached community. In stream, the syringe sampler tended to underestimate biomass, and the severity of this underestimation increased with water velocity under which the communities had grown. Thus, if it is necessary to use this sampler in streams, we recommend that for consistency within a study, it is used to collect all samples in that study.

6.5.7 Quantitative method 3 (QM-3): Sand/silt sampling

Equipment:

1. 20–30 m tape measure.
2. 2 pegs (> 20 cm long) and mallet.

3. Deep sided laboratory tray or container (e.g., an ice-cream container).
4. Labelled containers (e.g., 120 ml Elkays; 60 ml rigid clear plastic pottles).
5. Pipettes (small "eye-dropper" is sufficient).
6. Squirt bottle containing stream water.
7. Top half of a 47 mm diameter plastic petri dish.
8. Large stainless-steel spatula.

Procedure:

- Select a reference point in the middle of your site and on one bank drive a peg into the ground.
- Attach the tape measure to the peg and lay it out taut across the stream. Anchor the far end with the second peg.
- Divide the width of the stream (water's edge to water's edge) into 10 equally spaced intervals.
- Move out to the first point across the transect (this will be near the water's edge on one side of the stream). Bend down and gently press the petri dish lid into the top layer of sand / silt (to a depth of 5–7 mm).
- Slide the spatula blade under the petri dish to isolate the sediment in the dish.
- Gently bring to the surface, ensuring that as little material / water is lost from inside the petri dish as possible.
- Empty the petri dish into a tray / container, rinsing any residue from the petri dish, and then transfer into a labelled container.
- Finally rinse the tray into the sample container until no trace of periphyton remains.
- Store the labelled container of periphyton sample on-ice in a chilli-bin for transport to the laboratory.

6.6 Methods for sampling periphyton on artificial substrates

Artificial substrate sampling is used to measure net biomass production rates where the precise effects of water quality need to be assessed but where the natural substrates cannot be sampled. For example, the water may be too deep or the bed sediments may differ greatly among sites. Artificial substrate sampling is a type of in-stream "bioassay" of water quality conditions. This is because such substrata allow sampling surfaces and other habitat factors such as water velocities to be standardised, thereby allowing a more precise focus on differences in water chemistry. Factoring out confounding effects of habitat can be particularly important when evaluations cover broad geographic areas. Artificial substrata also allow easier and more reproducible sampling because they reduce heterogeneity in microhabitats. Artificial substrata are widely used for periphyton sampling in water quality surveillance, particularly in North America. However, artificial substrata sampling is not recommended if the study objective is to characterise local community composition, the effects of natural physical perturbations, the interaction between physical and chemical perturbations, or the effects of water quality perturbations on natural periphyton communities.

Various materials, sampler frames and sampling surface configurations have been used for periphyton artificial substrate samplers. Sampling surface materials commonly include etched acrylic and glass, but have also included slate, brick, glass microscope cover slips, clay tiles, filter papers, acrylic rods, and wooden dowels (Aloi 1990). The sampler frames also vary greatly, from bed-mounted samplers to fully floating devices. The samplers' frames should

reflect the type of environment to be monitored. For example, frames holding racks of glass microscope slides that float just below the water surface and that are tethered to the bed (like the Wildco periphyton sampler – see below) are commonly used in deep, sluggish rivers. However, a much more robust sampler comprising a rack of acrylic slides mounted on their edges in a steel caged that prevents debris damage (Figure 10), has been recommended for use in swift and shallow streams and rivers (Biggs 1988a).

While there are some advantages in using artificial substrate samplers, there are also several disadvantages. These have been summarised by Porter *et al.* (1993) as follows.

- Artificial sustrata require at least two visits to each sampling site (installation and retrieval), separated by a colonization period.
- The samplers can be susceptible to vandalism or washout during floods.
- The taxa found growing on the samplers can be biased toward those actively immigrating at the time of and in the location of the incubation.
- Artificial substrates may provide a less sensitive indicator than natural substrate communities of changes or differences in water quality associated with land disturbance, and changes in more general habitat conditions.

Also, biomass on artificial substrata is generally less than on natural rocks (Biggs 1988a). In relation to the taxonomic composition of communities, natural diatom assemblages are usually well simulated by those on artificial substrates, whereas green algae and cyanobacteria at high biomass can often be misrepresented (Cattaneo and Amireault 1992). Thus, data collected using artificial substrata should generally not be compared with data from natural substrata in assessments of possible effects of pollution. If a study is being carried out over a broad area (within a catchment or among streams) and artificial substrates are necessary at some times, then we recommend that such substrata be used at all sites so that valid comparisons of water quality can be made among the sites.

Changes in community composition can also occur during incubation. Indeed, duration of substratum exposure is critical to data interpretation and should be standardised among all sites within any one survey (Biggs 1988b). Rate of colonisation and succession varies as a function of time since the last major flood disturbance in the catchment (which affects density of available immigrants); degree of enrichment, water temperature and light (which affects growth rates), and local water velocities and invertebrate grazing (which affects delivery and losses of immigrants) (Biggs 1996). Suitable incubation periods can be from 1 to 2 weeks for maximum diatom diversity (Cattaneo *et al.* 1975) and for comparative studies of enrichment effects on growth rates across a gradient of nutrient concentrations, to 4–6 weeks for maximum biomass in mesotrophic and eutrophic habitats (Biggs 1988b). It is therefore important to be clear about your monitoring objectives and understand that artificial substrate sampling enables measures of relative differences; rarely do they enable assessments of absolute effects on natural communities.

Although using artificial substrates has advantages in relation to isolating water quality effects, there is a risk that data loss will occur through:

- vandalism;
- flooding;
- flotsam;
- stock crossing streams;
- recreational four-wheel-drive vehicles.

Careful sampler placement can help minimise such effects. For example, it is often possible to position samplers out of view (e.g., behind a willow tree) and away from "traditional" stock or vehicle crossings (preferably upstream!). Even taking such precautions, Biggs (1988a) reported a loss of 17% of samples due to vandalism over a 12 month study of nine rivers in Canterbury. Loss of data through vandalism can be reduced through replication of samplers at sites. Loss of samplers through flooding is always difficult to mitigate if sampling in foothills or mountain-fed streams or rivers. For example, Biggs (1988a) lost 19% of data due to flooding in the study of Canterbury rivers noted above. Conversely, flotsam from detached macrophytes is a problem in many low-gradient and spring-fed streams, particularly if a floating sampler is to be used.

The following are some general points to considered when designing an assessment programme using artificial substrate samplers.

- Local habitat conditions should be standardised among all samplers.
- The sampling surfaces should be moderately textured / etched to allow good colonisation and adhesion of the mat.
- The materials used for the sampling surfaces and holding frame should be inert so that no materials leach from the substrata which may influence community composition.

Standardising local habitat conditions among sampling sites can be difficult, but is essential to avoid erroneous conclusions about variations in water quality. For example, the data in Table 3 were obtained from three samplers exposed to habitat conditions that were as similar as could be obtained at a single site. There were no water quality differences between samplers, but significant differences were evident in the quantities of periphyton among the samplers. Had these samplers been placed individually at three different sites, as might have been done for a surveillance programme, erroneous 5-fold differences in productivity (and thus degrees of enrichment) might have been deduced. The sampling method is thus very sensitive to even minor differences in non-water quality habitat variables between samplers.

Table 3: Periphyton biomass on three artificial substrate samplers incubated at a single site in the Halswell River, near Christchurch. Eight replicates were collected per sampler and all samplers had biomass significantly different from each other ($P < 0.05$) (from Biggs 1985).

Sampler	Depth of sampler (m)	Velocity over sampler (m/s)	Mean biomass (mg chlorophyll *a*/m^2 + 95% C.I)
1	0.78	0.253	5.3 + 0.83
2	0.90	0.233	2.1 + 1.55
3	0.80	0.200	10.2 + 2.79

The type of frame used to mount the sampling surfaces is relatively unimportant provided that there is a good supply of light and water is free to flow across the surfaces without a high degree of turbulence or high velocities. If the samplers described below are considered inappropriate for your particular task, then three main factors should be evaluated when considering alternative designs (Biggs 1988a).

- The design must be appropriate to the physical conditions of the river being monitored.
- The sampler must hold substrates that will harbour a community as similar as possible to that normally found in the river so that major biases will not occur.

- The sampler should give reproducible results.

In the following sections we describe two types of artificial substrate samplers and one set of protocols for their deployment.

6.6.1 Sampler designs

Bed-mounted artificial substrate sampler

The bed-mounted sampler (Figure 10) has a oval acrylic (perspex) or marine-ply base-plate, fixed to a large concrete paving block (~ 10 kg) by masonry bolts which have been inserted into the block. This bolting system enables the sampler to be removed for sampling without disturbing the anchoring system. The block is fixed to the riverbed by wire and a stake which is driven in flush with the sediment to avoid catching debris.

A stainless steel wire cage is fitted to the front of the sampler base-plate to deflect debris. Two blocks of wooden or (preferably) acrylic slats are fixed to the base-plate under the cage. These slats hold vertical acrylic slide sampling plates, with the plates being held in place by wire rods which extend through a line of holes in the slats and base of the sampling plates. The leading edge of the plates is angled to avoid, or reduce, the collection of debris being washed downstream. The plates are first roughened by lightly sanding them with coarse sandpaper. The sampler can hold up to 28 plates, depending on the number of slats used.

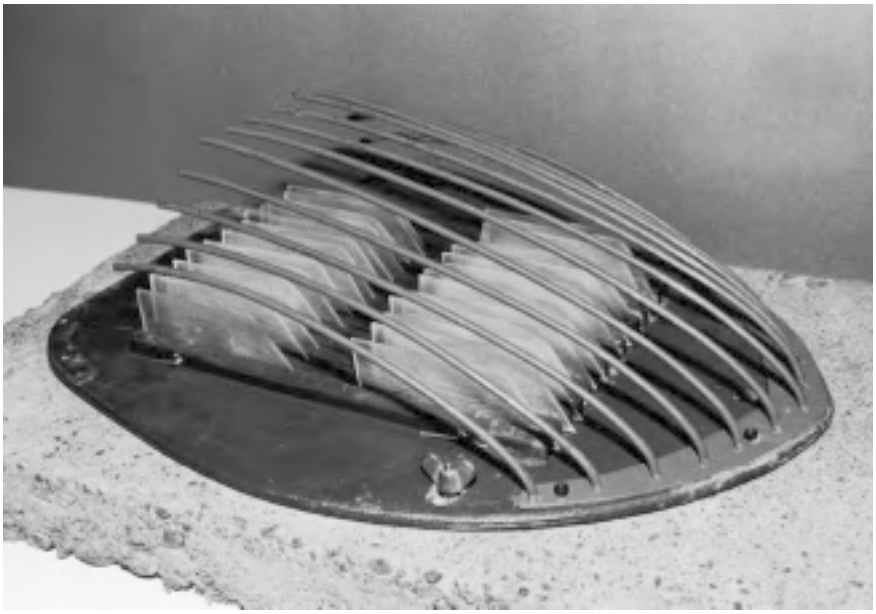


Figure 10: An artificial substrate sampler comprising a stainless-steel wire cage and edge mounted acrylic sampling plates. The sampler is attached to a large paving block to hold it in place on the stream bed (via bolts glued into the block and wing-nuts). The block can also be secured to the bed using long tent pegs and wire attached to the bolts on the block. The wing nuts allow easy removal of the sampler for periodic sampling and cleaning.

Floating sampler

Floating samplers have limited use in New Zealand because of our generally shallow, swift rivers and in deeper, low gradient rivers they tend to become clogged with macrophytes. There are a range of designs for floating samplers. These samplers can be purchased in North America (e.g., Wildlife Supply, Saginaw, Michigan – www.wildco.com / flowing.html#periph), but it might be cheaper to have them made (see diagram on p. 10-31 in APHA 1995). If you do

decide to construct your own, then the following points should be considered.

- The sampler frame should be constructed from perspex to avoid shadows on the sampling surfaces.
- Metal components should be corrosion-resistant (e.g., stainless steel).
- Ensure that there is a debris deflection guard.
- Position the floats away from the sampling surfaces.
- Rack the sampling surfaces vertically to minimise silt deposition.
- Use roughened acrylic or roughened glass microscope slides as sampling surfaces.

6.6.2 Sampler deployment

As noted in Sections 2.5.2, 4.2.3 and 6.6, placement of samplers is critical for:

- obtaining representative results;
- minimising variations among sites due to differences in physical habitat so that water quality effects can be isolated.

The second point is critical for valid comparisons of water quality among sites or habitats. As shown in Table 3, even minor differences in water velocity can result in highly significant differences in a number of periphyton metrics which are unrelated to water quality. Critical aspects of the physical habitat to standardise among sites are:

- degree of shading;
- water velocity;
- depth of sampling surfaces;
- proximity to the thalweg;
- influence of secondary currents.

It is usually necessary to carry out a preliminary physical survey of all sites where samplers are to be deployed before selecting exact positions for the samplers. Such a survey will help identify the constraints that might limit possible locations for deployment across the full range of sites. For example, water velocities might only cover a range of 0.1–0.3 m / s at one site, but range from 0.1 to 0.8 m / s at all other sites. In such a situation, you would need to standardise the placement of samplers at all sites at a velocity within the range of the first site (i.e., 0.1–0.3 m / s). Standardising for degree of riparian shade is usually quite difficult when monitoring in smaller streams.

When deploying bed mounted samplers, it is also important to ensure that the sampler is oriented so that the sampling surfaces are exactly parallel to the water flow. Otherwise, turbulence will be created around the slides which will cause a high degree of patchiness in the communities and siltation on the downstream side of the slides.

While it is very important to standardise for water velocity among samplers, depth is also an important factor. Variations in depth might influence growth particularly in more turbid streams and rivers where light attenuation is rapid. Therefore, where possible, attempt to also standardise depth among samplers.

6.6.3 Sampler retrieval

Samplers should be checked regularly to:

- clear any debris;
- check growth so that sloughing doesn't commence before retrieval. If growth at any one site appears about to slough from the substrates (i.e., it is loosely attached with small pieces

starting to break off), then the substrates should be removed at all sites.

It is important that incubation time be standardised among sites for any given sampling run so that differences in productivity and successional state can be adequately compared. Large variations, particularly in taxonomic composition, can occur with different rates of growth and leaving samplers at some sites to incubate longer than other sites for a particular sampling run will usually complicate data interpretation.

When retrieving the samplers:

- Approach them from downstream (i.e., move upstream to the sampler). It is often good to re-measure the depth and velocity of water immediately in front of the sampler to determine whether conditions have changed markedly since deployment.
- Detach the sampler from its "holdfast" (concrete block or attachment line).
- Gently lift the sampler from the water moving the sampler downstream with the current as you draw it through the water.
- When the sampler starts to clear the water, take a note of anything that might influence the periphyton communities and thus effect interpretation of the results. Write these down once on the river bank. Things to observe might include the amount of silt accumulated on the slides or substrates, any debris such as leaves, twigs or macrophyte stems that have caught on the sampler and might have brushed against the sampling surfaces or influenced local flow paths, numbers and types of invertebrates. A quick count of animals to the lowest taxonomic level that you can clearly recognise is often very useful in data interpretation and might provide another useful assessment of water quality (see the SHMAK rapid bioassessment protocol for a general grouping of invertebrates based on their pollution tolerances and ease of identification: Biggs *et al.* 1998c).
- On the river bank, carefully remove the sampling surfaces (e.g., perspex slides) and place them in individual labelled containers of stream water.
- Chill the samples to ~ 5°C, then return to the laboratory for analysis. Make sure that you keep the samples chilled and in the dark.

  *Notes:*The number of replicate substrates that you retrieve will depend on study objectives and the degree of precision. If you are assessing rates of production and need to construct a growth rate curve, then you may only retrieve three replicates at, say, 3-day intervals over a 2-week period (you will need to determine that you have started the incubation with enough replicate sampling surfaces to allow this). However, if you are assaying the effects of a perturbation on community composition (e.g., a discharge from a treatment plant), then one set of, say, five replicates might be sufficient. See Section 3 for statistical methods for determining sample replication.

6.7 Nutrient limitation assays

It is important to determine what nutrient might be limiting production of periphyton in streams, and the degree of limitation. Traditionally, this has been inferred from nitrogen and phosphorus concentrations and / or nutrient ratios in the water. More recently, nutrient limitation has been assessed by cellular nutrient quotas. However, these approaches have their drawbacks (Biggs 2000b), and the least ambiguous approach for establishing the nutrient limiting growth is the instream nutrient assay (Francoeur *et al.* 1999a). The simplest of these assays is the nutrient-diffusing substrate. These are artificial substrates of a porous material

placed over a container of agar impregnated with nutrients. The nutrients slowly diffuses out to the substrate providing a localised nutrient source. Periphyton grows on the substrate. After a defined time, the substrates are processed for chlorophyll *a* to compare periphyton growth in response to the different nutrient treatments. The treatment with the highest chlorophyll *a* indicates the most limiting nutrient for periphyton growth. Such assays have been used to determine the degree of enrichment caused by different landuses (e.g., Chessman *et al.* 1992, Corkum 1996), the effects of forest harvesting (e.g., Lowe *et al.* 1986), the effects of enrichment from point source discharges (e.g., Dube *et al.* 1997, Scrimgeour and Chambers 1997), and trophic interactions between nutrients, periphyton and invertebrates (e.g., Winterbourn 1990, Biggs and Lowe 1994).

Several types of nutrient-diffusing substrate have been used in periphyton monitoring and research. One type designed for use in New Zealand gravel-bed rivers comprises a large galvanised steel tray which holds plastic jars containing the nutrient enriched agar (Biggs and Lowe, 1994) (Figure 11). This steel-tray sampler is robust to floods, quick to set up and deploy, and has samples that are easily retrieved and analysed. Two incubations of approximately 2 weeks duration each can be obtained from each set of agar jars. Its disadvantages are that only chlorophyll *a* can be assessed from the substrates, large amounts of agar are required, the trays are difficult to fabricate by non-specialist engineers (they can be purchased or hired from NIWA, Christchurch), and the filter paper sampling surfaces sometimes rip. This design of substrata has been used extensively in New Zealand for both biomonitoring and research purposes (e.g., Francoeur *et al.* 1999a).

Other types of samplers are made from unglazed flower pots, through which the nutrients leak (e.g., Fairchild *et al.* 1985, Scrimgeour and Chambers 1997) and petri dishes containing the nutrient agar mix with a coating of sand set into the agar (or Nitex netting over the top) as collection surfaces (e.g., Pringle and Bowers 1984, Winterbourn 1990, Corkum 1996). This latter method uses much less agar and nutrients, but is also much less robust for use in flood prone and swift streams (e.g., Scrimgeour and Chambers 1997). Tate (1990) modified the flower pot and petri dish methods by using an inverted clay flowerpot saucer. This is more robust, does not protrude significantly into the flowing water where it might be damaged, and uses a substrate that is more analogous to natural hard rock surfaces. It also has the advantage over the steel-tray method that the samples can be analysed for a full suite of variables and not just chlorophyll *a*. Both the steel-tray and clay flowerpot saucer methods are described below.

Two problems occur with nutrient-diffusing substrate assays which may confound the interpretation of results. First, nutrients are released from the reservoirs at a negative exponential rate (Corkum 1996, Scrimgeour and Chambers 1997). This means that the supply concentration of nutrients that seeps from the agar and through the porous substrata reduces rapidly over time. Therefore, the volume of the agar reservoir is an important influence on how long samplers can be left out to incubate for. Corkum (1996) found that nutrients were depleted from 80-ml agar reservoirs in 6 days, with phosphate depleting at a much faster rate than nitrate. Similarly, Scrimgeour and Chambers (1997) found that nutrient release declined to relatively low levels after 32 days incubation of 325 ml nutrient-agar reservoirs.

Second, grazing by benthic invertebrates can have a major effect, and this may vary among sites. Such grazing influences are particularly acute in lowland or spring-fed streams where densities of snails may be high. The snails tend to gather on the patches of higher periphyton production and remove large amounts of the newly produced biomass. In such cases, the nutrient enhancement may not result in a significant difference in chlorophyll *a* compared

with controls, but an increase in consumer biomass may occur (e.g., Biggs and Lowe 1994). It is possible to reduce this effect by placing the substrates in relatively high velocities (e.g., > 0.5 m / s) where it is too harsh for snails to migrate onto the periphyton colonisation surfaces. Biggs and Lowe (1994) placed some of their substrates in riffles to dissuade snail colonisation. Another approach that has been used to control grazing is the use of the insecticide malathion in the agar which leaches out with the nutrients (e.g., Gibeau and Miller 1989, Winterbourn 1990, Peterson and Grimm 1992). However, recently, malathion has been found to inhibit algal growth and the optimal dose of malathion that will inhibit grazing without inhibiting periphyton growth has yet to be found (Francoeur *et al.* 1999b).

6.7.1 Steel-tray nutrient-diffusing substrate method (suitable for large streams and flood prone rivers)

Overview

The nutrient-diffusing substrates are galvanised steel trays (dimensions 600 mm x 400 mm x 200 mm), with a handle at each end and a drainage aperture at one end. An internal thick plastic frame holds 20 nutrient-diffusing reservoirs (Figure 11). Each reservoir is a 400 ml square plastic jar filled with nutrient agar.

The periphyton colonisation surfaces are hardened ashless filter papers placed over the top of the reservoir jars and held in place by push-fit plastic rings. A thick plastic lid fits over the entire box. Twenty holes cut in the lid fit exactly over the colonisation papers so that the stream current flows over a flat, unbroken surface. The lid is secured by tightening nuts onto four long bolts welded to the base of the box. On the surface of the lid, five 20 mm high longitudinal partitions maintain parallel flow and prevent diffusion of different treatments onto neighbouring rows. The trays and internal fittings are not easy to fabricate and detailed design notes are not given here. Samplers can be purchased or hired from NIWA Instrument Systems, P.O. Box 8602, Christchurch (phone 03 348 8987; e-mail g.elley@niwa.cri.nz).

For deployment on the stream bed, ideally the tray is placed in an excavated depression so that the surface is no more than 100 mm above the bed. Often this is not practical. Where possible, it is a good idea to secure the tray by wiring it to metal stakes (one at each end) driven into the bed, or by running anchor lines from a tree or post on the stream bank.

To assess periphyton growth, the entire hardened ashless filter papers are analysed for chlorophyll *a*.

The standard assay uses five replicates of each of four nutrient treatments:

- Phosphorus (0.05 molar solution of trisodium orthophosphate in agar)
- Nitrogen (0.5 molar solution of sodium nitrate in agar)
- Phosphorus + nitrogen (both the above in agar)
- Control (no nutrients added to agar).

Preparation of nutrient-agar solutions

Materials:

Standard chemicals used, with quantities, are:

- Granulated agar: 20 g per litre
- Sodium nitrate ($NaNO_3$) 42.5 g per litre
- Trisodium orthophosphate ($Na_3PO_4.12H_2O$) 19 g per litre

Each tray holds 20 jars, five each of:

Figure 11: Steel-tray nutrient-diffusing substrate sampler. *Top:* sampler without the lid attached showing the internal frame of the sampler. The four agar-filled jars at the rear show stages in securing the filter-paper colonisation surfaces to the jars. The phosphorus-enriched agar jars are dark in colour. *Bottom:* nutrient-diffusing substrate sampler after incubation with the lid attached (the upstream end is marked with red paint on the left). Treatments from the far side are: control, nitrate + phosphate, phosphate, nitrate .

- Plain agar
- Agar + nitrate
- Agar + phosphate
- Agar + phosphate + nitrate

The trays are designed to hold 400 ml Labserv jars with square bottoms and a round opening (Catalogue no. LBS 33030, 400 ml containers, natural cap, unlabelled). Allow a little extra agar solution for spillage.

Equipment:

1. Scales.
2. Measuring cylinder/jug.
3. Hotplate.

4. Large pot for boiling agar.
5. Mixing tools (glass rods).
6. Plastic jars for enriched agar.

Procedure:

- Measure out the required volume of water into the container.
- Sprinkle onto the water the required amount of granulated agar (20 g per litre).
- Heat the water and stir until the agar is completely dissolved.
- Weigh out the appropriate amounts of phosphate and / or nitrate and add to the warm agar solution. Heat and stir until completely dissolved.
- Bring the mixture to boiling point then allow it to cool down before pouring into the jars. If it's too hot the jars will buckle and collapse.
- Fill the jars to the brim. The growing surfaces for periphyton (filter papers) need to be in contact with the agar.
- Store the filled jars with their lids on in the refrigerator.
- Use coloured stickers or similar to distinguish jars of different media. (Those containing phosphate turn dark brown; nitrate and plain media are uncoloured.) We use the following colour codes:

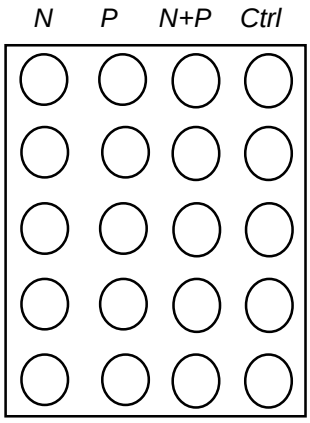
- N (nitrate) only	ORANGE or RED stickers
- P (phosphate) only	YELLOW stickers
- N and P together	PINK or WHITE stickers
- Control (agar only	GREEN stickers

Preparation of trays

Equipment:

1. NDS trays, including 20 rigid plastic rings for each.
2. Appropriate numbers of jars of nutrient agar.
3. Rubber bands (10 x 76 mm, no. 73).
4. Filter papers (Whatman no. 542, hardened, ashless, 9 cm diameter).

The jars of agar are always placed in the trays in the same configuration, i.e. four rows of five:



Position the jars in the tray, then:

- Place a rubber band around neck of each jar.

- Place a filter paper over the jar opening.
- Gently push one of the plastic rings supplied with the tray onto the jar over the top of the filter paper to fold the paper down over the neck and rubber band. The rubber band ensures a tight fit.
- Ensure the paper is stretched smoothly over the agar surface, with no creases or tears.

Notes:

- The lid of the tray fits over the top of the jars so that only the tops of the filter papers are exposed.
- Screw the lid down tightly.
- Each tray has a numbered tag attached to one of the handles. This number should match the one etched on the tray lid. Lids and trays are generally not interchangeable.

Deployment

Equipment:

1. Spanner for tightening nuts.
2. Metal stakes (warratahs).
3. Wire and wire cutters.
4. Mallet.
5. Spade (may be needed to dig a cavity in the stream bed for the tray).

Procedure:

- Prepare the trays with agar jars and filter papers shortly before deployment. This could be the night before, but don't do it much further in advance as the agar shrinks on exposure to air.
- Rinse off the lids and keep them handy. You'll need them if the media is to be re-used.
- Select a suitable location in the river. If monitoring at multiple sites, ensure that depth and velocities are the same at all deployment points (see Section 6.6.2).
- Once your limitations for water depth, velocity, shade, etc. have been set, then choose more-or-less stable locations on the stream bed.
- Make a depression in the bed (if bed material is suitable for this) and secure the trays, tags upstream, using a warratah wired onto the upstream handle. If in doubt, secure the downstream handle as well.

Notes:

- If you are deploying trays in pairs place them side by side, around 30–50 cm apart (or as convenient).
- Always deploy the trays with the numbered tag at the upstream end. For ease of recognition (and to allow for correct orientation of trays from photographs) the upstream end is also marked with brightly-coloured paint. As a further check, mark the nutrient rows at the upstream end of the inner plastic frame (N, P, N+P, C) with an indelible marker pen.
- Numbers 1–20 are systematically assigned to the jars. Always use these numbers as part of your labelling system for the containers into which you collect the filter papers, for example, site no. (or letter) + jar number.

Incubation time:

Normally, 14–17 days is enough to achieve good growth on the substrates before significant sloughing takes place. Of course this varies from river to river, and with season. After your first experiment you may need to adjust the length of any subsequent incubations. Keep the periods as consistent as is practical. It is best to avoid leaving the samplers to develop thick mats as sloughing may occur, and this would distort the results. Also large volumes of ethanol are required for chlorophyll extraction from thick mats.

We have found that the substrates give good growth responses when deployed for a second incubation with new filter papers. Beyond this time, results are doubtful.

Storage:

Freshly made media keep well for extended periods under refrigeration if the jar lids are kept tightly sealed. The media have limited "shelf life" between incubations, especially if not kept in a cool place.

Collection

Materials:

1. Collection containers for the filter papers (e.g., 60 ml. plastic containers, small whirl-pack or zip-lock plastic bags).
2. Permanent markers for labelling (can be done in advance).
3. Spanner to undo nuts.
4. Lids of agar jars for resealing (prior to re-use).
5. Paper towels.
6. Plastic bags.
7. Camera (possibly – see below).

Procedure:

- Gently remove the tray from the stream bed moving it downstream with the current as you lift it up. *Warning: the trays are very heavy when full of water.*
- Keep the tray close to horizontal while draining, especially if there is any chance of material becoming detatched from the filter papers.
- When the sampler starts to clear the water, take a note of anything that might influence the periphyton communities and thus effect interpretation of the results. Write these down once on the river bank. Things to observe might include the amount of silt accumulated on the slides or substrates, any debris such as leaves, twigs or macrophyte stems that have caught on the sampler and might have brushed against the sampling surfaces or influenced local flow paths.
- Count any invertebrates on each filter paper to the lowest taxonomic level that you can clearly recognise. These data are often very useful for interpreting the enrichment results and might provide another useful assessment of water quality (see the SHMAK rapid bioassessment protocol for a general grouping of invertebrates based on their pollution tolerances and ease of identification: Biggs *et al.* 1998c).
- Remove the lid and carefully slide off the plastic rings.
- Starting at the upstream left hand corner (the first N treatment), remove the whole filter paper from the agar substrate.
- Drain off as much water as possible.

- Fold the filter paper and sample in half (upper sides together).
- Clean off any periphyton growing on the underside of the filter paper with a soft toothbrush and discard.
- Fold the paper again, or roll up loosely, and place the whole paper in an appropriately labelled container.
- Put the 20 containers for each tray into a separate plastic bag – this greatly simplifies sorting out back in the lab.
- Transport the bag of samples back to the lab for analysis as quickly as possible. In the meantime, keep them frozen, if at all possible; otherwise keep them in a cool dark place.

Notes:

- Occasionally the papers become very fragile or are destroyed by invertebrate grazing.
- The most important part of the collection procedure is getting the filter papers into correctly-labelled containers.
- It is often very helpful for interpretation of the results to photograph each tray in the stream, and on the bank straight after removal. Make sure that the coloured paint is visible on the photo, as this means that you know for certain which treatment is which.

Post-collection analysis:

Chlorophyll *a* is analysed directly from the filter papers from the NDS trays using the method described in Section 7.3, but with slight variations. These are as follows:

- 60 ml containers are used for extraction of the chlorophyll because the NDS filter papers are so large.
- The 60 ml containers do not fit in standard tube racks so need to be placed in the water bath on a tray, while being held down with another tray on their lids.
- After boiling and leaving for 12 hours in the refrigerator, the containers with extractant are shaken and a 5 ml aliquot is removed which is then placed in a centrifuge tube for the balance of the analysis as described in Section 7.3.
- Blending is not required.
- Calculate chlorophyll *a* per square metre, assuming that the collecting surface is 65 mm in diameter (i.e. 0.003318 m^2).

6.7.2 Clay flowerpot-saucer nutrient-diffusing substrates (suitable for small streams and non-flood prone rivers)

Overview

The clay flowerpot-saucer nutrient-diffusing substrate technique is cheaper to construct than the steel tray method. However, more effort is required in the field for deployment and these substrates a more prone to disturbance during freshes and floods. The substrates comprise an inverted, unglazed, clay flowerpot saucer attached to a perspex base. Instructions for fabricating these samplers are given below. The saucer is filled with nutrient-agar solution through a hole in the top which is then sealed (e.g., with silicon caulk or a bung). Replicates of each treatment are then placed randomly on the streambed.

The nutrients diffuse out through the clay saucer and are absorbed by the adhering periphyton. The periphyton are removed at the end of the incubation period using a toothbrush. The sample is then analysed for chlorophyll *a*.

The standard assay uses five replicates of each of four nutrient treatments:

- Nitrogen (0.5 molar solution of sodium nitrate in agar)
- Phosphorus (0.05 molar solution of trisodium orthophosphate in agar)
- Nitrogen + phosphorus (both the above in agar)
- Control (no nutrients added to agar).

Fabrication of the sampler

Materials:

1. Unglazed clay flowerpot saucers (10–15 cm diameter) (purchase from garden suppliers).
2. Perspex (plexiglass) baseplates, cut in squares to be at least 1 cm larger than the diameter of the saucer (purchased as polycarbonate sheet; check Yellow Pages under "Plastics").
3. Silicon glue.

Procedure:

- Drill a small hole (e.g., 5–8 mm diameter) in the base of each saucer using a masonry bit and slow speed drill. Take care not to crack the saucer.
- Cut the perspex sheet to the appropriate size and drill holes in the corners for 10–15 cm galvanized nails (with flat heads) or ties to secure the samplers to the stream bed.
- Invert the saucer and glue it to the base plate using silicon fish-tank glue. Leave for several days to cure.
- Soak each sampler in water (preferably distilled) for a week to leach any mobile chemicals from the saucers. The samplers are now ready for filling with agar.

Preparation of nutrient diffusion solutions

Standard chemicals used, with quantities, are:

- Granulated agar: 20 g per litre
- Sodium nitrate ($NaNO_3$) 42.5 g per litre
- Trisodium orthophosphate ($Na_3PO_4.12H_2O$) 19 g per litre

For each site, prepare five substrates each of:

- Plain agar
- Agar + nitrate
- Agar + phosphate
- Agar + phosphate + nitrate

Allow a little extra agar solution for spillage.

Equipment:

1. Scales.
2. Measuring cylinder/jug.
3. Hotplate.
4. Large pot for boiling agar.
5. Mixing tools (glass rods).
6. Pre-prepared saucers.

Procedure:

- Measure out the required volume of water into the container.
- Sprinkle onto the water the required amount of granulated agar (20 g per litre).
- Heat the water and stir until the agar is completely dissolved.
- Weigh out the appropriate amounts of phosphate and / or nitrate and add to the warm agar solution. Heat and stir until completely dissolved.
- Bring the mixture to boiling point then allow it to cool down before pouring into the saucers.
- Fill the saucers to the brim. Any air trapped in the saucer will increase its buoyancy making it easier to dislodge.
- Store the filled saucers in the refrigerator.
- Use coloured stickers or similar to distinguish saucers of different media (those containing phosphate turn dark brown; nitrate and plain media are uncoloured.). We use the following colour codes:

- N (nitrate) only	ORANGE or RED stickers
- P (phosphate) only	YELLOW stickers
- N and P together	PINK or WHITE stickers
- Control (agar only	GREEN stickers

Deployment

- Select a suitable location in the river. If monitoring at multiple sites, ensure that depth and velocities are the same at all deployment points (see Section 6.6.2).
- Once your limitations for water depth, velocity, shade, etc. have been set, then choose more-or-less stable locations on the stream bed.
- The replicate substrates can be deployed in 5 groups of 4 treatments (N, P, N+P and Control) or haphazardly around the site. Given the constraints of depth, velocity shading and the main flow path, it is usually very difficult to deploy these substrates in a truly random arrangement.
- Secure the samplers using a several large galvanised nails driven into the gravels with a hammer. Alternatively a long tent peg (or short warratah) may be driven into the bed upstream and the substrate wired to the peg. Try to ensure that the pegs are driven down far enough so that the head is flush with the surrounding sediment to avoid catching debris which may then brush against the substrates. If in doubt about how well secured the substrates may be then secure the downstream end as well.
- Take care that the substrates do not influence one another (i.e., nutrients diffusing from an N treatment does not diffuse directly onto a P treatment). If substrates must be downstream of another because, say, the stream is very narrow, then attempt to have at least a 1.5 m gap between substrates.

Notes:

- Always deploy the saucers with a number on the upstream end (this helps for photo identification and later analysis).
- Numbers 1–20 are assigned to the saucers. Always use these numbers as part of your labelling system for the containers into which you collect the periphyton.

Incubation time

Normally, 14–17 days are enough to achieve good growth on the substrates before significant sloughing takes place. Of course this varies from river to river, and with season. After your first experiment you may need to adjust the length of any subsequent incubations. Keep the periods as consistent as is practical. It is best to avoid leaving the saucers to grow very thick mats as sloughing may commence which will distort the results.

Collection

Materials:

1. Collection containers for the periphyton removed from the samplers (e.g., 60 ml plastic pottles).
2. Toothbrush (flathead, medium hardness).
3. Scalpel.
4. Small pippette.
5. Permanent markers for labelling (this can be done in advance).
6. Paper towels.
7. Laboratory tray.
8. Plastic bags.
9. Camera.

Procedure:

- Detach the securing pins or wires first.
- Gently lift the substrates from the streambed moving then in a downstream direction with the current.
- When the sampler starts to clear the water, take a note of anything that might influence the periphyton communities and thus effect interpretation of the results. Write these down once on the river bank. Things to observe might include the amount of silt accumulated on the saucers, any debris such as leaves, twigs or macrophyte stems that have caught on the sampler and might have brushed against the sampling surfaces or influenced local flow paths.
- Pace the sampler on a laboratory tray.
- Count any invertebrates on each saucer to the lowest taxonomic level that you can clearly recognise. These data are often very useful for interpreting the enrichment results and might provide another useful assessment of water quality (see the SHMAK rapid bioassessment protocol for a general grouping of invertebrates based on their pollution tolerances and ease of identification: Biggs *et al.* 1998c).
- Scrape off as much periphyton growth as possible with the scalpel into an appropriately labelled container.
- Scrub the saucer with a toothbrush transferring the slurry into the sample container using the small pipette.
- Rinse the saucer with a minimal amount of water. Remove any surplus water using a pipette and transfer into the sample container. Thoroughly rinse the brush into the container.
- Store the labelled container of periphyton sample on-ice in a chilli-bin for transport to the laboratory.

- Group the samples into separate bags for each site – this greatly simplifies organisation back in the laboratory.

Notes:

- The most important part of the collection procedure is getting the periphyton thoroughly removed from the saucer and placing it in the correctly labelled container of water.
- It is often very helpful for interpretation of the results to photograph groups of saucers in the stream, and then on the bank straight after removal. Make sure that label is visible in the photo.
- You should probably count invertebrates before removing them from the samplers.
- Make notes on any unusual features such as the spatial distribution of periphyton, parts that may have been abraded etc.

Post-collection analysis

Analyse the slurry collected from each replicate sampler for biomass and / or taxonomic composition as required. See Section 7.

6.8 Community metabolism (maximum primary productivity and community respiration) using benthic respirometry

Primary productivity and respiration are two important process variables of periphyton communities. These measures are based on the oxygen evolved during photosynthesis and oxygen consumed by algae and heterotrophic organisms during respiration.

The method described below enables maximum primary productivity and community respiration to be measured; it does not allow net nor gross primary production to be measured (these latter variables require use of the diurnal oxygen curve method– see Bott 1996). The method described here can be used to indicate rates of carbon assimilation (a surrogate for growth rates), oxygen uptake rates / heterotrophic activity, and the relative balance between autotrophic and heterotrophic activity in the periphyton community. Such variables can be important for understanding the more detailed mechanisms resulting in response of periphyton to natural and artificial perturbations. Respirometers can also be used for nutrient limitation / uptake experiments, assessments of responses to different light levels (i.e., developing photosynthesis vs. irradiance curves), and toxicity assays.

Briefly, the method involves placing a sample of substrates (stones, gravel or artificial substrates) into a container with a perspex / plexiglass lid (the respirometer). The respirometer is then submerged in a shallow pool in the stream / river, the system sealed, and then stream water is pumped across the surface of the substrates and then recirculated back into the chamber through a short return hose with a 12-V DC submersible pump. Oxygen concentrations in the water within the respirometer are monitored over time (10–15 min.). The output from the dissolved oxygen (DO) meter recorder port is routed via a cable through a scale expander to amplify the DO signal within its working range and then this signal is routed to a field chart recorder. A black rubber sheet is placed over the respirometer for measuring respiration. The rate of change in oxygen during each treatment is used to estimate maximum photosynthetic rates (P_{max}) and community respiration (CR).

A significant trend in DO is difficult to obtain with low biomass and when the stream is near DO saturation. This leads to spontaneous degassing with the result that there is accumulation of air bubbles under the lid of the respirometer. Such degassing results in a major bias in

recordings and very noisy data. Ways to avoid this are explained below including purging the water in the respirometer with nitrogen gas. Respirometers are also prone to resource (CO_2 and / or nutrient) limitation. However, the method described below uses only short incubation times which greatly reduces the potential for this problem. Non-uniform velocity fields within the chambers are another potential problem. Water flow is often centered in the middle of the chamber. Baffles are inserted in the entrance region in an effort to create more uniform flow across all the substrate. Regardless of this, flow is always quite turbulent, which is probably more realistic if test substrates are from shallow, cobble bed streams. Dodds and Brock (1998) have recently designed a new chamber configuration in an attempt to reduce the problem of non-uniform velocity fields.

If biomass is low, there is little that can be done except attempt to fill the chamber with as much substrate as possible. Sometimes there can be difficulties detecting trends in oxygen evolution or consumption. It is important that each analysis is monitored carefully as it progresses so that problems or lack of trends are quickly identified and remedied. These measurements can be quite problematical at times.

P_{max} is usually influenced by both biomass and temperature (Boston and Hill 1992, Hill 1996, Biggs *et al.* 1999). It is therefore essential that both these variables are measured. Temperature should be recorded at the beginning and end of each respiration and photosynthesis run. If the respirometer is fully submerged in water with a moderate current (e.g., 0.2–0.4 m / s) then changes in temperature should be minimal during each incubation. At the end the substrates brushed clean (using sampling Method 1a, Section 6.5.4) to remove the periphyton and the slurry is analysed for both chlorophyll *a* and ash-free dry mass.

The following chamber design and methodology is based on that developed and tested by Hickey (1988).

6.8.1 Equipment

1. Benthic respirometer (Figure 12), consisting of:
   - A 25 cm x 40 cm internal dimensions stainless steel base tray,
   - A perspex / plexiglass chamber that clips over the top of the tray and seals around the edges with rubber flanges. The chamber has baffles at the inlet end to dissipate the flow.
   - A re-circulation hose with an in-line 12-V DC submersible pump and a housing for the dissolved oxygen probe.
   - 12 V DC deep cycle truck battery to power the submersible pump.
2. Precision dissolved oxygen meter with a recorder output port and a 0–10 V range for the output signal.
3. A scale expander to amplify the DO meter signal within a specific range.
4. Thin rubber sheet with a weighting chain attached to the perimeter.
5. A 1000–1500 watt quartz halogen lamp mounted on a stand with adjustable legs.
6. Portable generator capable of > 1500 watts and isolating transformer to power the lamp.
7. Large rectangular plastic bin (~ 0.8 m x 0.5 m x 0.3 m).
8. Large finely graduated plastic measuring jug (2–3 litres).
9. Small bottle of nitrogen gas (optional).

Figure 12: Benthic respirometer on adjustable legs. The submersible pump is mounted on the left end and dissolved oxygen probe is insert through a port on the lid of the main chamber. An in-line electromagnetic flow meter (not shown) can be inserted in the return flow line (foreground). Behind the respirometer are the (from left): 12-V DC marine grade battery to power the pump, voltage controller, chart recorder, scale expander and dissolved oxygen meter. The overhead lighting system is not shown (see Figure 13).

Figure 13: Undertaking P_{max} measurements with the benthic respirometer. A 1500 Watt lamp is mounted over the respirometer to provide standardised, high light for incubations. The light is power by a generator on the bank (out of photo).

Notes:

- Respirometers and scale expanders can be purchased from NIWA through the authors. These would be made up on demand. Customised designs could also be considered, or alternative designs such as that proposed by Dodds and Brock (1998).
- Most perspex / plexiglass filter out UVB wavelength light and thus creates a modified light environment for the periphyton. If it is important that these wavelengths are not altered, then special UV transparent perspex / plexiglass needs to be used for the chamber lid.
- The return hose length and depth in the chambers needs to be minimised to reduce the volume of water being re-circulated. This increases the sensitivity of the method.
- The volume of the respirometer needs to be known exactly to enable the photosynthesis / respiration rate calculations to be completed.
- We have designed a variation on the above respirometer that is quite small to accomodate unglazed ceramic tiles (up to 5 tiles) for experimental studies. This respirometer is used in the laboratory under broad wavelength artificial light (~ 1000 $\mu E\ s^{-1}\ m^{-2}$).

6.8.2 Procedure

- Locate a suitable swift pool or run (> 0.3 m deep, velocity 0.2–0.4 m / s) at your study site and set-up the respirometer in the water. It may be necessary to excavate a small pit to place the respirometer if the stream is quite shallow. Ensure that the chamber is level.
- Start the motor and check that it is pumping water through. If it is not then the pump probably has air trapped inside it. This can usually be removed by raising one of the respirometers. Then ensure that all air bubbles are purged from the system then disconnect the pump.
- Connect the DO probe to the meter, connect the DO recorder output line to the scale expander and then connect the expander line to the chart recorder. Check that all are working.
- Check the calibration of the DO probe (ensure that it is properly warmed up first – this can take longer than 10 minutes). Then connect the probe to the housing in the chamber re-circulation hose.
- Remove the stainless steel base tray of the respirometer and collect stones from the stream bed according to the requirements of the study (this will normally be from a transect across a specific riffle or run).
- Place the stones in the base tray so that their upstream–downstream orientation is maintained.
- Reconnect the battery to start the pump and check that the water is flowing through the respirometer and that there are no further air bubbles. Adjust taps on the inflow lines to get a reasonably even inflow of water. Then close the lid (you may need to wipe the underside of the lid to remove any attached bubbles. Run for pump for 2–5 minutes before starting DO measurements.
- Carry out the respiration measurement first by placing the black blanket over the chamber. Ensure that there are no gaps around the side where light could penetrate.
- Turn on the chart recorder (set it to run at ~ 1 cm / min and 1 mm = 1 mg / l scale). Set the scale expander to the appropriate range. Note the temperature, start time, begin-

ning oxygen concentration from the meter readout, and chart recorder speed on the chart.

- Track the change in DO. The line should trend down after a few minutes. Note the oxygen concentration on the chart recorder chart at several different concentrations to ensure a correct calibration of the chart divisions with DO.
- Run the respirometer until a monotonic linear trend has been recorded for at least 8 minutes. There should be only minor high frequency fluctuations in DO (1–2 mm maximum on the chart).
- Once a clear trend has been established, note the temperature again, then remove the rubber blanket to record photosynthetic rates.
- Place the light over the respirometer and start the generator to power it. The downward trend in DO should be reversed within a few minutes. Note the DO concentration regularly during the upward trend.
- Run the respirometer again until a clear upward linear trend occurs for at least 8 minutes.
- When a satisfactory record has been obtained, record the temperature, remove the light and carefully remove the tray of rocks.
- Collect all the periphyton from the surfaces of the rocks using collection Quantitative method 1A (Section 6.5.4).
- Measure the *x*, *y* and *z* axes dimensions of the cobbles/stones.
- Determine the volume of the rocks using water displaced in a finely graduated measuring jug.

Notes:

- In some streams the water may be near or at saturation with oxygen. This can result in spontaneous de-gassing of the water going through the pump with the result that large quantities of oxygen bubbles accumulate in the chamber. It helps to run the sealed chamber for about 5 minutes then clear the bubbles before doing a measurement. Doing the respiration measurement first also helps. However, in super-saturated water it is necessary to purge any excess oxygen by bubbling a small amount of N_2 gas into the system (through the bung hole in the chamber lid). Take the O_2 down to 8–9 mg/l. Release the bubbles through the bung hole on the lid at the downstream end. If you overdo it, then exchange a little stream water with chamber water through the lid bung holes.
- As the recording proceeds, write any notes on the chart paper that may be relevant in interpreting the results or calculating rates of change in oxygen.
- Carry out a blank run (i.e., no rocks in the chamber) for both respiration and productivity at the beginning of each day of measurements to ensure that the system is running well and there is no drift in the DO meter. If there is drift, and this cannot be fixed, then the results of the real measurements need to be corrected for the rate of drift. If you suspect problems, then re-run blanks at regular intervals.
- It pays to switch the chart recorder off for a few seconds when recording temperature from the DO meter because there is a major change in strength of signal and the recorder immediately goes off scale.
- Regularly check the membrane on the DO probe. It should be replaced every few days if doing many measurements.

- Wipe the underside of the lid after each run to remove the very small oxygen bubbles that sometimes accumulate there.
- To avoid electrical shocks always wear rubber gumboots in the water, and avoid putting your hand in the water while running the overhead light. It is a good policy to not enter the water while the generator is running in case the light falls over. Also, use a restricted current device (RCD isolating transformer) between the light and the generator.

6.8.3 Calculations

- Determine the rate of uptake and evolution of oxygen for the respiration and photosynthesis measurements, respectively, from the chart record by converting the graph scale into DO units (mg/l) and the linear distance into time in minutes, then calculating the amount of change in DO divided by time. The respiration rate will be a negative number, but the sign is dropped as it is inherent in the definition of community respiration (CR) that it is an oxygen uptake (i.e., loss) rate. Now convert the rate to mg O_2/l/hr by multiplying by 60.
- Subtract the volume of rocks used in the chamber from the gross chamber volume (chamber plus hose-line volumes) to give net chamber volume for the analysis. Multiply the rate of oxygen change by the net chamber volume (in litres). This gives rates for CR and net maximum productivity (Pn_{max}) in mg/l/hr.
- To normalise the rate measurements to surface area of the substrate, use:

$$\text{Stone surface area (cm}^2) = 1.59 + 0.811\,(xy + yz + xz)$$

  where *x*, *y* and *z* are the lengths of the three main axes of the stones in centimetres. This formula has been adjusted to only include the area of the stone normally protruding into the water on which the periphyton can colonise (~ 65 % of the total surface area). This gives the results in mg/min/m^2.
- Calculate gross maximum productivity (P_{max}) as:

$$P_{max} = CR + Pn_{max}$$

Notes:

- When determining the rate of change in DO off the chart paper, adopt a systematic procedure in relation to how you draw a line through the readout. If there is some noise in the signal, then attempt to average this out by drawing a line up through the mean of the short-term fluctuations.
- At the beginning of the CR section of the incubations, there is often a non-linear reduction in oxygen. Ensure that you only use the linear portion of the output for your calculations.

6.8.4 QA/QC for metabolism measurements

This method does not lend itself particularly well to the development of formal QA/QC procedures. However, the following points should always be borne in mind when carrying out these measurements:

- The largest source of interference is the accumulation of gas bubbles under the chamber lid. This can cause high frequency noise with large magnitude variations in DO. Ensure that the system is as free of bubbles as possible at the beginning of a run. If possible avoid opening the system up between CR and productivity sections of the run. If you

find that this is needed, then you may need to use N_2 gas to lower the background concentration of dissolved oxygen in the water.

- Probe drift and temperature variations within runs is a common source of error in results. As noted above, always run a blank for both productivity and respiration at the beginning of a set of measurements. The blank is just stream water without any substrates. Both lines of DO output should be flat without any upward or downward trend. If temperature increases by more than 3ϒC over both the CR and productivity sections of the incubation, then the results are probably subject to unacceptable error.
- Ensure that all calculations are checked.

6.9 Sample transport, storage and registration

6.9.1 Sample transport

Ideally all types of samples should be transferred to the laboratory as soon as possible after collection. Pack samples into chilly bins. Use crushed ice or slicka pads to keep the bins cool. Pack any spaces with crumpled newspaper or equivalent. It is important to keep samples chilled and in darkness.

If transport cannot be arranged within 18 hours of sample collection, the samples may be frozen and dispatched later at a convenient time (see below). Ensure that samples sent chilled are not in transit for more than 6 hours and those sent frozen are not in transit for more than 18 hours.

6.9.2 Sample storage

Three methods can be used to store periphyton samples:

- Freezing, for samples that are to be analysed for biomass, diatom composition and semi-quantitative abundance of soft-bodied taxa such as green algae and cyanobacteria. This is the only method that is practical for large biomass samples.
- Gluteraldahyde, for small samples (or sub-samples) to be analysed quantitatively for taxonomic composition. This method of preservation should not be used if biomass is to be analysed.
- Lugol's iodine / acetic acid, for small samples (or small sub-samples) to be analysed quantitatively for taxonomic composition. This method of preservation should not be used if biomass is to be analysed.

Freezing is widely used in New Zealand. Periphyton samples will remain intact for several months if kept at <–10ϒC, but probably should not be kept for more than 3 months if chlorophyll *a* is to be analysed. We find that freezing periphyton samples does not significantly affect ash-free dry mass measures (Biggs 1987). While diatom form is not affected by freezing, the contents of filamentous green algae may distort after freezing. However, the common types retain a characteristic appearance.

Gluteraldehyde and Lugol's iodine enables the soft-bodied algae to be preserved. Glutaraldehyde is now most commonly used for preservation of algae for detailed examination of soft-bodied forms because it generally does not degrade the structure of the organelles or colouration. This preservative is active indefinitely. Lugol's solution facilitates sedimentation (particularly of very small diatoms), stains starch bodies, maintains fragile cell structures. However, this preservative is generally only active for 1–2 years and samples must be stored in the dark. If detailed taxonomic and biomass analyses are planned, then the samples should be blended and a small sub-sample removed (see Section 7.2) for preservation in

gluteraldehyde or Lugol's iodine.

Check the sample containers before freezing. If you are using rigid plastic pottles, ensure that there is some air space in the container (1 cm at the top is safe). Otherwise the containers will crack on freezing. This costs valuable time when you have to decant the sample on thawing and re-label another container. It also increases the chances of mis-labelling. Elkays are flexible and will rarely crack on freezing.

Method:

- Glutaraldahyde (this is available as 50% stock solutions from Biolab Scientific).
  - Assess the volume of sample to be preserved.
  - Under a fume hood, add sufficient gluteraldehyde to give a 3–5% final concentration. **NB: glutaraldehyde is a toxic solution and you should avoid skin contact or breathing the fumes.**
- Lugol's iodine:
  - Under a fume hood, dissolve 25 g of iodine and 50 g of potassium iodide in 50 ml of glacial acetic acid.
  - Add distilled water to bring the volume to 500 ml.
  - Store in an amber glass bottle.
  - Assess the volume of sample to be preserved.
  - Under a fume hood, add sufficient Lugol's solution to give 3–5% final concentration. **NB. Lugol's solution can irritate your eyes and nose. Avoid breathing fumes and minimise skin contact.**

6.9.3 Sample labelling and registration

- Often it is most convenient to pre-label sample containers. Store sets of containers for a site separate from those for other sites. For example, hold each set in a labelled plastic bag. Before you begin sampling, satisfy yourself that the containers are the correct ones for this site.
- Labelling containers on-site is OK, but again double check that you are using the correct name / number for the site.
- Enter the samples onto a sample registration system as soon as they arrive in the laboratory. This will ensure that a record exists that they are on the premises and await analysis by a certain date.

7 Biomass analyses

7.1 Overview of biomass analyses

Two standard measures of biomass are used:

Chlorophyll a*:* this gives an indication of the total amount of (predominantly) autotrophic organisms in the sample.

Ash-free dry mass (AFDM): this is a measure of the total amount of organic material in the sample, and includes living autotrophic and heterotrophic microorganisms, plus dead periphyton, micro-invertebrates, and usually some terrestrial leaf debris.

It is preferable to analyse for both of these variables because: (a) they provide complementary information; (b) they can be combined to form a ratio (i.e., AFDM in mg / m^2:chlorophyll a in mg / m^2) called the "autotrophic index – AI" (Weber 1973), which itself is an informative measure. The AI is indicative of the proportions of the community composed of heterotrophic and autotrophic organisms. Stream periphyton communities unaffected by labile organic pollution, and dominated by algae, usually contain 1–2% (by weight) of chlorophyll *a*. Therefore, AI values of 50–100 are characteristic of non-polluted conditions with little organic detritus (where autotrophy dominates) (Biggs 1989). Values greater than 400 are taken to indicate communities affected by organic pollution (Collins and Weber 1978) (but see caution in Section 7.4.6, bullet #8).

In cases where periphyton growth is extremely sparse, the material collected may be insufficient to carry out an analysis of ash-free dry mass (errors in sample measurement are larger than the actual measurement). In this case analyse for chlorophyll *a* only. If available resources only allow one measure of biomass then chlorophyll *a* is recommended (and is the most commonly used measure) because it is cheaper to perform and more sensitive at low levels. Also, it is less prone to error associated with allochthonous organic matter.

7.2 Sample preparation

Most periphyton samples will contain clumps of filamentous algae or diatoms. This creates difficulties in sub-sampling to obtain representative portions for analysis. Representative sub-sampling is essential for accurate assessment of periphyton communities. Homogenising the sample with a simple, hand-held laboratory or kitchen blender can significantly reduce variability in sub-sample analyses, thus increasing the precision of the results. Biggs (1987) found that the assessed density of cells in a periphyton sample could increase by up to 500% and coefficients of variation decrease by nearly 90% following blending compared with just shaking the sample to achieve mixing. The highest increase in precision can be expected with samples that are dominated by filamentous algae. The blending process generally does not greatly damage cells, although some colonial species tend to break apart and long, lightly silicified diatoms and chrysophytes can be damaged. Most filamentous taxa break apart into much smaller fragments at inter-cellular connection points. Certain green filamentous algae do not break apart easily (e.g., *Cladophora*) during blending and tend to get wrapped around the cutting blades. The use of sharp blades on the blender, only a small volume of sample, and a slightly longer blending time usually overcomes this problem (Biggs 1987).

7.2.1 Equipment

1. Glass beaker.

2. Stoppered bottles with narrow necks (marked at 50 ml intervals).
3. Kitchen or laboratory tissue blender.
4. Squirt bottle with distilled water.

7.2.2 Procedure

- Tip the contents of the sample container into a glass beaker (the width of the beaker should be only slightly greater than the width of the housing holding the cutter blades of the blender). Rinse out any sample residue from the container and lid into the beaker. Distilled water is preferred for rinsing and making up the sample volume. However, tap water can be used providing the water isn't heavily chlorinated.
- Pick out any invertebrates, pieces of gravel, leaves, moss, etc. from the sample.
- Ensure that there is enough water to fully cover the blender-blade housing.
- Blend for about 30 seconds or until the mixture is free of obvious clumps of material. If the sample contains much filamentous algae, break the strands up by repeated cutting with a pair of sharp scissors. Every 10 seconds, or so, stop the blender and free any filaments that may have become caught on the blades or blender housing using a squirt of water. You need to end up with a homogeneous solution suitable for sub-sampling.
- Transfer the blended liquid to a stoppered bottle. Rinse out the beaker and make up the solution to a known volume (usually 50, 100 or 150 ml). Do a quick calculation of how much solution is required for all your sub-samples (variables to be analysed x aliquots x replicates) and ensure that you will have at least 50% more volume than this to allow for re-analysis of variables where there may have been some analytical errors or problems – see Sections 7.3.6, 7.4.6, and 8.7. Enter this volume onto your lab analysis sheet.
- If the sample is frozen between blending and different sets of analyses, then it should be re-blended briefly before sub-sampling for the next analysis because freezing tends to coagulate some filamentous and mucilaginous periphyton.

7.3 Chlorophyll *a*

While different taxonomic groups have distinctive assemblages of pigments, it has become standard procedure to assay the quantity of chlorophyll *a* as a measure of live biomass because this pigment is abundant in most periphytic algae including cyanobacteria. However, there are several important sources of bias with this parameter. First, the amount of chlorophyll *a* does vary among taxonomic groups and this can result in apparent biomass differences among communities when quantities of organic matter vary only a little. Second, the chlorophyll degrades naturally as communities age and cells die. This results in the formation of degradation products called phaeopigments which occurs through the loss of a magnesium ion from the chlorophyll molecule. Indeed the periphyton mat is also a sink for dead algal cells from in-situ growth, upstream deposition and also terrestrial plant debris. These phaeopigments interfere with the analysis of “live” chlorophyll *a* because they have an absorption peak the same as for chlorophyll *a*. It is therefore essential to carry out an acidification step as a second part to the analysis analysis to correct the measurements for phaeopigments. Acidification removes the magnesium ion from the chlorophyll molecules converting the whole sample to phaeopigment.

Three main methods have been developed for the determination of chlorophyll *a* in algae:

- Spectrophotometry
- Fluorometry, and
- High performance liquid chromotography (HPLC).

Fluorometry and HPLC are more precise than spectrophotometry. For example, spectrophotometry has been found to overestimate pigment concentrations in natural phytoplankton by 13–46% (Sartory 1985). This overestimation has been attributed to interference by carotenoids in the assessment of phaeopigment concentrations (Riemann 1978). However, a number of studies have also shown that the differences between spectrophotometry, fluorometry, and HPLC are not systematic (e.g., Jacobsen and Rai 1990). Fluorometry and HPLC techniques have a major advantage with very low concentrations of algae in that they are far more sensitive (Lorenzen 1966, Sartory 1985). However, this is rarely a problem with stream periphyton analyses. We describe the spectrophotometric technique here, because of the much wider availability of spectrophotometers.

A second issue that has varied widely among researchers internationally, is what method to use to extract the chlorophyll from the algae for analysis. Four solvents have been commonly used:

- Acetone
- Methanol
- Ethanol
- Dimethyl sulfoxide (DMSO).

Acetone has been the most popular method, but many studies have shown that alcohol solvents (particularly ethanol) are more efficient than either acetone or DMSO at extracting pigment from many algae (e.g., Holm-Hansen and Reimann 1978, Riemann and Ernst 1982, Sartory 1982). For example, Jacobsen and Rai (1990) reported that methanol and ethanol extractions were almost complete after 4 h, but were still incomplete after 24 h with acetone from a culture of *Scenedesmus* (a colonial green alga). The final acetone extraction efficiency was only 50% of the ethanol extraction. Maceration of the sample using a tissue grinder can greatly assist extraction (e.g., Marker 1972), but may not totally compensate for acetone's poor extraction efficiency. Grinding also results in an additional step in the analysis that is not required for ethanol extraction (Sartory and Grobbelaar 1984). The use of ethanol over methanol has now been recommended by a number of researchers (e.g., Nusch 1980, Sartory 1982, Sartory and Grobbelaar 1984, Webb *et al.* 1992). Because ethanol is a very effective solvent, there is only a minor shift in the absorbance peak with acidification, interference from chlorophyll *b* is less, ethanol presents a much lower health and safety risk, and it is cheaper. We have carried out a number of our own tests on extraction efficiency of various solvents with periphyton communities from New Zealand streams and these support the above results. Thus, since 1984, we have adopted the spectrophotometric ethanol method for analysis of chlorophyll *a* for general use in New Zealand.

The procedure detailed below is based on Sartory (1982) and Sartory and Grobbellar (1984), where absorption coefficients and acid ratios were re-defined based on the work of Nusch (1980). The method involves the following steps:

- Concentrate a sub-sample of homogenised periphyton on a glass-fibre filter.
- Extract the chlorophyll in a solution of 90% boiling ethanol (i.e., 78 °C. Boiling increases extraction efficiency and helps to “fix” the chlorophyll by destroying enzymes thus

making the chlorophyll relatively stable when in storage).

- Leave to complete extraction in a refrigerator overnight.
- Centrifuge to remove particulates from the solution.
- Read background turbidity and pigment concentration on a spectrophotometer.
- Acidify sample to convert the chlorophyll *a* to phaeopigments and re-read absorbances
- Correct for the presence of turbidity and phaeopigments
- Calculate chlorophyll *a* concentrations by multiplying by an absorbance coefficient.

If you have collected whole rocks for direct extraction of chlorophyll *a*, then a larger quantity of the ethanol extractant is put into the zip-lock bags with the rocks. The bags and rocks are then placed in the hot water bath for extraction.

Notes:

- The absorbance peak for chlorophyll *a* is very narrow and varies slightly among spectrophotometers, so it is important that you locate the wave length of the peak exactly by scanning absorbance of a standard solution. This is done as follows:
  - Make up a dilute standard solution of chlorophyll *a* in 90% ethanol (concentrated chlorophyll is available from some laboratory supply companies) and bring to the boil at 78°C for 5 minutes
  - Cool the standard to room temperature and then measure the absorbance of the standard at wavelengths ranging from 660 to 668 nm. This will enable you to determine where the peak in absorbance is for your spectrophotometer. This will normally lie between 663 and 665 nm. Use this waveband for all future measurements on your spectrophotometer.

7.3.1 Equipment

1. Data sheets.
2. Ethanol, 95 or 96% ex-stock, diluted to 90% with distilled water.
3. Glass fibre filters, 47 mm Whatman GFC.
4. Polythene centrifuge tubes, stoppered, numbered and stored in order in racks.
5. Forceps / tweezers.
6. Vacuum filtration apparatus.
7. Pipettes set to 5 ml and 0.1 ml (the 5 ml pipette needs to have a wide aperture ~ 2 mm diameter).
8. Squirt bottle with distilled water.
9. 0.30 molar HCl.
10. Water bath.
11. Spectrophotometer.

7.3.2 Sub-sample filtering

- Prepare tubes by placing 5 ml of 90% ethanol in each and re-capping the tube firmly.
- Set up filtering apparatus.
- Place fresh filters in each filtering chamber.
- Apply suction pressure (be careful not to have a high pressure as this will rupture cells releasing the chloroplasts; <10 mm Hg vacuum is recommended).

- The filtered sub-sample comprises three smaller aliquots of the blended sample which are pooled. To take these aliquots, shake the bottle of blended sample and withdraw 5 ml with an automatic pipette from half-way down the solution while the liquid is still agitated. Release this solution into the filtering chamber. Repeat this twice more to give the full sub-sample (i.e., the total sub-sample volume = 15 ml) (see QA / QC recommendations).
- If there is not an obvious colouring from periphyton on the filter, then you should filter more aliquots. However, if you intend to do other analyses as well, ensure that you have enough sample left (AFDM analyses aren't as sensitive as chlorophyll *a* and require larger sub-samples where there is a low concentration of periphyton).
- Check for any fragments of leaves, mosses, invertebrates etc. on the filter paper and remove these with forceps.
- Record the volume of sub-sample (i.e., number of 5 ml aliquots used).
- Rinse the pipette by sucking up and discarding some clean water.
- If you are sub-sampling for both ash-free dry mass and chlorophyll *a*, get into a routine of always doing them in the same order. We take sub-samples for chlorophyll *a* first, then for AFDM.
- Remove the filter from the filtering apparatus, fold in half, loosely roll up and place in its numbered tube. Make sure it is completely covered with the ethanol and the tube is firmly sealed.

Notes:

- Always check that clumps of algae or a leaf fragment, etc., hasn't blocked the intake to the pipette.
- If the aliquots are taking a very long time to filter for each sub-sample, you probably need to dilute your sample or take a smaller volume aliquot (e.g., 2 ml). Ensure that you record the degree of dilution and aliquot volume so that this can be used in the calculations of AFDM concentration.
- Remember to have sufficient sample solution for multiple analyses as required (N and P content, species analysis, repeat analyses, etc.).
- Throughout the filtering process, periodically check that the centrifuge tube numbers you use correspond to the numbers and the sample identifiers marked on the data sheets.

7.3.3 Analysis

- Pre-heat the water bath to 78 °C (boiling point of ethanol). Immerse the racks of tubes in the bath for exactly five minutes.

  NB. Make sure the tubes are held firmly in the racks. Loosen the tops to prevent them popping off as the ethanol boils. But leave the tops ON, otherwise you will lose ethanol through evaporation.
- Place the racks in the refrigerator overnight.

Notes:

- At this stage, if any of the chlorophyll seems to be really concentrated (i.e., a very deep green), then dilute the extractant with 90% ethanol. The aim is to keep the absorbance readings to less than 1.000. Readings higher than this indicate concentrations which may no longer be linearly proportional to absorbance.

- To dilute, first add an extra 5 ml of 90% ethanol to the tube. Push down the stopper firmly and shake well. Note on the data sheet that the volume of extractant is 10 ml (instead of the usual 5 ml). If the solution still looks very concentrated, centrifuge the solution (as described above), then withdraw 5 ml from the tube, add another 5 ml of 90% ethanol and shake well. Note on the data sheet that the (effective) volume of extractant is now 20 ml.

7.3.4 Absorbance readings

Equipment:

1. Spectrophotometer including cuvettes, printout paper.
2. 90 % ethanol for washing out cuvettes and for blank solutions.
3. Pipettes set to 4 ml and 0.1 ml.
4. Tissues.
5. 0.30 molar hydrochloric acid (6.25 ml concentrated HCl made up to 200 ml with distilled water).

Procedure:

- Switch on the spectrophotometer, wait for initialisation and for the machine to completely warm up (warm-up time will vary among brands).
- Set the wavelengths at the wavelength peak for chlorophyll *a* for your spectrophotometer and 750 nm.
- Ensure that the outside surfaces of your cuvette are clean and free of drips and dirt. Take blank readings using 90 % ethanol to check this.
- Using a glass rod, or metal forceps, push the filter papers as far as possible to the bottom of the centrifuge tubes, and re-close firmly.
- Centrifuge at a speed of 6000 rpm for 10 minutes.
- Take the first centrifuged sample and pipette out 4 ml into the cuvette.
- Read absorbances at 665 and 750 nm.
- Remove the cuvette, inject 0.1 ml of 0.3 M HCl in the cuvette, place a cap on the cuvette and invert to mix. Wipe the side of the cuvette again with a clean tissue.
- Place the cuvette back into the spectrophotometer and re-read absorbances at 665 and 750 nm, leaving a 30 second delay.
- Remove the cuvette, discard the acidified sample and rinse it thoroughly with 90% ethanol. Invert and tap dry on a clean tissue.

Notes:

- Always make sure that the spectrophotometer is adequately warmed up and has stabilised before analyses. This may take up to an hour for some machines.
- Always use the same cuvette for each sample batch, and always use it facing the same way (e.g. always place it in the compartment with the “L” facing to the left.). Make sure that the outside of the cuvette is clean and dry before each reading.
- Always hold the cuvette by the frosted side panels.
- Acidification occurs within a few seconds, but ensure that there has been a delay of at least 30 seconds before re-reading to ensure that acidification is complete before taking the next reading.
- Make sure you clean the cuvette thoroughly after each sample has been acidified to

avoid contaminating the next sample with an acidic solution.

- Be careful not to over-acidify the extract because this may cause the development of products which interfere with the phaeopigment absorbance peak.
- Always close the sliding door over the cuvette compartment before taking a reading.
- The optimal band pass width for the spectrophotometer is 1–2 nm, with a slit width of ~ 0.2 nm.

7.3.5 Calculations

$$\text{Chlorophyll } a \text{ (mg per sample)} = [(\text{absorbance}_{665\ \text{before}} - \text{absorbance}_{665\ \text{after}}) \times 28.66 \times \text{sample vol.} \times \text{extractant vol.}] / (\text{filtered sub-sample volume})$$

where:

- $\text{absorbance}_{665\ \text{before}}$ and $\text{absorbance}_{665\ \text{after}}$ are the absorbance readings at a wavelength of 665 nm before and after acidification (having already subtracted the respective turbidity blanks read at 750 nm);
- 28.66 is the absorption coefficient for chlorophyll *a* as defined by Sartory and Grobbelaar (1984) (based on the specific absorption coefficient for chlorophyll in ethanol of 83.4 g/l/cm and an acid ratio for chlorophyll *a* in ethanol of 1.72 (Sartory 1982));
- all volumes are in litres.

Phaeopigment concentrations may be calculated as follows:

$$\text{Phaeopigments (mg per sample)} = [(1.72 \times \text{absorb.}_{665\ \text{after}}) - \text{absorb.}_{665\ \text{before}}] \times 28.66 \times \text{sample vol.} \times \text{extractant vol.}] / (\text{filtered sub-sample volume})$$

where all volumes are in litres.

It is then necessary to normalise the chlorophyll or phaeopigment concentration for the area from which the sample was collected. If whole stone sampling was used (i.e., from sampling Method 1a; Section 6.5.4), you need to calculate the surface area of stone from which the sample was obtained:

$$\text{Stone surface area (cm}^2) = 1.59 + 0.811\ (xy + yz + xz)$$

where *x*, *y*, and *z* are the lengths of the three main axes of the stones in centimetres. This formula has been adjusted to only include the area of the stone normally protruding into the water on which the periphyton can colonise (~ 65 % of the total surface area). Otherwise, calculate the area based on the diameter of the sampling circle used (i.e., from sampling Method 1b).

If samples have been collected from a set area on top of the stones (Quantitative Methods 1b, 2 and 3: Sections 6.5.5, 6.5.6, 6.5.7), or from artificial substrates (Section 6.6), then calculate the surface area of the individual replicate and multiply by the number of replicates collected.

Finally, calculate the concentration of chlorophyll *a* or (phaeopigments) in mg/m^2 as follows:

$$\text{Chl. } a \text{ (mg/m}^2) = \text{Chlorophyll } a \text{ (mg/sample)/area of sample (m}^2)$$

(Ensure that you have converted your sampling area from cm^2 to m^2 for this final calculation.)

7.3.6 QA/QC for chlorophyll *a*

Sources of error

Two main areas in the chlorophyll *a* analysis can introduce errors. These are:

- sub-sampling;
- analysis.

Variation associated with sub-sampling is generally <10% (as % coefficient of variation), but may be as high as 25% for samples of communities that are difficult to break up during blending (Biggs 1987). Higher levels of sub-sampling precision are expected with diatom dominated communities than with filamentous communities (particularly mat forming cyanophytes). It is common to only remove and analyse one sub-sample. If you only wish to analyse one sub-sample then we strongly recommend that this sub-sample be composed of at least 3 ↔ 5-ml aliquots. This considerably reduces sub-sampling variability.

When sub-sampling and filtering a large batch of samples it is easy to loose track of how many aliquots you have put through the filters. Also, it can be easy to get centrifuge tube numbers and sample numbers interchanged when working between the tubes and laboratory sheets. Establish a clear, systematic, routine for the filtering process. Don't attempt to carry out the sub-sampling / filtering procedure too fast. Ensure that the sample bottle labels are clearly readable, the bottles are arranged in order on the lab bench, and the samples are listed in the same order on the laboratory bench sheet. Never label the sample bottles on their lids only (these can be swapped too easily!). Mislabeling or accidental swapping of samples and or sample labels is one of the easiest and most significant sources of error in these analyses.

Analytical error should, in most cases, be small relative to variability associated with the patchy distribution of mats on the stream bed (i.e., sampling error). For chlorophyll *a* analyses, several sources of analytical error are possible:

- Incorrectly measuring the 5 ml aliquot of 90 % ethanol. The viscosity of the ethanol is relatively low, so sometimes you don't withdraw the exact 5 ml from the working solution. Also, the ethanol often drips from the automatic pipette as the solvent is being transferred to the centrifuge tubes. These problems can introduce large errors.
- Not shaking the sample thoroughly to ensure a homogeneous mixture when sub-sampling. Be very careful to ensure that the blended mixture is homogeneous. Do not swirl the container as this distributes the heavier cells / filaments to the perimeter of the container. Shake the container and take the sub-sample from approximately the middle of the container.
- Incorrectly measuring the 5 ml aliquots that are pooled for the sub-sample. When processing large numbers of sub-samples it is easy to become a little sloppy in the sub-sampling technique. This usually occurs through incorrectly releasing the pressure on the automatic pipette so that the sub-sample gets sucked up too fast. This results in incomplete withdrawal of the aliquot. Always check that clumps of algae or a leaf fragments haven't blocked the intake to the pipette.
- Not allowing the spectrophotometer to warm up properly.
- Not correctly locating the peak in absorbance for chlorophyll *a* on your spectrophotometer. This can introduce very large errors because the peak is very narrow.
- Not allowing sufficient time for acidification.
- Errors in calculations for dilutions. These calculations must be done very carefully and

checked because you can double, quadruple, half, or quarter the apparent concentrations very easily without knowing it.

For overall errors associated with sub-sampling and analyses, Biggs (1987) reported mean co-efficients of variation of 10.6% for chlorophyll a based on 258 sets of analyses of three sub-samples. The samples covered a wide range of biomass and community types including diatoms, filamentous green algae and Cyanobacteria.

Ethanol dissolves ink from marker pens, so be sure that any drips created by pouring the chlorophyll extract from the centrifuge tube to the cuvette don't wash the identifying mark off the outside of the tube. If this does happen, then ensure that the drip with the associated ink doesn't land in the cuvette. Wipe the outside of the tube immediately and re-apply the identifier.

Formal quality control procedures

The following steps should be included in a formal QA / QC system for chlorophyll *a* analysis.

- Check the location of the chlorophyll absorbance peak on the spectrophotometer using a pure chlorophyll a in 90% ethanol solution at least twice per year (see page 79).
- Check the calibration, and volume setting, on the automatic pipette used for sub-sampling before each batch of analyses by weighing sub-samples of distilled water on a precision balance.
- All field sample labels should be checked and signed off as being clearly readable.
- All samples should be registered in a properly prepared and maintained electronic or hard copy register as they arrive in the laboratory. Information should include unique sample identifiers (this would normally be a site + replicate sample number), date of collection, date of receipt in laboratory, method of preservation, analysis required, person who collected sample, person responsible for the job, and job number.
- Check the transcribing of sample numbers / labels onto the laboratory bench sheet and ensure that the samples on the bench are in the same order as on the laboratory bench sheet. Prepare data sheets before you begin the analyses. Enter each site / sample number in a logical order (e.g., sites in upstream to downstream sequence).
- Prepare racks of numbered centrifuge tubes for extraction of chlorophyll *a* and enter these numbers onto the data sheets. Start numbering from 1 for each sampling run and ensure that the tubes are arranged in their racks in the correct order.
- Results, calculations, and transposition of data should all be checked and signed off by someone other than the analyst. All errors should be logged in a laboratory registry of errors. Apart from helping ensure the quality of the analysis, this registry also helps isolate problem areas in the analytical process.
- For analyses where the results are likely to be used for a resource consent hearing or the Environment Court, then 3 sub-samples should be analysed from each sample and the results averaged. If the value for any one sub-sample is >2 x the mean of the two nearest sub-samples, then the outlier is considered to be a sub-sampling or analytical error and the result of the outlier should be discarded.
- The scientist responsible for analysing the data and reporting must check the results and sign them off as being acceptable. The balance of all samples should be retained (frozen) until the data has been approved in case re-analysis is required.

Notes:

- It is difficult to develop Quality Control charts to allow "benchmarking" of batch analyses for chlorophyll *a* as has been suggested for AFDM (see below). This is because we are, as yet, uncertain of the stability of frozen chlorophyll samples or ethanol extracts if the sub-samples are kept for more than a few months. Preserving samples for later analysis and benchmarking is an essential component of a QC chart.
- A good quality assurance system in a laboratory is dependent on feedback. Feedback between:
  - laboratory staff and laboratory manager, regarding problems with samples, techniques etc.;
  - laboratory staff and scientists regarding expected capabilities, variability, and expected levels for the results, and required levels of precision, etc.;
- Feedback helps to ensure:
  - the objectives of the project are being met;
  - variability of results is reduced and thus confidence in the results is maintained;
  - all participants in the analyses know what is expected of them in the QA system.

7.4 Ash-free dry mass (AFDM)

In brief, the method involves drying a sub-sample to drive off all water, determining the dry weight, ashing the sample and then re-weighing. The difference between these two weights gives the ash-free dry mass value (sometimes called loss-on-ignition or ash-free dry weight).

As with analyses for chlorophyll *a*, many methods have been used and no one standard method has been adopted internationally. Variations in technique have arisen mainly in the temperature and time used for ashing. Ridley-Thomas *et al.* (1989) assessed the effects of different sample sizes, ignition time and ignition temperature on AFDM results for a set of stream periphyton samples. They found significant variation as a function of differences in all these aspects, but did not develop a recommended procedure.

Most commonly it has been recommended to ash samples at 500–550ϒC for 1–2 hr (e.g., APHA 1995). However, at such high temperatures there is a risk of dehydroxylation of the clay minerals (Mook and Hoskin 1982) which tend to accumulate in periphyton mats (sometimes to high concentrations; Davies-Colley *et al.* 1992). If biomass is low, this can potentially result in a very high biasing of results. An ignition temperature of 400ϒC for 4 hr has been most commonly used for analysis of stream periphyton in New Zealand in an effort to reduce such problems (e.g., Biggs 1987, Biggs and Close 1989, Biggs 1990, Davies-Colley *et al.* 1992, Biggs 1995). This method is described in detail below.

7.4.1 Equipment and materials

1. Data sheets.
2. Glass-fibre filters, 47 mm Whatman GFC.
3. Crucibles, with number/ID mark, on metal trays.
4. Forceps/tweezers.
5. Vacuum filtration apparatus.
6. Glass beakers, stoppered bottles (marked at 50 ml intervals).
7. Blender.

8. Automatic pipettes set to 5 ml (with a wide aperture ~ 2mm diameter).
9. Squirt bottle with distilled water.
10. Desiccator.
11. Muffle furnace, drying oven, and balance capable of measuring to 0.1 mg.

7.4.2 Procedure

- Place one filter in each crucible. Do not fold or tear.
- Pre-ash in the muffle furnace at 400°C for two hours – this is necessary in case any traces of combustible material in the GFC filters interfere with the ash-free dry weight measurements.
- If dry weight of the samples is required, then transfer the crucibles and filters to a desiccator to cool. Allow expanded hot air to escape for a few seconds through the desiccator lid valve, then close the valve to create a vacuum seal. About 30 minutes is usually enough for cooling.
- Record the weight of each crucible and filter paper after they have cooled (if sample dry weight is to be determined).

Notes:

- Always handle the crucibles with forceps / tweezers to avoid contamination with grease, etc., from your hands.
- If dry weight is not needed, then allow the crucibles to cool on trays.
- Replace the lid on the desiccator between each weighing. Once out in the open, the filter papers start to absorb moisture, which increases their “dry” weight.
- Allow the crucibles to cool fully. Weigh the crucibles at room temperature. If they are warm or hot, you get spuriously low values.

7.4.3 Sub-sample filtering

- Set up the filtering apparatus.
- The filtered sub-sample comprises at least three smaller aliquots of the blended sample which are pooled. To take these aliquots, shake the bottle of blended sample and withdraw 5 ml with an automatic pipette from half-way down the solution while the liquid is still moving. Release this solution into the filtering chamber. Repeat this twice more to give the full sub-sample (i.e., the total sub-sample volume = 15 ml).
- If there isn't a significant accretion of periphyton on the filter then you should filter more aliquots.
- Check for any fragments of leaves, mosses, invertebrates etc. on the filter and remove these.
- Apply suction pressure to the filtering apparatus.
- Record the volume of sub-sample (i.e., number of 5 ml aliquots used).
- Rinse the pipette by sucking up and discarding some distilled water.
- If you are sub-sampling for both ash-free dry mass and chlorophyll *a*, get into a routine of always doing them in the same order.
- Replace the filter in its crucible when filtering is complete.

Notes:

- If the aliquots are taking a very long time to filter for each sub-sample, you will probably need to dilute your sample or take a smaller volume aliquot (e.g., 2 ml). Ensure that you record the degree of dilution and / or smaller aliquot volume so that this can be used in the calculations of AFDM concentration.
- Pipette tips can be widened slightly to overcome persistent blockage.
- Remember to have sufficient sample solution for multiple analyses as required (N and P content, species analysis, repeat analyses etc.).
- Throughout the filtering process, periodically check that the crucible numbers you use correspond to the numbers and the sample identifiers marked on the data sheets.

7.4.4 Ashing of samples

- Dry the sub-samples (crucibles + filters with filtered material) for 24 hours at 105 °C.
- Weigh each crucible after cooling in a desiccator, as before.
- Ash for 4 hours at 400°C, cool in the desiccator, and weigh for the final time.

7.4.5 Calculations

Ash-free dry mass (g per sample) =
[{(weight of crucible + filter + sample after drying) – (weight of crucible + filter + sample after ashing)} x sample volume]
/ [volume of filtered sub-sample]

Dry mass (g per sample) =
[{(weight of crucible + filter + sample after drying) – (weight of crucible + filter after drying)} x sample volume]
/ [volume of filtered sub-sample]

All volumes are in litres.

If whole stone sampling was used (i.e., sampling Method 1a; Section 6.5.4), you need to calculate the surface area of stone from which the samples were obtained:

$$\text{Stone surface area (cm}^2\text{)} = 1.59 + 0.811\,(xy + yz + xz)$$

where x, y, and z are the lengths of the three main axes of the stones in centimetres. This formula has been adjusted to only include the area of the stone normally protruding into the water on which the periphyton can colonise (~ 65 % of the total surface area).

If samples have been collected from a set area on top of the stones (Quantitative Methods 1b, 2 and 3: Sections 6.5.5, 6.5.6, 6.5.7), or from artificial substrates (Section 6.6), then calculate the surface area of the individual replicate and multiply by the number of replicates collected.

Finally, calculate the AFDM or dry mass in g / m^2 as follows:

$$\text{AFDM or DM (g / sample)} = \text{AFDM or DM (g) / area of sample (m}^2\text{)}$$

Ensure that you have converted your sampling area from cm^2 to m^2 for this final calculation (x cm^2 / 10,000 = y m^2).

Additional variables may be calculated as follows:

$$\% \text{ organic matter} = (\text{ash-free dry mass} \times 100) \ / \ (\text{dry mass})$$

$$\text{Autotrophic index (AI)} = \text{AFDM (in mg/m}^2) \ / \ \text{chlorophyll } a \text{ (mg/m}^2)$$

7.4.6 QA/QC procedures for ash-free dry mass

Sources of error

There are two main areas in the ash-free dry mass analysis that can introduce errors. These are:

- sub-sampling;
- analysis.

Variation associated with sub-sampling is generally < 10% (as % coefficient of variation), but may be as high as 25% for samples of communities that are difficult to break up during blending (Biggs 1987). Higher levels of sub-sampling precision are expected with diatom dominated communities than with filamentous communities (particularly mat forming cyanophytes). It is common to only remove and analyse one sub-sample. If you wish to analyse only one sub-sample then we strongly recommended that this sub-sample be composed of at least 3 x 5-ml aliquots. This considerably reduces sub-sampling variability.

When sub-sampling and filtering a large batch of samples it is easy to loose track of how many aliquots you have put through the filters. Also, it can be easy to get crucible numbers and sample numbers interchanged when working between the sample containers and laboratory sheets. Establish a clear systematic, routine for the filtering process. Don't attempt to carry out the sub-sampling/filtering procedure too fast. Ensure that the sample bottle labels are still clearly readable, the bottles are arranged in order on the lab bench, and the samples are listed in the same order on the laboratory bench sheet. Never label the sample bottles only on their lids (these can be swapped too easily!). Mislabelling or accidental swapping of samples and or sample labels is one of the easiest and most significant sources of error in these analyses.

Analytical error should, in most cases, be small relative to variability associated with the patchy distribution of mats on the stream bed (i.e., sampling error). For AFDM analyses, several sources of analytical error are possible:

- Not shaking the sample thoroughly to ensure a homogeneous mixture when sub-sampling. Be very careful to ensure that the blended mixture is homogeneous. Do not swirl the container as this distributes the heavier cells/filaments to the perimeter of the container. Shake the container and take the sub-sample from approximately the middle of the container.
- Incorrectly measuring the 5 ml aliquots that are pooled for the sub-sample. When processing large numbers of sub-samples it is easy to become a little sloppy in the sub-sampling technique. This usually occurs through incorrectly releasing the pressure on the automatic pipette so that the sub-sample gets sucked up too fast. This results in incomplete withdrawal of the aliquot. Always check that clumps of algae or a leaf fragment, etc., hasn't blocked the intake to the pipette.
- Errors in calculations for dilutions. These calculations must be done very carefully and checked because you can double or half the apparent concentrations very easily without knowing it.
- Non-uniform heat distribution within ovens and the muffle furnace. Ovens should have an internal fan to mix the heated air.

- Dehydration of the inorganic fraction. The ashing temperature has been set at a relatively low level of 400°C to help reduce the effect of dehydration of the clay fraction in the sample. However, if clays are a large fraction of the sample and precise results are required, then it is recommended that the mineral fraction is re-hydrated after ashing (but before final weighing). This is done by cooling the sample after ashing, adding a few ml of water to the sample, allowing it to stand overnight, drying again at 105°C for 4 h, cooling in a desiccator and then taking the final weight.
- Re-hydration of the organic component can occur after the initial drying step if the sample is not cooled in a desiccator. This can result in positive biases to the results. Ensure that the silica gel in the desiccator is fully dry.
- Weighing errors can occur. This is a particular problem when biomass is very low. Indeed, AFDM is not nearly as accurate as chlorophyll *a* as a measure of biomass at low levels. The magnitude of error introduced may be illustrated by taking the ratio of AFDM:chlorophyll *a* (both in mg/m^2, which gives the Autotrophic Index [AI] – see above), and making the assumption that the chlorophyll measurement is a more sensitive and accurate analysis. Healthy communities in unpolluted streams normally have an AI of 100–200. However, for healthy low-biomass communities dominated by, say, diatoms the error associated with weighing will usually result in spurious AI values of 0.1–2,000. Occasionally there appear to be net gains in biomass during ashing. Therefore, if you are attempting to discriminate differences among sites or treatments in an experiment where biomass levels are all low, then use chlorophyll *a* as the measure of biomass. Autotrophic indices should not be calculated for samples where AFDM < 2 g/m^2.

For overall errors associated with sub-sampling and analyses, Biggs (1987) reported mean coefficients of variation of 8.9% for AFDM based on 258 sets of analyses of three sub-samples for each variable. The samples covered a wide range of biomass and community types including diatoms, filamentous green algae and cyanobacteria.

Formal quality control procedures

The following steps should be included in formal QA/QC procedures for AFDM:

- Balances and temperatures for the ovens and muffle furnace should be calibrated at least annually.
- The calibration and volume settings on the automatic pipette used for sub-sampling should be checked before each batch of analyses by weighing sub-samples of distilled water on a precision balance.
- All sample labels should be checked and signed off as being clearly readable.
- All samples should be registered in a properly prepared and maintained electronic or hard copy register as they arrive in the laboratory. Information should include unique sample identifiers (this would normally be a site + replicate sample number), date of collection, date of receipt in laboratory, method of preservation, analysis required, person who collected sample, person responsible for the job, and job number.
- Check the transcribing of sample numbers/labels onto the laboratory bench sheet and ensure that the samples on the bench are in the same order as on the laboratory bench sheet. This checking should be done by another analyst and signed off.
- Results, calculations, and transposition of data should all be checked and signed off by someone other than the analyst. All errors should be logged in a laboratory registry of

errors. Apart from helping ensure the quality of the analysis, this registry also helps isolate problem areas in the analytical process.

- For analyses where the results are likely to be used for a resource consent hearing or the Environment Court, three sub-samples should be analysed from each sample and the results averaged. If the value for any one sub-sample is > 2 x the mean of the 2 nearest sub-samples, then the outlier is considered to be a sub-sampling or analytical error and the result of the outlier should be discarded. For such analyses, you should also carry out the re-hydration step after ashing.
- Use QC charts to benchmark the analyses. With every new batch of analyses three sub-samples are included from low, medium and high biomass samples with known means and error. The sub-samples must fall within two standard deviations of the known mean for the batch results to be accepted. The following gives detailed instructions:
  - Collect three large samples (from the field from areas 2–3 times bigger than usual) covering low (e.g., < 10 g / m^2 AFDM), medium (20–40 g / m^2 AFDM) and high biomass (> 40 g / m^2 AFDM) communities.
  - Transport the samples to the laboratory and analyse while still fresh.
  - Blend the samples thoroughly as described in Section 7.2. Ensure that there is sufficient blended solution for removal of 30 sub-samples, each 15 ml (i.e., at least 450 ml of blended solution for each sample).
  - From each sample, remove and filter 30 sub-samples through pre-ashed filters as described in Section 7.4.3.
  - Randomly select five of these sub-samples and place them in labeled crucibles. Fold the filter papers for the remaining 25 sub-samples, place in individual small zip-lock plastic bags, and label each with a unique identifier consisting of the QC batch, biomass group, and sub-sample number. Store in a freezer at < –20°C.
  - Analyse the five QC sub-samples for AFDM as described above and calculate the results. Because the results of these analyses are then used to benchmark the QC checks in subsequent routine analyses, it is very important that they be carried out very carefully. It is recommended that two analysts work on carrying out the QC benchmark analyses, to review each other's manual operations (if possible, one analyst should have considerable experience), and fully check the calculations.
  - Prepare a QC chart for each of the low, medium and high biomass samples by calculating the mean and standard deviation of the five sub-samples from each sample and then plotting the mean and 2 x the standard deviation of the mean as lines across the chart (Figure 14).
  - Every time a batch of analyses is run, remove one of the QC sub-samples from storage for each of the levels of biomass and analyse these three sub-samples with the batch.
  - Plot the results of the QC sub-samples on the QC chart. The value should lie within the two standard deviation boundaries around the mean of the original five QC sub-samples. If one of the three QC results falls outside these limits you should re-check all your calculations and methodology. If there are no errors in the calculations flag the results for the whole batch of samples and check with the person using the results whether there is a requirement for high precision (in which case the batch should be re-analysed). If two or more of the QC sub-samples fall outside the 2 standard deviation limits and there are no errors in the calculations, then you

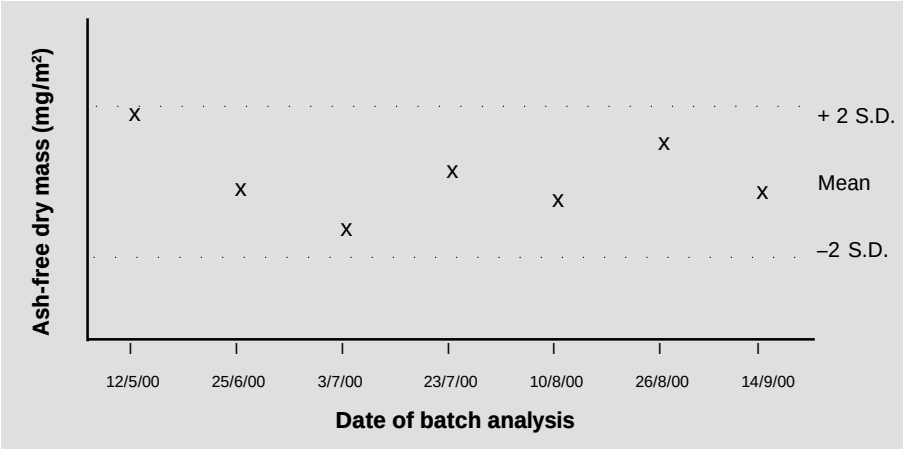


Figure 14: Example of a quality control chart for ash-free dry mass analysis. Preserved QC sub-samples from a given QC batch are analysed with each batch of project samples and the values are plotted and compared with the mean and 2 x the standard deviation (2 S.D.) for the initial analysis of the QC group of sub-samples.

should repeat the whole set of analyses for that batch as a matter of course.

- The scientist responsible for analysing the data and reporting must check the results and sign them off as acceptable. The balance of all samples should be retained (frozen) until the data has been approved, in-case re-analysis is required.

Notes:

- The frozen AFDM QC sub-samples should not degrade appreciably over time. However, look for any trends when you plot up individual results over time. If you detect a trend (expected to be downward) then a new set of QC sub-samples should be prepared. As a general rule, QC sub-samples should not be kept for more than a year.
- A good quality assurance system in a laboratory is dependent on feedback. Feedback between:
  - laboratory staff and laboratory manager, regarding problems with samples, techniques, etc.
  - laboratory staff and scientists regarding expected capabilities, variability, and expected levels for the results, and required levels of precision, etc.
- Feedback helps to ensure that:
  - the objectives of the project are being met;
  - variability of results is reduced and thus confidence in the results is maintained;
  - all participants in the analyses know what is expected of them in the QA system.

7.5 Biomass accrual and growth rate estimates

7.5.1 Net biomass accrual rates

It is often useful to determine the rates per unit area at which the periphyton community accumulates on substrates. This provides an approximation of growth rates in situations where losses due to factors such as detachment and invertebrate grazing are low. However, the term "accrual rate" is preferred over "growth rate" because the variable being calculated is area specific biomass change (not biomass specific or cell specific biomass change) and

reproduction rates are not accurately estimated. Net accrual rate measures the rate of accumulation of community biomass on the substrate per day (Biggs 1990, Stevenson 1996). Net accrual rates are particularly useful for assessing differences in the degree of enrichment of sites (e.g., to assess the effects of a waste discharge; see Biggs 1990) and to assess the effects of differences in habitat variables within sites (e.g., Biggs and Stokseth 1996).

Net accrual rates are most easily determined using artificial substrates, whereby a large number of replicate plates (say 20) are loaded onto a sampler at the beginning of the assay, then subsets (3–5 replicates) are randomly selected and removed at progressively greater time intervals (e.g., see Biggs 1990). Natural substrates can also be sampled if you can commence sampling immediately after a flood which has reduced stream bed biomass to near zero (e.g., see Biggs and Stokseth 1996). It is recommended that you sample at least four times during the exponential phase of growth to accurately define the rate of change of biomass (Figure 15). With artificial substrates in a stable flowing river, the time interval between sampling during the exponential phase may need to be as little as 3 - 4 days. However, the time to the first sampling can be much greater if colonisation is slow (e.g., soon after a flood disturbance when the pool of colonists is low). If possible, sampling should continue until peak biomass. This may only take several weeks in some spring-fed streams, but several months in some foothills streams if they have been recently disturbed by high flows (e.g., Biggs and Stokseth 1996).

Net accrual rate is calculated according to the following model:

$$B = a \exp(kT) \qquad (9)$$

where B is the biomass measure per square metre at day T, a is the initial biomass concentration and k is the net accrual rate during the exponential growth phase. The way we calculate k is to perform a $\log_e (B + 1)$ transformation on the data which changes the exponential part of the accrual curve into a straight line (Figure 15, plot B). We then use these points on the curve in a regression of B against T (using a statistical calculator or statistical software). The resulting coefficient for T is the value we use for k. Low values for k are < ~ 0.10 and high values for k are >~ 0.35. Since k is the natural log exponent for the rate of change, it has no units except day^{-1}.

An example of accrual rate results used to determine the enriching effects of a discharge from a domestic sewage oxidation pond is given in Figure 16. Artificial substrate samplers were placed upstream and downstream of the discharge point in the South Brook near Rangiora, North Canterbury. The samplers were incubated for up to 15 days on three occasions. No significant differences were found between upstream and downstream accrual rates even though phosphorus concentrations were a factor of 10 higher in the downstream reaches (nitrogen was available in excess quantities). The results indicated that the waters had sufficient P to not be limiting accrual rates at both upstream or downstream sites and therefore that the discharge was unlikely to be stimulating periphyton growth. For more details see Biggs (1990).

While we usually use chlorophyll *a* as a measure of biomass accrual, it is also possible to use AFDM and cell densities. If cell densities are used, and immigration, emigration, death and consumption losses can be quantified (or estimated), then the approach outlined above can be used to calculate growth rates for specific taxa (see Stevenson 1996 for more discussion on this topic).

7.5.2 Specific and relative specific growth rates

If losses by emigration, death and invertebrate grazing appear to be minimal (as usually

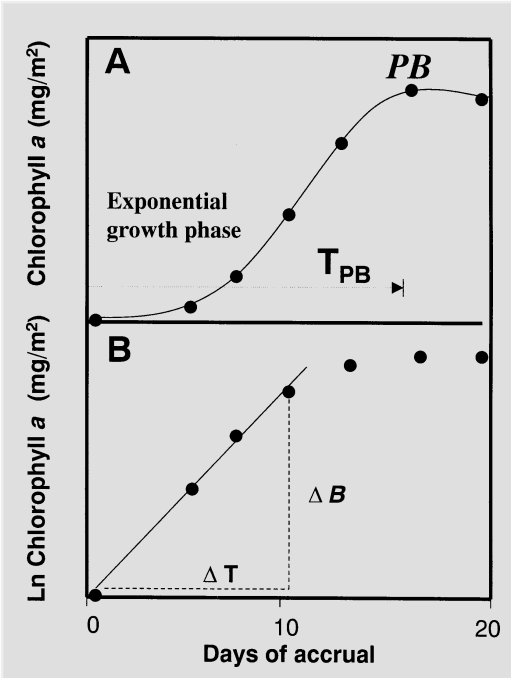


Figure 15: Idealised periphyton biomass (*B*) accrual curve (as chlorophyll *a*) on artificial substrates for (A) untransformed chlorophyll data, and (B) $\log_e$ transformed data. Each data point represents the mean of 5 replicates collected for different lengths of accrual time (T). PB = peak biomass for the accrual cycle, T_{PB} = time to peak biomass.

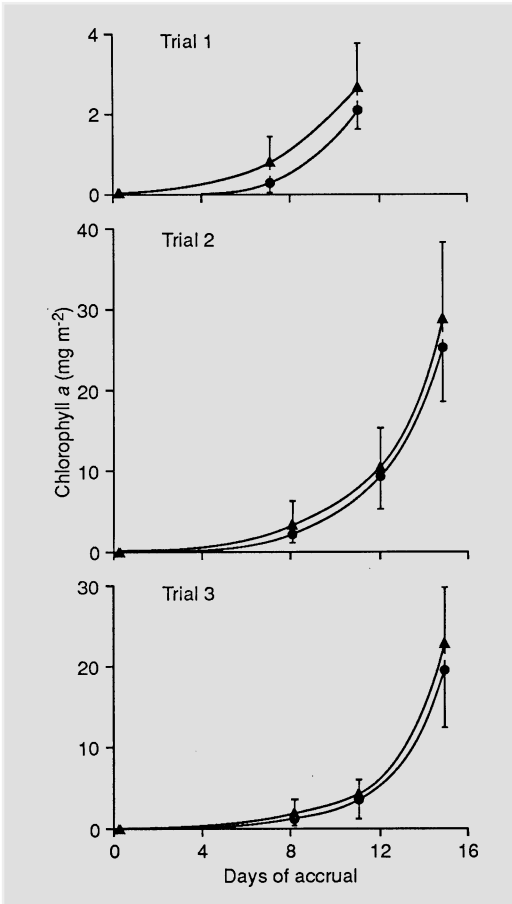


Figure 16: Periphyton chlorophyll *a* accrual curves (and 95% confidence limits) for three trials to test the effects of an oxidation pond discharge on periphyton accrual in the South Brook, North Canterbury (•, above the discharge; ▲, below the discharge). Trial 1: Upstream *k* = 0.180, Downstream *k* = 0.338; Trial 2: Upstream *k* = 0.351, Downstream *k* = 0.310; Trial 3: Upstream *k* = 0.411, Downstream *k* = 0.385 (reproduced from Biggs 1990 with permission of the *New Zealand Journal of Marine and Freshwater Research*).

occurs on artificial substrates raised off the stream bed in slow moving water), then specific growth rates (μ) can be calculated. To do this we use the value for *k* derived from analysis of chlorophyll *a* accrual in the preceding section and apply a correction to convert the value to $\log_2$:

$$\mu = k / 0.693 \qquad (10)$$

where μ is expressed as cell divisions per day (Bothwell 1988). This is the measure for growth more commonly used in phytoplankton studies.

The advantage of calculating μ is that it is then possible to use μ as a nutrient availability indicator by normalising μ to μ_{max} – the nutrient-saturated, maximum specific growth rate. Goldman (1980) promoted the use of this concept as a means of removing the effects of temperature on algal growth rates and thus to isolate growth as a function of limiting nutrient concentration (this is because growth is mainly a function of temperature if nutrients and light are not limiting). We can estimate the P-saturated maximum specific growth rate as a function of temperature using the model of Bothwell (1988):

$$\mu_{max\text{-}P} = 0.189 + 0.0278 \leftrightarrow \text{temperature (°C)} \qquad (11)$$

The "P" subscript with $\mu_{max\text{-}P}$ denotes the communities used to calculate the relationship were P-unlimited, but some slight limitation by other nutrients could not be ruled out by Bothwell (1988). If one assumes that growth is unlimited by any other nutrients, then the degree of nutrient limitation is simply calculated as the relative specific growth rate:

$$\mu{:}\mu_{max\text{-}P}. \qquad (12)$$

Bothwell (1985, fig. 3) provides data which indicate that P-deplete relative specific growth rates occur when $\mu{:}\mu_{max\text{-}P}$ is < 0.3, and P-replete growth rates occur when $\mu{:}\mu_{max\text{-}P}$ is > 0.8. Biggs (1990) used a series of other nutrient limitation assays such as alkaline phosphatase activity, cellular nutrient quotas and phosphorus uptake rates to test the applicability of this method for enrichment monitoring in New Zealand streams. While highly nutrient-limited conditions did not occur in the test communities, the results for moderately P-limited and P-saturated periphyton communities conformed well with the criteria of Bothwell (1985).

Note that in making these calculations we assume that nutrient limitation is the only factor affecting biomass reduction. However, other factors – such as light, grazing and emigration – could potentially reduce biomass independently. Therefore the conversion of biomass accrual to relative specific growth rates and the use of these as indicators of nutrient limitation should be used with caution.

8 Taxonomic analyses

8.1 Overview of taxonomic analyses

Finding out what taxa comprise a periphyton community is fundamental in most periphyton studies. It sets the scope for interpretation and evaluation in any resource or pollution monitoring investigation. For some investigations this can be adequately carried out in the field using small portable microscopes (particularly where communities are dominated by distinctive macro-algae). However, for most investigations it will be necessary to bring samples back to the laboratory for analysis. Methods for sampling will vary depending on the objective of the work as discussed in Sections 2 and 6.

Where possible, samples should be returned to the laboratory and analysed fresh if any taxa except diatoms are of interest. This is because the colouring and cell shape in many "soft-bodied" taxa is lost with preservation. Where analysis is delayed for less than 48 h it is usually adequate to store samples in a cool, dark place. Most commonly, though, it will be necessary to preserve samples for later analysis. Longer preservation is easily achieved with preservatives or through freezing. Freezing can result in the contraction of some organelles such as chloroplasts in *Spirogyra*, but this is generally easier and less of a health risk than many other methods based on toxic preservatives such as gluteraldahyde. Freezing also enables later analysis of chlorophyll *a*; this is not possible if chemical preservatives are used. For diatoms, sub-samples may need to be cleaned in acid or peroxide to remove organic coatings and clearly reveal the morphology of the silica frustule, from which detailed identifications are made. As with biomass analyses, sub-sampling is usually carried out after the samples have been homogenised to break up large clumps and very long filaments (see Section 7.2). Sub-samples of some green algae may need to be stained to assist with identification of important bodies within the cells such as starch granules.

There are several possible levels of enumeration. One is a semi-quantitative determination of the relative abundance of taxa based on their contribution to sample biovolume. A rapid scan technique is used for this. This method does not allow for the calculation of densities of the different taxa, or diversity indices, but it does allow a robust assessment of what is mainly contributing to overall community biomass (which is usually also measured using chlorophyll a or ash-free dry mass). The method is based on the Braun-Blanquet approach that is widely used for terrestrial vegetation analysis (e.g., see Kershaw 1973).

A second method is fully quantitative and involves a detailed count of the number of individuals of each taxa. This enables the calculation of cell densities (usually expressed as number of cells / mm^2), diversity indices and the proportion of individuals of different taxa in a given sample. For this method, sub-samples are systematically scanned and all cells counted in a series of randomly located fields until 300 cells have been enumerated. A calculation involving the area of the sample, the proportion of the sample used as a sub-sample, and the number of fields analysed then enables densities to be determined. It is often necessary to move to a further level of detail by calculating the size / biovolume of each of the common taxa and multiplying the densities of individual taxa by their mean biovolumes so that the data are appropriately weighted by the proportion of the biomass made up by each taxon. This step is usually necessary because of the great range in sizes of the different taxa.

Quantitative analyses, however, are very time consuming. An average analysis, for example, will generally take 1–2 hours depending on how much silt is in the sample and how familiar you are with the taxa. Because of eye strain while working on the microscope, and the high

potential for developing repetitive strain injuries, it is unwise to spend more than six hours a day doing analyses. Thus, it can take a considerable time (and therefore expense) to analyse a group of 50–100 samples. In practice, we are often only interested in the most abundant taxa and somewhat less than four taxa (often only one or two) usually comprise > 70 % of biomass in samples from most New Zealand gravel bed streams. Therefore, we most frequently use the semi-quantitative method. This method is up to six times faster than the cell count - biovolume method which then allows many more samples to be analysed and a better range of information to be generated than detailed enumerations for a given budget. The semi-quantitative method is appropriate for impact assessment and state of the environment reporting, where major differences in periphyton are expected between sites or sampling dates. However, there can be inter-operator variations in evaluations.

It is highly recommended (and an essential part of a formal quality assurance system: see Section 8.7) that every laboratory develops a photographic register of the taxa that have been encountered and the taxonomic designations given. Attach the photographs to filing cards and note on the cards the literature source used to obtain the designation. This will enable you to be consistent with your designations and to have them checked by an expert. Indeed, in taxonomic analyses it is essential that identification of taxa is done based on consistent criteria. It is also good practice to have a voucher collection of preserved sample from each study so that identifications can be verified in the future.

We recommend that you do a preliminary scan of samples if they have been collected from a site not previously sampled, or you are expecting some changes in community composition. During this scan it is a good policy to attempt to identify every cell to the lowest taxonomic level you can. If you distinguish two taxa which you later find are the same, then it is easy to combine them. However, the reverse is not possible. Always photograph any new taxa that you encounter and add them to your photographic library so that there is a record of your designation. If you discriminate one taxon as being clearly different, but cannot determine a species or genus name, then identify the specimen to the lowest level you can and give it a unique number (e.g., *Phormidium* sp. 1).

8.2 Sample preparation

Most periphyton samples will contain clumps of filamentous algae or diatoms. This creates difficulties in sub-sampling to obtain representative portions for analysis. Representative sub-sampling is essential for accurate assessment of periphyton communities. Homogenising the sample with a simple, hand-held laboratory or kitchen blender can significantly reduce variability in sub-sample analyses, thus increasing the precision of the results. Biggs (1987) found that the assessed density of cells in a periphyton sample could increase by up to 500 % and coefficients of variation decrease by nearly 90 % following blending compared with just shaking the sample to achieve mixing. The highest increase in precision can be expected with samples that are dominated by filamentous algae. The blending process generally does not greatly damage cells, although some colonial species tend to break apart and long, and lightly silicified diatoms and chrysophytes can be damaged. Most filamentous taxa break apart into much smaller fragments at inter-cellular connection points. Certain green filamentous algae do not break apart easily (e.g., *Cladophora*) during blending and tend to get wrapped around the cutting blades. The use of sharp blades on the blender, only a small volume of sample, and a slightly longer blending time usually overcomes this problem (Biggs 1987).

8.2.1 Equipment

1. Glass beaker.
2. Stoppered bottles with narrow necks (marked at 50 ml intervals).
3. Kitchen or laboratory tissue blender.
4. Squirt bottle with distilled water.

8.2.2 Procedure

- Tip the contents of the sample container into a glass beaker (the width of the beaker should be only slightly greater than the width of the housing holding the cutter blades of the blender). Rinse out any sample residue from the container and lid into the beaker. Distilled water is preferred for rinsing and making up the sample volume. However, tap water can be used providing the water isn't heavily chlorinated.
- Pick out any invertebrates, pieces of gravel, leaves, moss, etc. from the sample.
- Ensure that there is enough water to fully cover the blender-blade housing.
- Blend for about 30 seconds or until the mixture is free of obvious clumps of material. If the sample contains much filamentous algae, break the strands up by repeated cutting with a pair of sharp scissors. Every 10 seconds, or so, stop the blender and free any filaments that may have become caught on the blades or blender housing using a squirt of water. You need to end up with a homogeneous solution suitable for sub-sampling.
- Transfer the blended liquid to a stoppered bottle. Rinse out the beaker and make up the solution to a known volume (usually 50, 100 or 150 ml). Do a quick calculation of how much solution is required for all your sub-samples (variables to be analysed x aliquots ↔ replicates) and ensure that you will have at least 50% more volume than this to allow for re-analysis of variables where there may have been some analytical errors or problems – see Sections 7.3.6, 7.4.6 and 8.7. Enter this volume onto your lab analysis sheet.
- If the sample is frozen between blending and different sets of analyses, then it should be re-blended briefly before sub-sampling for the next analysis (freezing tends to coagulate some filamentous and mucilaginous periphyton).

8.3 Relative abundance assessments using an inverted light microscope

In the following, we describe a relative abundance method based on scores ranging from 8 (“Dominant”) to 1 (“Rare”). This method is used for a rapid evaluation of community composition and the main taxa comprising biomass.

8.3.1 Equipment

1. Inverted microscope with a 12.5x eyepiece and 10x, 40x and 63x objectives.
2. Ocular and stage micrometers for cell measurements.
3. 0.5–2 ml automatic pipette with a ~ 2 mm wide bore at the tip to allow the free passage of cells.

8.3.2 Procedure

- Blend your sample to get a homogenous solution as described in Section 7.2. If the sample has already been blended and then stored frozen, we recommend that you re-blend it. Freezing can result in coagulation of the cells (particularly filaments).

- Transfer the freshly blended sample to a narrow-necked bottle.
- The sub-sample for analysis comprises at least three smaller aliquots of the sample which are pooled. To take these aliquots, shake the bottle well and withdraw 2 ml with an automatic pipette from half-way down the solution while the liquid is still moving. Check that the liquid is not swirling around the perimeter of the bottle - this will cause density separation of the cells and prevent the removal of a representative sub-sample. Release the sub-sampled solution into the settling chamber. Repeat this procedure twice more to give the full sub-sample (i.e., the total sub-sample volume = 6 ml).
- Attempt to obtain a thin covering of cells on the bottom of the settling chamber – not so thick that they overlap frequently.
- Ensure that the cells are evenly distributed across the entire bottom of the settling chamber. This requires some practice and can be done by gently shaking the chamber from side to side. Don't swirl the sample as it will just result in the cells being redistributed to the perimeter of the chamber.
- If your first sub-sample is too dense, then dilute the sample by 2x or 4x, or whatever is appropriate. Rinse out the chamber with water then sub-sample again.
- If the first sub-sample is too dilute, then leave the sample to settle for several hours (most periphyton cells are relatively heavy and settle quickly) then decant off the supernatant to reduce the original volume by half or quarter, or whatever is appropriate. The amount of dilution or concentration of the cells is unimportant for this type of analysis.
- Allow the sample to settle in the microscope chamber for 2–5 minutes.
- First, scan the whole sub-sample at low power (about 100x) and write down the names of taxa you can see. You may get an idea at this stage of which contribute most to the volume of cells. Only consider those cells containing chlorophyll (i.e., live cells), but the diatoms are often easier to identify to species level by viewing dead cells of a similar size and shape from which the organic matter has decayed.
- Scan again at dry high-power (400–700x), check your original identifications made at low power and note down the small taxa present.
- Rank the main taxa in decreasing order of importance of their contribution to the volume of cells in the chamber relative to the most common taxon. The DOMINANT taxon contributes the most volume to the sample. Note that you can make 2 or even 3 taxa CO-DOMINANT if you find it impossible to decide on a single one being dominant.
- Rank all the other taxa you have listed in relation to the dominant taxon (taxa) as follows:

8	Dominant
7	Abundant
6	Common – abundant
5	Common
4	Occasional – common
3	Occasional
2	Rare – occasional
1	Rare

Notes:

- You don't have to include all ranking categories in every assessment. For example, if a sample is overwhelmingly dominated by a single taxon, and the next most common one is much less common, then you should rank the second one as “common”, for example, rather than abundant.
- The number of ranks we recommend is quite high (i.e., 8). We have found that with practice it is quite easy to use an 8-rank scale and gives much more information (it is also more satisfying as an analyst). High numbers of ranks are also advocated, and used, for modified Braun-Blanquet assessments of terrestrial vegetation cover (e.g., see Kershaw 1973, Table 1.5). However, some analysts for varying reasons, may prefer to use fewer ranks (e.g., 3; rare, common, abundant). This is quite acceptable. In general terms the equivalence with our scale would be as follows:

Recommended ranks		**Alternative (reduced) ranks**	
8	Dominant	3	Abundant
7	Abundant		
6	Common – abundant		
5	Common	2	Common
4	Occasional – common		
3	Occasional		
2	Rare – occasional	1	Rare
1	Rare		

- If adopting a reduced ranks system, then it is important that you still use the enumeration protocol defined in the preceding method (i.e., the taxa / taxon that dominate the biovolume of the sample are given a ranking of 3 – Abundant, and everything else is ranked in relation to this score). The use of a reduced, but semi-quantitative, ranking system has been advocated by some workers. In such an approach, the above ranks are intuitively given boundaries in terms of absolute cell densities (i.e., a few cells = rare, dense cells = common, too dense to count = abundant). However, we strongly discourage this approach because it is almost impossible to keep a mental benchmark of absolute densities among samples, sampling occasions and analysts. It also doesn't take into account different sample sizes. Such an approach would only be possible if a fixed area of substrate is always sampled; the sample and sub-sample volumes are always identical, and a system is setup where by taxa are ranked according to a frequency of occurrence for a given number of microscope fields. If you are going to this trouble then you are better off doing a quantitative assessment, but with a reduced count (say, 100 cells).
- When using size as a diagnostic feature, refer to identification texts for typical sizes. The microscope should have an eyepiece micrometer and you should keep handy a conversion table which lists units on the scale and their equivalent actual lengths for the magnifications.
- Refer to Section 10 (an identification guide to common periphyton taxa in streams and rivers) to assist with identifications. See Section 10.5 for a selection of other published texts.

- If resources allow, you should complement relative abundance assessments with some quantitative measure of biomass such as chlorophyll *a*.
- If you are a novice, repeat your analysis on the same sub-sample and then different sub-samples until you are confident that you are getting consistent results (see QA / QC procedures).
- The most efficient way to record the scores is to use a standard taxa list. These can be made up for a range of monitoring programmes and you should select a list made up from samples in the region being monitored. Leave spaces on the sheets to add new taxa as required. With such recording sheets you end up with a matrix of sites vs. periphyton taxa, with blank cells wherever a listed taxon did not occur in a sample.
- It is preferable to use fresh material for microscope assessments of periphyton. However, this may be impractical due to the need to process blocks of samples that have been collected elsewhere or over time. Such samples usually require storage (freezing or preservatives) to prevent decay.
- Sub-samples may be preserved in gluteraldahyde, which retains the cellular form of both green algae and diatoms (see Section 6.9.2).

8.4 Quantitative analysis (cell counts) using an inverted or compound microscope

The quantitative microscope method involves counts of cells in a known number of microscope fields in a sample and sub-sample of known volumes. It provides the most accurate assessments of the taxonomic composition of a periphyton sample, providing enough cells are counted and that sub-sampling is representative. Its drawback is that it is very time-consuming (therefore expensive). In addition, the level of detail obtained from a full count is often superfluous.

This quantitative method may be employed using an inverted microscope or a normal compound microscope. The difference is in how the samples are prepared for examination. In general, if there are large quantities of filamentous taxa (e.g., *Cladophora*, *Vaucheria*, *Batrachospermum*) it is recommended that an inverted microscope be used because many of the filamentous cells are so large that it is difficult to mount them properly for examination with a compound microscope.

In brief, an aliquot of the homogenised sample is placed into a settling chamber (inverted microscope) or Palmer-Maloney chamber (compound microscope). The cells settle to a glass plate which forms the bottom of the chambers. This base plates are the thickness of a cover-slip. For the inverted microscope, up to 5 ml of sub-sample is placed in the counting chamber. The objective lens is oriented to capture the image from below the chamber and therefore permits the examination of cells and filaments that have settled on the glass base-plate without disrupting them. For the compound microscope, the objective looks down through the sample in the Palmer-Maloney chamber. This chamber is much smaller than for an inverted microscope (it is only as deep as a microscope slide) so a much smaller sub-sample is used (e.g., 0.1 ml). With both techniques it is possible to examine the samples at up to 790x magnification using dry lenses which is sufficient to identify all soft-bodied algae and most diatoms. However, for samples with large quantities of very small diatoms, >1000x magnification is required with oil immersion lenses. This is possible with the inverted microscope, however, it is recommended that such enumerations be carried out after samples have been cleaned and mounted on a slide (see Section 8.5), so that oil immersion analysis using a high power compound microscope can be used. This will give much greater optical resolution and,

therefore, greater certainty with identifications.

Cells and 10 µm long units of filamentous taxa with very small cells (mainly cyanobacteria) are counted in fields that are located according to a set of random numbers and a stage grid. Counting in strips across the microscope chamber has often been carried out, but is not recommended here because random fields are less prone to error due to non-random settling of cells in chambers (Sandgren and Robinson 1984). We are recommending that a 300 cell / unit count be carried out as the standard for quantitative enumeration. This number of cells / units and random fields gives good reproducibility / precision, is in line with the standard U.S. Geological Survey protocol for their National Water-Quality Assessment programme (S.D. Porter, US Geological Survey, pers. comm.), and has been recommended in most recent methods descriptions in the international literature (e.g., Lowe and LaLiberte 1996).

8.4.1 Concentrating and diluting samples

Ideally, you want about 30–50 cells per field at 500x magnification for efficient and / or accurate quantitative enumeration. Often the cells in the samples will be too dilute, or too concentrated. In such cases it is necessary to alter the volume of water that they are suspended in after the sample has been homogenised to give a "workable" concentration of cells. It is usually best (but not critical) to perform dilution or concentration of the samples before any sub-sampling (e.g., for analysis such as chlorophyll *a*).

Dilution is simply achieved by adding more water to the sample, while concentration is achieved by siphoning off the supernatant after the sample has been left to settle for at suitable time in a measuring cylinder (usually allow about 1 hour per centimetre of settling depth). When transferring the sample to the measuring cylinder, make sure that you thoroughly rinse the original container to remove cells adhering to the walls. It is useful to add or dilute water in multiples of the original sample volume (e.g., doubling or halving the volume). This makes it much easier to perform calculations for cell density and to calibrate your eye on the microscope when determining how much dilution / concentration a sample might need. Always record the final sample volume.

If you are already working with a sub-sample that has been previously removed from a larger sample (e.g., used previously for biomass analyses), then we treat the sub-sample as "the sample" in terms of volumes and calculations. This is because the concentration of cells in the taxonomic "sample" should be the same as the original, larger, sample (assuming that it was properly homogenised before sub-sampling).

8.4.2 Materials

1. An inverted microscope or compound microscope with 10x, 40x, 63x and 100x objectives and 12.5x oculars.
2. An ocular micrometer and calibration slide for measuring cell size.
3. Settling chambers (generally 5 ml is adequate for inverted microscopes) or a Palmer-Maloney chamber for a compound microscope.
4. Settling board or leveled table. A settling board can be made easily by inserting a leveling screw in each corner of a 20 cm x 20 cm flat board. The screws are adjusted until the board is level. Check with a small spirit level.
5. Pipettes: 1 ml serological, or accurate automatic pipettes with a ~ 1 mm wide bore at the tip to allow the free passage of cells.
6. Measuring cylinder (~ 1 litre) (required only if a sample needs to be concentrated).

Notes:

- The inverted microscope settling chamber is a plastic or glass cylinder of variable height (the height determines the volume of sub-sample that can be settled) and that screws into a metal ring that fits into a movable stage of the microscope. The metal ring has a lip on which a thin glass base-plate sits (equivalent to the cover slip of a compound microscope). The cylinder is then screwed into the base-plate glass to make the settling chamber. Silicone grease or vaseline may be required to make a water-tight seal between the cylinder and base-plate. The base-plates are easily broken when the chambers are being washed, so ensure that you always have a good supply of plates from the microscope supplier.
- Palmer-Maloney counting chambers can be purchased from specialist biological instrument suppliers (check the Web for your most convenient source or try the "Biology Shop" in Australia; fax: +61 247 586 806). They can also be fabricated easily by making a clean hole in the middle of a plastic microscope slide using a cork borer, then gluing a cover slip to the underside. A small cut is made on the edge of the well across the lip to allow excess water to escape when sealing off the top of the sample with a second cover slip. Calculate the volume of the chamber from its dimensions.

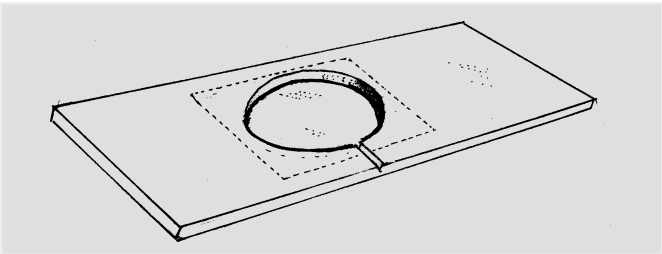


Figure 17: Palmer-Maloney counting chamber.

8.4.3 Preparation procedure

- Blend the sample to get a homogenous solution (see Section 7.2).
- Transfer the sample to a narrow-necked bottle that is graduated for volume measurements. Rinse any residue from the original container into the bottle.
- Record the volume of the blended sample. This is essential for calculating cell densities.
- For the inverted microscope, shake the sample and using a precision pipette remove a 0.1 – 0.5 ml aliquot from half-way down the sample and place it into the centre of a clean settling chamber. Repeat this step twice more (shaking the sample between each) to give a total sub-sample of three pooled aliquots in the chamber. You may need to add a few extra drops of water to the chamber after the sub-sample to give enough liquid for the cells to be evenly distributed (Note: this extra liquid doesn't affect the volume calculations for density estimates). Shake the chamber from side to side to distribute the cells. Be careful not to swirl the chamber because this will re-distribute the cells to the perimeter of the chamber. Record the total sub-sample volume.
- For the compound microscope, shake the sample and using a precision pipette remove a 0.1 ml aliquot from half-way down the sample and gently place it into the Palmer-Maloney chamber. Then slip a cover slip over the chamber ensuring that there are no air bubbles trapped under the cover slip. Record the sub-sample volume.
- Allow the sample to settle for 2 – 5 minutes in the chambers.

Notes:

- Try to obtain a *thin* covering of cells on the bottom of the chambers – not so thick that they overlap frequently. Check the density of cells. If your first sub-sample is too dense, then dilute the sample as described above (ensure that you record the new sample volume). Rinse out the chamber with water then add a sub-sample from the diluted sample. Don't forget to shake the sample between the withdrawal of each aliquot. Re-record the sub-sample volume.

8.4.4 Examination procedure

- First scan the whole sub-sample at low power (about 100 x). Check the distribution of cells to ensure that they are reasonably evenly spread across the base plate. If they aren't, then gently shake the chamber from side to side to re-suspend the material then allow to settle. Avoid swirling.
- If the cells are evenly distributed, then continue with the analysis.
- Change to a magnification of 400–500x. This is adequate for most enumerations.
- Locate your first field for the cell counts using a random number table: one number to give the horizontal setting and one number to give the vertical setting on the movable stage. (These numbers can be generated from most scientific calculators or obtained from statistics texts. Compile a table that covers the range of numbers and degree of movement on the stage rack so that all positions are within the area of the base-plate. If there is a low density of cells then you will need many pairs of random numbers. This table can be part of your laboratory bench data sheet as shown in Table 4).
- Count all cells for each taxon within the first field. Only count those cells with chloroplasts (however, the diatoms are often easier to identify to species level by viewing empty frustules of a similar size and shape from which the organic matter has decayed). Only include cells which are > 50% within view. For filaments with very fine cells that are difficult to discern (mainly filamentous cyanobacteria), count the number of 10 µm long units.
- When all cells / units in the field have been enumerated, use another pair of random numbers to locate the next field. Count all cells as for the first field. Continue this procedure until at least 300 cells / units have been counted.
- Record the number of fields examined.

Notes:

- Set out your lab bench sheet so that it is easy to keep a running check on the total number of cells / units that have been counted (e.g., groups of five ticks).
- If you reach 300 cells / units before a full field has been completed then carry on to finish the field. This is necessary so that the densities can be accurately determined. Counting more cells / units than the recommended number just improves the precision of the density estimates.
- It can be useful to also count the number of empty (dead) frustules. However, these should not be included as part of the 300 cells / units for the primary analysis.
- Instead of manually logging your counts, there are several software programs that run on laptops to allow you to register counts as you examine the samples (e.g., see www.bgsu.edu / department / biology / algae / count / count.html). These programs give a range of running statistics such as the total number of cells / units, diversity indices and confidence limits on the counts. However, it is relatively easy to develop your own

Table 4: Example of a laboratory sheet for quantitative taxonomic analysis of periphyton samples. The following are entered: sample ID, sample and sub-sample volumes; sub-sample number. Each taxon is entered as it is encountered and the number of cells or 10-µm long units of fine-celled filaments in each field is registered as ticks. These ticks are sorted into groups of five for ease of summation. Each set of random numbers for the stage settings is crossed out as it is used. This enables the total number of fields that have been counted to be determined. This number is entered at the end of the analysis.

Sample/ Lab. ID	**Sample (ml.) =** **Sub-sample no. =** **Sub-sample (ml) =** **No. of fields =**	**Comments:**					
Stage setting (x, y)	**Taxa**	**No. cells/units**					
		5	**10**	**15**	**20**	**25**	**30**
39, 113							
41, 113							
39, 108							
29, 112							
43, 113							
39, 109							
40, 114							
31, 110							
35, 121							
29, 109							

Etc.

customised entry systems using Microsoft Excel and Access if you are familiar with these programmes. Direct entry onto spreadsheets or databases is a significant time saving and reduces the potential for transposition errors.

- Permanent mounts of samples dominated by soft bodied taxa can also be made and used for quantitative analysis using Taft's media (see Lowe and LaLiberte 1996 for instructions on preparing Taft mounts). It is not recommended that quantitative analyses be carried out under a compound microscope by just placing the coverslip directly over the sub-sample on a slide. This usually results in the material being forced out the sides of the coverslip, thus changing the volume of the sub-sample to an unknown degree which can introduce large errors in the analysis.

8.4.5 Calculations

The density of cells for given taxa or total cell density is calculated as follows (all volumes in ml):

Cells per sample =
(total number of individuals x sample volume x area of the counting chamber or base or coverslip)
/ (area of each field x number of fields counted x volume of sub-sample)

If whole stone sampling was used (i.e., sampling Method 1a; Section 6.5.4), you need to calculate the surface area of stone from which the samples were obtained:

$$\text{Stone surface area (mm}^2) = [1.59 + 0.811\ (xy + yz + xz)] \times 100$$

Where x, y, and z are the lengths of the 3 main axes of the stones in centimetres. The "x 100" is to convert the area from cm^2 to mm^2. This formula has been adjusted to only include the area of the stone normally protruding into the water on which the periphyton can colonise (~ 65 % of the total surface area).

If samples have been collected from a set area on top of the stones (Quantitative Methods 1b, 2 and 3: Sections 6.5.5, 6.5.6, 6.5.7), or from artificial substrates (Section 6.6), then calculate the surface area of the individual replicate in mm, then multiply by the number of replicates collected.

Finally, calculate the density of cells follows:

$$\text{Cell density / mm}^2 = \text{Cells per sample / area of sample (mm}^2).$$

8.5 Procedures for cleaning diatoms and making permanent mounts

Some diatoms (particularly very small taxa) will be difficult to identify with confidence from fresh or frozen material, even under high power dry objectives. In particular some genera such as *Nitzschia* sp., *Synedra rumpens*, and some *Gomphonema* spp. can be difficult to distinguish. For these cases it is advisable to clean and mount a sub-sample of the algal suspension for examination under an oil immersion objective at ≥1000x magnification.

8.5.1 Cleaning methods

Ashing

The ashing method is relatively straightforward and suitable for samples that comprise mostly diatoms and little other organic material. It is also good for preparing fine or delicate diatom frustules (particularly plankton samples).

Equipment:

1. Muffle furnace.
2. Pipettes (disposable glass droppers are fine).
3. Forceps.
4. Coverslips (No. 1 thickness).

Procedure:

- Pipette a few drops of the well-mixed suspension onto a clean coverslip.
- Sit the coverslip on a slide (with the solution facing up) and view under 40x magnification to determine whether the dilution or concentration of the sample is necessary to easily observe the cells for the enumeration.
- Once the appropriate density has been determined, allow the sub-sample to air-dry on the coverslip overnight.
- Using fine forceps, transfer the coverslip to a crucible and ash at 400°C for about an hour. This burns off the organic matter leaving behind only the silica frustules ("skeletons") of the diatoms. Allow to cool. Alternatively, heat the coverslip on a hotplate at about 600°C for up to 10 minutes.

Acid cleaning

Many samples will contain all sorts of material as well as diatoms – other algae, detritus, plant fragments. In these cases, cleaning with acid (or some other strong oxidising agent) is recommended because it is more effective at removing the larger amounts of organic matter without leaving an ash residue. There are numerous methods involving the use of various types of acid, and hydrogen peroxide, with and without heating. We have found the following to be effective for most periphyton samples.

Equipment:

1. Concentrated sulphuric acid.
2. Hydrogen peroxide.
3. Clean glassware (tall beakers or conical flasks, 600 to 800 ml).
4. Fume hood.
5. Distilled water.
6. No. 1 cover slips.
7. pH papers.
8. Glass transfer pipettes.

Procedure:

- Allow the sample to settle for a few hours, then drain off as much water as possible.
- Place a small amount of sample – say a quarter of a teaspoonful – in a labelled container. You could allow the sample to dry out further at this stage.
- Working under a fume hood, add a small amount of concentrated sulphuric acid to the container. The amount you use depends on how much material you have. Usually 10–20 ml should be sufficient.
- Add a little ~25% hydrogen peroxide. Both additions will set off a reaction, with generation of heat and sulphur dioxide. Keep adding a few drops of hydrogen peroxide until there is no more fizzing.
- When the reaction has completely subsided and the container has cooled down, top up the container with distilled water and leave to settle.
- Rinse the residue of diatom valves until the supernatant is neutral. This normally takes up to eight changes of distilled water. After each addition, allow the residue to settle for at least 4 hr, then carefully pour off as much supernatant as possible without losing any of the settled sample. Top up the container with the next rinse of distilled water and repeat until the pH is 7.
- Using a forceps, pick out any remaining large fragments.
- The rinsing process can be speeded up by centrifuging the sample between each rinse. However, this could possibly damage some diatom valves.
- When rinsing is complete, drain off as much supernatant as possible. Transfer the sample to a smaller container and continue to draw off water until the sample is at a suitable concentration. Check this by examining a small drop (on a slide) under the microscope.
- Pipette the samples onto coverslips ready for drying and mounting (see Section 8.5.2). Pipette on enough sample to cover each slip but not so much that the surface tension is broken.

Notes:

- Always take safety precautions when handling sulphuric acid and hydrogen peroxide. Use protective clothing including gloves and facemask. Always carry out the cleaning under a fume hood.
- The heat generated in the reaction causes most diatom frustules to dissociate into their two valves.
- It is advisable to use a new transfer pipette for each diferent sample, to prevent cross-contamination.
- The slips can be arranged on trays for drying and should be carefully labelled.
- If you wish to keep the cleaned sample for any length of time, add 95% ethanol to the storage container – about equal in volume to the sample. This will prevent fungal growths.

8.5.2 Mounting

Equipment:

1. Slide mounting medium (Naphrax – this is available from only a few outlets world-wide. One is: Northern Biological Supplies, U.K.; fax +44 1473 612 148). Instructions for preparation of the Naphrax with toluene come with the product.
2. Clean slides.
3. An electric hotplate, preferably with no ridge around the perimeter.
4. Glass dropper (for dispensing Naphrax)
4. Forceps.
5. Slide labels and / or permanent marker (e.g., a diamond-tip glasscribe).

Procedure:

- Drop a small amount of Naphrax (in toluene) fixative onto the centre of a clean glass slide.
- Place the slide onto a hotplate at ~ 120ϒC and heat until the Naphrax–toluene solution begins to bubble gently.
- Place the coverslip (with ashed or cleaned diatoms facing down) onto the Naphrax–toluene solution and ease down very gently.
- Continue heating until bubbling subsides then remove the slide from the hotplate.
- Gently press down the coverslip with forceps until all air bubbles have been squeezed out.
- Allow to cool then label the slide.
- Examine the slide under oil immersion as described in Section 8.4.

Notes:

- Always ensure that all glassware is kept clean. Dried diatoms can easily stick to slides, beakers, etc. and then contaminate other samples. Also, keep laboratory benches and preparation areas clean and free of dust. This dust could also include diatom frustules.
- *Always* use No. 1 coverslips (the thinnest available). This is especially important if you are intending to use a 100x objective because of the very small working distance between the sample and the lens.
- Heating the Naphrax drives off the toluene solvent after which the mountant hardens.

Some practice will be required to judge the right amount of heating necessary. Too little, and the Naphrax stays sticky; too much and the mountant becomes yellow and brittle.

- Toluene is toxic (carcinogenic). When mounting slides, always have an extractor fan above the hotplate or use a facemask.
- When processing multiple samples, ensure that slips, crucibles and slides are correctly labeled throughout.
- Slides prepared in this way can form the basis of a reference collection of samples from different sites and at different times.

8.6 Biovolume estimates

The most meaningful quantification of periphyton communities is given by converting density information into biovolumes. This enables a more accurate analysis of the biomass dominance of the different taxa. For example, a filamentous green alga may dominate a site in terms of the biomass (and what you see), but have fewer cells than taxa such as very small diatoms in the sub-canopy. By calculating representative biovolumes for a sample of each of the main taxa, the data can be corrected for the contribution of each taxon to the total amount of organic matter at the site.

Biovolumes are usually determined using the calibrated eyepiece micrometer by measuring the length (A), width (B) and depth (C) dimensions of 10–15 randomly selected cells of all the common taxa and then inserting the data in the geometric formula that best represents the shape of the taxa. Note that the volume of each specimen should be calculated individually and then the volumes averaged for each taxa. Calculating the mean dimensions first and then calculating the volume gives a biased estimate of the mean volumes.

Alternatively, Dr D. Kirchtel (University of Vermont, USA) has written software that allows an even more accurate assessment of biovolume. This is available free from: www.uvm.edu/~kirschtel/biovol/

8.7 QA/QC procedure for taxonomic enumerations

8.7.1 Sources of interference and error

Quantitative analysis of periphyton is prone to several sources of interference:

- Periphyton often accumulates silt within their matrix. This may result in many of the smaller taxa being obscured.
- Previously used sample bottles, settling chambers and slides need to be thoroughly cleaned before re-use. Many taxa (particularly diatoms) tend to stick very well to glass surfaces.
- Convection currents and air bubbles in the settling chambers can interfere with sedimentation. The distribution of material needs to be checked before any analysis to ensure that cells are randomly distributed across the bottom of the settling chamber.

There are three main areas of the analysis that can introduce error. These are:

- Sub-sampling,
- Counting (a lower-scale than sub-sampling), and
- Identifications.

Variation in cell density estimates with sub-sampling is generally <15% (as % coefficient of

variation), but may be as high as 50% (Biggs 1987). Less precision generally occurs with filamentous communities (particularly mat-forming cyanophytes) because fine clumps still often exist after blending. Generally only one sub-sample is withdrawn from the sample for analysis. However, we have recommended that 3 x 0.1–0.4 ml aliquots be removed and pooled for each sub-sample analysed (if using the inverted microscope) to reduce this sub-sampling variability. However, this isn't possible using the Palmer-Maloney chambers because of their small volume. Thus, analysis of multiple sub-samples will probably be required if using a Palmer-Maloney chamber to obtain the levels of precision commonly required for quantitative analyses. Assessments of error and sub-sampling precision can be carried out as has been detailed for sampling error and precision (see Section 3).

There should be minimal chance of mislabelling results for taxonomic analyses as the person carrying out the analyses will normally work from the original sample container. Double check at the end of each taxonomic assessment that you have entered the correct site number on the data sheet.

As mentioned in Section 8.1, it is recommended that every laboratory sets up a photographic library of the taxa encountered and the designations given. Identifications should be regularly checked against this library to ensure that there is consistency with designations. If you later find a mis-classification, it will be necessary to return to the earlier databases and correct them. To assist tracing the location of re-classified taxa in older datasets, it is valuable to develop a spreadsheet of taxa encountered at the lab and the studies in which they were found (this is an essential part of a formal quality assurance system). It is also recommended that a collection of preserved or mounted samples from each study be set up. This enables identifications, relative abundance assessments, and density estimates to be checked later (as with a photographic library, this is an essential part of a formal quality assurance system).

Errors can also arise from failure to discriminate distinct taxa, microscope calibration errors, errors in calculating densities and transcription errors.

8.7.2 Formal quality control procedures

The following steps should be carried out if formal QA / QC procedures are required for taxonomic enumerations:

- The calibration on the automatic pipette used for sub-sampling should be checked before each batch of analyses.
- All sample labels should be checked and signed off as being clearly readable.
- All samples should be registered in a properly prepared and maintained electronic or hard copy register as they arrive in the laboratory. Information should include unique sample identifiers (this would normally be a site + replicate sample number), date of collection, date of receipt in laboratory, method of preservation, analysis required, person who collected sample, person responsible for the job, and job number.
- Check the transcribing of sample numbers / labels onto the laboratory bench sheet and ensure that the samples on the bench are in the same order as on the laboratory bench sheet.
- Results, calculations, and transposition of data should all be checked and signed off by someone other than the analyst. All errors should be logged in a laboratory registry of errors. Apart from helping ensure the quality of the analysis, this registry also helps isolate problem areas in the analytical process.

- For analyses where the results are likely to be used for a resource consent hearing or the Environment Court, 3 sub-samples should be analysed from each sample and the results averaged. If the value for any one sub-sample is > 2x the mean of the 2 nearest sub-samples for a given taxon or total density, then the outlier is considered to be a sub-sampling or analytical error and the result of the outlier should be discarded.
- A comprehensive photographic library to record all designations used by the laboratory must be kept. The photographs need to be high quality colour images using a fine grained film. Attach the photographs to filing cards and note on the cards the literature source used to obtain the taxonomic designation. Where possible, include examples of fresh and preserved material, and images covering the range of sizes / shapes for each taxon. For diatoms, there should also be examples of cleaned material in valve and girdle view. Include dimension data with each image. A register of when new designations are added to the library should also be prepared and kept up to date.
- A voucher collection of preserved, representative sub-samples from each study should be maintained. To do this, pool a sub-sample from every sample collected for a particular site or habitat on each sampling occasion. This composite sample should be preserved in gluteraldehyde, labelled with the site and study details, date of collection, and the number of samples the sub-sample was derived from. On a reference sheet, record the volume of the samples and sub-samples, and the surface area of substrate from where the samples were collected so that mean densities can also be checked.
- When doing batches of routine analyses, it is standard practice to only analyse one sub-sample per sample unless pre-determined requirements for precision dictate more sub-sample replication. If only one sub-sample per sample is being analysed then three replicates from one sample in every 10 should be analysed as an estimate of variability for the batch. This QC sample should be selected randomly from the batch and the data sheet for the extra two replicates labelled with the addition of a QC identifier. The data from the three replicates (either as total density or abundance / relative abundance of specific taxa) is then used to calculate a measure of variability such as the standard error (see Section 3). This measure is reported to the client or project leader who will then decide whether the sub-sampling variability is acceptable in relation to study objectives or the batch needs to be re-analysed.
- No samples are to be disposed of until the data have been checked and signed off by the client / project leader.

Notes:

- The voucher collection sub-samples should not degrade appreciably over time. However, ensure that the containers are air tight so that the preservative doesn't evaporate off.
- A good quality assurance system in a laboratory is dependent on feedback between:
  - laboratory staff and laboratory manager, regarding problems with samples, techniques, etc.
  - laboratory staff and scientists regarding expected capabilities, variability, and expected levels for the results, and required levels of precision, etc.
- Feedback helps to ensure:

- the objectives of the project are being met;
- variability of results is reduced and thus confidence in the results is maintained;
- all participants in the analyses know what is expected of them in the QA system.

9 Data analysis and reporting

9.1 Introduction

In the following section we briefly summarise some approaches and precautionary notes that might assist in analysis of periphyton monitoring data. The idea is not to try to give a comprehensive guide on how to do the analyses, but to give pointers on good practice. This will reduce the range of possibilities for data analysis and help to standardise approaches while improving analytical quality. We assume that you have access to suitable spreadsheet software such as EXCEL and specialised statistical software such as SYSTAT, GENSTAT, SPSS, STATISTICA, etc. (note that some caution needs to be used with non-standard data or experimental designs if using the statistical procedures in EXCEL). We also assume that you have access to several of the many excellent books which give statistical details for analyses such as Elliott (1977), Green (1979), Sokal and Rohlf (1981), Zar (1996) and Underwood (1997). There are a number of journal papers / book chapters that should also be consulted as they give reviews of approaches and specific recommendations for analysing biological monitoring data from streams (e.g., Norris and Georges 1993, Lowe and Pan 1996).

9.2 A general philosophy for data analysis

Any biological data analysis is potentially a minefield of difficulties and errors. This is particularly true for periphyton data because it is usually high in:

- temporal variability (community dynamics and processes are strongly controlled by stochastic processes as well as rapid colonisation potential and fast species turnover);
- spatial variability (community structure, biomass and function are strongly controlled by spatial variations in local controlling variables such as velocity and turbulence, substrate particle size and texture, history of substrate stability, nutrient and light resource supply, and herbivore activity) (for further details see chapters in Stevenson *et al.* 1996 and Biggs 2000a).

In carrying out data analysis, it is necessary to distill the data to a form that is understandable and useful. This is often not a simple process. It is also necessary to reconcile your data with the issues which spawned the study and this is often not as easy at it might seem. It is also important to be able to determine and understand the causes and mechanisms for changes / differences that may have been recorded.

We have three suggestions that should assist in data analysis with the goal of obtaining "information from data". The first point has already been discussed in some detail (see Section 2) and relates to study planning; the second cuts across all processes of data analysis.

1. **"Begin with the end in mind"** – thorough study planning will almost always facilitate speedy and meaningful data analysis. Ultimately, we usually want to test for some difference or impact…in effect we are hypothesis testing. So, set up your hypothesis and sampling programme with the test in mind (preferably written down as part of your planning documentation). Then ensure that you do everything correctly to enable that test to be valid and meaningful. Enough is known about the ecology of periphyton communities that it would be rare that even a basic survey of "what's there" would need to be carried out without first guessing (based on the literature and your experience) "what might be there" before you start, and then "why might it be there". Once you do guess, it then becomes a simple and logical step to set up a hypothesis. This

hypothesis then drives your whole sampling, data analysis and reporting procedures. Embedded in the data is the answer to the first basic question "what's there" (you just need to extract this information specifically). However, you learn so much more about the system and controlling processes by having thought about the potential results and processes of causation, and then testing for something specific.

2. **"Use simple and meaningful metrics"** – this applies particularly to taxonomic results. There is a strong tendency in ecological sciences (and particularly in biomonitoring) to want to distill community data down to indicator metrics and multivariate component scores. The intent is to facilitate understanding. However, in the effort to provide simplification, the biology is often lost and we are (unwittingly) starting to provide ammunition for "single number environmental management". For example, single number invertebrate community indicators are widely used for assessing pollution effects in streams both in New Zealand and internationally. While they do have their place, we have sometimes witnessed discussions with water managers in which such data have been summarised and then the presenters criticised for using such approaches because the non-specialist has more difficulty seeing the issues or effects. The following dialogue is an example:

   Water manager or tribunal member – *"Well Dr Bugs, what does this all mean?"*

   Biologist – *"Quite simple really ... all the diatoms and other algae that like clean water to live, and are found abundantly upstream of the discharge, are being killed off in downstream areas and replaced by sewage fungus and green filamentous algae that thrive in polluted waters."*

   Water manager or tribunal member – *"Then, why didn't you say that in the first place? So what are the implications of this change for us?"*

   Biologist – *"These slime communities downstream of the discharge are not good habitat for large insects such as mayflies and stoneflies so these insects will also be displaced from downstream reaches. Trout like to feed on such insects so we expect that this could also reduce trout growth and numbers in the impact reach".*

   Such simple, and informative, depiction of results isn't possible with complex metrics and many pollution indices. A far more powerful story is conveyed using a simple biologically based description of the system and effects. As Hynes (1974, p.163) has stated:

   > *"If numerical data are collected and tabulated, or drawn as histograms, the effects of pollution are clearly shown even when it is very slight. There is neither need of, nor advantage in, a formal classification into zones, which in any event are not clearly defined, nor is anything to be gained by elaborate graphical methods."*

   We believe that Hynes' advice is as salient today … particularly when attempting to convey information to the public and water managers.

   As a result of the above scenario, we have been very hesitant to develop periphyton pollution and eutrophication indices for New Zealand. Indeed, we don't believe that such indices would facilitate a better incorporation of periphyton data into the decision making processes. We have, however, developed a stream-health indicator score system based on percentage cover of the bed by different periphyton growth forms (Biggs *et al.* 1998c; see also Section 6.4.3). This has been to assist untrained biologists to get some information from their data as part of community monitoring programmes. Once identifications to generic or species level are made, then the corresponding ecological data (e.g., see Table 9 of Biggs 2000a) should be used to understand and interpret

results in relation to specific components of the community.

A further problem with employing multi-component metrics is that it becomes much more difficult to discern "what is an ecologically important difference?". The multi-metric may change significantly (say from 120–100), but how does this relate to the important taxa and wider ecological processes? We seldom know the answer. However, if we clearly depicted data showing that clean water diatom taxa dominated at a control site and filamentous green algae such as *Cladophora* (that require high nutrient concentrations) dominated at the impact site just downstream, then we would have a good basis for interpreting the possible wider ecological effects of the perturbation (as illustrated in the earlier example). This issue of "ecological importance" biomonitoring results is discussed further in Section 3.7 in relation to constraints on sampling replication.

3. **"First, plot your data"** – a visual representation of your results in relation to the hypothesis being tested is an essential first step to analysis and reporting of any study. Use the plots as the initial assessment of your hypothesis. For many of us, this is one of the most exciting and interesting parts of the whole scientific process: are we right or wrong with our predictions and associated hypotheses? These plots then usually form the basis for statistical testing of trends and differences to determine if what you see in the plots is statistically significant. While it is good to look for overall patterns in your data, avoid going on "fishing expeditions" to create hypotheses based on what you see plotted up. Be careful about jumping to early conclusions about causes for observed patterns. This is particularly important if doing correlation plots.

9.3 Some common data analysis approaches

In the following sections we will briefly discuss three families of data analysis procedures that we would commonly use in relation to periphyton monitoring or experimentation in streams. Full statistical background for most procedures is given in texts such as Zar (1996). Here, we present examples illustrating two methods for assessing differences among treatments, in order to clarify the principles behind the methods. The third procedure is for assessing trends over tim. An worked example is presented because the method is not yet available in standard software programs.

9.3.1 Assessing differences among "treatments"

Treatments might refer to sites in streams with different landuses, sites above and below waste discharges, or experimental application of different nutrients such as described for the nutrient-diffusing substrate assays (Section 6.7). The most widely adopted method for discriminating treatment differences is the use of bar plots (with standard error bars) or "box and whisker" plots (e.g., Figure 5). Such plots are used to show a periphyton response variable on the vertical axis for various category states of a treatment on the horizontal axis. For many analyses a simple analysis of variance (ANOVA) will assist to determine the magnitude of differences among the treatments relative to the sampling variability. This may be carried out using parametric analyses (after first checking for assumptions of normality in the distribution of data – and correcting if necessary – and approximately equal variances among the treatments) or using the non-parametric Kruskal-Wallis procedure which requires fewer assumptions; in particular it does not require normality in the sample data. In relation to assumptions required for tests such as ANOVA, it should be noted that they are robust to departures from assumptions if the experiment / data sets are "relatively large". This is par-

ticularly true where data are balanced (i.e., sizes of samples for the different populations are the same). For ANOVA, Underwood (1997) suggests that "relatively large" means having more than about 5 treatments, with sample numbers exceeding about 6 per treatment.

A number of situations can occur when a more complex form of the ANOVA is required. This is when there is some "structure" to the way the treatments have been applied. The most common of these is nesting of secondary effects within the overall variations being assessed among treatments (a "nested" ANOVA) and secondly when the effects of two treatments and their interaction are being assessed simultaneously (a "factorial experiment" ANOVA). An example of a nested design might commonly be when attempting to discriminate the effects of a point source perturbation such as a nutrient-rich discharge from a waste water treatment plant using multiple sites above and below the discharge point (e.g., Figure 5). The structure of this data is summarised in Table 5. This table also represents how the data need to be arranged and coded in the data file to allow the categorical treatment effects to be tested in the ANOVA model.

For such an analysis, the ANOVA model statement for this would need to be:

$$\text{Chlorophyll } a = \text{Constant} + \text{Location} + \text{Site}\{\text{Location}\}$$

The results of this analysis are summarised in Table 6. They show that chlorophyll *a* biomass

Table 5: Summary of data structure for an analysis of the effects of a point source discharge to a river using multiple sites above and below the discharge point. This is a "nested" ANOVA structure. The actual data are depicted in Figure 5. In reality each site had 10 replicate samples.

"Treatments"		Response
Site (and replicate samples within each)	**Location** (relative to discharge point and replication within)	**Chlorophyll *a*** (mg/m^2)
1_1	$Above_1$	52.3
1_2	$Above_1$	167.6
1_3	$Above_1$	110.0
etc.	etc.	etc.
2_1	$Above_2$	153.2
2_2	$Above_2$	112.7
2_3	$Above_2$	80.2
etc.	etc.	etc.
3_1	$Below_1$	187.5
3_2	$Below_1$	378.5
3_3	$Below_1$	232.5
etc.	etc.	etc.
4_1	$Below_2$	317.2
4_2	$Below_2$	164.0
4_3	$Below_2$	266.0
etc.	etc.	etc.
5_1	$Below_3$	292.0
5_2	$Below_3$	265.0
5_3	$Below_3$	113.5
etc.	etc.	etc.

did differ significantly as a function of location above and below the discharge, but within each location there weren't any inter-site differences.

Table 6: Summary ANOVA results for data in Table 5. Data were $\log_e$ transformed prior to analysis.

Source	Sums-of-squares	Degrees of freedom	Mean-squares	F-ratio	*P* - value
Location	5.939	1	5.939	19.727	< 0.001
Site{Location}	0.507	3	0.169	0.562	0.643
Error	13.548	45	0.301		

An example of a 2-way ANOVA data structure is summarised in Table 7. In this analysis, we were interested in determining whether the effect of higher nutrient enrichment of periphyton chlorophyll *a* biomass was dependent on degree of stream shading. The nutrient treatments were 19 vs. 188 ppb nitrate-N, and light treatments were 400 vs. 1,200 $\mu E\ m^{-2}\ s^{-1}$.

In reality, this study was done as an experiment so that all conditions except those of the treatments were held constant among the artificial stream channels. Thus, we can specify an interaction term as well as "main-effects" terms in the ANOVA:

$$\text{Chlorophyll } a = \text{Constant} + \text{Nutrients} + \text{Light} + \text{Nutrients} \leftrightarrow \text{Light}$$

Table 7: Summary of data structure for an analysis of the effects of two nutrient concentrations (low and high) and light levels (low and high) on periphyton chlorophyll *a* in experimental streams. This is a two treatment "factorial" ANOVA structure.

"Treatments"		**Response**
Nutrients (and replicate samples within each)	**Light** (and replicates within each sample)	**Chlorophyll *a*** (mg/m^2)
Low_1	Low_1	1.16
Low_2	Low_2	0.33
Low_3	Low_3	0.26
Low_4	Low_4	1.36
Low_5	$High_1$	0.76
Low_6	$High_2$	1.59
Low_7	$High_3$	1.50
Low_8	$High_4$	0.37
$High_1$	Low_5	56.9
$High_2$	Low_6	38.3
$High_3$	Low_7	94.0
$High_4$	Low_8	64.2
$High_5$	$High_5$	135.0
$High_6$	$High_6$	77.2
$High_7$	$High_7$	55.2
$High_8$	$High_8$	76.0

The output table will define whether nutrients and light are statistically significant effects on their own. If the Nutrients x Light interaction is also statistically significant, it would mean that the effects of nutrients is not uniform over the range of light intensities tested. Table 8 summarises the results. It identifies that the periphyton responded only to the nutrient treatment, and that the interaction term with light was not statistically significant, meaning that the nutrient effect was not modified by light within the tested levels.

Table 8: Summary ANOVA results for data in Table 7. Data were $\log_e$ transformed for the analysis.

Source	Sums-of-squares	Degrees of freedom	Mean-squares	F-ratio	*P* - value
Nutrients	82.708	1	82.708	226.268	< 0.001
Light	0.489	1	0.489	1.338	0.270
Nutrients x Light	0.010	1	0.010	0.027	0.873
Error	4.386	12	0.366		

One more important aspect of ANOVA can be illustrated here. This is the presence of another environmental variable which may also be changing among the treatments, but which we cannot control. This is called “co-variance”. Such phenomena can be accommodated in the ANOVA using most modern statistical software so long as the state of the co-varying variable has been measured. In the above experiment on the effects of nutrient enrichment under different light conditions, it is plausible that the high light treatments were warmer than the low light treatments which could confound interpretation of the results (i.e., temperature differences alone could explain the results). By including temperature as a co-variate (this is defined in different ways in the model statement for the various statistical packages) the analysis will first remove the effect of temperature by accounting for the temperature correlation with chlorophyll and then assess the relationship between the residual variance in chlorophyll with light. This process isolates “main effects” from secondary effects and provides a much more robust analysis and set of conclusions.

In carrying out any of the above assessments it is also important to remember to try and recognise what might be ecologically important (see Section 3.7). Periphyton communities at most sites or patches on the streambed can be shown to be statistically significantly different just by collecting more samples. But … so what? Think about differences and what they might mean for wider issues such as ecosystem functioning, trophic interactions etc., before concluding whether treatment differences are important.

9.3.2 Assessing trends over time

There are many situations, such as routine monitoring for SOE reporting, where we may need to determine whether the periphyton communities have changed over time as a result of, say, a change in landuse or other management decision. When using the term “trend” we are adopting a reasonably strict definition in that we are referring to multiple points collected over a time series. There are a number of situations where a site might be sampled only on two occasions. Analysis of such data can be easily done as bar or box and whisker plots followed by a Student's *t*-test or Mann-Whitney U-test. There are several methods by which longer time series of data can be analysed.

As already emphasised, the data must first be plotted. These plots just consist of the response variable being plotted as a function of date of collection. An example is given in

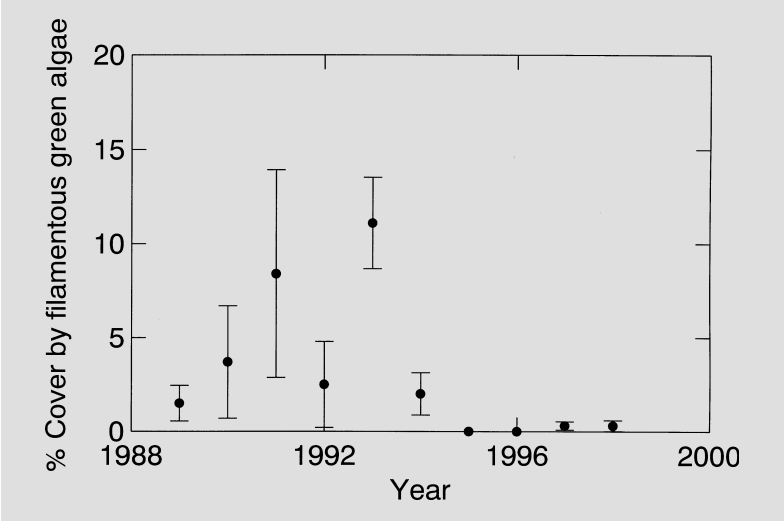


Figure 18 Time series of percentage cover (± 1 SEM) of filamentous green algae during summer low flows in the Manawatu River (data from the NIWA National Water Quality Monitoring Network courtesy of Dr J.M. Quinn).

Figure 18. This depicts 10 years of summer percent cover of the river bed by filamentous green algae. It depicts what appears to be a reduction in algal cover over the duration of the monitoring. The plot also illustrates a difficulty we are often confronted with when analysing such time series. This is that simple, linear trends rarely occur. Nonetheless we will use the standard approach to determining whether a trend is occurring in periphyton cover over time. This involves assessing the significance of the slope on a linear regression of the dependent variable as a function of time:

$$Y = c + b(t) \qquad (13)$$

Where Y is the dependent variable (say percentage cover of filamentous green algae), c = a constant (the intercept on the Y axis), and b = coefficient giving the average rate in Y for a given increment in time (t).

This approach assumes that the change is monotonic (i.e., only proceeds up or down) and that each observation is independent of the previous. The second assumption is generally easy to fulfill with periphyton data if they are sampled at intervals of > 4 weeks because of the rapid turnover time for the community. The results of this analysis do not indicate a clear monotonic trend. Indeed, the slope of the regression line between percentage cover and time is not significant (P = 0.194). Instead percentage cover appears to increase from 1989–1993, then is suddenly very low again through to 1998.

This plot also illustrates a common difficulty in such trend analysis in which periphyton data are collected very infrequently: variability over time is so great that it is difficult to distinguish clear trends. An alternative approach to analysing this data for trends in slope over time is to divide the time periods up according to any clear discontinuities. In this example we might want to compare cover pre-1994 vs. 1994 and after using a Student's t-test or Mann-Whitney U-test. This indicated that there was a significant difference in cover (p = 0.016 for a Mann-Whitney test) between the two time periods suggesting that there has been a reduction in cover of filamentous green algae in the Manawatu River during late summer since 1994.

Several other approaches are available such as the Mann-Kendall test for trend (Gilbert 1987) which is a non-parametric test for monotonic trend that does not assume linearity, but does

assume independence of samples. This approach has been developed further to account for seasonality, with the option of removing flow dependence, and is widely recommended for trend analysis in water quality monitoring (Ward *et al.* 1990, Loftis and Ward 1994, Manly 1999). A software package (WQStat-Plus) is now available to enable such calculations (contact NIWA Instrument Systems: g.elley@NIWA.cri.nz).

9.3.3 Correlation and regression analyses

These are probably the most familiar type of statistical analyses to most stream ecologists. They are the "bread and butter" tools of our trade. While ANOVA focuses on defining the statistical significance of differences in responses to set levels in a controlling variable (often just as binary treatments), correlation and regression analysis have their strengths in establishing graduated responses to gradients in the independent variables. Such "gradient analysis" is often much more useful to management. Also, biotic responses to changes in the environment are seldom linear and it is usually important to define the shape of this response. For example, the biotic responses may be asymptotic, Gaussian (i.e., bell-shaped), or display critical thresholds whereby the biota can resist the effects of changes in an environmental variable and then there is a sudden response. Correlation and regression analysis can be used to analyse and model such non-linear effects providing the appropriate equations are adopted for curve fitting. Thus, these analyses provide core tools for developing simple predictive models that can be useful to water managers.

Correlation and regression are closely related techniques and have similar roots. With correlation analyses we are generally only interested in whether one variable changes in harmony with another variable (i.e., there is no implied causative relationship between one variable and the other). There are no statistical assumptions to be satisfied in order to compute the correlation coefficient, however there are assumptions underlying testing of hypotheses about, and the determination of confidence intervals for, correlation coefficients (Zar 1996). Parametric (Pearson's correlation) and non-parametric (Spearman's rank order) methods can be used. The parametric method assumes linearity, whereas the non-parametric method does not. The non-parametric test is particularly appropriate to use when the distribution of the data for each variable is far from normal. It is important to remember that a non-significant correlation using the parametric test does not necessarily mean that there isn't a relationship between your variables. It may just mean that the relationship isn't linear. Thus, to reiterate our earlier advice … always plot your data first and inspect the relationship before designing an appropriate data analysis approach.

Regression analysis is used to define the dependence of one variable (a response, or "dependent" variable) on another variable (the variable causing that response – usually environmental – called the "independent" variable). The general form of the linear model is summarised in Equation 14. As with correlation analysis, most statistical software will offer a range of approaches for regression analysis from simple bivariate regression to complex stepwise procedures for use when attempting to isolate the best set of predictors for the dependent variable. The statistical engine of these analysis is defined in texts such as Zar (1996) and Underwood (1997). General procedures for fitting complex curves are given in Snedecor and Cochran (1980). We will not repeat any of this background information here, but it is useful to reiterate the main assumptions of regression analysis. These are:

- Independence of data,
- Homogeneity of variables,

- The independent variable is measured without error, and
- The errors in the dependent variable for any given value of the independent variable are normally distributed (see Underwood 1997, p. 424 for discussion of these assumptions).

One of the critical issues in using regression analyses in studies of stream ecology (particularly periphyton), is that some thought be put into the processes or mechanisms that might be expected to result in a given relationship. If the study has been well planned (see Section 2), then possible causal mechanisms will have already been isolated and the investigator will find it much easier to draw sound conclusions from a significant relationship. However, if regression (or correlation) analysis is used to search for patterns in large datasets, then it is often difficult to interpret relationships in any meaningful way and, indeed, many absurd linkages can be defined. Use some thought and common sense before embarking on such analyses.

One of the difficulties in regression analysis that we are often confronted with is finding an appropriate model to fit non-linear relationships. In practice, what we would normally do is adopt a general equation that best simulates the shape of our relationship, apply the transformations given by the models and then use least-squares regression to estimate the parameters. A summary of general types of curves and their equations is given in some statistical texts (e.g., Steel and Torrie 1960).

10 Identification guide to common periphyton in New Zealand streams and rivers

10.1 Introduction

This identification guide covers many of the simple photosynthetic organisms (algae) that occur in the periphyton of streams and rivers in New Zealand. It is intended to provide rapid and practical assistance for non-specialist biologists and stream ecologists wishing to identify the most common taxa in samples of periphyton. For completeness we also include some less common taxa because they can be abundant regionally.

The guide is organised into broad groups based on easily distinguishable features. These groups do not necessarily correspond to those used in taxonomic classification.

10.2 Nomenclature and classification

The term "algae" has traditionally been used to encompass all simple organisms that are capable of photosynthesis and that are not included in the bryophytes (mosses and liverworts) or vascular plants. Therefore algae do not form a single evolutionary group. For example, the prokaryotic "blue-green algae" (see page 124) are only distantly related to the rest of the algae, which are eukaryotic organisms (see page 122) and it is now usual to refer to the blue-green algae as cyanobacteria. Nevertheless, the term "algae" remains a useful ecological grouping of chlorophyll *a*-containing organisms that occur in mixed communities in aquatic habitats.

The classification of algae is based on a wide range of characteristics, the more obvious of which are listed in Table 9. Using various combinations of these features it is possible to distinguish the main groups, as shown in the key (Table 10). The groups normally encountered in stream and river periphyton are shown highlighted in colours, with their common names in **BOLD CAPITALS**. Sections 10.2.1 to 10.2.6 give more information about each of the groups.

Each description in this manual is based on a genus and includes basic taxonomic information (e.g., Division, Order, Genus). The colours highlighting each group in Table 10 are repeated throughout the guide as a quick indication of the algal group to which each genus belongs. Note that, at the level of Order, you will find a slightly different classification scheme in almost every phycology guide. For the eukaryotic algae we follow the orders used by Prescott (1978) and Round *at al.* (1990) (for the diatoms). For cyanobacteria, the groups used are those proposed by Anagnostidis and Komàrek (1985) (see Section 10.2.1). For an up-to-date account of algal classification see Graham and Wilcox 2000.

Table 9: Characteristics of algae used in classification and identification

Cellular organisation:	The simple photosynthetic organisms found in periphyton are either: • "prokaryotes", in which the cell inclusions are not bound by membranes and pigments are distributed throughout the cell, giving a diffuse appearance (these are the "blue-greens" (Cyanobacteria)); or • "eukaryotes", in which cell inclusions such as nucleus, mitochondria and chloroplasts are distinguishable and are enclosed by membranes.
Cell pigments	All possess the photosynthetic pigment chlorophyll *a*, but chlorophylls *b* and *c*, and phycobilins also occur in certain groups.
Flagella	Flagella (singular: flagellum) are long, thin flexible appendages that allow many algal cells to move around. Some algae have no flagella; sometimes they occur only at the reproductive stages; in some cases they are always present and there may be one, two or four. Where there are two or more flagella they may be the same or different lengths. Flagellated species are mainly planktonic and are rarely encountered in stream periphyton.
Colour	Cell colour ranges from red to brown through yellow and green to blue-green and even blue. The major taxonomic groups are named on the basis of colour but, because many taxa contain a mixture of pigments, colour is not always a reliable guide to the groups. Colour may vary with environmental conditions and may also vary according to the type and duration of preservation.
Food reserves	Some of these organisms have food reserves of starch and therefore a positive reaction to the iodine starch test. Others store food as leucosin or oil and do not react to the starch test.
Cell wall type	Cyanobacteria often possess a muco-polysaccharide sheath; green algae have cellulose cell walls laid down in a criss-cross pattern; diatoms have rigid walls made of silica.
Reproduction	Some algae and cyanobacteria reproduce by cell division, each new cell being independent of all others. Most algae also produce specialised reproductive bodies, or spores, and in some groups the form of these is the main character that distinguishes species. Sexual reproduction is also common.
Gross structure	This is the group of characteristics that is of most practical use in *identifying* algae. For example, is the alga single-celled, filamentous, colonial or sheet-like? Does the alga have different types of cells – e.g., heterocysts? Many of the same structural characteristics occur in different groups, and thus do not reflect natural relationships.
Ecology	Aquatic algae including cyanobacteria are capable of inhabiting almost all damp to wet habitats in existence, from sea water through to hot springs. However, many types occur only within a certain range of conditions.

Table 10: Key to the major groups of common freshwater periphyton

1a	Cells without chloroplasts (though granules in the cells could be mistaken for chloroplasts), diffuse blue-green, olive or red-brown. Cells often very small.	
	Division: **Cyanobacteria (BLUE-GREENS) [see page 124]**	
1b	Cells with chloroplasts (discrete structure in which the cell pigment is concentrated).	**2**
2a	Chloroplasts pale to deep grass green.	**3**
2b	Chloroplasts some other colour.	**4**
3a	Single-celled, filamentous or colonial form, normally with one or two chloroplasts arranged as sheets inside the cell wall or along the length of the cell. Sometimes with many chloroplasts. Where flagella are present there are two or four and their lengths are equal. Positive reaction to starch test.	
	Division: **Chlorophyta**; Class: Chlorophyceae **(GREEN ALGAE) [see page 124]**	
3b	Large, erect plants, typically 4-50 cm long, with regular branches. Rooted by rhizoids in soft sediment. Cells large with numerous chloroplasts.	
	Division: **Chlorophyta**; Class: Charophyceae	
3c	Single-celled, colonial or filamentous, normally with two or more small, discoid chloroplasts per cell. Where flagella are present there are two, of unequal length. Negative reaction to starch test.	
	Division: **Xanthophyta (YELLOW-GREEN ALGAE) [see page 125]**	
3d	Single cells with one long, thick flagellum emerging from a depression at the end of the cell. Two-to-many discoid chloroplasts. Cell wall can be elastic and striated.	
	Division: **Euglenophyta**	
4a	Cells with a rigid, ornamented silica wall composed of two halves, sometimes forming filaments. Often motile, but no flagella. One to many brownish chloroplasts.	
	Division: **Bacillariophyta (DIATOMS) [see page 126]**	
4b	Cells not as above.	**5**
5a	Cells with two flagella, partly within two furrows on the cell surface, one around the cell, the other at right angles. Cell walls of smooth or angular plates, flat or with projecting horns. Positive reaction to starch test.	
	Division: **Dinophyta (= Pyrrophyta {dinoflagellates})**	
5b	Cells without two deep furrows	**6**
6a	Chloroplasts pale yellow to brown, usually 1 or 2 per cell. Cells single, colonial or (rarely) filamentous. If flagella are present they are either one long one, or one long and one short.	
	Division: **Chrysophyta (GOLDEN-BROWN ALGAE) [see page 128]**	
6b	Chloroplasts some other colour, no cysts, flagella (if present) not strongly unequal.	**7**
7a	Single-celled, bean-shaped with two slightly unequal flagella arising from a small depression of furrow. One or two chloroplasts, coloured olive, red or blue.	
	Division: **Cryptophyta**	
7b	Plants filamentous and frequently with complex structure, or flattened and encrusting, or (rarely) single-celled. One or more chloroplasts per cell, coloured olive, red or blue. Usually attached to rocks and mosses in streams. No flagella.	
	Division: **Rhodophyta (RED ALGAE) [see page 128]**	

Throughout the idenification guide, the above colours are used to indicate the major algal group to which each genus belongs.

10.2.1 Cyanobacteria

Cyanobacteria are easily distinguished from other algae by the absence of chloroplasts – the discrete organelles that contain the photosynthetic pigments in eukaryotes. Instead, these pigments are diffused throughout the cell protoplasm. Additional pigments found in this group are responsible for the range of colours they exhibit, most notably the blue-green appearance. The orders of cyanobacteria represented in this guide are listed below. These groups are those used by Anagnostidis and Komàrek (1985), with details in Anagnostidis and Komàrek (1988a, b) and Komàrek and Anagnostidis (1986, 1989). This classification system follows the traditional botanical approach, being based on "Geitler's" system (see Geitler 1925, 1932, 1942) but now incorporating morphological, cytological and ecophysiological characters. Note that several other classification schemes have been proposed based on the bacteriological approach (Stanier 1977 and Rippka *et al.* 1979) or on "Drouet's" system (Drouet and Daily 1956 and Drouet 1981), where the number of taxa was reduced considerably.

Chroococcales: Unicells, colonies, pseudoparenchymatous colonies or pseudofilamentous colonies. Trichomes, heterocysts and akinetes are lacking. Cell division in one, two or more perpendicular planes. Examples include *Gloeothece* and *Chamaesiphon*.

Oscillatoriales: Cells forming trichomes (a single row of cells connected by cross walls); false branching, gas vesicles and sheaths lacking or facultatively present. Heterocysts, akinetes and true branching absent. Reproduction occurs by "hormogonia" formation through trichome fragmentation. Examples are *Phormidium*, *Oscillatoria* and *Lyngbya*.

Nostocales: Cells forming trichomes with a wide or narrow mucilaginous sheath. Trichomes unbranched or falsely branched (initiated at a heterocyst or between two vegetative cells). Specialised nitrogen-fixing cells (heterocysts) and spore cells (akinetes) may be present. Reproduction mainly by hormogonia or hormocysts. Examples are *Nostoc*, *Tolypothrix*, *Calothrix* and *Rivularia*.

Stigonematales: Cells forming true trichomes, sometimes combined with pseudotrichomes. True branching always present while false branching may occur. Akinetes rarely present while heterocysts occur facultatively in several genera. Reproduction mainly by hormogonia and hormocysts. Best known example is *Stigonema*.

10.2.2 The green algae (Division: Chlorophyta)

The orders listed below belong to the class Chlorophyceae and are represented in the periphyton of New Zealand streams and rivers. These are traditional orders (largely as used in Prescott 1973). See Graham and Wilcox (2000) for recent classification schemes.

Tetrasporales: These occur in a non-motile vegetative form, usually in colonies held together by mucilage (e.g., *Gloeocystis*, *Palmella*). The cells can reproduce by simple cell division. The chloroplasts are usually described as cup-like – that is, they are curved so that they line part of the inside of the cell.

Chlorococcales: These can look similar to the Tetrasporales (e.g., *Oocystis*). The main difference is that the cells do not reproduce by simple cell division in the vegetative state, though they do divide to form spores. Single-

celled or colonial. Examples found in periphyton are *Pediastrum*, *Ankistrodesmus*, *Scenedesmus*.

Ulotrichales: Unbranched simple filaments with mostly cylindrical cells containing a single band-like chloroplast similar to that in the Chlorococcales. Most species are attached when young, but become free-floating later. The best known filamentous alga in this order is *Ulothrix*. Others include *Geminella*.

Ulvales Many cells in a sheet-like arrangement, e.g., *Enteromorpha*, in which the sheets form hollow filaments.

Microsporales: Unbranched filaments of cylindrical cells with walls in sections with a cell wall at the centre – so that broken-up filaments comprise H-shaped pieces. The chloroplast covers the whole wall of the cell and may be thin or dense. The only genus in this order is *Microspora*.

Cylindrocapsales: Another order with only one genus – *Cylindrocapsa*. Usually filamentous with dense, large chloroplasts.

Chaetophorales: Branched filaments that arise from a holdfast. Cells forming branches often smaller than those nearer the base. Chloroplasts are parietal, sometimes completely covering the cell wall. Examples are *Chaetophora*, *Draparnaldia* and *Stigeoclonium*.

Cladophorales: (also known as Siphoncladales) Repeatedly branched filaments, cylindrical cells, thick walls. Chloroplast parietal and net-like in young, healthy specimens, but sometimes appearing as many small disks. Often the cell walls are very thick and the filaments frequently carry many diatom epiphytes, e.g., *Cladophora*, *Rhizoclonium*.

Oedogoniales: There are both unbranched and branched forms in this order, which is characterised by occasional ring-like scars at the front end of cells, caused by cell division. Genera described in this guide are *Oedogonium* (unbranched) and *Bulbochaete* (branched).

Zygnematales: Unbranched filaments of long or short cylindrical cells with a cellulose cell wall; end walls are separated by a middle lamella. The chloroplasts are large, with 1–2 per cell usually. Species in this order have no motile reproductive cells. Instead, cells transform into amoeboid gametes, two of which fuse to form the zygospore (via "conjugation" of cells). Genera represented in this guide are *Mougeotia*, *Spirogyra* and *Zygnema*.

Desmidiales: The desmids are single-celled forms (occasionally in filaments) related to the Zygnematales through having a similar mode of reproduction. Most desmids are divided into two equal halves. The chloroplasts are variable and can be complex. Examples found in periphyton are *Cosmarium*, *Closterium* and *Staurastrum*.

10.2.3 The yellow-green algae (Division: Xanthophyta)

The yellow-green algae can be difficult to distinguish from the green algae. The main features that separate the two divisions are a predominance of yellowish pigments (e.g., carotenoids) in the yellow-greens, and leucosin or oils as food reserves, rather than starch. Just two orders are included in this manual.

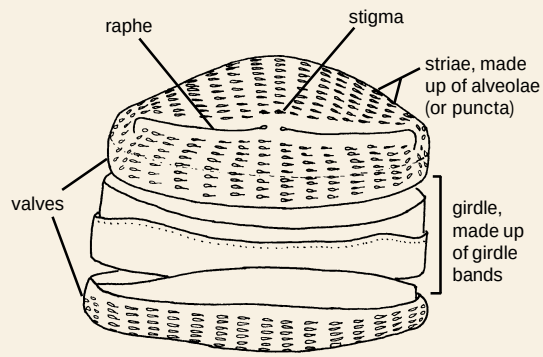
Tribonematales	Branched or unbranched filaments, e.g., *Tribonema.*
Vaucheriales	Branched, cylindrical filaments, no cross walls, e.g., *Vaucheria.*

10.2.4 The diatoms (Division: Bacillariophyta)

The structure of diatoms means that it is relatively easy to identify many of them to species or even variety. In this guide, we include descriptions of many *common* species – particularly those that are useful indicators of environmental conditions. This should enable you to distinguish them from other species that are similar in appearance, but that you won't necessarily be able to identify beyond genus level. Some identifications should be possible using fresh or frozen material.

For detailed studies on diatoms it is necessary to examine acid-cleaned specimens at magnifications of up to x1000, using an oil-immersion objective on a light microscope in order to see the ornamentation of the cell walls. In addition, you need an understanding of their structure and a comprehensive collection of specialised diatom taxonomy texts because, as yet, there is no complete guide to diatoms in New Zealand.

Structure of a generalised diatom frustule



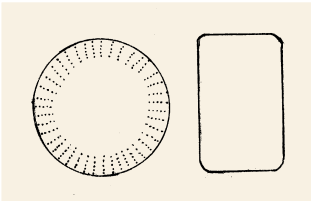
(drawing: Liz Bergey)

Briefly, the diatom cell wall is made of silica and the basic construction is of two halves (valves) that fit together with one half overlapping the other so that the whole structure resembles a chocolate box. The two valves together are called the frustule. Classification is based on the arrangement of various surface features and etchings on the frustule. A major feature is the raphe, a pair of slits in the valve face. When present, the raphe may be on both valves or on one valve only. The two valves may be connected by a girdle or a series of girdle bands. The valve faces may carry rows (called striae) of openings or depressions (known as puncta or areolae). Larger holes are called "stigmata" (singular: stigma). Most descriptions of diatoms are based on the view from the top ("valve view"). The view from the side ("girdle view") can look quite different.

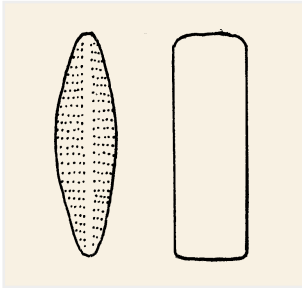
The structure of the chloroplasts and other cell inclusions may also be considered in descriptions. For more details about the fine structure of diatoms refer to Barber and Haworth (1981), Round, Crawford and Mann (1990) and Cox (1996).

As for the algae in general, various classification systems have been proposed for diatoms (see Bourelly 1981, Krammer and Lange-Bertalot 1987–1997, Round *et al.* 1990) and we follow the system of orders of Round *et al.* 1990. For descriptive purposes, these orders can be placed in seven groups (as used by E. Stoermer, pers. comm.) that are convenient though not necessarily natural (i.e., made up of related organisms). The illustrations below show representative valve and girdle views in each group.

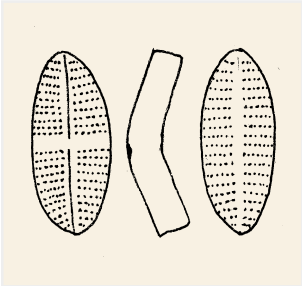
Centrics: Radially symmetric valves. No raphe system.
Thalassiosirales: *Cyclotella;*
Melosirales: *Melosira;* Aulacosirales: *Aulacoseira.*



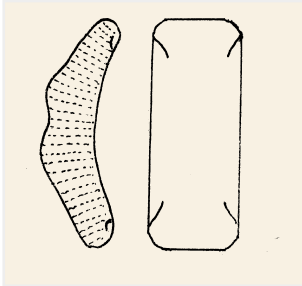
Araphid pennates: Usually symmetrical along the all axes of the valve (exceptions: *Meridion, Asterionella*). No raphe system on either valve, but usually a "pseudoraphe" present (i.e., a break in the striae, as if a raphe were present).
Fragilariales: *Fragilaria, Fragilariaforma, Staurosira, Diatoma, Meridion, Synedra;*
Tabellariales: *Tabellaria.*



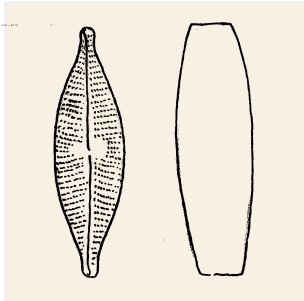
Monoraphid pennates: Usually symmetrical along at least two axes. A raphe system present on one valve only. The second valve may or many not have a pseudoraphe. The ornamentation on the two valves can be quite different. (Illustration shows raphe valve, girdle view and pseudoraphe valve.)
Achnanthales: *Achnanthes, Achnanthidium, Cocconeis.*



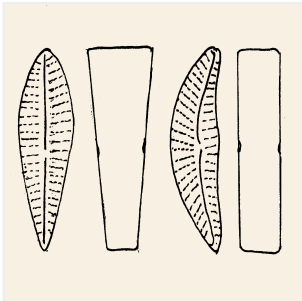
Eunotioids: A rudimentary raphe system on both valves, with the two valves usually similar. There may be asymmetry along all three axes.
Eunotiales: *Eunotia, Eunophora, Actinella.*



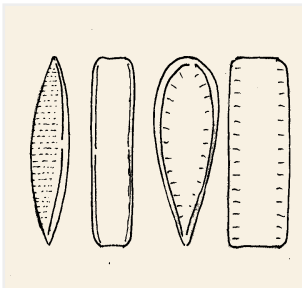
Biraphid pennates: Symmetric naviculoids: These are usually more or less symmetric along both the long and cross-valve axes (exception: some *Pinnularia* species). A fully developed raphe system present on both valves. The two valves are usually identical.
Mastogloiales: *Mastogloia;*
Naviculales: *Brachysira, Frustulia, Navicula, Neidium, Pinnularia, Stauroneis.*



Biraphid pennates: Asymmetric: Lacking symmetry along at least one axis. A raphe system present on both valves. The two valves are usually identical (an exception is *Rhoicosphenia*).
Cymbellales: *Cymbella, Rhoicosphenia, Encyonema, Gomphonema, Gomphoneis, Reimeria;*
Thalassiophysales: *Amphora.*



Biraphid pennates: "External" raphe: The raphe lies partly or wholly along the edge of the valve, sometimes within a raised canal. The structure can be quite complex. Some diatoms in this group are nitrogen-fixing because of a symbiotic relationship with cyanophytes, and can therefore tolerate nitrogen-limited situations. Bacillariales: *Nitzschia;*
Rhopalodales: *Epithemia, Rhopalodia;*
Surirellales: *Surirella, Stenopterobia.*



10.2.5 The golden-brown algae (Division: Chrysophyta)

Mostly motile cells, though the division includes some filamentous and sheet-like forms. This Division is more commonly found in lake habitats than in flowing waters. Chrysophytes are characterised by possessing a very large chloroplast, and by their particular combination of pigments. The one example described is *Dinobryon* (Order: Chrysomonadales).

10.2.6 The red algae (Division: Rhodophyta)

The red algae are distinguished from the other algal divisions by their methods of reproduction and life histories. The "red" comes from the predominance of the red pigment phycoerythrin in many species. Most red algae are marine. The examples in this manual represent the two subclasses of the Rhodophyta. See Bourelly (1985) for a version of classification finer than this. See Entwistle and Kraft (1984) for an account of freshwater red algae in south-east Australia, including notes on the New Zealand flora.

Bangiophycidae	Freshwater forms have a branched, unbranched or plate-like thallus, with thick cell walls. Asexual reproduction is by non-motile spores. Sexual reproduction rare. Represented by *Chroodactylon*, *Compsopogon*.
Florideophycidae	The more "advanced" group, with a wide range of morphologiesfrom slightly branched filaments to more complex growths. The female sex organs (carpogonia) are characteristic. Representatives are: *Audouinella*, *Batrachospermum*, *Bostrychia*.

10.3 How to use this guide

To aid identification, each taxon described in this guide is assigned to a group on the basis of cell type and growth form. These groups do not necessarily correspond to the traditional classification outlined above.

Photosynthetic periphyton may be subdivided into two **cell types**:

- Containing "organelles" (distinguishable structures like chloroplasts and a nucleus), but may not have cell walls. These are the green, yellow-green and red algae, and the diatoms.
- Cell contents diffuse, granular, with no distinguishable organelles (but take care not to interpret granules as chloroplasts). These are the cyanobacteria.

These groups can then each be divided into four broad **growth forms** that are easily distinguished:

- flamentous, unbranched;
- filamentous, branched;
- single-celled;
- colonial or multi-celled (e.g., sheets).

Since diatoms are readily recognised from their silica frustules they are placed in groups on their own.

A further obvious feature of algae is colour. In periphyton this can be:

- green (various shades);
- brown – golden-brown;
- pale bluish green, olive-green or pinkish.

However, even though colour is used to name the major taxonomic groups, the colour of a particular algal genus or species can vary quite a lot depending on, for example, the environmental conditions of the habitat, the health of the cells, preservatives used, and the light source and filters on your microscope. Therefore, you should be cautious in using colour as a diagnostic feature. Just remember that it is often helpful, but sometimes can be misleading.

The groups used in this guide are as follows.

(a) Group C: cells containing chloroplasts (eukaryotic algae)

1. **C: unbranched filaments**. All of these are green algae, except one representative from the yellow-greens. 11 genera
2. **C: branched filaments**. This is a more diverse group, including genera from the green, yellow-green and red algae. 10 genera
3. **C: unbranched filaments, or as single cells (diatoms)**. This group includes diatoms that grow in filaments. They are grouped separately from the unbranched filaments above because all of them are also frequently seen in periphyton as single cells. 7 genera, some with individual species described.
4. **C: single cells (diatoms)**. Because diatoms are relatively easy to recognise from their solid "skeletons", and because many species are found in periphyton, they are all grouped together. 35 genera, with individual species described for most.
5. **C: single cells (non-diatom)**. This much smaller group includes single-celled green algae. 3 genera.
6. **C: colonial**. Included here are cells that are always found growing in colonies of 4 cells or more. 6 genera (5 green, 1 golden-brown).
7. **C: colonial (sheet-like)**. This includes just one distinctive green alga.

(b) Group BG: cells with diffuse cell contents (prokaryotic algae: Cyanobacteria)

8. **BG: unbranched filaments**. Filaments growing in masses or singly. mixed with other algae. 7 genera.
9. **BG: filaments in gelatinous masses**. These are filamentous forms that always grow in cohesive gelatinous clumps. 2 genera.
10. **BG: branched filaments**. Filaments growing in masses or singly, mixed with other algae. 5 genera.
11. **BG: single cells**. One example of a single-celled blue-green alga in periphyton.
12. **BG: colonies**. Two examples of colonial cyanobacteria occasionally found in periphyton.

To use the guide, decide on the category to which your specimen belongs, then look for possible matches in the relevant section. All genera included are listed on page 131.

The coloured bars alongside the descriptions refer to the algal group (e.g., Division) to which the genus belongs (see Table 11 on page 123 for a key to the groups).

As noted above, each description refers initially to a *genus*. In each section the genera are usually in alphabetical order. Most green, yellow-green and red algae require detailed studies – including examination of the reproductive bodies – in order to get past genus level. Many diatoms, on the other hand, can be identified to *species* relatively easily. Common species within many of the genera are described (in words and/or photographs), with the more

frequently encountered species first – though these may not be the most common in all samples. In environmental studies species identification can be important because some diatom species are known to have particular environmental preferences that are not shared by other species in the same genus. Their identification can add considerable value to the interpretation of taxonomic data.

Notes on *macroscopic appearance* and *microscopic details* are included. The former is included only where a taxon has some recognisable feature, rather than being part of a mixed periphyton community. For microscopic examination we assume the use of a good quality light microscope capable of magnifications of at least 400x. An oil-immersion objective allowing magnifications of >1000↔ is also desirable for examining the smaller algal taxa. Ideally you will have an eyepiece micrometer on your microscope so that you can measure the dimensions of your specimens. This should be calibrated using a stage micrometer.

A typical *size range* is given for most taxa.

For a few genera we include notes on the *appearance of algae following freezing*, with illustrations where the changes are particularly noticeable. Many types of algae – particularly diatoms – are not greatly altered by freezing other than some shrinkage of the chloroplasts.

Although examination of frozen material is not ideal, it is often impractical to examine fresh material in routine environmental monitoring programmes. Normally part of the sample needs to be analysed for chlorophyll *a* (a measure of total live biomass). Unless this can be done immediately, the samples must be temporarily preserved. Freezing is the most effective way of doing this. Preservatives such as glutaraldeyde retain the cellular structure of algae, but interfere with the chlorophyll content (see Section 8).

For each taxon there are notes on typical habitat and environmental preferences (if known), as well as observations on abundance and distribution. These comments are based on published notes on algal distributions in New Zealand (Cassie 1984a,b, Biggs and Price 1987), on information in the literature on habitat preferences (Cassie 1989, Cox 1996, Winter and Duthie 2000), and on personal observations.

We also note taxa that might be confused with the one being described. In addition, for some of the diatoms we include notes on recent name changes.

All the photographs in this guide are of periphyton from New Zealand rivers and streams. Magnifications are approximate.

Following the illustrated guide, Sections 10.5 to 10.8 comprise:

- A glossary explaining the technical terms used in the descriptions.
- A table summarising recent new diatom genera that are becoming generally accepted. These genera do not appear in older identification texts, though many of the species can be found under their traditional genus name.
- A list of general references used for identification of algae. (See Section 11 for a full list of literature cited.)
- A list of the diatom species described, with authorities, and reference(s) to one or more source(s) from which the identification may be made.

Definitions

Conductivity:	high, >200 µS/cm
	medium, 80–200
	low < 80

LM = light microscope

SEM = scanning electron microscope

µm = micrometre (1 mm = 1000 µm)

cf. = “compare with” (i.e., looks like)

10.4 Guide to periphyton genera

Contents (names in parentheses indicate a partial description only)

C: unbranched filaments (pages 132–139)

Cylindrocapsa	*132*	*Mougeotia*	*134*	*Spirogyra*	*136*
Geminella	*132*	*Oedogonium*	*135*	*Tribonema*	*137*
Klebsormidium	*133*	*Rhizoclonium*	*134*	*Ulothrix*	*138*
Microspora	*133*	(occasional branches)		*Zygnema*	*139*

C: branched filaments (pages 140–147)

Audouinella	*140*	*Chaetophora*	*142*	*Draparnaldia*	*145*
Batrachospermum	*141*	*Chroodactylon*	*143*	*Stigeoclonium*	*146*
(Bostrychia)	*143*	*Cladophora*	*144*	*Vaucheria*	*147*
Bulbochaete	*142*	*Compsopogon*	*143*		

C:unbranched filaments, or as single cells (diatoms) (pages 148–155)

Aulacoseira	*148*	*Fragilaria*	*151*	*(Pseudostaurosira)*	*152*
Diatoma	*149*	*Fragilariforma*	*152*	*Tabellaria*	*154*
Eunotia	*150*	*(Frustulia)*	*155*	see also *Staurosira*	*184*
(Encyonema)	*155*	*Melosira*	*153*	*Staurosirella*	*185*

C: single cells (diatoms) (pages 156–188)

Achnanthes	*156*	*Epithemia*	*168*	*Pinnularia*	*179*
Achnanthidium	*157*	*(Eunophora)*	*160*	*Placoneis*	*181*
Actinella	*159*	*Frustulia*	*169*	*Reimeria*	*181*
Amphora	*160*	*Gomphoneis*	*170*	*Rhoicosphenia*	*182*
Asterionella	*159*	*Gomphonema*	*171*	*Rhopalodia*	*182*
Brachysira	*161*	*Gyrosigma*	*173*	*Sellaphora*	*183*
Cocconeis	*162*	*(Hantzschia)*	*177*	*Stauroneis*	*184*
Cyclotella	*163*	*Mastogloia*	*173*	*Staurosira*	*184*
Cyclostephanos	*163*	*Meridion*	*174*	*Staurosirella*	*185*
Cymbella	*164*	*Navicula*	*174*	*Stenopterobia*	*185*
Diatomella	*165*	*Neidium*	*176*	*Surirella*	*186*
Diploneis	*166*	*Nitzschia*	*177*	*Synedra*	*187*
Encyonema	*166*				

C: single cells (non-diatom) (pages 189–190)

Cosmarium	*189*	*Closterium*	*189*	*Staurastrum*	*190*

C: colonial (pages 190–193)

Ankistrodesmus	*190*	*Dinobryon*	*191*	*Pediastrum*	*192*
(Chrysocapsa)	*191*	*Gloeocystis*	*191*	*Scenedesmus*	*192*
(Crucigenia)	*193*	*(Palmella)*	*191*	*Tetrastrum*	*193*

C: colonial (sheet-like) (page 193)

Enteromorpha	*193*				

BG: unbranched filaments (pages 194–198)

Anabaena	*194*	*Cylindrospermum*	*195*	*Lyngbya*	*196*
Calothrix	*194*	*(Heteroleibleinia)*	*196*	*Oscillatoria*	*197*
(occasionally branched)		*Leptolyngbya*	*195*	*Phormidium*	*198*

BG: filaments in gelatinous masses (pages 199–200)

Nostoc	*199*	*Rivularia*	*200*		

BG: branched filaments (pages 200–203)

Dichothrix	*200*	*(Fischerella)*	*202*	*Stigonema*	*202*
Coleodesmium	*201*	*Scytonema*	*202*	*Tolypothrix*	*203*

BG: single cells (page 203)

Chamaesiphon	*203*				

BG: colonies (page 204)

Aphanocapsa	*204*	*Merismopedia*	*204*		

Cylindrocapsa

C: unbranched filaments

Division: Chlorophyta, Order: Cylindrocapsales

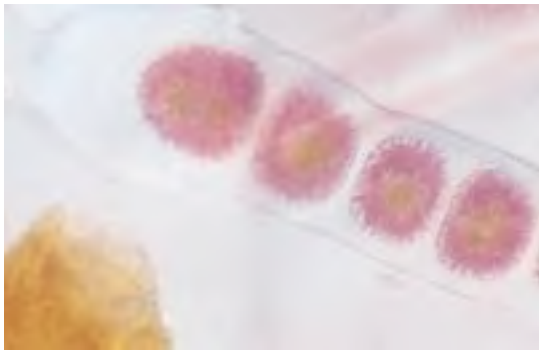
How to recognise the genus:

Macroscopic: Cylindrocapsa grows as dark green-brown masses.

Microscopic: Thick cell wall with a many-layered clear gelatinous sheath between cells. Each cell contains a single large dense chloroplast. In the example illustrated the cells have a distinctive purple colouration, which occurs during reproduction. Normally cells are green. The filaments are about 25 µm across

Habitat and distribution: The genus *Cylindrocapsa* is found mostly in boggy areas but also in slow-flowing shallow streams downstream of wetlands. It has been reported rarely in New Zealand and we have found it only in high altitude, pristine areas. The specimen photographed was collected in the Arthur's Pass region, South Island.

Possible confusion: Distinctive, therefore unlikely to be confused.



Cylindrocapsa *filaments. top, x 200, bottom, x 800.*

Geminella

C: unbranched filaments

Division: Chlorophyta, **Order:** Ulotrichales

How to recognise the genus:

Macroscopic: Geminella has no distinctive macroscopic features.

Microscopic: Small, separate cells, each with a single folded chloroplast, embedded in a thick mucilaginous sheath to form filaments. Individual cells are about 8 µm across and 10 µm long. The entire sheath is around 30 µm in diameter.

Habitat and distribution: *Geminella* is typically a lake-dwelling genus but has been found in the periphyton of lake-fed rivers, usually close to the lake outlet.

Possible confusion: Distinctive, therefore unlikely to be confused.

Geminella. *Note the sheath, just visible. x 800*

Klebsormidium

C: unbranched filaments

Division: Chlorophyta **Class:** Charophyceae

How to recognise the genus:

Microscopic: Narrow filaments (less than 10 µm across). The curved chloroplast covers a relatively small proportion of the cell wall.

Habitat and distribution: Not certain.

Possible confusion: The chloroplasts in *Ulothrix* (page 138) are similarly curved but occupy much more of the cell wall.

Note: *Klebsormidium* was recently placed in the Charophyceae (see Margulis *et al.* 1990)

Klebsormidium. *x 550 (photo: Stephen Moore, Otago Regional Council)*

Microspora

C: unbranched filaments

Division: Chlorophyta **Order:** Microsporales

How to recognise the genus:

Macroscopic: Microspora forms long, deep-green filaments, often trailing in water and entangled around other algae or vascular water plants.

Microscopic: Unbranched filaments of regular, oblong or, usually, squarish cells; the chloroplast is reticulate (net-like) and parietal (arranged against the cell walls). Through the microscope the chloroplast appears as a dense layer around the inside of the cell wall.

The clearest distinguishing feature of this genus is the H-shaped join between cells, although this is not always obvious. Try to find the end of a filament; the cells normally break at the end of the H rather than at the dividing wall. Under high power (1000x) it should be possible to see the where successive sections of cell wall overlap. Filaments range from about 10 to 40 µm across.

Habitat and distribution: *Microspora* is found in a range of conditions, but often in clean, gently flowing streams. The genus is common and widespread. It may proliferate in enriched cold streams.

Possible confusion: *Tribonema* (see page 137). This also has H-joins. However, the two genera are easy to distinguish as *Tribonema* has several discoid chloroplasts rather than a dense net.

from top:

Microspora *growing amongst macrophytes in a spring-fed stream.*

Microspora *filaments (with diatoms). x 150*

Fragment of Microspora *in which the cells are dividing. Note the new H-shapes alternating with the older (outer) pieces. x 350*

Microspora *sp. x 680*

Mougeotia

C: unbranched filaments

Division: Chlorophyta, **Order:** Zygnematales

How to recognise the genus:

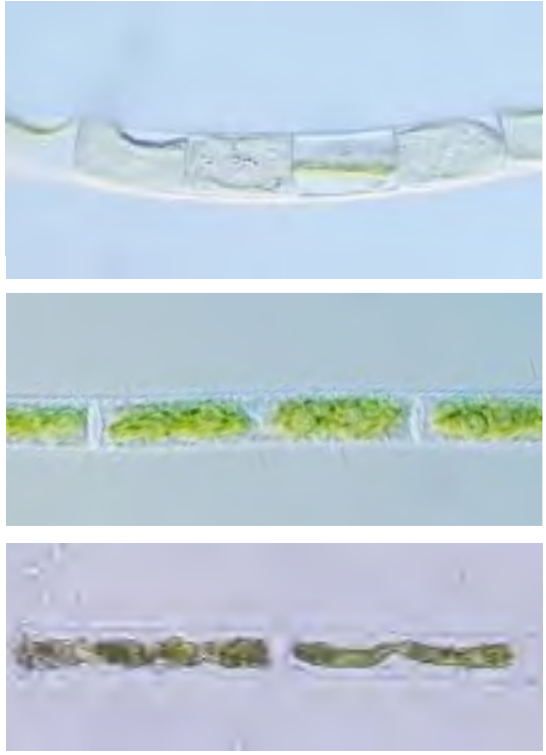
Macroscopic: Mougeotia grows in light green cottony-looking masses that feel slimy and are often mixed with other types of algae.

Microscopic: Unbranched filaments of oblong cells, usually much longer than they are wide. The chloroplast is a ribbon-like and runs along the cell. It may be twisted in places. Pyrenoids often visible at regular intervals along the chloroplast. In many cases, between each cell is a well-defined lens-shaped (or "rice-grain" shaped) area.

Typically filaments are 25–40 µm across, with cells up to 150 µm long.

Habitat and distribution: *Mougeotia* is typically found in moderately enriched to highly enriched slow flowing streams. It has been observed as the dominant taxon during low flows in an enriched lowland river in Canterbury.

Possible confusion: After freezing, *Mougeotia* and *Spirogyra* (see page 136) can be confused, though the lens-shape between cells (if present) reamins clear. The two genera are easily distinguishable when fresh.



from top:

Mougeotia *from the Waiau River. Note the sheet-like chloroplasts, in some cases folded over. x 500*

Mougeotia *filament from the Waipara River, Canterbury. x 650*

Filament following freezing. x 650

Rhizoclonium

C: unbranched filaments

Division: Chlorophyta, **Order:** Cladophorales

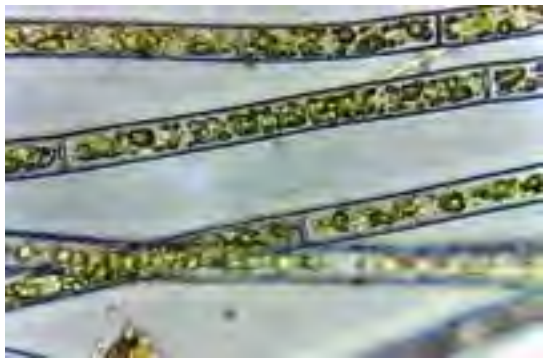
How to recognise the genus:

Macroscopic: Long, coarse, tough filaments, dull-green coloured.

Microscopic: Cells from 10 to over 50 µm wide, usually much longer than wide. Chloroplast is a dense or loose network.

Habitat and distribution: *Rhizoclonium* is found only in high conductivity, warm rivers and streams, Encountered most often in Hawkes Bay (usually very large species). Also found in tidal rivers in Otago (Stephen Moore, pers. comm.)

Possible confusion: Possibly with *Cladophora*, which is branched, though sometimes *Rhizoclonium* puts out rhizoids.



Rhizoclonium *sp. x 450 (photo: Stephen Moore, Otago Regional Council)*

Oedogonium

C: unbranched filaments

Division: Chlorophyta, **Order:** Oedogoniales

How to recognise the genus:

Macroscopic: Oedogonium sp. grows in dull green masses and can form massive blooms under warm, stable, low-flow conditions.

Microscopic: Unbranched filaments attached to the substrate at one end. The cells are fairly regular and square to oblong and sometimes slightly barrel-shaped. The chloroplast is a light green parietal network with a lacy appearance. Pyrenoids are scattered throughout the network.Look for the scarring that occurs as a result of cell division – one or more rings encircling the outer cell wall at the end of some cells. This, with the non-branching form, is diagnostic for the genus.

In frozen material, the chloroplast shrinks away from the cell wall but remains attached at the perimeter at each end of the cell. Although it looks quite different from the fresh cells, it is fairly distinctive.

There are many species of *Oedogonium*. To distinguish them it is necessary to consider a combination of size of the vegetative cells, and the size and form of the reproductive bodies. Filaments range from about 10 to 40 µm across.

Habitat and distribution: *Oedogonium* is normally associated with fairly enriched conditions but may also occur in streams with stable, low flows, sometimes with little apparent enrichment. It is extremely common and widespread and occasionally forms blooms.

Possible confusion: Usually there is no difficulty in identifying this genus, although the branched genus *Bulbochaete* (closely related) has similar cells (see page 142).

from top:

Oedogonium *bloom in the Hakataramea River, North Otago, 1996.*

Fresh Oedogonium *filament. Nore the scarring on the left hand cell. Specimen from clean, foothillls stream, Canterbury. x 400*

A different Oedogonium *species, from an enriched lowland river. Note the lacy chloroplasts and cell-scarring. x 600*

Filament following freezing. This is the typical appearance: cell walls collapse and the chloroplast shrinks to the middle of the cell. x 300

Spirogyra

C: unbranched filaments

Division: Chlorophyta, **Order:** Zygnematales

How to recognise the genus:

Macroscopic: Clouds of fine, bright green filaments often with a foamy look (bubbles at the water surface). Very slimy to touch.

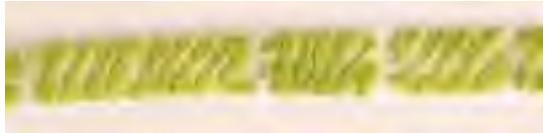
Microscopic: Smooth-sided filaments with cells varying from almost square to very much longer than they are wide. Cells up to 70 µm wide and 100 µm long, or more. Within the cells, one or more long ribbon-like chloroplasts are arranged in a spiral or series of overlapping spirals. Pyrenoids are dotted along the chloroplasts.

The walls between successive cells are usually more or less flat; sometimes somewhat convex (into the cell). Some species have "replicate" end walls that are evident under the microscope as an H-shape on the dividing wall.

In frozen material the spiral chloroplasts shrink together to form a dense mass at the centre of each cell.

Habitat and distribution: *Spirogyra* is found most often in slow-flowing backwaters in open (unshaded) situations in a range of environments from pristine mountain rivers to lowland streams and often appears in response to point sources of nutrients such as from groundwater inputs. The genus is extremely common and widespread. Species with replicate ends seem to occur mostly in lakes.

Possible confusion: With *Mougeotia* (when frozen) (see page 134). In both cases the chloroplast shrinks to the centre of the cell.



from top:

Spirogyra *growing along the margins of the Ashley River, Canterbury.*

Dense Spirogyra *from Siverstream, Canterbury. x135*

Three species with different spiral patterns. Note the pyrenoids (first specimen). nuclei (second and third specimens) and replicate ends (third specimen) x 270

Spirogyra *filaments following freezing.*

Tribonema

C: unbranched filaments

Division: Xanthophyta, **Order:** Tribonematales

How to recognise the genus:

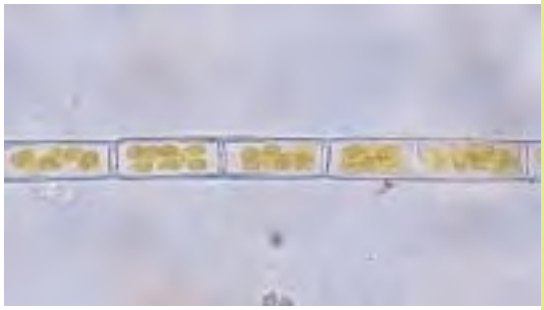
Macroscopic: Light green masses of filaments.

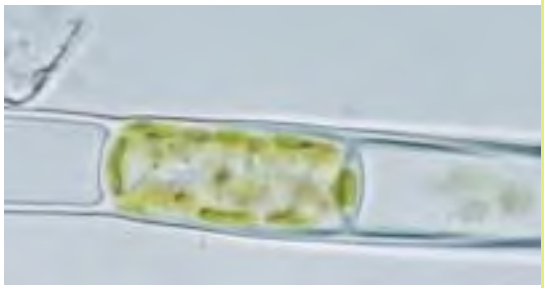
Microscopic: Cells longer than wide, usually slightly inflated at the central part of the cell (barrel-shaped). The cell walls are thin and successive cells are joined at the central area (rather than at the cell dividing walls) where cell walls overlap. Thus when the cells dissociate, they break into H-shaped pieces – halves of adjacent cells. There are several disc-shaped pale green chloroplasts dotted through each cell.

Tribonema filaments are usually quite fine, ranging in size from 3 to 10 µm in diameter and 15 to 50 µm long.

Habitat and distribution: This genus occurs in open situations in a range of conditions, from clean headwater streams (mixed with filamentous diatoms, during low-flow conditions), to lowland high-conductivity streams.

Possible confusion: *Microspora* also has H-shaped cell walls. However, in *Microspora* the chloroplasts are much denser and are single sheets rather than several separate bodies (see page 133).





from top:

A fine filament of Tribonema*, showing the disc-like chloroplasts. Cell joins not obvious. x 500.*

Tribonema *filament clearly showing H-joins between calls, and also the yellow-green colour of the disk-like chloroplasts. x 500.*

Higher power photo of a Tribonema *cell separating at the H-join. x 1400*

Ulothrix

C: unbranched filaments

Division: Chlorophyta, **Order:** Ulotricales

How to recognise the genus:

Macroscopic: Vivid green skeins of fine filaments.

Microscopic: Unbranched filaments of cells ranging in length from noticeably shorter than they are wide to (occasionally) longer. Each cell has a single chloroplast that forms either a complete or partial ring around the inside of the cell wall. The width of the ring varies from almost as long as the cell to very narrow. The filaments may be attached to the stream substrate with a holdfast.

Cells of *Ulothrix zonata* – a very common species in New Zealand – may be up to 45 µm in diameter. Other species are smaller – up to 20 µm in diameter.

Habitat and distribution: *Ulothrix* sp. (especially *U. zonata*) is very widespread. Much growth occurs in spring and, under suitable flow conditions (i.e., low, stable flows) blooms of *Ulothrix* are common in many lowland rivers. It also dominates the periphyton communities of many mountain streams in spring and late summer.

Possible confusion: Perhaps could be confused with sparsely branched types of *Stigeoclonium* (see page 145), in which the chloroplasts are similar.

from top:

Spring growth of Ulothrix *sp. in a steep headwater stream (Avoca catchment, Canterbury).*

Three examples of Ulothrix *(probably* U. zonata*). Note the air bubbles in the cells in the top two photographs. x 270*

Two examples of Ulothrix *at high power showing the different extent of the chloroplast. x 680*

Zygnema

C: unbranched filaments

Division: Chlorophyta, **Order:** Zygnematales

How to recognise the genus:

Macroscopic: Light green slimy filaments.

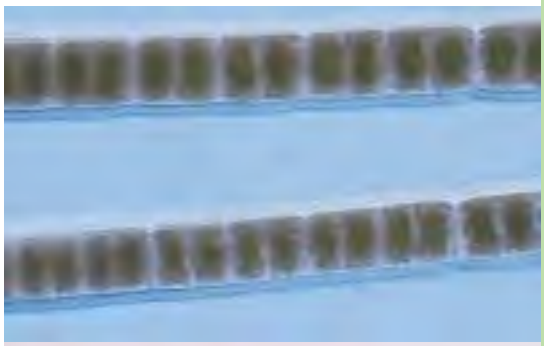
Microscopic: Unbranched filaments of cylindrical cells with straight or rounded end walls. The cells vary in length from almost square to oblong. There are two star-shaped chloroplasts per cell, arranged side-by side. Sometimes this arrangement is extremely clear. Where the chloroplasts more or less fill the cell, the arrangement is not quite as obvious. In fresh material, often the cell nucleus can be seen between the chloroplasts, as a greyish, more-or-less circular body.

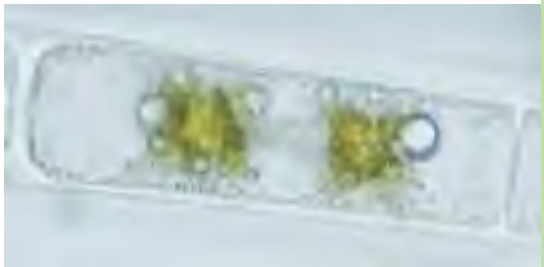
In frozen material the chloroplasts shrink and lose their star-like appearance but it is usually still possible to discern the double side-by-side arrangement.

Filaments vary from about 20 to 50 µm wide.

Habitat and distribution: *Zygnema* is usually found in relatively still waters in lakes, rather than rivers. However it has been collected from slow-moving backwater areas in rivers in a range of environmental conditions: pristine to lowland. This genus is widespread but is not commonly found in periphyton.

Possible confusion: *Zygnema* is usually easy to recognise though there may be confusion with *Spirogyra* in frozen material (see page 136) as the chloroplasts clump together in both genera.





from top:

Zygnema *growing in the Waipawa River, Hawkes Bay*

Filaments with various densities of cell contents: note the muclaginous covering to the filaments in the top two filaments. x 270

Lower photo: x 600.

Audouinella

C: branched filaments

Division: Rhodophyta, **Subclass:** Florideophycidae

How to recognise the genus:

Macroscopic: Pink–brown patches, like felt, sometimes forming a continuous, firmly attached mat, on rocky substrates.

Microscopic: Tufts of branched filaments growing from a basal holdfast. Thick cell walls and square to oblong cells, sometimes with constrictions at the cell walls. The filaments branch repeatedly with often no clear "main" filament; tips usually rounded or slightly tapering. The chloroplasts are a dull greyish green sometimes with a pink or purple tinge. There are no pyrenoids.

The most common species in New Zealand is *Audouinella hermanii.* Cells are 10–25 µm in diameter.

Habitat and distribution: *Audouinella* can be very common in clean foothills and lowland rivers, where the reddish brown mats may be visible particularly on very stable substrates like bedrock and large stones. Also found in shady forest streams. Widespread.

Possible confusion: Usually none, though note that *Batrachospermum* (page 141) goes through a young stage that resembles *Audouinella.*

from top:

Audouinella *growing amongst moss and green algae, recently dried out.*

Audouinella *from the Cass River, Canterbury. x 220 (photo: Nelson Boustead)*

Audouinella*. x 680*

Batrachospermum

C: branched filaments

Division: Rhodophyta, **Subclass:** Florideophycidae

How to recognise the genus:

Macroscopic: Red–brown to green–grey fronds growing in streaming brown jelly-like masses up to about 15 cm long. The main branches are clearly visible to the naked eye and have a beaded appearance.

Microscopic: Batrachospermum comprises a thick central filament bearing closely spaced whorls of much branched filaments. Individual cells are bulbous with rounded cells at the branch tips. The central filament is also branched.

Habitat and distribution: Clean, cool streams, often spring fed, or shady forest streams.

Possible confusion: Unlikely, though note that *Batrachospermum* goes through a young stage that resembles *Audouinella* (page 140). If you see both genera in a sample, there is a good chance that it is all *Batrachospermum*. However, without culturing the sample you cannot be certain.

from top:

Batrachospermum *sp. from a spring-fed creek, Canterbury. x 70*

A different species of Batrachospermum *sp. from the same spring-fed creek. x 135*

As above. x 270

Bulbochaete

C: branched filaments

Division: Chlorophyta, **Order:** Oedogoniales

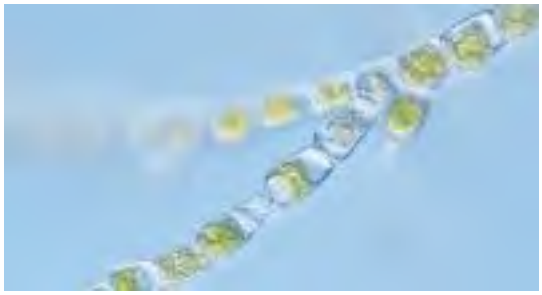
How to recognise the genus:

Macroscopic: Green filaments with no particular distinguishing features.

Microscopic: Bulbochaete has branched filaments of somewhat bulbous cells, most bearing a long hair – a "seta" (thugh these can detach). Cells are 20–30 µm across. *Bulbochaete* is closely related to *Oedogonium* (see page 135) and has a similar chloroplast – a lacy network lining the cell.

Habitat and distribution: *Bulbochaete* is encountered most often in lakes and pools, however it does occur in the periphyton of outlet streams and rivers.

Possible confusion: Usually none; a very distinctive genus.



from top:

Bulbochaete*, from a lake outlet, Canterbury. x 135*

Bulbochaete *with setae missing Note the resemblance of the cells to* Oedogonium*. x 270.*

Chaetophora

C: branched filaments

Division: Chlorophyta, **Order:** Chaetophorales

How to recognise the genus:

Macroscopic: Bright green firm jelly-like blobs up to 10 mm across, or elongated branching fronds in mucilage.

Microscopic: Chaetophora is closely related to *Stigeoclonium* and is distinguished by being enclosed in firm mucilage (hence its macroscopic appearance) and by its much denser branching. Each tuft arises from a flattened mass of cells. The chloroplast is a parietal band covering most of the cell wall.

Habitat and distribution: *Chaetophora* seems to prefer cool, clean and moderate- to fast-flowing streams. It is not often found in periphyton but may possibly be more common than recorded because of confusion with *Stigeoclonium* (see below).

Possible confusion: Maybe with *Stigeoclonium*, though growth form differs: *Chaetophora* grows in compact masses, *Stigeoclonium* in streaming tufts. The growth form of *Chaetophora* can be preserved quite well even after samples have been blended and frozen.

from top:

Chaetophora *cf.* incrassata *(branching fronds). x 70*

Chaetophora*, a type that forms firm globules. x 80*

Chroodactylon

C: branched filaments

Division: Rhodophyta; **Subclass:** Bangiophycidae

How to recognise the genus:

Microscopic: Single rows of vivid blue-green, oval cells, usually isolated from each other, within a thick, clear sheath. Branching is irregular. The chloroplast is star-shaped, though this is not always evident. .

Habitat and distribution: *Chroodactylon* is found occasionally in periphyton, and can be common within individual samples. Distribution not known.

Possible confusion: Distinctive. The genus could be mistaken for a cyanobacteria because of its colour. However, discrete chloroplasts are usually obvious.

Chroodactylon, *showing branching (top, x250) and the striking blue-green colour (bottom, x 450). (Photos: Nelson Boustead)*

Compsopogon

C: branched filaments

Division: Rhodophyta; **Subclass:** Bangiophycidae

How to recognise the genus:

Macroscopic: Forms coarse, stringy red–brown or bleached filaments, sometimes very long ("like fishing line").

Microscopic: Main filaments can be 400 µm wide or more and comprise a "cortex" (layer) of angular cells overlying a central axis of large, clear cells. Branches reduce to a single row of disc-like cells at the tips.

Habitat and distribution: Usually reported from tropical areas. In New Zealand, most records of *Compsopogon* have been from lowland rivers in the North Island.

Possible confusion: Distinctive, though it could possibly be confused with another red alga, *Bostrychia* (Subclass: Florideophycidae) which also has multi-cellular branched filaments (see photo). In *Bostrychia* the cells are arranged in longitudinal lines (i.e., a bundle of several filaments with the outer layer forming a cortex of flattened cells), clearest in young filaments. The branch tips tend to curl. In *Compsopogon*, the cells are not clearly in rows. *Bostrychia* is mainly a marine genus but one species occurs in fresh water.

Compsopogon. *Top: SEM of main stem showing the cortex of flattened, angular cells overlying rounded axial cells. x 120. Bottom: drawing showing main stem and branch.*

Bostrychia. *x 70 (photo: Nelson Boustead)*

Cladophora

C: branched filaments

Division: Chlorophyta, **Order:** Cladophorales

How to recognise the genus:

Macroscopic: Deep or dull green to brown, tough filaments in mats or masses; individual filaments sometimes thick, stringy and khaki brown because of epiphytes.

Microscopic: Large branched filaments with very long cells. Branches originate from cell cross walls and are usually smaller than the main stem. Filaments taper slightly towards rounded ends. The cell contents appear granular – a combination of the lace-like chloroplast lining the inside of the cell walls (often appearing as many small chloroplasts) and numerous pyrenoids.

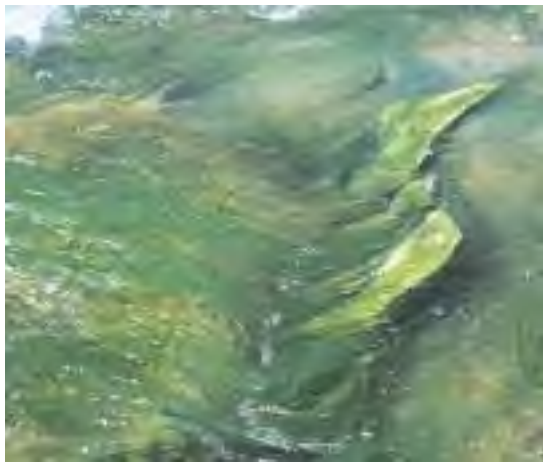
There is no mucilaginous layer on the outside of the cell walls, accounting for the coarse feel and appearance of *Cladophora*, and the ease of colonisation of the filaments by epiphytes – mainly diatoms.

In frozen material the cell walls may collapse and become distorted.

In New Zealand, *Cladophora glomerata* has been identified as the most common species. Cells on the main axes may be up to 75 – 100 µm across, on branches they are about half that size and also shorter.

Habitat and distribution: *Cladophora* is often found in high-conductivity streams and rivers and can form large blooms in summer and autumn under stable flow conditions. The taxon is widespread in soft-rock areas of North Island (naturally high conductivity) and occurs elsewhere in response to other sources of enrichment.

Possible confusion: *Rhizoclonium* (see page 134). *Rhizoclonium* has large, long, cylindrical cells, very occasionally branched, but not with the tree-like pattern in *Cladophora*. There is a slight chance that *Cladophora* filaments could be confused with *Vaucheria*, which lacks cross walls (see page 147).



from top:

Cladophora, *Wairoa River, Hawkes Bay.*

Cladophora *sp.: top: x 70; bottom: x 270. Note the pattern of branching.*

Cladophora glomerata*, with epiphytes (diatoms). x 270*

Draparnaldia

C: branched filaments

Division: Chlorophyta, **Order:** Chaetophorales

How to recognise the genus:

Macroscopic: Light green, soft masses, up to about 5 mm across, slimy because of copious mucilage.

Microscopic: Draparnaldia is closely related to *Stigeoclonium* and *Chaetophora*. It is distinguished by the thick central filament that carries densely branching tufts of filaments whose ends taper to fine hairs. The large central cells have a single parietal chloroplast that extends part way around the cell and is much narrower than the cell. The central stem is up to 100 µm across. Branch cells are up to 10 µm across, and longer than they are wide.

Habitat and distribution: *Draparnaldia* occurs mostly in clean, cool streams, often spring-fed. Distribution is uncertain.

Possible confusion: Usually no problem identifying this genus.

from top:

Draparnaldia *from a spring-fed stream, Canterbury. x 80*

Note the very wide main branches. x 200

Stigeoclonium

C: branched filaments

Class: Chlorophyceae, **Order:** Chaetophorales

How to recognise the genus:

Macroscopic: Bright green tufts growing on stony substrates, often dotted amongst growths of brown algae (diatoms).

Microscopic: Branched alga with small, squarish to elongated cells, sometimes inflated at the centre and constricted at the cross walls. The cross-walls are straight. Within the cells, the chloroplast partly lines the inside of the cell wall. Each tuft is attached to the stream substrate by a holdfast structure of similar cells in a sheet-like arrangement. Branching varies among species and can be quite sparse. The filaments taper either to a point or to a more blunt end. Cell diameter is typically up to about 15 µm for main axes and less for the branches.

Stigeoclonium does not change greatly in overall appearance following freezing. The branching habit and tapering filaments of this genus make it easy to recognise, fresh or frozen.

Habitat and distribution:

Stigeocloneum seems to prefer moderate- to fast-flowing streams in a range of environments, from clean to enriched waters. The genus is common and widespread and may dominate the periphyton.

Possible confusion:

In very sparsely branched specimens the filaments may be mistaken for *Ulothrix*, because of the similar arrangement of the chloroplast. If you identify a doubtful *Ulothrix*, check along the filaments to see if there is any branching. Also watch out for confusion with *Chaetophora*, which forms firm mucilaginous clumps (see page 143).

from top:

Tuft of Stigeoclonium *with diatoms.*

Stigeoclonium *from the Hokitika River, West Coast. x 70.*

Stigeoclonium *from the Cust River, Canterbury. x 270*

Vaucheria

C: branched filaments

Division: Xanthophyta, **Order:** Vaucheriales

How to recognise the genus:

Macroscopic: Dark green round or oval "cushions" – up to about 20 cm across – attached to stones or other stable substrate. When squeezed free of water the cushion has a tough "woolly" feel.

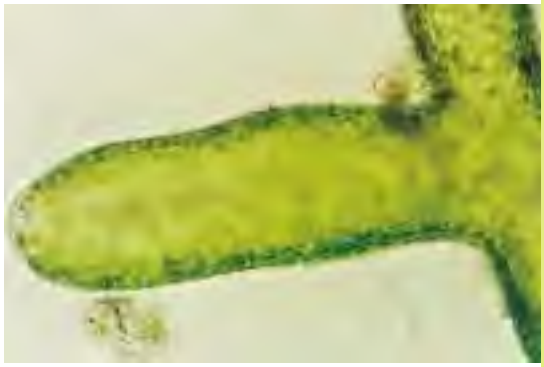
Microscopic: Large branched filaments with rounded tips and no cell cross walls. Multiple small, disc-like chloroplasts line the cell wall. Diatom epiphytes are often present.

In frozen material, the cell walls collapse and become distorted and the chloroplasts clump together.

The filaments are up to 150 µm in diameter and decrease in size with branching. Note that branching can be infrequent and the clearest diagnostic feature is the lack of cross walls.

Habitat and distribution: Found in a wide range of conditions, from clean, cool headwater and spring-fed streams, to more enriched lowland locations.

Possible confusion: The macroscopic appearance of *Vaucheria* is very characteristic. It is possible that isolated filaments under the microscope could be mistaken for *Cladophora*. Therefore in cells with apparently multiple small chloroplasts, you should always check for the presence of cross walls.



from top:

Vaucheria *growing in tufts.*

Vaucheria *from the Avon River, Christchurch. x 70*

Showing branching, x 135

Showing many small chloroplasts. x 270

Aulacoseira

C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta **Order:** Centrales

How to recognise the genus:

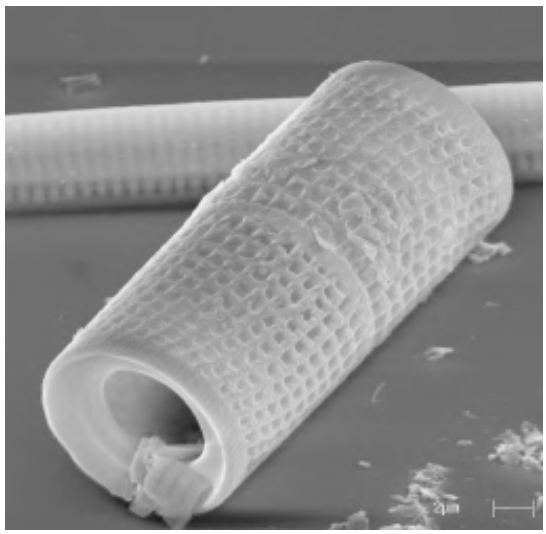
Microscopic: Aulacoseira forms distinctive short filaments in which the ornamentation of the valves is often visible even under relatively low power (e.g., x 400). Several irregular chloroplasts.

Possible confusion: Usually none.

Aulacoseira species

The most common species of *Aulacoseira* in New Zealand is probably *A. granulata* (and its varieties). The species is is characterised by widely spaced long spines linking successive valves. These cannot be seen easily in live material. The valves range from 5 to 25 µm wide and can be short and fat or very elongated (*A. granulata* var. *angustissima*). The specimens illustrated are tentatively identified as this species.

Habitat and distribution: *Aulacoseira* species are most often found in lakes as part of the phytoplankton. However they may appear occasionally in the periphyton of lake-fed streams and rivers.



Note: The old genus name *Aulacoseira* has recently been resurrected to include several species previously placed in *Melosira*, but separated because of their very different cell wall structure (see Round *et al.* 1990).

from top:

Aulacoseira *filament, Tarawera River. x 270*

Cleaned specimen, Waiau River. x 680

Aulacoseira *sp. SEM. Cells separated at the girdle.*

Diatoma

C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta, **Order:** Fragilariales

How to recognise the genus:

Macroscopic: Brown shiny, slimy covering on rocks, though often *Diatoma* species are mixed with other algae – both diatoms and green algae.

Microscopic: Diatoma species form ribbon-like filaments or zig-zag or star-like colonies. They are also seen as solitary cells. Each cell contains several chloroplasts. In cleaned material *Diatoma* cells lie in either valve or girdle view. Thick ribs of silica across the valve face ("costae") are visible as dark lines (valve view) or knobs at the sides (girdle view).

Possible confusion: The two *Diatoma* species described below are fairly distinctive.

Diatoma hiemale var. *mesodon*

Short ribbon-like filaments of square cells each containing several irregular, brown-coloured chloroplasts; sometimes seen from the top. Often seen as single cells. Two or 3 ribs across the valve face. Cells 15–20 µm long and 5–12 µm across (valve view).

Habitat and distribution: Common and widespread; "occasional" or less in many periphyton samples. This species often dominates the periphyton in cold, clean headwater streams.

Diatoma tenuis

Solitary or in zig-zag colonies; cells elongated. In valve view, the two ends may bulge out slightly (sometimes in different directions). In girdle view you see a rectangle with dark dots down each long edge. Cells are typically 15–30 µm long and up to 4 µm wide.

Habitat and distribution: *D. tenuis* has been identified mainly from the periphyton of southern South Island rivers, from the lowlands to the headwaters. This species has been recorded as dominant in the periphyton, e.g., in some locations on the Waiau River, Southland, in summer 2000.

from top:

Filaments of D. hiemale *growing in a backwater of a pristine mountain stream. (The green is* Spirogyra*.)*

Live Diatoma hiemale *chains. x 450*

Diatoma hiemale *valve and girdle views. x 1000*

Joined cells of Diatoma tenuis*, girdle view, plus valve view (right). x 1200*

Eunotia

C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta, **Order:** Eunotiales

How to recognise the genus:

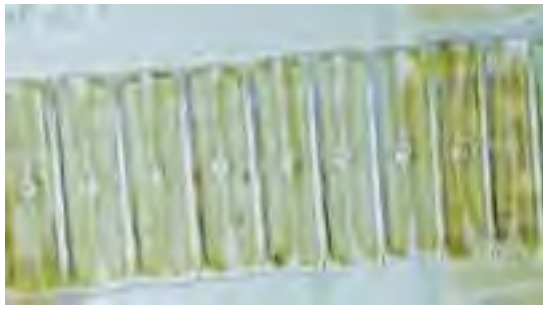
Microscopic: In valve view *Eunotia* species are mostly variations on a crescent shape; the girdle view is rectangular, sometimes rhomboidal. Each part of the raphe system is very short. Often all four raphe ends may be seen in girdle view. Some species of *Eunotia* form ribbon-like filaments in which they lie in girdle view. There are two sheet-like chloroplasts in each cell. Species range in size from 10 µm up to 150 µm long.

Possible confusion: Single cells of *Eunotia* are usually recognisable, especially if lying in valve view. In filaments, there could be confusion with *Fragilaria.* Look for single cells in valve view that are likely to be the same species as the filaments (from their size, etc). In wet samples you can agitate the sample to get cells to turn over. Check the chloroplasts: *Fragilaria* has irregular plates; *Eunotia* usually has two larger sheets per cell. Under high power LM, focus through to find the raphe system in *Eunotia.*

Eunotia species

Identification of many *Eunotia* species is difficult without access to a good microscope and to cleaning and mounting facilities. Therefore we do not provide any individual descriptions. For characterising periphyton it is more important to be able to distinguish between filaments of *Fragilaria* and *Eunotia.*

Habitat and distribution: *Eunotia* species tend to be very common in low-conductivity, acid still waters, e.g., in lakes and upland bogs and tarns. The genus can also be an important part of stream periphyton, especially as filaments in relatively clean, spring-fed lowland streams, or mountain-fed streams, e.g. *Eunotia* cf. *serpentina* (illustrated).



from top:

Eunotia *filaments from a spring-fed stream, Canterbury. Note the chloroplasts. This is probably* Eunotia serpentina*, which has undulating valve margins when seen in valve view, discernible here as faint shadows running along the filament. x 375*

Girdle view of a single valve of Eunotia *cf.* serpentina*. x 800*

Valve view, Eunotia serpentina*. x 800*

Valve view of Eunotia cf. incisa *(from a small stream, Stewart Island). x 2000*

SEM showing structure of valve of Eunotia*. Note short raphes extending over the edge of the valve face.*

Fragilaria

C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta, **Order:** Fragilariales

How to recognise the genus:

Microscopic: Fragilaria species form ribbon-like colonies in which cells lie in girdle view. Some species are commonly seen as single cells. An elongated chloroplasts lies against the face of each valve. Currently, the genus *Fragilaria* is reserved for species that have certain structural features that cannot usually be seen under LM (including, in many species, spines around the margins of the valve face, which allow them to form chains).

Possible confusion: Single cells of some *Fragilaria* species cannot be distinguished from *Synedra* under LM. Some *Synedra* species also have spines around the valve edge and form chains (page 187). *Fragilaria* filaments could be confused with *Eunotia* (see page 150).

Fragilaria vaucheriae

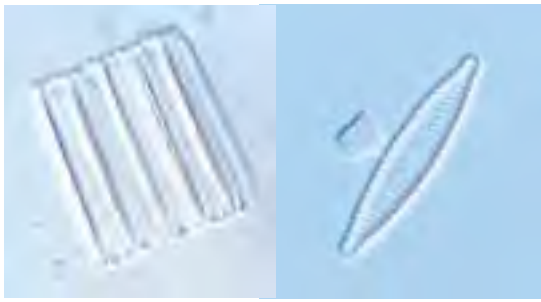
Small spindle-shaped cells, sometimes with capitate ends, rectangular in girdle view. Often seen as single cells but also in filaments. The species is distinguished by the presence of a slightly swollen area, without striae, to one side of the valve centre. There are two narrow chloroplasts. Up to 30 µm long and 6 µm wide.

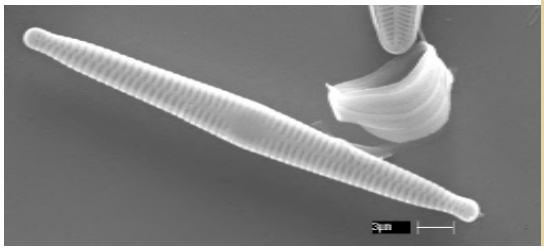
Habitat and distribution: Very common; seems to occur in a wide range of conditions.

Fragilaria capucina

Pencil-shaped cells generally longer than *F. vaucheriae* (up to over 100 µm long, but often only 25 µm). Central area spans the whole width of the valve, clear axial area. Fine striae usually, though some varieties have a coarser arrangement with striae clearly alternating on either side of the valve (see photo).

Habitat and distribution: Probably wide-spread. When not in chains, some varieties of this species may be identified as *Synedra rumpens* (see page 188).





from top:

Fragilaria filaments growing over a thick diatom mat.

Filament of Fragilaria vaucheriae*. Fresh material. x 500.*

Fragilaria vaucheriae*, cleaned frustules, valve view (left), girdle view (right). x 2000*

Fragilaria capucina (left); Fragilaria capucina *var.* distans (right – *note the widely-spaced striae). x 2000*

SEM , Fragilaria capucina*. Note the small spines at the valve margin.*

Fragilariforma

C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta, **Order:** Fragilariales

How to recognise the genus:

Microscopic: Fragilariforma is a new genus based on *Fragilaria viriscens*. It is distinguished from *Fragilaria* by the type of puncta and its very narrow pseudoraphe, i.e., striae almost span the whole valve width. Other diagnostic features are visible only under SEM. *Fragilariforma* species form ribbon-like colonies in which they lie in girdle view.

Possible confusion: With other *Fragilaria* species and also with *Eunotia* species in chains (see page 150). Always try to find a valve view in the sample.

Fragilariforma viriscens

Small elongated cells (10–20 µm long by about 10 µm wide) usually seen as filaments. The cells have fine striae, a very narrow axial area and no central area.

Habitat and distribution: Occasionally found in South Island periphyton samples. Reported to prefer low-conductivity spring and stream waters (Cox 1996).

from top:

Fragilariforma cf. viriscens*: live filament. x 1000*

Cleaned material, valve and girdle views; from a South Island West Coast stream. x 850.

Note on the re-classification of *Fragilaria*

The genus *Fragilaria* has recently been downsized by splitting off newly established genera including *Fragilariforma*. The other new genera represented in this guide include species that are most often seen as single cells, though they may also form short filaments. They are covered on pages 184 (*Staurosira*, based on *Fragilaria construens*) and 185 (*Staurosirella*, based on *Fragilaria lapponica*).

Pseudostaurosira (based on *Fragilaria brevistriata*) is also seen occasionally (see photo, right)

See Williams and Round (1987, 1988) for a full account of the new genera.

Pseudostaurosira brevistriata *valve view and short chain, Okuku River, Canterbury. x 1300*

Melosira

C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta, **Order:** Centrales

How to recognise the genus:

Macroscopic: Medium brown fluffy masses, though often *Melosira* is mixed with other algae – both diatoms and green algae. Can sometimes form whitish to iridescent green mats as a monoculture.

Microscopic: Cylindrical filaments with cells approximately as long as they are wide. There is no ornamentation on the cell walls either in valve or girdle view. Several to many irregular, brown-coloured, chloroplasts line the cell walls. Sometimes the chloroplasts fit together in a neat jigsaw pattern. Cells are loosely joined and the filaments break up easily into single cells, especially following blending. It is common to see *Melosira* only as single cells unless the sample is dominated by the genus.

Possible confusion: *Aulacoseira* (page 148) forms similar chains but the valves have noticeable ornamentation. If Melosira is not recognised as a diatom filament, the species may be confused with a range of non-diatom filaments.

Melosira varians

This is the only *Melosira* species known in New Zealand. Cleaned material viewed under high power shows barrel-shaped frustules with little ornamentation. Easy to identify. *M. varians* ranges from 10 to 40 µm in diameter.

Habitat and distribution: *Melosira varians* is found throughout the country in slow to medium flowing open lowland streams. It can dominate the periphyton community in moderately enriched situations. It is reported as both a "cleanwater species" and "moderately polluted water species" in Cassie (1989).

Note: Other species of *Melosira* listed in some texts have been transferred to the genus *Aulacoseira*, notably *A. granulata* (see page 148).

from top:

Masses of Melosira varians *growing on a stream bed.*

Melosira varians*-dominated periphyton community, photographed following storage (freezing). x 400*

Healthy Melosira varians *filament showing chloroplasts in two planes of focus. x 480*

Tabellaria

C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta, **Order:** Tabellariales

How to recognise the genus:

Microscopic: *Tabellaria* species form zig-zag or straight filaments and are also often seen as single cells. The cells usually fall in girdle view – oblong to square with prominent dark projections running towards the centre of the cell ("septa" – sheets of silica partly filling in the girdle bands). Valve views – narrow with swollen ends and centre – are usually seen in cleaned preparations where frustules have broken apart into the two valves and several girdle bands. This is an araphid genus. There are several small chloroplasts throughout the cell.

Possible confusion: Unlikely.

Tabellaria flocculosa

Tabellaria flocculosa forms zig-zag chains of valves in girdle view. Each valve is square to oblong in girdle view and usually about 10–40 µm wide by 30–100 µm long. There are several (up to 7 or 8) irregular looking septa at each side, usually with short rudimentary septa opposite. In valve view there is a prominent central bulge and expanded ends.

Habitat and distribution: This is the only *Tabellaria* species found widely in New Zealand periphyton. Although widespread, it is not often common in stream periphyton communities. It seems to do best in low-conductivity, clean waters. (see Cassie (1989), p. 18). We have found the elongated form illustrated mostly in clean, high-altitude outlets from tarns.

Note: The elongated *Tabellaria* species illustrated is most likely a form of *Tabellaria flocculosa*. A widespread species that has the same shape in girdle view is *Tabellaria fenestrata*. However, in *T. fenestrata* there are usually four septa only and no rudimentary septa. Some the the cells shown here appear to have both rudimentary septa and sometimes more than four full-sized septa.

from top:

Chains of Tabellaria flocculosa*, typical cells, more or less square (top), and elongated oblong cells (bottom). x 350*

Tabellaria flocculosa*, valve views. Left: typical form. x 1500. Right: elongated form. x 700.*

The following two genera form "filaments" in mucilage tubes in which the cells are not joined into true filaments. Both genera are frequently seen in single-celled form, and are described in more detail under C: single cells (diatoms)

Encyonema

C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta, **Order:** Cymbellales

How to recognise the genus:

Microscopic: See page 166 for a full description. Some species of *Encyonema* typically form long "filaments" of somewhat irregularly arranged frustules in mucilage tubes.

Possible confusion: Single cells of *Encyonema* are easily confused with *Cymbella*, though the latter tend to grow on stalks (like *Gomphonema* or *Gomphoneis*) and do not occur in mucilage tubes.

Encyonema caespitosa *packed into a mucilage tube, forming a filament. x 400*

Frustulia

C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales

How to recognise the genus:

Microscopic: See page 169 for a full description. *Frustulia* may occur scattered in long mucilage tubes, but is normally encountered as single cells.

Possible confusion: Single cells of *Frustulia* may be confused with other naviculoid diatoms but this is the only naviculoid genus you will find in mucilage tubes.

Frustulia *cells in a mucilage tube. x400*

Achnanthes

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Achnanthales

How to recognise the genus:

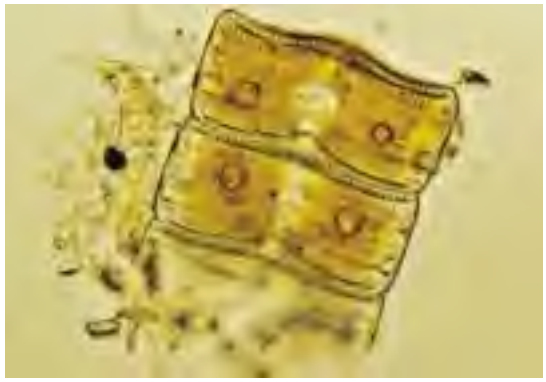
Microscopic: This is a monoraphid genus. In girdle view the valve is slightly bent into a V-shape with the raphe valve on the inside of the V (i.e., concave). The raphe and pseudoraphe are somewhat off-centre and the areolae are large and clearly seen at 1000 x.

Possible confusion: Unlikely. Most representatives of this genus are marine, and the freshwater species are large and distinctive.

Achnanthes inflata

Microscopic: The only common representative of *Achnanthes* in periphyton in New Zealand is *A. inflata*, which is recognised from its large size (up to 80 µm long) and distinctive shape.

Habitat and distribution: Normally found mainly in clean, cool, shaded streams and never in great numbers. This species seems to be able to withstand some salt-water intrusion (Patrick and Reimer 1966) and has been reported as common in an Otago estuary (Steven Moore, Otago Regional Council, pers. comm.)



from top:

Achnanthes *cf.* inflata*, valve view (raphless valve). x 1000*

Achnanthes *cf.* inflata*, live cells in girdle view. x 1000*

A note on the re-classification of *Achnanthes*

Until recently many monraphid diatom species with a "bent" valve were included in *Achnanthes*, but many freshwater species have now been transferred to the genus *Achnanthidium* [and subsequently to other genera, see Ross *et al.* 1990] (see page 157). The genus *Achnanthes* is currently reserved for species with a particular type of areola and raphe structure. The chloroplast is also different.

Achnanthidium

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Achnanthales

How to recognise the genus:

Macroscopic: Achnanthidium is usually part of a mixed community of periphyton. When dominant these diatoms form a dense, dark brown slimy layer.

Microscopic: A monoraphid genus. Most *Achnanthidium* species are very small and live attached by mucilage to substrates or aquatic plants. The valve is bent so that in girdle view they are slightly V-shaped. In valve view they are oval, elongated or captitate (i.e., with a "head" at the ends). In many species the raphe and pseudoraphe valves differ in their ornamentation. There is one chloroplast.

Possible confusion: Other small diatoms. Look for the V-shape in girdle view.

Achnanthidium minutissimum

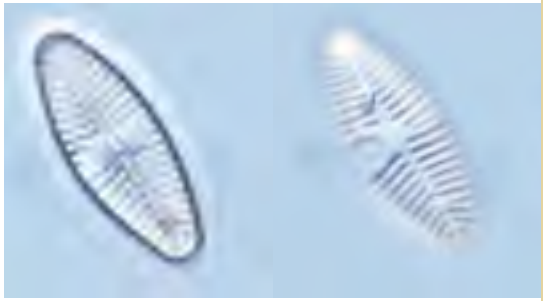
A very small species (5–25 µm long), narrow with slightly pinched ends. In girdle view the V is flattened out a little at each end.

Habitat and distribution: Common and widespread in a range of ecological conditions but does best in clean, low-conductivity streams. The species often dominates the periphyton following flooding, growing in dense masses attached to substrates. In more stable conditions other species take over.

Achnanthidium lanceolatum (now transferred to *Planothidium lanceolatum*, see note page 158)

Planothidium lanceolatum has an obvious "empty area" on the pseudoraphe valve on one side of the central area, formed by a flap of silica. The cells can be relatively large (up to 35 µm long) compared with other species in this genus. Each stria is made up of more than one row of puncta (visible only under SEM).

Habitat and distribution: *P. lanceolatum* is widespread and occasionally abundant. It occurs in the periphyton of a wide range of river and stream types, is tolerant of moderate levels of organic pollution and is reported to favour alkaline waters (e.g., see Round and Bukhtiyarova 1996).



from top:

Dense brown coating of diatoms on a river stone. Achnanthidium minutissimum *dominated this community from the Selwyn River, Canterbury.*

Achnanthidium minutissimum, *valve and girdle views (g.v. in centre). x 1400*

Planothidium lanceolatum, *showing the raphe valve (left) and rapheless valve (right). Note the silica "flap". x 1100*

Achnanthidium linearis (now transferred to *Rossithidium linearis*, see note below)

A very small diatom (10–20 µm long), elongated oval in shape and often seen in girdle view as a straight-sided shallow "V". Striae are quite close together and usually parallel, with no break at the centre except sometimes a slight gap on the raphe valve.

Habitat and distribution: Common, widespread and seems to tolerate a wide range of conditions.

The following related species have yet to be formally transferred to an appropriate genus.

Achnanthes oblongella (= *A. saxonica* in Foged 1979)

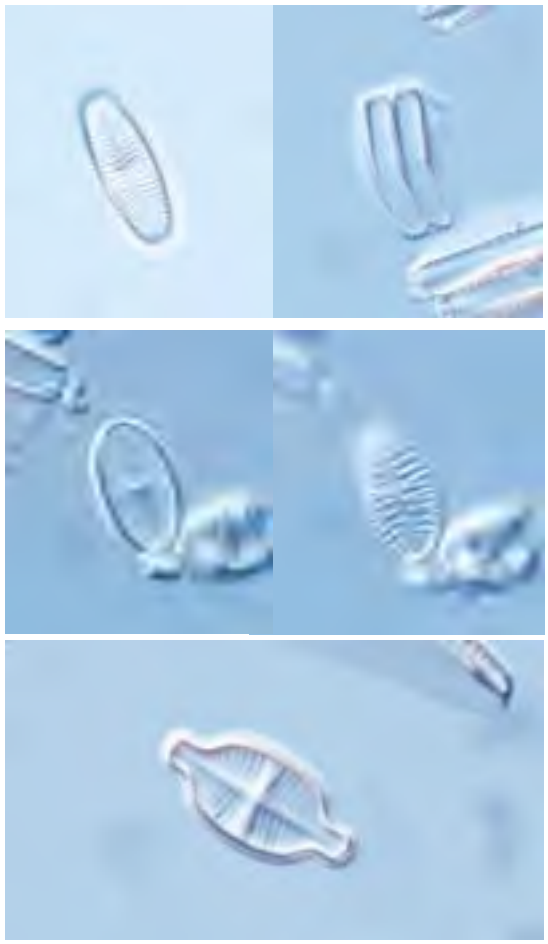
Another small species, 10–20 µm long. The raphless valve has quite prominent and slightly irregular striae.

Habitat and distribution: Widespread but not usually common.

Achnanthes exigua

Small, 10–20 µm long, with noticeably pinched ends.

Habitat and distribution: Not very common. Habitat unknown.



from top:

Rossithidium linearis*, valve view (left) and girdle view (right). x 1400*

Achnanthes oblongella*, raphe valve (left) and raphless valve (right). x 1400*

Achnanthes exigua*, rapheless valve. x 1400*

A note on the re-classification of *Achnanthidium*

Some species included in *Achnanthidium* have been re-assigned to new genera, proposed by Round and Bukhtiyarova (1996) on the basis that the original genus lacked any unifying diagnostic characters except for the "bent" frustule and for being monoraphid. The genera commonly found in the New Zealand periphyton are represented by the first three species described above:

Achnanthidium (e.g., *A. minutissima*)

Planothidium (e.g., *A. lanceolata*)

Rossithidium (e.g., *A. linearis*)

Actinella

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Eunotiales

How to recognise the genus

Microscopic: Elongated cells expanded at one end, and living attached to plants or substrate by the other (narrow) end. Some species are wedge-shaped in girdle view (like *Gomphonema).* Closely related to *Eunotia, Actinella* species also usually have an inconspicuous raphe system that lies to one side of the valve.

Possible confusion: *Actinella* species are often very distinctive, however small forms that are wedge-shaped in girdle view could easily be mistaken for *Gomphonema* under low power LM. Check for raphe endings at the centre of the valve in *Gomphonema* (visible as a slightly thickened area in the middle of each long side in girdle view, see page 171).

Actinella species

Until recently *Actinella* had been rarely reported in New Zealand. The genus has now been found in several locations and these finds include new species that are still being described.

Habitat and distribution: *Actinella* seems to be confined to clean, acid habitats. It can be extremely common locally. Distribution not certain. Most recent findings have been in the South Island and Stewart Island.

Actinella *sp. valve and girdle views. From the outlet stream of a high altitude tarn, South Island. x 1400*

Asterionella

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Eunotiales

How to recognise the genus

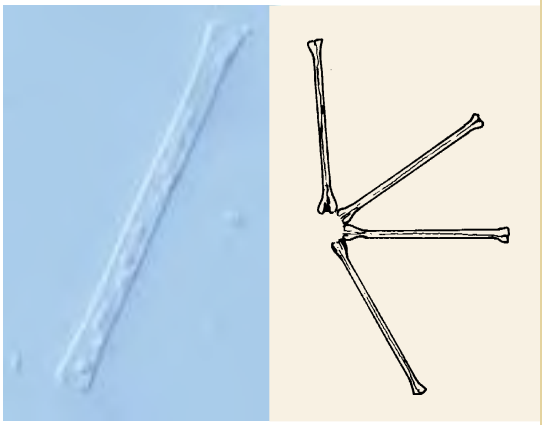
Microscopic: An araphid genus with long thin cells inflated at one or both ends, often forming star-shaped colonies.

Possible confusion: Should be no problem recognising this genus.

Asterionella formosa

Long cells, inflated at both ends, more at one end than the other. Often seen joined at one end to make a star-shape.

Habitat and distribution: *Asterionella formosa* is most common in lake plankton, but can be common in lake-fed rivers and streams.



Asterionella formosa*. Left: single cell from periphyton (Waiau River). x 600. Right: drawing of part of star-shaped colony.*

Amphora

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Thalassiophysales

How to recognise the genus:

Microscopic: An asymmetrical biraphid diatom. *Amphora* species in valve view are roughly crescent-moon-shaped, like *Cymbella*. However, because the girdle band is much wider on the convex side of the valve than on the concave side, the two valves are usually visible side-by-side, i.e., the girdle and valve views are the same. Single valves are seen when the frustule has broken apart. There is an H-shaped chloroplast. In many *Amphora* species, the ornamentations on the valve are too fine to be seen except under very high power.

Possible confusion: The valve views of *Amphora* and *Cymbella / Encyonema* are similar in shape: *Amphora* tends to be finer. In *Cymbella* and *Encyonema* the girdle may be also wider at the top (curved) side of the valve than at the bottom so that in girdle view, both valve faces are visible obliquely. You'll need to look for cells in valve view to be sure of your identification. Also note that *Eunophora* (see photo below) looks superficially like *Amphora*, but is actually closely related to *Eunotia*. *Eunophora* (a recently described genus, see Vyverman *et al.* 1998) is common in pristine, high-altitude lakes but is rarely found in streams and therefore is not described separately in this manual.

Amphora veneta var. *capitata*

A very small, fine diatom in which the valve markings are difficult to see. Size: 20–40 µm long and up to 15 µm wide (whole valve).

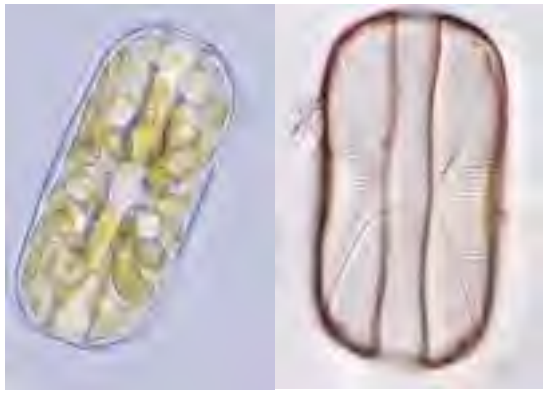
Habitat and distribution: *A. veneta* has been reported as common in a moderately enriched North Island stream and has occurred occasionally elsewhere. It is reported (Cox 1996) to occur in enriched waters and to be pollution tolerant.

Amphora ovalis

Much larger, 30–100 µm long and up to 50 µm wide.

Habitat and distribution: Not seen very often to date. We have found some in the Waiau River. This species is reported to be widespread in medium conductivity waters overseas.

Compare with *Eunophora*:



from top:

Amphora veneta *var.* capitata, *single valve (left) and whole frustule. Cleaned material, from the Waitekauri River, Coromandel. x 1000*

Amphora ovalis, *from the Waiau River, Southland. x 1000*

Eunophora *sp. (Order: Eunotiales). As in* Amphora *the valves lie side by side; however the genus has four to many chloroplasts and features in common with* Eunotia. *Left: live cell , x 700 ; right: cleaned frustule x 1000*

Brachysira

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales

How to recognise the genus:

Microscopic: A symmetrical biraphid diatom. *Brachysira* species are naviculoid in valve view and more or less rectangular in girdle view. Instead of the regular radial striae seen in *Navicula* species, the valve decorations are more irregular, generally appearing as dashes that line up to form wavy lines down the valve face. There is single, lobed chloroplast.

Possible confusion: The shape is similar to that of *Navicula* (see page 174). However, in the latter, the paired chloroplasts should be distinctive. Under medium to high power (over 400x) it should be possible to see clearly the irregular markings on cleaned frustules of *Brachysira*.

Brachysira vitrea (= *Anomoeoneis vitrea*)

Slender naviculoid shape with pinched ends, 15-40 µm long and about 5 µm wide. Striations not clear on the valve face, even at high power.

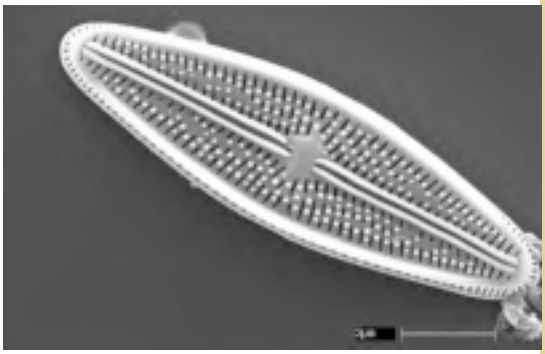
Habitat and distribution: Widespread mostly in low to medium conductivity waters. Most commonly found in lakes and tarns, but has has been found in periphyton samples from lake-fed streams and rivers, e.g., it is quite common in the Waiau River in Southland.

Brachysira serians (= *Anomoeoneis serians*)

Cells larger: 50–100 µm long and 10–20 µm wide. Pronounced naviculoid shape. Variety *acuta* is smaller and has drawn out ends. Identification not certain.

Habitat and distribution: Less often seen in periphyton. More likely to occur in the bottom sediments of clean, acid-water lakes and tarns.

Note: Until recently, *Brachysira* species were included in the genus *Anomoeoneis*. Species remaining in the latter genus are typically found in high conductivity waters. *A. sphaerophora* has been recorded in New Zealand but has not been noted in any of our periphyton samples.



from top:

Brachysira vitrea*, from the Waiau River. x 1400*

Brachysira *cf* serians *var. acuta, live specimen showing the form of the chloroplast (compare with* Navicula*); also note the large oil droplets (food storage). x 1000*

Brachysira *sp. (unknown), Waiau River. x 1400*

*SEM (*Brachysira *sp.) showing markings on the valve face.*

Cocconeis

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Achnanthales

How to recognise the genus:

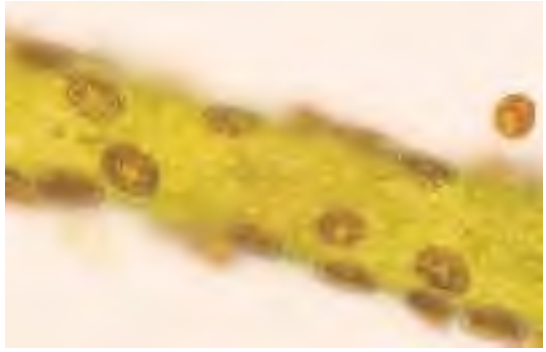
Microscopic: A monoraphid diatom. *Cocconeis* species are oval in shape and virtually always seen in valve view. Commonly epiphytic. There is a single flattened or lobed chloroplast, and the puncta – especially around the edge of the frustule – are often clearly visible. Examining cleaned material under high power (x 1000) should allow you to see both the raphe and rapheless valves, which can be quite different.

Possible confusion: Usually none

Cocconeis placentula

A oval-shaped diatom that varies considerably in size – from 10 to 90 µm long and 8 to 40 µm wide. Look for the distinctive ridge around the perimeter of the raphe valve. The ornamentation of the raphless valve varies in different varieties.

Habitat and distribution: *C. placentula* is common and widespread, occurring in a range of conditions from clean to moderately enriched to very enriched waters. This species sometimes dominates the periphyton following high flows because its flattened shape allows it to withstand turbulent conditions. Found on stones or growing as an epiphyte on wood, filamentous algae or vascular plants.



Cocconeis pediculus

C. pediculus differs from *C. placentula* in that it is distinctly curved (so that it is like a bowl) and lacks an extra ring around the outer edge of the raphe valve. Its size range is smaller: 10 to about 30 µm long and up to 20 µm wide.

Habitat and distribution: Grows as an epiphyte on filamentous algae (e.g., *Cladophora*) or on vascular plants. Tends to occur in high conductivity waters. Not as widespread or as common as *C. placentula.* All of our examples have come from periphyton from North Island rivers.

from top:

A filament of the green alga Cladophora *colonised by* Cocconeis *as an epiphyte. x 300*

Cocconeis placentula*, raphe valve (left), rapheless valve (right). x 1200*

Cocconeis *cf.* pediculus*. x 1200*

Cyclotella

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Thalassiosirales

How to recognise the genus:

Microscopic: This is a centric diatom. In valve view it is round with radial striae around the edge that differ from the pattern in the middle. The girdle view is rectangular (with rounded corners). There are numerous small chloroplasts.

Possible confusion: Many small centric diatoms are difficult to identify. However, the common *Cyclotella* species (below) is one of just a few that turn up regularly in periphyton samples, and it is fairly easy to recognise.

Cyclotella cf. *stelligera*

Microscopic: Cyclotella stelligera is typically 5–40 µm in diameter and circular in valve view, with radial striae around the margin and a few raised bumps in the centre – the overall effect a bit like a daisy. In girdle view the cells are rectangular with rounded edges.

Habitat and distribution: *Cyclotella* is normally planktonic, in lakes. *C. stelligera* is seen occasionally in the periphyton of lake-fed streams and rivers.

from top:

Cyclotella stelligera *in two planes of focus. x 1200*

Cyclotella *cf.* meneghiniana*, another species found in habitats similar to those of* C. stelligera*. x 1400*

Cyclostephanos

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Thalassiosirales

How to recognise the genus:

Microscopic: Another centric diatom. It differs from *Cyclotella* (above) in having a pattern of radiating markings on the valve face that is continuous between the edge and centre, though you'll need to view cleaned specimens in different planes of focus. Other ultra-structural details separate the two genera including the presence of spines in *Cyclostephanos*.

Possible confusion: Other centric diatoms (see above); however this genus is clearly distinguishable from *Cyclotella* (above).

Cyclostephanos *sp. in two planes of focus; note that the striae are continuous from the edge to the centre of the valve but the valve is not flat. Common in the Moawhango River, North Island. x 1200*

Cymbella

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Cymbellales

How to recognise the genus:

Macroscopic: Cymbella kappii can accumulate as mats of light brown-green streaky slime.

Microscopic: An biraphid diatom that is asymmetric across the valve. Most species are more or less half-moon shaped in valve view. Some grow on mucilage stalks.

Possible confusion: *Cymbella* resembles *Encyonema* (page 166), and the differences are obvious only under high power (1000 x). In *Cymbella* the raphe ends turn upwards at their outer ends and are deflected downwards in the middle of the valve. In *Encyonema*, it is the other way around. Features of the chloroplast are also reversed in the two genera.
Other half-moon shaped genera include *Epithemia* (page 168) and *Amphora* (page 160). These are fairly easy to separate from *Cymbella*.

Cymbella kappii

Cymbella kappii is typically 25–35 µm long and about 10 µm wide. Because this species is so common in New Zealand periphyton, you can be reasonably confident from the shape alone that the identification is correct. If in doubt, check that there are two stigmata (isolated holes) in the central area, below the raphe endings (see photo).

Habitat and distribution: Very widespread in New Zealand and has been recorded as dominant or abundant in several lowland streams and rivers.

Cymbella aspera

C. aspera is a large, easily recognised diatom, typically 70 to 150 µm long and 20 to 45 µm wide. The striae and puncta are large enough to distinguish and count easily. There are no stigmata in the central area of the valve.

Habitat and distribution: This species is seen in the periphyton fairly often, and can be abundant in low-conductivity streams. Widespread.

from top:

Cymbella *slime in the Wangapeka River, near Nelson*

left: Cymbella kappii. *x 1000; right:* Cymbella *sp., showing girdle view. x 800*

Cymbella aspera: *live specimen showing chloroplast.Note the "fold" at the dorsal (top) side of the cell. In* Encyonema*, this is at the bottom. x 350*

Cymbella aspera, *cleaned specimen. Note lack of stigmata. x 350*

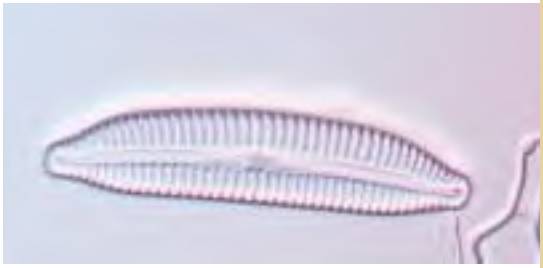
Cymbella tumida

A larger, chunkier diatom than *C. kappii*, typically 40–80 µm long and 15–20 µm wide. Slightly bulbous ends; striae radiating from a round central area with a single stigma.

Habitat and distribution: *Cymbella tumida* seems to occur in rivers with moderate to high conductivity. It has been noted as common in some North Island samples.

Other *Cymbella* species

Stream periphyton may contain various other species of *Cymbella*. To distinguish these from the three common species described, examine cleaned material under high power and look for: overall shape and size, density and direction of the striae, shape and size of the central area and number of stigmata present.



from top:

Cymbella tumida*, from the Maowhango River, North Island. x 1200*

Cymbella cistula *var* gracilis*. Note 4 stigmata. x 700*

Cymbella *sp. (unknown). x 1000*

Diatomella

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales

How to recognise the genus:

Microscopic: A symmetric biraphid genus with noticeable "septa" present, each a plate with three large holes. In girdle view the solid sept show up as short thick lines.

Possible confusion: A distinctive genus.

Diatomella balfouriana
Diatomella parva

Diatomella balfouriana is up to 30 µm long; *D. parva* around 10 µm.

Habitat and distribution: Possibly confined to clean mountain-fed streams. Distribution not certain. Specimens found in a stream in Abel Tasman National Park (*D. balfouriana*) and in the Waiau River, Southland (*D. parva*).

Diatomella balfouriana*, valve view focussed to show the raphe (lop) and the septum (bottom). x 1400*

Diatomella parva*, girdle view. x 1400*

Diploneis

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales

How to recognise the genus:

Microscopic: A symmetric biraphid diatom. Usually oval in shape, but sometimes with a constriction in the middle of the valve. The raphe lies in a thick rib of silica. Obvious rows of spots (holes) run parallel to the outer edges of this rib. The striae are easy to see even under low power. Two chloroplasts.

Possible confusion: A fairly distinctive diatom genus.

Diploneis elliptica

Diploneis elliptica is basically oval, usually between 20 and 80 µm long. It is distinguished from other species by its shape, rounded central area and the coarse striae and puncta. *Diploneis ovalis* is similar in shape but has finer striae and puncta.

Habitat and distribution: Usually inhabits lakes, though we have found this diatom in the periphyton of lake-fed rivers; can be occasional to common.

Diploneis *cf.* elliptica. *x 1800*

Encyonema

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Cymbellales

How to recognise the genus:

Macroscopic: Some species of *Encyonema* grow in mucilage tubes (see page 155) and can accumulate as extensive mats of light brown slime.

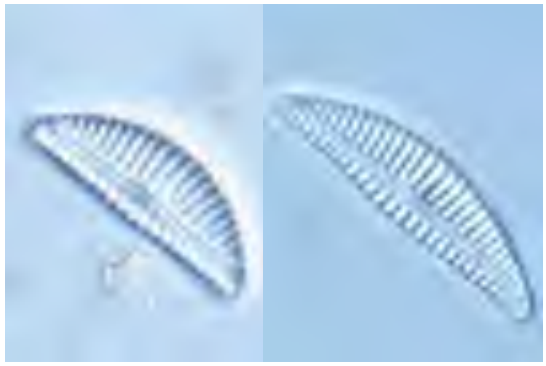
Microscopic: A biraphid diatom, asymmetric across the valve with most species roughly half-moon shaped. Look for upward-pointing central raphe endings and downward-pointing terminal endings. The chloroplast in valve view is more or less H-shaped.

Possible confusion: *Cymbella* (see page 164).

Encyonema minutum (= *Cymbella minuta* = *Cymbella ventricosa*)

Small (15–30 µm long, 5–7 µm wide) with a more or less flat ventral (bottom) side and a strongly rounded dorsal side. The striae can be seen clearly at 400↔ and the raphe runs close to the ventral (bottom) margin. There are no stigmata.

Habitat and distribution: *E. minutum* is common and widespread throughout New Zealand. It has been recorded as dominant or abundant in periphyton from streams draining moderately developed catchments.



Encyonema minutum, *cleaned specimens showing range of size. x 1500*

Encyonema gracile (= *Cymbella gracilis* = *Cymbella lunata*)

Narrow cells, slightly rounded on the dorsal (upper) side and more or less flat beneath, from 30 to 50 µm long and 5 to 7 µm wide. The rounded ends sometimes seem to point slightly downwards. Fine striae.

Habitat and distribution: This species seems to occur mostly in the periphyton of clean-water lake-fed rivers. It has also been recorded in the periphyton of high altitude lakes and tarns (acid conditions?). Most examples seen in South Island samples.

Encyonema prostratum (= *Cymbella prostrata*)

Chunky cells with prominent markings, often seen as filaments in mucilage tubes. Cells are 40–80 µm long and 20–30 µm wide. The striae contine right around the ends of the valve.

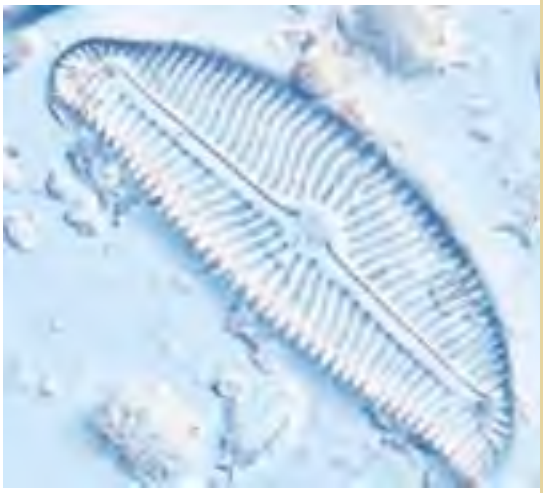
Habitat and distribution: Seems to occur usually in relatively high conductivity streams. This species does not appear to be widespread but it has been reported in abundance in the Shotover River (S. Moore, pers. comm.) and in a stream near Murchison.

Encyonema caespitosum (= *Cymbella caespitosa*)

Microscopic: Smaller than *E. prostratum*, with more pointed ends; 20–40 µm long and 10–15 µm wide. The cells often have a somewhat asymmetrical appearance. Again the striae are quite prominent. Occurs as filaments in mucilage tubes (see illustration page 155).

Habitat and distribution: Found in the periphyton of large rivers. Distribution unknown.

Note: The old genus *Encyonema* has now been expanded to include many species placed in *Cymbella*, including the examples above. (See Round *et al.* 1990.)



from top:

Encyonema gracile. *x 1400*

Encyonema prostratum *cells in a mucilage tube. x 350*

Encyonema prostratum. *x 1400*

Encyonema caespitosum. *x 1400.*

Epithemia

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Rhopalodiales

How to recognise the genus:

Microscopic: Epithemia has an "external" raphe. In valve view the cells are typically shaped like an orange segment (side view) or half-moon; the girdle view is roughly rectangular. Thickened areas (costae) on the valve wall show up as obvious dark lines under LM. The raphe runs in a "canal" along the ventral (i.e., the bottom, concave) edge of the valve, curving up at the centre. A single deeply lobed sheet-like chloroplast lies at the ventral side of the valve. All cells contain symbiotic cyanobacteria (visible as small spheres mainly in the girdle view of live specimens).

Possible confusion: Possibly *Cymbella* and *Encyonema* (see pages 164, 167). However, the structure of *Epithemia* is quite different from either of these genera.

Epithemia sorex

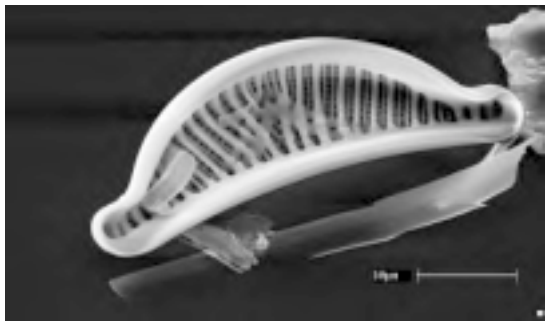
Epithemia sorex is 20–60 µm long and 6–16 µm wide. In valve view it looks like a "Napolean's Hat". In girdle view it is oval with squared-off ends (because of the way the valve bulges out at the sides). In valve view the entire raphe canal is visible.

Habitat and distribution: Common, found in lowland (enriched) streams and rivers throughout New Zealand, often growing attached to plants and other algae. It occasionally dominates periphyton, usually in moderately enriched streams after prolonged low flows.

Epithemia cf. *adnata* *(= Epithemia zebra)*

Larger than *Epithemia sorex*, up to 150 µm, with rounded ends, coarser markings and costae usually more widely and less regularly spaced. Rectangular in girdle view.

Habitat and distribution: Seems to be widespread, though not usually common in periphyton. Often occurs with *Epithemia sorex*. Reported to prefer moderate to high conductivity streams, possibly extending to brackish water (Cox 1996).



from top:

Epithemia sorex, *girdle view (live cell). x 850, valve view (cleaned specimen). x 1400*

SEM showing internal "costae" and raphe canal.

Epithemia *cf.* adnata, *girdle view (live). x 500; valve view (cleaned specimen). x 1000*

Frustulia

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales

How to recognise the genus:

Microscopic: Frustulia is a symmetrical biraphid naviculoid genus, rectangular in girdle view. The raphe is distinctive: instead of a simple slit (or line), there is a ridge at either side (actually on the inside of the valve) so that the raphe appears to lie in a channel. The striae are very fine, barely visible under LM. Two chloroplasts, as in *Navicula*, but usually extending futher across the valve face.

Possible confusion: Other naviculoid genera. Look for the form of the raphe, especially the outer ends, and the extent of the chloroplasts. In *Navicula* (see page 174) the two lateral chloroplasts do not extend the width of the valve.

Frustulia rhomboides

A fairly large species, somewhat angular in shape, from 70 to 150 µm long and to 30 µm wide (at the widest point).

Habitat and distribution: Usually occurs only occasionally in stream periphyton, but this species can be abundant. It can also be very common in clean-water lake sediments. Widespread.

Frustulia rhomboides var. *crassinerva*

Smaller than *Frustulia rhomboides*, 30–50 µm long. This variety has capitate (pinched) ends and very slightly undulating margins.

Habitat and distribution: Not certain. Again may be abundant in streams in some areas (e.g., West Coast). May be more common in cleaner (acid) waters.

Note: Other varieties of *Frustulia rhomboides* have more rounded sides, or are longer and more slender.

Frustulia vulgaris

Up to 60 µm long, 12 µm wide. The cell has barely capitate ends and slightly rounded sides. The valve face sometimes looks slightly asymmetrical, with the raphe tending to one side.

Habitat and distribution: This species occurs quite often in periphyton samples over a range of conditions; can be common.

Note: Some species of *Frustulia* form "filaments" of cells in mucilage tubes. See page 155.

from top:

Frustulia rhomboides, *live specimen. x 450*

Frustulia *cf.* rhomboides *var.* crassinerva*. x 1300*

Frustulia *cf.* vulgaris. x 1300

Gomphoneis

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Cymbellales

How to recognise the genus:

Macroscopic: Gomphoneis can form thick, glistening, light-brownish mats on river substrates.

Microscopic: An asymmetric biraphid diatom, *Gomphoneis* is club-shaped in valve view, with the raphe lying along the centre of the long axis. Under high power (in cleaned specimens) there seems to be a discontinuity in the density of the striae about half way between the valve margin and the raphe. This occurs where the puncta go right through to the inside (see SEM below). Double rows of striae may just be visible. In girdle view the genus is wedge-shaped. *Gomphoneis* grows attached to the substrate by long branching mucilage stalks. The chloroplast seems to be H-shaped, with sheets flattened against each valve face and joined at the centre.

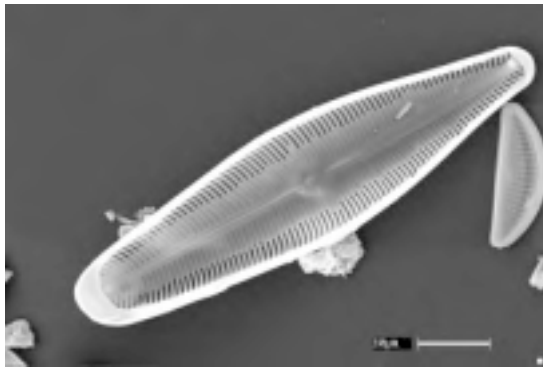
Possible confusion: *Gomphonema* spp. (page 171). Look for the larger size, double striae (barely visible under LM) and long stalks in *Gomphoneis.*

Gomphoneis minuta var. cassieae

Gomphoneis minuta var. *cassieae* ranges in size from 60 to 100 µm long and about 20 µm wide (at the widest point).

Habitat and distribution: This species occurs all over New Zealand and often dominates periphyton communities in moderately enriched to enriched waters.

Note: This species has formerly been identified as *Gomphoneis herculeana* and was reassigned by Kociolek and Stoermer 1988. We assume here that the New Zealand form of *Gomphoneis* is *G. minuta* var. *cassieae.* However, the difference between the two species is subtle.



from top:

Thick Gomphoneis *growth on a stone, with* Fragilaria *(the darker strands in the middle).*

Mass of live Gomphoneis*, showing stalks. x 125*

Gomphoneis minuta *var.* cassieae*, cleaned specimen, x 820*

The interior structure of Gomphoneis*. SEM.*

Gomphonema

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Cymbellales

How to recognise the genus:

Microscopic: There are many species of *Gomphonema* in New Zealand, some of which still await description. Obvious features of this asymmetric biraphid genus are longitudinal asymmetry and a wedge shape in girdle view. Many live attached to the substrate – either rocks or water plants, for example, and sometimes growing in masses – by long or short mucilage stalks that enable them to withstand water currents.

Possible confusion: *Gomphoneis* (see page 170).

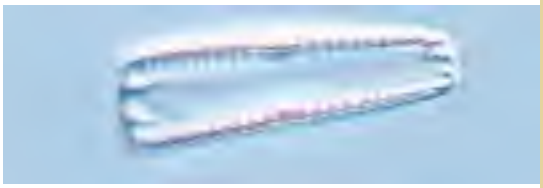
Gomphonema parvulum

Distinguished mainly by size and shape: 10–25 µm long; narrow leaf-like with pinched ends (slightly pinched or markedly so). Under high power, cleaned specimens are identified by the more-or-less parallel and relatively densely spaced striae (though the latter is very variable), a space on one side of the central area, and a single "stigma" opposite the space.

Habitat and distribution: *G. parvulum* is generally considered to be pollution tolerant, and if common to dominant in a sample, this often indicates that there is some enrichment (particularly organic) to the water.

Note: Most *Gomphonema* specimens of the shape and size illustrated are likely to be *G. parvulum*. However, another species, *Gomphonema angustatum* , is similar and it can be difficult to decide which one you have, even under high power. Many specimens seem to have spaced out central striae characteristic of *G. angustatum*, but also have striae that are close to parallel rather than radiate. The former is characteristic of *G. parvulum*). *G. parvulum* and *G. angustatum* are clearly distinguished in, for example, Foged (1979, p. 57/59, pl 38). However, illustrations in other publications (e.g., Krammer and Lange-Bertalot 1997) are not as clear cut.

Note: Don't confuse the similarly named species *Gomphonema angustatum* and *G. angustum* (see page 172)



from top:

Small Gomphonema *sp., growing massed together attached to substrate, mostly in girdle view.*

Two specimens of G. *cf.* parvulum. x 1600

Left: Uncertain species, possibly a variety of G. *cf.* parvulum, *wider, with more densely spaced striae than we see normally. Right:* G. parvulum *tending towards* G. angustatum? *x 1600*

Gomphonema *sp., girdle view. Note the thickened areas on each side, indicating the raphe endings.*

Gomphonema truncatum *(= G. constrictum)*

"Jelly-baby"-shaped with a rounded end. 20–70 µm long, striae radiate at the centre with a space at centre on both sides of valve and a single stigma.

Habitat and distribution: A fairly common epiphyte, usually in moderate to high conductivity streams.

Gomphonema acuminatum

A very distinctive shape, like *Gomphonema truncatum* but with an extra projection at the end. 30–100 µm long.

Habitat and distribution: Sensitive to moderate levels of pollution (Cox 1996). Widespread and can be common.

Gomphonema cf. *minutum* *(= G.tenellum)*

Small, narrow species, up to 25 µm long and about 5 µm wide, with quite widely spaced striae.

Habitat and distribution: This small species seems to be widespread and can be very common. Often in fairly clean streams. Identification is uncertain.

Gomphonema cf. *angustum* *(= G.intricatum)*

Narrow, elongated cell, up to 50 µm long and 8 µm wide, with a wide axial area.

Habitat and distribution: Reported to prefer clean streams.Seems to be widespread and can be common.

Gomphonema clavatum *(= G. longiceps)*

Club-shaped, blunt ends, 25–100 µm long. narrow axial area with central stria shorter, wider spaced, one stigma.

Habitat and distribution: Encountered occasionally. Distribution not certain.

Note: The above are some of the more obvious species of *Gomphonema* found in stream periphyton. There are many others. In processing periphyton samples, the best way to deal with them is to note the different forms in a sample, assign any to the above species, then simply assign numbers to the others (see Section 8.1).

from top:

Gomphonema truncatum*, live cells. x 1000*

Gomphonema acuminatum. *x 1250*

Gomphonema *cf.* minutum. *x 1250*

Gomphonema *cf.* angustum. *x 1250*

Gomphonema clavatum. *x 1250*

Gyrosigma

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales

How to recognise the genus:

Microscopic: Gyrosigma has a sigmoid shape, with fine striae. It is biraphid, like *Navicula*. There are two chloroplasts, one along each side of the girdle bands.

Possible confusion: Another genus, *Pleurosigma*, has a similar shape, but is rarely seen in fresh water in New Zealand. In *Pleurosigma* the puncta often appear to lie in diagonal rows, while in *Gyrosigma*, if visible, they lie parallel to the raphe.

Gyrosigma cf. *scalproides*

Parallel sides, with the ends turning in opposite directions to form an S-shape. 60–100 µm long and about 15 µm wide. Central raphe endings are T-shaped (but this can be difficult to make out).

Habitat and distribution: This species has been recorded as abundant in lowland streams in North Island, but does not seem to be widespread.

Note: *Gyrosigma spencerii* (not illustrated) is similar in size but tapers more gradually towards the ends. It seems to be quite widespread, but not common. Reported to occur in high-conductivity streams.

Gyrosima *cf.* scalproides *from the lower Waitekauri River, Coromandel. live specimen, x 700; cleaned valve, x 700*

Mastogloia

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Mastogloiales

How to recognise the genus:

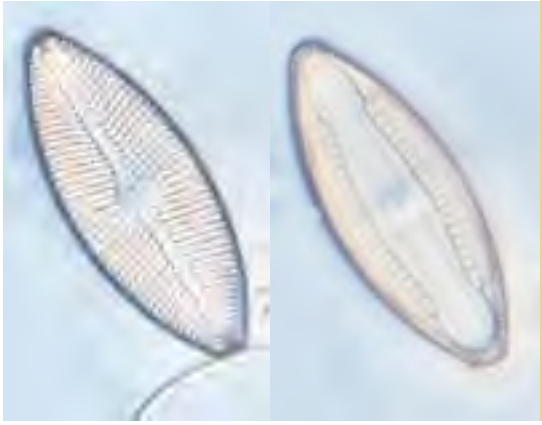
Microscopic: Mastogloia is biraphid and fairly "chunky" (i.e., heavily silicified). Naviculoid in valve view with a distinctive wavy raphe. Focussing through the specimen reveals a series of chambers on each side of the valve, which is part of the girdle. In valve view the frustule is rectangular, with the chambers visible. Two chloroplasts, one at each end.

Possible confusion: Normally very distinctive, but when separate from the girdle, the valve faces could be mistaken for *Navicula* (see page 174). Look for the wavy raphe.

Mastogloia elliptica

Microscopic: Roughly oval, with slightly pinched ends, 30–70 µm long and 10–20 µm wide.

Habitat and distribution: *M. elliptica* can be common in the periphyton of lake-fed rivers in both North and South Islands.



Mastogloia elliptica*, focussing on the valve face (left) and the internal chambers (locules) (right). x 1200*

Meridion

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Fragilariales

How to recognise the genus:

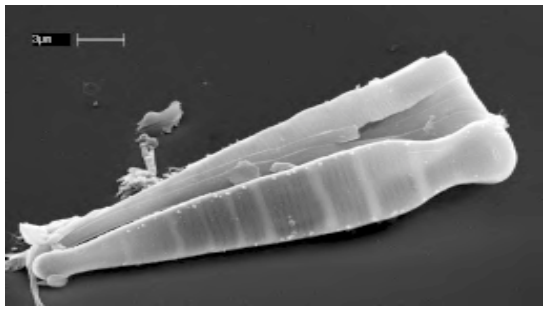
Microscopic: An araphid genus, *Meridion* forms semi-circular or radial colonies of valves joined face to face. In girdle view, the valves are wedge shaped. Like *Diatoma*, thick ribs ("costae") cross the valve face and these are visible in girdle view as small knobs down each long edge. There are several small chloroplasts dotted around the cell. This is a small genus with only one main species.

Possible confusion: Unlikely.

Meridion circulare

From 20 to about 80 µm long.

Habitat and distribution: *Meridion circulare* seems to prefer clean, cool streams, in which cells attach themselves to the substrate with a mucilage pad. It is usually not very common. A "cleanwater species" in Cassie (1989).



from top:

Meridion circulare *colony. x 900*

SEM of a single frustule of M. circulare.

Navicula

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales

How to recognise the genus:

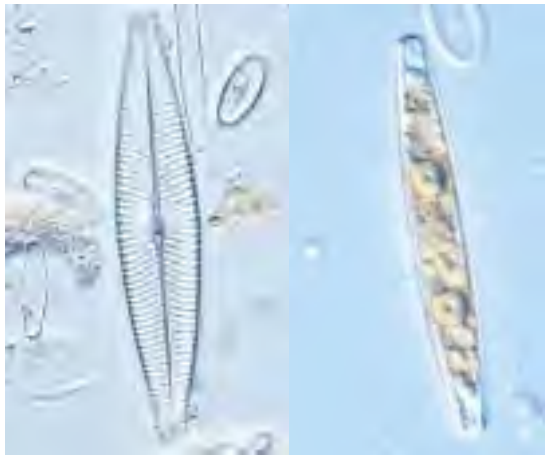
Microscopic: Navicula occurs in almost all periphyton samples containing diatoms. The genus is biraphid, with basically lens-shaped ("naviculoid") frustules. Two narrow chloroplasts are located laterally in valve view. Striae usually radiate from the centre. In fresh samples *Navicula* species tend to be noticeably motile.

Possible confusion: Other naviculoid diatoms include *Frustulia, Mastogloia, Neidium, Brachysira, Stauroneis.* Look for the narrow paired chloroplasts in healthy *Navicula.*

Navicula lanceolata (= *N. avenacea*)

Up to about 70 µm long, with noticeable striae radiating from a round central area, becoming convergent at the ends. which are blunt and barely capitate.

Habitat and distribution: *N. lanceolata* is widespread, common and can dominate the periphyton in streams of low to moderate conductivity. Often called *N. avenacea.*



left. Navicula lanceolata *x 900. right, Girdle view of a live specimen. x 900*

Navicula cryptocephala

Small, slender, with capitate ends; 20–40 µm long and 5–7 µm, wide. Narrow axial area expanding to a rounded area at the centre of the valve. In live material look for fine striae, and the arrangement of the two narrow chloroplasts exactly opposite each other.

Habitat and distribution: Widespread and common; occasionally dominates the periphyton in relatively clean streams.

Navicula capitoradiata

Similar in size to *N. cryptocephala*, but a little fatter with a smaller central area. The chloroplasts are slightly offset from each other (see Cox 1996).

Habitat and distribution: Cox (1996) reports that *N. capitoradiata* prefers high-conductivity streams and is pollution tolerant. Thus it may be important to separate it from *N. cryptocephala.* Distribution in NZ uncertain.

Navicula rhynchocephala

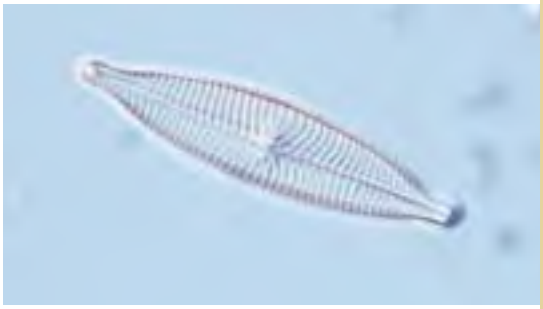
Resembles a bigger (35–80 µm long) version of *N. cryptocephala.* The striae are coarser and the central area rounded (like *N. avenacea*). The chloroplasts are wider and rather ragged looking.

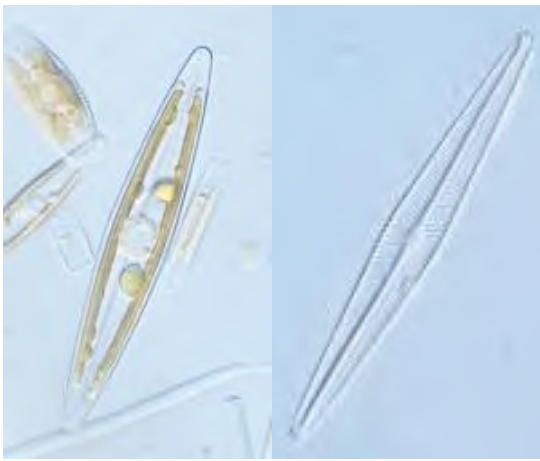
Habitat and distribution: Widespread in low to moderately enriched streams draining foothills, but not usually common. Reported as a "grossly polluted water species" (Cassie 1989).

Navicula radiosa

Microscopic: Longer and narrower than either *N. avenacea* or *N. rhynchocephala*, up to 100 µm long. Ends definitely not capitate. Striae radiate out from the centre. Central area smaller than *N. avenacea.* Long thin chloroplasts lie opposite each other.

Habitat and distribution: Found in cool, clean streams. Widespread but not usually common. Reported to be sensitive to moderate pollution (Cox 1996).





from top:

Navicula cryptocephala, *x 1500*

Navicula capitoradiata, *x 1500*

Navicula rhynchocephala, *x 1500*

Navicula radiosa; *(left) live specimen x 600; (right) cleaned specimen, x 600*

Navicula cf. *margalithi*

Blunt cigar-shaped typically 40–50 µm long and 10 µm wide. Distinctive in its almost parallel striae.

Habitat and distribution: This species has been recorded as common in some North Island locations. Not yet found in South Island yet. (Tentative identification.)

Note: Many other species of *Navicula* occur in stream periphyton usually as "occasional" or less, e.g., :

N. gregaria: about 15 µm long, pinched ends, striae parallel, chloroplasts markedly offset.

N. cincta: 15–25 µm long, smooth-sided (like a rugby ball), chloroplasts opposite.

(Both species reported to occur in high-conductivity streams. *N. cincta* may be pollution-tolerant.)

Navicula *cf.* margalithi*, Moawhango River, North Island. left, live cells x 700; right, cleaned specimen x 1400.*

NB. Navicula *cf.* gregaria *appears on the photo of* Meridion*, page 174.*

Neidium

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales

How to recognise the genus:

Microscopic: Biraphid, symmetric naviculoid cells, rectangular in girdle view. Very fine striae that lie more or less parallel across the valve. In most species, at the centre of the valve the raphe endings curve in opposite directions. There are four chloroplasts per cell.

Possible confusion: Usually easy to recognise by its size and shape, the distinctive central raphe endings (if present) and the four chloroplasts.

Neidium affine

Usually 40–80 µm long and about 20 µm wide, with rounded capitate ends and slightly curved margins, not quite parallel.

Habitat and distribution: Fairly widespread but not very common. Usually found in clean streams.

Neidium iridis

A larger species, 50–90 µm long. Slightly rounded sides curving smoothly to the ends.

Habitat and distribution: Reported usually from very slow moving streams or ponds. Widespread but not common.

left: Neidium affine*, from the Waiau River, Southland. x 1100*

right: Neidium iridis*: live specimen from a pond, Wairau Valley, Canterbury. x 700*

Nitzschia

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Bacillariales

How to recognise the genus:

Microscopic: A large genus with an "external" raphe system. Most *Nitzschia* species are narrow needle-like cells. Some are slightly sigmoid (S-shaped). The raphe lies on a ridge (the "keel") near or at one edge of each valve. A series of internal ribs across the keel appear as a row of dots (the "keel puncta") under the microscope. This is very obvious in some species. There are two chloroplasts, arranged along the valve – a good indicator of this genus.

Possible confusion: Small *Nitzschia* species may be confused with some small *Synedra* species, especially where the chloroplasts are not clearly visible (see page 187). Look for the more delicate structure (less silicon) of many *Nitzschia*. If possible, examine specimens at 1000x and look for the keel puncta. The genus *Hantzschia* (not in this manual) is closely related; the main *visible* difference from *Nitzschia* is strong asymmetry across the valve. *Nitzschia* has many species that are difficult to identify. All identifications below are tentative.

Nitzschia cf. *palea*

Usually 15–60 µm long and up to 5 µm wide. Delicate-looking sometimes with a slightly asymmetric appearance. The keel edge is rounded and the opposite edge is slightly rounded, flattened or even slightly concave. The striae are very fine – virtually invisible except at very high power. The keel puncta can also be difficult to make out under low power (400 x and less).

Habitat and distribution: Widespread, common and well-known as a pollution tolerant species.

Nitzschia cf. *amphibia*

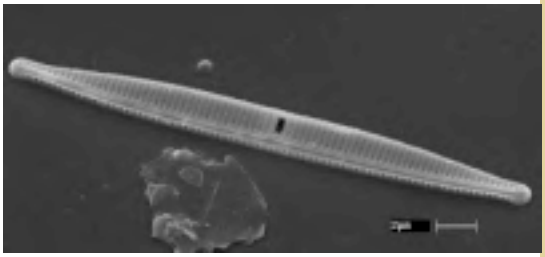
From about 10 to 40 µm and 4–6 µm wide. The striae and keel puncta are conspicuous compared with those of *N. palea.*

Habitat and distribution: Widespread and common in a wide range of conditions; may not as pollution-tolerant as *N. palea*, but possibly does best in high-conductivity streams.

Nitzschia cf. *inconspicua*

A minute diatom, 3–15 µm long, with rounded ends. Valve markings not visible except under high power.

Habitat and distribution: Common in high-conductivity streams. Tolerates moderate pollution levels.



from top:

Nitzschia *cf.* palea*, x 1800*

Nitzschia *cf.* amphibia, *x 1800.*

Nitszchia *cf.* inconspicua, *several specimens. x 1800*

Scanning electron micrograph of Nizschia *sp. to illustrate structure. (NB. valve broken at centre.)*

Nitzschia cf. *dissipata*

Usually small but can be up to 70 µm long and 3 to 6 µm wide, with tapering ends. The elongated keel puncta are set in from the valve edge. Very fine striae (not visible under LM).

Habitat and distribution: Seems to be widespread, but not common in stream periphyton samples. Usually in higher conductivity waters.

Nitzschia cf. *linearis*

Typically large (over 90 µm long), with obvious, elongated keel puncta and fine striae.There is a notch at the centre of the valve where the two raphe ends meet (see photo). Long chloroplasts. In fresh samples usually lies in girdle view.

Habitat and distribution: Reported overseas as being tolerant of polluted conditions (Cox 1996). Seems to be widespread in New Zealand. In fresh or frozen material could be confused with *N. intermedia* (below).

Nitzschia cf. *intermedia*

Another large species, up to 150 µm long. The keel puncta are dot-like, more widely spaced than in *N. linearis*, and there is no obvious central notch. Long chloroplasts.

Habitat and distribution: Found widely in periphyton from lowland rivers, and can be common. This species is reported to prefer moderate to high conductivity, but is not particularly tolerant of organic pollution (Cox 1996).

Nitzschia cf. *acicularis*

A delicate species, 20–60 µm long, including fine, drawn-out ends. The keel puncta and striae not visible under the light microscope.

Habitat and distribution: Encountered widely in stream periphyton, but usually not very abundant. This species is reported overseas as being tolerant of moderately polluted conditions (Cox 1996).

from top:

Nitzschia *cf.* dissipata*. Small specimen from the Avon River, Christchurch. x 1400*

Nitzschia *cf.* linearis*, cleaned material. Top: valve view – note notch; lower: girdle view. x 800*

Nitzschia *cf.* intermedia*, x 350, live specimen (girdle view) from the Ashley River, Canterbury, showing chloroplasts and continuous keel puncta.*

Nitzschia *cf.* acicularis*. Live specimen. x 900.*

Nitzschia cf. *gracilis*

Fine, elongated, up to 100 µm long. Ends drawn out and rounded at the tips. Keel puncta and striae visible only under high power.

Habitat and distribution: Not known.

Note: *Nitzschia sigmoidea* (not illustrated) is a large species (150 –500 µm long and up to 15 µm wide) which is S-shaped in girdle view. In other smaller species the valve is have a sigmoid shape in valve view, e.g., *Nitzschia nana* (= *N. ignorata*, about 50 µm long and 4 µm wide). None of these are common in stream periphyton.

Nitzschia *cf.* gracilis. *live specimen x 1100. Note the two small chloroplasts.*

Pinnularia

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales

How to recognise the genus:

Microscopic: A biraphid symmetric naviculoid genus. Each stria is made up of many tiny holes opening into a chamber, so that the striae appear wide and continuous rather than made up of a series of puncta. This is especially visible in large species. The raphe endings curve strongly in the same direction and some species seem almost asymmetrical across the valve (like *Cymbella*). The two chloroplasts lie one each side of the frustule. In valve view you see both; in girdle view you see a single sheet. *Pinnularia* species are fairly motile.

Possible confusion: Can sometimes look like *Navicula* sp. (see page 74). Note that there are many species of *Pinnularia*, species can be variable and identification can be difficult. Most of the following identifications are tentative.

Pinnularia viridis

A large diatom (up to 150 µm long and 25 µm wide), cigar-shaped with prominent striae that are slightly radiate in the middle of the valve changing to parallel and then convergent at the ends.

Habitat and distribution: Occurs occasionally in stream and river periphyton. Along with many other *Pinnularia* species, the preferred habitat is clean, acid lakes and ponds. Widespread.

from top:

Live Pinnularia. *cf.* viridis *in girdle view, showing the large sheet-like chloroplast. x 500*

Pinnularia viridis, *cleaned specimen. x 700*

Pinnularia cf. *gibba*

Large (80–120 µm long and about 15 µm wide), with very slightly capitate ends. Quite short striae, sometimes with a gap at the centre of the valve; wide axial and central areas.

Habitat and distribution: Habitat uncertain. Not usually common in stream periphyton. Widespread.

Pinnularia cf. *microstauron*

Smaller – up to 70 µm long and 15 µm wide with slightly undulating margins and blunt rounded ends. A gap in the striae at the central area, but much less empty space in the central and axial areas than *P. gibba*.

Habitat and distribution: Probably widespread, usually in low conductivity.

Pinnularia cf. *mesolepta*

Margins definitely undulating, with rounded ends, from 25 to 80 µm long and 15 µm wide.

Habitat and distribution: Probably widespread and found in a range of stream types.

Pinnularia cf. *subcapitata*

Usually small (25 to 60 µm long and are 6–7 µm wide); slightly undulating sides, and rounded slightly capitate. There is a gap in the striae each side of the central area. The axial area can be quite wide.

Habitat and distribution: Occasionally found in stream and river periphyton. Most findings from South Island samples, especially high country streams.

Note: *Pinnularia* species are typically abundant in acid wetland areas. Note also that there are many small species of *Pinnularia*. To distinguish between species look at the cell shape and arrangment of the striae. Is there a gap at the central area? Do the striae radiate from this area? Do they change direction part way along the valve (as in the specimen in the bottom photograph)?

from top:

Pinnularia *cf.* gibba, *Waiau River. x 1200*

Pinnularia *cf.* microstauron*. Tentative identification. x 1200*

Pinnularia *cf.* interrupta*, live cell from a lowland stream, Banks Peninsula, Canterbury. x 1200*

Pinnularia *cf.* subcapitata *variety?. This example has a very wide axial area so the identification is tentative only. x 1200*

Pinnularia *sp. found in a stream on Stewart Island. x 1200*

Placoneis

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Mastogloiales

How to recognise the genus:

Microscopic: Placoneis species are biraphid symmetric naviculoids, usually with fat, rounded sides and capitate ends. The main diagnostic feature is the single lobed chloroplast. Cleaned specimens show strongly radiating striae; individual puncta may be visible under high power.

Possible confusion: *Navicula*: check the chloroplast structure if possible. There are other differences but these are clearly visible only under SEM. *P. placentula* is distinctive.

Placoneis placentula (= *Navicula placentula*)

Usually quite large, up to 60 µm long and noticeably wider than most naviculoid diatoms (up to 25 µm wide).

Habitat and distribution:

Reported to be a clean-water species, but usually in moderate to high conductivity streams (Cox 1996). Widespread but usually not common.

Placoneis placentula *from the Moawhanga River, central North Island. x 1300*

Note: *Placoneis* was formerly included in *Navicula*, but is now reinstated as a genus in its own right (see Cox 1987).

Reimeria

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Cymbellales

How to recognise the genus:

Microscopic: Reimeria is a biraphid asymmetric genus. Features are a small, slightly curved valve view (like *Cymbella*) with a bulge on the concave side, widely spaced striae and small size. Rectangular in girdle view and quite motile.

Possible confusion: With other small diatoms such as *Fragilaria* especially in girdle view.

Reimeria sinuata (= *Cymbella sinuata*)

This is the only species known in New Zealand. *R. sinuata* varies in size from 10 to 25 µm long.

Habitat and distribution: Quite common and widespread in New Zealand stream periphyton. It has been recorded as abundant in lowland rivers, e.g., in Westland, during summer low flows.

Reimeria sinuata*, with girdle view above. x 1900.*

Note: The genus *Reimeria* was established in 1987, based on *Cymbella sinuata* (see Kociolek and Stoermer 1987).

Rhoicosphenia

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Cymbellales

How to recognise the genus:

Microscopic: Rhoicosphenia is a small biraphid genus, asymmetric in three planes. In valve view it is club-shaped (like *Gomphonema*); in girdle view it is bent (like *Achnanthidium*) and also wider at one end. Under high power (x 1000) the valve view shows a "thickening" at each pole where an internal layer of silica extends into the cell. The concave valve face has a more highly developed raphe than the convex face. *Rhoicosphehia* grows on short stalks often as an epiphyte on other algae.

Possible confusion: *Gomphonema* (see page 171) – though the girdle view is distinctive.

Rhoicosphenia abbreviata (= *R. curvata*)

Only one species of *Rhoicosphenia* is found commonly in streams. *R. abbreviata* varies in size from 20 to 60 µm long and up to 8 µm wide.

Habitat and distribution: Very common and widespread in New Zealand periphyton. It has been recorded as dominant (growing massed together on substrates) in small lowland streams with moderate to high water velocities. It seems to be pollution tolerant.

Rhoicosphenia curvata *from the Tarawera River; left: valve view; right: girdle view. x 1800*

Rhopalodia

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Rhopalodiales

How to recognise the genus:

Microscopic: In this genus the raphe system lies close to the valve edges, and the valve faces are rotated so that they lie roughly in the same plane (as in *Amphora*, for example). so you normally see both valve faces at the same time. Thick ribs of silica (costae) may run across the inside of the valves, visible as heavy lines or stripes under LM. There is a single large chloroplast. Some *Rhopalodia* species contain symbiotic bacteria which enable them to fix inorganic nitrogen.

Possible confusion: Unlikely.

Rhopalodia novae-zealandiae

A large diatom up to 200 µm long and 25 µm wide (both valves). Cells have rounded ends and a bulge at the middle of each side, with a slight notch where the raphe ends meet. Striae should be visible at 400x in fresh material,There are no thicker ribs (costae).

Habitat and distribution: Very abundant in lakes but can also be common in clean lake-fed rivers and streams.

Rhopalodia novae-zealandiae, *live specimen showing chloroplast. x 500*

Rhopalodia operculata

Smaller than *R. novae-zealandiae* (20–40 µm long and about 20 µm wide). Valves are rounded on their outer margins and have thick costae running across them.

Habitat and distribution: This species is encountered occasionally in river periphyton. Distribution and habitat preferences uncertain.

Note: May be the same as *R. musculus* in Foged 1979.

Rhopalodia operculata. *x 1200*

Sellaphora

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales

How to recognise the genus:

Microscopic: A biraphid genus with rounded ends (valve view). The raphe lies in a sort of raised plate running along the centre of the valve. There is often a clear area at each end of the valve. A single chloroplast, with lobes lying against each side of the valve and joined in the middle to form an "H" shape.

Possible confusion: With other naviculoids, though the shape is fairly distinctive, and the chloroplast different.

Sellaphora pupula

S. pupula is 30–70 µm long and 5–20 µm wide. Elongated shape with rounded, slightly capitate ends.

Habitat and distribution: *Sellaphora pupula* seems to be quite widespread in stream periphyton in New Zealand, though never abundant. Overseas, it is reported as being tolerant of a wide range of ecological conditions (Cox 1996).

Sellaphora bacillum

Similar in size to *S. pupula* but has parallel sides.

Habitat and distribution: *Sellaphora bacillum* has been recorded throughout New Zealand (Cassie 1984). It is reported overseas as being tolerant of some pollution.

Note: Species in *Sellaphora* were formerly placed in *Navicula* but have now been transferred back to their original genus (see Mann 1989).

from top:

Sellaphora pupula, *cleaned specimen from the Tarawera River. x 2000*

Sellaphora bacillum, *cleaned specimen from a stream, West Coast, South Island . x 2000*

Stauroneis

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales

How to recognise the genus:

Microscopic: A symmetric biraphid naviculoid. Some species can be quite large (over 100 µm long). The main diagnostic feature is the thickened band of silica across the middle of the valve (this may be difficult to see). There may also be internal flaps of silica at the ends of the valve (cf. *Rhoicosphenia*). Fine striae in which the puncta may be visible. Two chloroplasts lie against the girdle (one each side) and extend under the valve face.

Possible confusion: *Navicula (see page 174)*, other naviculoids.

Stauroneis cf. anceps

Up to 75 µm long and 15 µm wide. Rouded margins terminating in rounded captitate ends. Striae radiate throughout the valve.

Habitat and distribution: Widespread, mostly in ponds and lakes or slow-moving stream outlets. Reported to occur over a wide ecological range (Cox 1996).

from top:

Stauroneis *sp., live specimen showing chloroplasts. x 450*

Stauroneis anceps, *cleaned specimen from the Waiau River. x 1100*

Staurosira

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Fragilariales

How to recognise the genus:

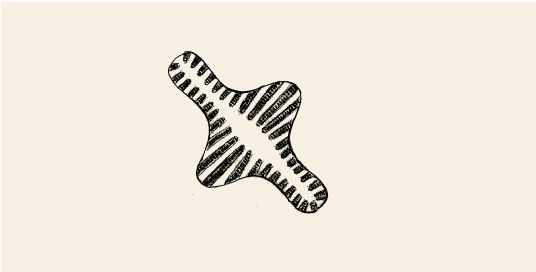
Microscopic: A small araphid genus formerly included in *Fragilaria*. Small cells often cruciform (shaped like a cross), with a prominent striae and a pseudoraphe that usually widens at the centre of the cell. Rectangular in girdle view.

Possible confusion: Other small araphid genera formerly placed in *Fragilaria*, especially *Staurosirella*, which has a very different stria structure (see page 185). In *Staurosira* the striae comprise single rows of small round or oval puncta.

Staurosira construens (= Fragilaria construens)

Cruciform to oval cells 8–25µm long, up to 12 µm wide, rectangular in girdle view, sometimes forming short chains.

Habitat and distribution: Has been recorded as common in clean, spring-fed streams. Widespread.



Drawing of Staur osira construens *showing features visible under LM. x 2000.*

Staurosirella

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Fragilariales

How to recognise the genus:

Microscopic: An small araphid genus formerly included in *Fragilaria*. Small cells cruciform or oval with wide striae; pseudoraphe may be wider at the centre. Rectangular in girdle view.

Possible confusion: Cruciform cells of *Staurosira* (page 184). Difficult to distinguish except under high-power LM, although *Staurosira* is usually smaller. In *Staurosirella* the coarse striae are spaced farther apart than the finer striae of *Staurosira*.

Staurosirella leptostauron *(= Fragilaria leptostauron)*

Cruciform cells 15–35µm long and up to 20 µm across, s ometimes forming short chains. Coarse striae widely spaced (less than 9 in 10 µm). Under high power fine lines across the striae may be visible.

Habitat and distribution: Usually occurs in clean rivers and streams, and can be locally common. Distribution not certain.

Staurosirella leptostauron *from the Okuku River, Canterbury. x 2000*

Stenopterobia

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Surirellales

How to recognise the genus:

Microscopic: Elongated cells, sometimes S-shaped, with an "external" raphe system. As in *Surirella* (page 196), the raphe runs around the entire margin of the valve so that the two"distal" raphe ends are adjacent to each other, and the central join is at the opposite end of the cell. There are small ribs around the whole cell margin. A single chloroplast has a large lobe lying against each valve face.

Possible confusion: The S-shaped species could be confused with some *Nitzschia* species that have a similar shape and also *Gyrosigma*. Check the chloroplast.

Stenopterobia species

Stenopterobia delicatissima is spindle shaped with noticeable ribs around the margin. Size: from 30 to 100 µm long.

Stenopterobia curvula is longer, slender, sigmoid with somewhat angular ends (rather than rounded). Marginal ribs faint. Size: up to about 250 µm long.

Habitat and distribution: Both these species have been found in pristine streams draining small lakes and wetlands. Distribution uncertain.

left: Stenopterobia delicatissima. *x 1100*

right: Stenopterobia curvula *with chloroplast partly intact. x 350*

Surirella

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Surirellales

How to recognise the genus:

Microscopic: Surirella species have an "external" raphe. They can be oval to rounded, sometimes with a "waist" at the middle. The "keel" (containing the raphe) encircles the entire valve. Prominent ribs lead onto it. Valves are generally rectangular in girdle view with the ribs visible at each side. There is a single large lobed chloroplast arranged as a plate against each valve face, joined by a narrow "bridge".

Possible confusion: The valve views of *Surirella* and *Epithemia* (page 168) species are occasionally confused.

Surirella linearis

Elongated oval, more or less symmetrical, sometimes with a slight "waist" in the middle. From 40 to 100 µm long and 10 to 20 µm wide. Ribs round the edge quite large and widely spaced.

Habitat and distribution: Seems to occur in a range of conditions, from low-conductivity to enriched. Widespread, can be quite common.

Surirella angusta

Smaller with parallel (or slightly rounded) sides, conical ends and closely spaced ribs. Typical size 20-30 µm long and about 10 µm wide.

Habitat and distribution: Widely found in nutrient-rich lowland rivers and streams.

Surirella brebissonii var. *kuetzingii*

Small and rounded, slightly pointed at one end. Usually 10-20 µm wide.

Habitat and distribution: Not certain, but has been recorded as common in a stream in farmland in the Catlins region.

Note: *S. minuta* (not illustrated) also occurs in enriched streams. It is elongated egg-shaped with parallel ribs, 10-45 long and 10 µm wide.

S. tenera (not illustrated) occurs occasionally in stream periphyton. This species is much larger (up to 200 µm long), an elongated oval shape, rounded at one end and much sharper at the other. The ribs are noticeably closer together at the ends of the valve than in the middle. (See Foged 1979 for illustrations.)

Many species of *Surirella* are extremely abundant in freshwater wetland areas.

from top:

Surirella linearis, *live cell showing large chloroplast. x 900*

Surirella *cf.* linearis, *showing ribs. x 900*

Surirella angusta, *live cell and cleaned frustule. x 1200*

Surirella cf. brebissonii var. kuetzingii, *live cell and cleaned frustule. x 1200*

Synedra

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Fragilariales

How to recognise the genus:

Macroscopic: Sometimes *Synedra* species can dominate the periphyton and may have the appearance of masses of short, thick, brown "filaments".

Microscopic: An araphid genus. *Synedra* are thin pencil-like cells, solitary or occasionally in fan-shaped colonies where the frustules attach to the substrate by one end. A couple of species form filaments like *Fragilaria*. A "pseudoraphe" runs the length of the cell on both valves often with a clear central area in the middle. In the larger species under high power LM, a single "rimoportula" (a small hole) is visible at each end of the valve. This is diagnostic for the genus. Two long chloroplasts, one lying against each valve face.

Possible confusion: The small species of *Synedra* can sometimes be confused with *Nitzschia* (page 177): check the chloroplasts, or examine under higher power. Also some types of *Synedra* and *Fragilaria* (page 151) may be impossible to distinguish without the use of an SEM.

Note: A range of different schemes has been published for classifying *Synedra* species. The following are suggested to cover the common forms found in New Zealand periphyton.

Synedra ulna

Very variable length, up to 200 µm long. Regular parallel striae and parallel margins tapering or rounded at the apices. (Some varieties differ in shape.) There is usually a clear central area (absent in some varieties).

Habitat and distribution: *Synedra ulna* seems to grow well in response to nutrients. It can dominate the periphyton in enriched lowland streams. The species is widespread and extremely common.

Synedra ulna *var.* contracta

As for *S. ulna*, except that the centre of the valve is slightly concave. Size, up to about 120 µm long.

Habitat and distribution: Frequently found in the periphyton of large lowland rivers. Widespread.

from top:

Synedra ulna *cf. var.* biceps*, growing in a colony joined face-to-face. x 270*

Synedra *cf.* acus, *from the Avon River, Christchurch, growing in star-shaped colonies, each cell attached at one end. x 270*

Synedra ulna*, x 900*

Synedra ulna *var.* contracta*. x 900*

Synedra ulna var. *biceps*

Very long cells (up to as much as 500 µm). No central area. Small spines along the valve margins allow the formation of colonies joined face-to-face (cf. *Fragilaria*).

Habitat and distribution: Found dominating enriched streams in summer. Probably widespread.

Synedra cf. *ulna* var. *ramesi*

Short, stubby with pinched ends and a narrowing at the centre of the valve. Striae fine and slightly irregular-looking. Size: up to 60 µm long.

Habitat and distribution: Seems to occur mostly in clean-water streams and rivers. It is can be quite common.

Synedra acus

The valve definitely tapers from the central area to the ends and there is no narrowing at the centre. 90–180 µm long.

Habitat and distribution: Common. Seems to be mostly in moderate conductivity streams.

Synedra rumpens

Small fine cells, 25–50 µm long and 2–4 µm wide. Central area apears to be very slightly swollen.

Habitat and distribution: Can be very abundant locally, but its small size and lack of detail make it difficult to discern. There may be confusion with *Fragilaria capucina* (see page 151). Indeed Williams and Round (1987) consider *S. rumpens* and *F. capucina* to be synonomous.

Synedra delicatissima

Very fine, needle-like cells tapering to the apices; up to about 200 µm long and 3–5 µm wide

Habitat and distribution: Not certain.

from top:

Synedra ulna *var.* biceps. *Half of valve. Note spines around the valve margin. Also note the rimoportula (islolated hole) near the tip of the valve. One at each end is characteristic of* Synedra *species. x 700*

Synedra ulna *var* ramesi. *x 1000*

Synedra acus, *live specimen. x 800*

Synedra rumpens. *x 1400*

Synedra delicatissima. *x 400*

Cosmarium

C: single cells (non-diatom)

Division: Chlorophyta, **Order:** Desmidales

How to recognise the genus:

Microscopic: Like other desmids, *Cosmarium* is clearly divided into two parts, visible in "face view" (see photographs). From the top, the shape is usually rounded or oval. There are many species. Those encountered in periphyton range from relatively small (around 25 µm across) to larger species such as *C. margaritatum* (illustrated).

Habitat and distribution: *Cosmarium* is most abundant in lakes and wetland areas. However some species regularly occur in the periphyton of streams and rivers and can be common.

Possible confusion: Potential confusion with some species of *Staurastrum* (see page 190) which have a triangular top view but may resemble *Cosmarium* in face view.

from top:

Cosmarium *cf.* margaritatum *from the Waitekauri River , Coromandel. x 400*

left: Cosmarium *cf.* impressulum *from the Waitekauri River , Coromandel. x 400; right: Cosmarium sp. x*

Closterium

C: single cells (non-diatom)

Class: Chlorophyta, **Order:** Desmidales

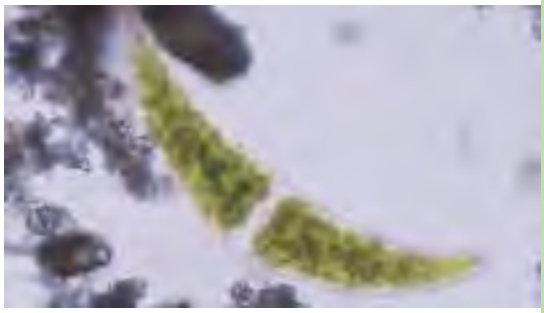
How to recognise the genus:

Microscopic: Crescent-shaped cells with an obvious division of chloroplasts at the centre (but no constriction to the outside of the cell). In live material you should be able to make out vacuoles at each end containing moving granules. Usually large – up to 500 µm long and 40 µm wide.

Habitat and distribution: The genus as a whole is more typical of lake than river habitats, but a large form often occurs in the periphyton. It can be quite common in moderately enriched (e.g., lowland agricultural) streams, in a mixed periphyton community.

Possible confusion: Unlikely.

Note: For a full account of Desmids in New Zealand, see Croasdale and Flint (1986, 1988) and Croasdale, Flint and Raine (1994).



from top:

Closterium *sp. from the Wairau River, North Canterbury. x 270*

A smaller species of Closterium. *x 270*

Staurastrum

C: single cells (non-diatom)

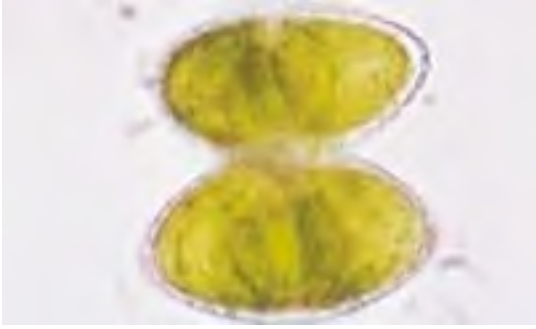
Division: Chlorophyta, **Order:** Desmidales

How to recognise the genus:

Microscopic: Similar to *Cosmarium*, but the top view is three-sided rather than rounded. The form found most often in periphyton resembles a solid triangle rather than the more delicate three-pronged planktonic form. Up to about 40 µm across.

Habitat and distribution: Very common planktonic genus in enriched ponds and lakes. The solid triangular forms can be common in the periphyton of lowland sreams and rivers.

Possible confusion: Possibly with *Cosmarium* (see page 189).



from top:

Side view of Staurastrum *sp., a "solid" type often encountered in periphyton. The top view is triangular. x 400*

The more delicate type of Staurastrum *normally seen as part of the plankton communitiy in ponds and lakes. x 400*

Ankistrodesmus

C: colonial

Division: Chlorophyta, **Order:** Chlorococcales

How to recognise the genus:

Microscopic: Small colonies that look like loose bundles of spindle-shaped cells, though the bundles seem to break apart easily and you often seen individual cells scattered among other taxa. Cells may be up to 100 µm long but are often much smaller. The chloroplast covers most of the cell wall and there is usually a pyrenoid.

Habitat and distribution: *Ankistrodesmus* can be common in the periphyton of low- to moderately enriched streams.

Possible confusion: There are other fine, spindle-shaped green algae, but these generally are not a common component of periptyon communities.

Ankistrodesmus *sp. x 650*

Dinobryon

C: colonial

Division: Chrysophyta, **Order:** Chrysomonadales

How to recognise the genus:
Microscopic: colonies of cup-like individuals, each containing an oval cell with a light yellow-green chloroplast. This is one of the golden-brown algae.

Habitat and distribution: Usually part of lake phytoplankon, but has occurred in periphyton of lake outflows.

Possible confusion: Unlikely.

Note: Another representative of the golden-brown algae sometimes found in periphyton is *Chrysocapsa* (Order Chrysocapsales), which forms colonies of round cells embedded in mucilage.

from top:

Dinobryon *sp. x 500*

A brown gelatinous coating of a colonial alga identified as Chrysocapsa*, found as dominant in summer/autumn low flows in a foothills Canterbury river with moderate conductivity.*

Gloeocystis

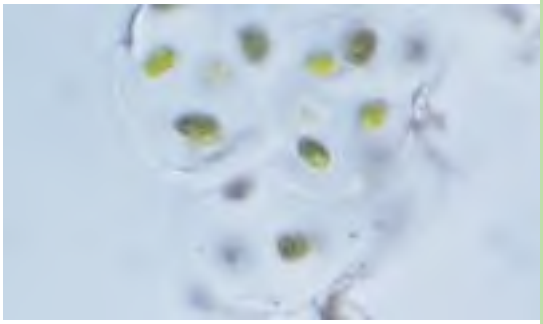
C: colonial

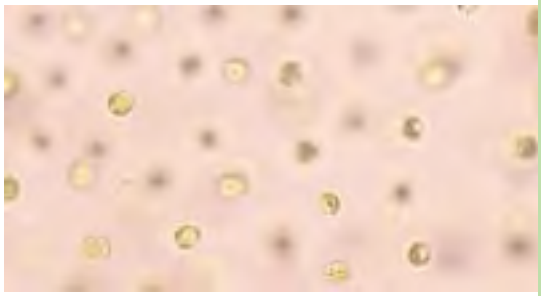
Division: Chlorophyta, **Order:** Tetrasporales

How to recognise the genus:
Microscopic: Small colonies of 4 or 8 green, spherical cells around 10 µm in diameter, each enclosed in a mucilage "envelope". The cells may separate during blending. The single chloroplast can cover the entire cell wall.

Habitat and distribution: Not certain. Has been found in abundance in a stream on the Coromandel Peninsula.

Possible confusion: There are many kinds of green colonial cells similar to *Gloeocystis* and all are difficult to identify. For example, the lower photograph is possibly *Palmella* (Order: Tetrasporales) in which many spherical cells (up to 10 µm across) are arranged in an amorphous mass with cell sheaths not always visible. It is often only possible to identify such green cells as "unknown colonial greens" or "unknown green unicells".





from top:

Gloeocystis*. Note the definite sheaths around groups of 4 cells. x 500*

Possibly Palmella*: smaller cells scattered in a mass of mucilage. x 500*

Pediastrum

C: colonial

Division: Chlorophyta, **Order:** Chlorococcales

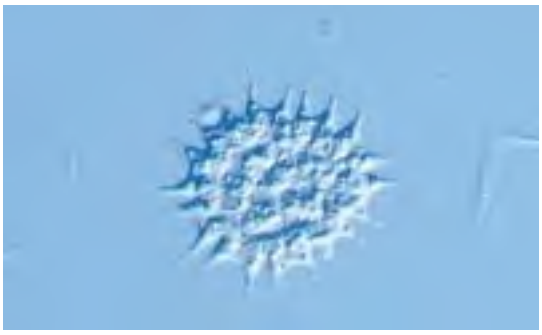
How to recognise the genus:

Microscopic: More or less circular colonies of lobed cells, up to about 100 µm across.

Habitat and distribution:

Mostly found in slow-moving streams. Not usually common.

Possible confusion: Unlikely.



Pediastrum*: a relatively small colony (about 40 µm across). x 600*

Scenedesmus

C: colonial

Division: Chlorophyta, **Order:** Chlorococcales

How to recognise the genus:

Microscopic: This genus grows as small colonies that look like stacks of oblong cells. Sometimes there are two stacks side-by-side and there are usually 4, 8 or 16 cells. The many species in this genus are distinguished on the basis of size and shape of the cells and the presence and form of spines. Cells may be up to 35 µm long. The chloroplast covers most of the cell wall and each cell has one pyrenoid.

Habitat and distribution: *Scenedesmus* can be extremely common in the periphyton of low- to moderately enriched streams, especially in the absence of other green algae. It occurs as "rare" or "occasional" in many periphyton samples.

Possible confusion: Unlikely.

Two different species of Scenedesmus*. The upper one is often common in periphyton. x 700*

Tetrastrum

C: colonial

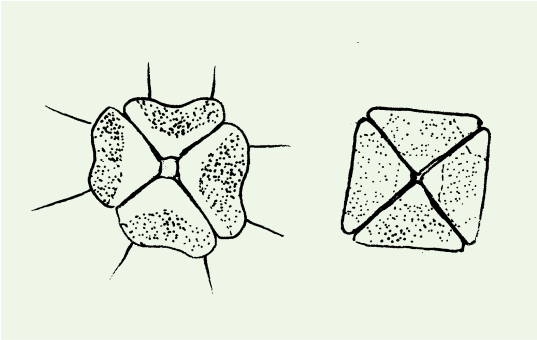
Division: Chlorophyta, **Order:** Chlorococcales

How to recognise the genus:

Microscopic: Colonies of four cells arranged in the shape of a cross. About 30 µm across.

Habitat and distribution: Not often found in periphyton, but has been recorded as common in a moderately enriched stream (Waitekauri River)

Possible confusion: Similar to *Crucigenia*. *Tetrastrum* has "spines" protruding from the cells, *Crucigenia* does not. Also *Tetrastrum* does not tend to form multiple colonies. In blended material it may be difficult to decide between them.



Diagrams of Tetrastrum *(left) and* Crucigenia *(right). Note that* Crucigenia *is often found growing in multiple groups.*

Enteromorpha

C: colonial (sheet-like)

Division: Chlorophyta, **Order:** Ulvales

How to recognise the genus:

Macroscopic: Thin, bright-green fronds, like a tiny seaweed.

Microscopic: Sheets of cells organised into hollow tubes a single cell thick, often with branches.

Habitat and distribution: Most species of *Enteromorpha* inhabit brackish or marine habitats. We have found the genus in high conductivity streams, for example in the Waipara River, Canterbury during summer low flows (measured conductivity about 180 µS/cm).

Possible confusion: Unlikely.

from top:

Enteromorpha*, from the Waipara River, Canterbury.*

Enteromorpha*. x 90*

Enteromorpha*. x 300*

Anabaena

BG: unbranched filaments

Division: Cyanobacteria, **Order:** Nostocales

How to recognise the genus:

Macroscopic: Usually no distinguishing macroscopic features but in lakes, some species of *Anabaena* can form severe toxic blooms.

Microscopic: Unbranched trichomes (filaments) may be straight or curved to spiral. Individual cells are rounded or barrel-shaped, and there are frequent intercalary heterocysts (appearing between vegetative cells), approximately the same size as the vegetative cells. Akinetes (reproductive cells), where present, are often much larger ellipsoidal to cylindrical, highly granular cells.

Habitat and distribution: We have found *Anabaena* species in slow-flowing high-country streams, mixed with green filamentous algae, diatoms and other cyanobacteria.

Possible confusion: *Anabaena* looks very similar to *Aphanizomenon* (not in this manual). However, the end cells of *Aphanizemenon* are elongated and sometimes narrowed.

from top:

Anabaena *sp., from an inland Canterbury stream, curved species. x 270*

Anabaena *sp. x 680*

Calothrix

BG: unbranched filaments

Divison: Cyanobacteria, **Order:** Nostocales

How to recognise the genus:

Microscopic: Relatively short trichomes growing from a heterocyst at the base of the filament and usually tapering to a fine point. The trichome is enclosed in a firm sheath. *Calothrix* may grow in star-like tufts or as solitary trichomes.

Habitat and distribution: *Calothrix* often grows amongst other algae and can be epi-phytic. It is not seen often in periphyton.

Possible confusion: *Rivularia* (page 200) also has trichomes tapering from a basal heterocyst, though these are smaller than *Calothrix*, and always arranged side-by-side in a macroscopic colony.

Note: *Calothrix* can have limited false branching, and merges into *Dichothrix* (see page 200).

Calothrix. *x 700 (photo: Donna Sutherland)*

Cylindrospermum

BG: unbranched filaments

Division: Cyanobacteria, **Order:** Nostocales

How to recognise the genus:

Microscopic: Light blue-green symmetrical trichomes with terminal heterocysts. Akinetes develop just beside the terminal heterocyst at both ends of the trichome in most species.

Habitat and distribution: Mostly in still waters but has been found in slow-flowing pool outlets.

Possible confusion: Could be confused with *Cylindrospermopsis*. However, the position of akinetes in *Cylindrospermum* is adjacent to the terminal heterocyst while in *Cylindrospermopsis* they are always slightly distant from the heterocyst.

Cylindrospermum. *x 400*

Leptolyngbya

BG: unbranched filaments

Division: Cyanobacteria, **Order:** Oscillatoriales

How to recognise the genus:

Microscopic: This genus includes most of the very narrow species (0.5–3 µm wide) previously included in *Phormidium* or *Lyngbya*. Little detail will be visible except under very high magnification. The filaments do not taper and are enclosed within a fine but distinct sheath The colour varies from pale blue-green to brown. The cylindrical cells are often shorter than they are wide, but can be longer. There are no specialised cells. The thallus comprises a free cluster of tangled trichomes.

Habitat and distribution: Clean-water upland streams and rivers. Probably widespread in New Zealand.

Possible confusion: *Leptolyngbya*, *Leibleinia*, and *Jaaginema* are all very similar in appearance. However, *Leibleinia* differs by its special epiphytic mode of life (see photo) and *Jaaginema* (not in this manual) has no sheath.

from top:

Masses of Leptolyngbya. *Outlet from tarn, Richmond Range, Nelson/Marlborough. x270*

Leibleinia *growing as an epiphyte on* Tolypothrix. *x 700 (photo: Donna Sutherland)*

Lyngbya

BG: unbranched filaments

Division: Cyanobacteria, **Order:** Oscillatoriales

How to recognise the genus:

Microscopic: Straight filaments that do not taper, usually blue-green, and with a definite sheath which is often evident beyond the tip of the trichome. The cells are usually disc-like, i.e., wider than they are long, and there are no heterocysts. Cell division is rapid, and often new cell walls can be seen forming even before the walls in the previous division are completed. Size varies from 5 µm to 25 µm across.

Habitat and distribution: Clean-water upland streams and rivers. *Lyngbya* is widespread in New Zealand.

Possible confusion: Some *Lyngbya* species resemble *Oscillatoria* (see page 197) and *Phormidium* (see page 198). *Oscillatoria* seldom has a sheath and the cells are disc-like; *Phormidium* usually has a sheath and the cells appear square.

Note also the appearance of *Heteroleibleinia* (formerly included in *Lyngbya*).

from top:

Lyngbya *from a clean mountain stream (Arthurs's Pass). x 270.*

Lyngbya*. x 600 (photo: Donna Sutherland)*

Possibly Heteroleibleinia*, previously included in* Lyngbya*. This can be quite common in clean streams. x 250 (photo: Nelson Boustead)*

Oscillatoria

BG: unbranched filaments

Division: Cyanobacteria, **Order:** Oscillatoriales

How to recognise the genus:

Macroscopic: Slimy, dark-coloured layers, or undistinguishable, mixed with other periphytic algae.

Microscopic: The trichomes are cylindrical, straight or slightly wavy. Akinetes and heterocysts are always absent. Sheath absent for most part, but some species will rarely produce sheaths under sub-optimal conditions. Trichomes can move by a waving (oscillation) action. The cells are characteristically discoid (i.e., cells wider than long) and are between 6–23 µm wide. A thickened cap (calyptra) sometimes forms on the end of older trichomes (this feature is age-related). Cell division occurs rapidly and often new cell walls can be seen forming before the walls in the previous division are completed.

Habitat and distribution: Widespread in New Zealand, and found in a wide range of conditions.

Possible confusion: *Lyngbya* (see page 196), *Phormidium* (see page 198) and *Oscillatoria* all look somewhat similar. *Phormidium* and *Lyngbya* typically have sheath (most easily seen extending beyond the end of the trichome).

from top:

Oscillatoria*, showing the thickened cap (calyptra) at the end (found in older trichomes). x 500*

Note the new cell walls forming within each narrow cell. x 270 (photo: Donna Sutherland)

As above. x 540 (photo: Donna Sutherland)

Phormidium

BG: unbranched filaments

Division: Cyanobacteria, **Order:** Oscillatoriales

How to recognise the genus:

Macroscopic: Forms expanded, gelatinous, dark green to black mats growing on stable substrates – stones, other alga, plants. These mats can be fine and thin to compact and leather like. Very rarely, single trichomes are scattered throughout a mixed periphyton community.

Microscopic: The cylindrical trichomes are slightly to intensely waved, and unconstricted or slightly constricted at the cross-walls. The sheaths when present, are firm and adherent to the trichome, never lamellated. Trichomes move with a gliding, trembling or waving action. Cells are typically box-like, that is as long as they are wide. Ther are no heterocysts.

Habitat and distribution: The genus is widespread in New Zealand, often very abundant in high-conductivity streams and rivers.

Possible confusion: *Phormidium*, *Lyngbya* (see page 196) and *Oscillatoria* (see page 197) all look somewhat similar. *Phormidium* has square, box-like cells, rather than the disk-like cells seen in the other two genera. Specimens that can not be distinguished between these three genera are typically provisionally placed in *Phormidium*.

from top:

Phormidium *mat. Waipapa River, Hawkes Bay.*

Phormidium *filaments (from the Waipara River, Canterbury). x 270*

Phormidium*. x 400*

Phormidium*. x 1400*

Nostoc

BG: filaments in gelatinous masses

Division: Cyanobacteria, **Order:** Nostocales

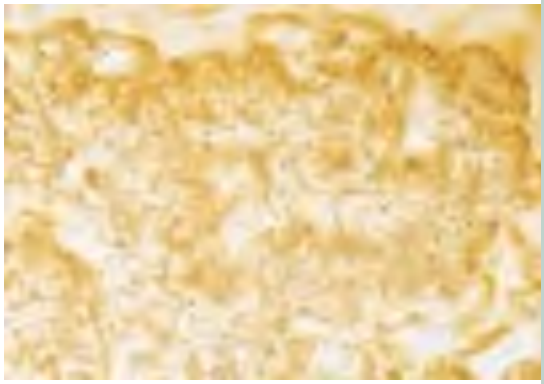
How to recognise the genus:

Macroscopic: Tough, irregularly shaped or rounded, gelatinous nodules, clear to yellow-brown to dark brown in colour; usually growing on stone substrates.

Microscopic: Under low power (up to 200x) *Nostoc* appears as a compact, cohesive mass in which numerous looped and twisted filaments are embedded. These may be denser around the outsides of the colony. Under higher power (up to 1000x) you can see that individual filaments are unbranched and comprise chains of bead-like rounded cells. (Don't mistake overlapping trichomes for branches.) The cells can be up to 8 µm in diameter, while the heterocycts are often slightly larger. Akinetes, where present, develop between adjacent heterocysts.

Habitat and distribution: *Nostoc* is usually found in fast-flowing relatively clean-water upland streams and rivers, growing attached to rocky substrates. Some species also grow on damp soil. The alga can become abundant and quite noticeable. It is widespread in New Zealand.

Possible confusion: Macroscopically, *Nostoc* is quite characteristic, though some types of green algae grow as tough nodules in similar environmental conditions. These are usually much darker than typical *Nostoc*. Under the microscope *Anabaena* (see page 194) and *Wollea* (not in this manual) look very similar to *Notsoc*. These three genera are distinguished on the basis of growth form – *Anabaena* never forms gelatinous colonies while the colonies of *Wollea* are long and tube-like with parallel trichomes.



from top:

Nostoc *growing on a stone taken from a fast-flowing hill-country stream in Otago.*

Nostoc colonies on Cladophora.

Nostoc *sp., showing edge of nodule. x 135*

Nostoc *trichomes. x 270.*

Rivularia

BG: filaments in gelatinous masses

Division: Cyanobacteria, **Order:** Nostocales

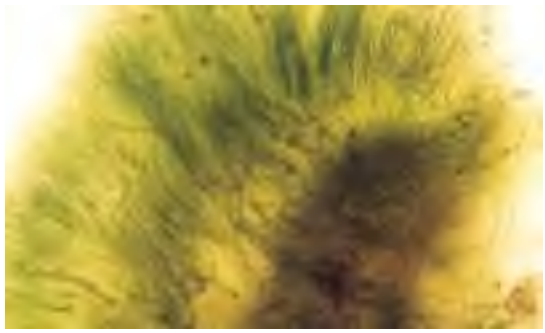
How to recognise the genus:

Macroscopic: Small dark raised hemi-spherical colonies attached firmly to substrates, up to 3 mm across.

Microscopic: Masses (colonies) of short tapering trichomes arranged parallel to each other, sometimes with false branching. Each trichome is enclosed within a sheath and a heterocyst is located at the base of each.

Habitat and distribution: *Rivularia* is usually found in fast-flowing relatively clean-water upland streams and rivers, growing attached to rocky substrates. Some species also grow on damp soil.

Possible confusion: *Gloeotrichia* (not included in this manual) is similar to *Rivularia* except that it has an akinete next to each heterocyst. *Gloeotrichia* colonies tend to be less firm than *Rivularia* and are usually found floating in lakes rather than in streams.



Rivularia*, Waiau River. x 70 (top) and x 135 (bottom). Note tapering filaments with a heterocyst at the base.*

Dichothrix

BG: branched filaments

Class: Cyanobacteria, **Order:** Nostocales

How to recognise the genus:

Macroscopic: Brown gelatinous layer.

Microscopic: Brown to blue-green branching trichomes. The heavily pigmented sheaths are either thin and firm or lamellated and several times wider than the trichome. Each trichome originates with a heterocyst and gradually tapers towards the end forming a hair-like structure. False branching always originates at a heterocyst (compare with *Scytonema*).

Habitat and distribution: Found mostly in lakes but also in clean-water streams. Probably widespread.

Possible confusion: *Tolypothrix* (see page 203) has a similar type of false branching, but the new branches part immediately from the original trichome. The hair-like tapered ends are absent in *Tolypothrix*.

from top:

Dichothrix. *Note false branching with trichomes running parallel before branching off. x 135*

Note thick sheath and tapering ends. x 270

Coleodesmium

BG: branched filaments

Division: Cyanobacteria, **Order:** Nostocales

How to recognise the genus:

Macroscopic: Tiny blue-green tufts up to 3 mm across, atttached to substrates in streams.

Microscopic: Bundles of trichomes that lie side by side and entwined around each other within a common sheath, then gradually branch off (with additional false branching) to form an expanded fan-like thallus. At the ends of the branches, each trichome lies within an individual, finer sheath and often can be seen breaking up to form rounded fragments. Heterocysts seem to be both intercalary and at the origins of false branches. The cells are slightly constricted at the cross walls. Trichomes typically 10 to 15 µm in diameter.

Habitat and distribution: *Coleodesmium* was first found in New Zealand in 1996 by Steven Moore of Otago Regional Council, and identified by Dr Paul Broady (University of Canterbury). It is apparently common in Otago, and has been found in Taranaki. We have recently (May 2000) found the genus in clean foothills streams in both Canterbury and Nelson/Marlborough. The genus may well be widespread.

Possible confusion: *Schizothrix* (not illustrated in this manual) also has multiple filaments within a sheath, with progressive branching. However, there are no heterocysts and spores do not form. (See Bourelly 1985 for a description of both genera).

from top:

Coleodesmium. *Note typical branching fan-like growth. x 70*

Note the heterocysts. x 270

Filaments showing sheath and individual cells. x 270

Scytonema

BG: branched filaments

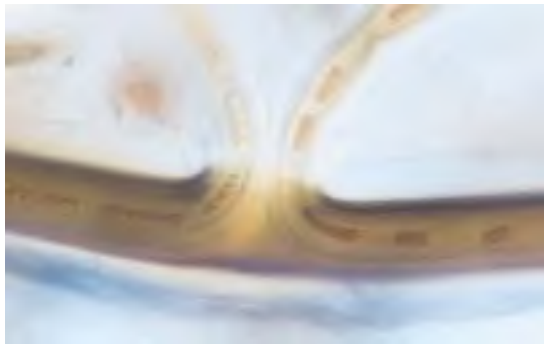
Division: Cyanobacteria, **Order:** Nostocales

How to recognise the genus:

Microscopic: Long trichomes with a definite base and tip, always enclosed in a usually heavily pigmented sheath. False branches always arise between two vegetative cells. Usually two branches arise beside one another, rarely only one. Heterocysts are always intercalary.

Habitat and distribution: Most often found in still waters, but can occur in outlet streams and rivers.

Possible confusion: *Tolypothrix* and *Scytonema* are habitually very similar. However, the false branches of *Tolypothrix* arise from heterocysts.



from top:

Scytonema *amongst other periphyton, Waiau River, Southland. x 90*

Branching in Scytonema*. Note wide sheath. x 680*

Stigonema

BG: branched filaments

Division: Cyanobacteria, **Order:** Stigonematales

How to recognise the genus:

Macroscopic: Tough, irregularly shaped, gelatinous nodules, yellow-brown to dark brown; usually on stone substrates.

Microscopic: Trichomes divided into main filament and branches, with the rounded cells generally in multiple rows. Heterocysts are either intercalary or absent. True branching occurs, i.e., a new branch grows directly from cells in the main stem (compare with false branching in *Dichothrix* and *Tolypothrix*).

Habitat and distribution: *Stigonema* is often found in damp habitats rather than in flowing water. The specimen photographed was from a shallow, clean, upland stream near Motueka, South Island, growing with other cyanobacteria on marble bedrock. Distribution not known.

Possible confusion: *Stigonema* is unlikely to be confused with any other taxon in this manual. A similar taxon, *Fischerella* (see photo) has cells in single rows.

from top:

Stigonema: *note the true branching, and multiple rows of cells. x 250*

Probably Fisherella, from a spring-fed stream, Canterbury. *There is true branching but the cells are mainly in single rows. x 70*

Tolypothrix

BG: branched filaments

Class: Cyanobacteria, **Order:** Nostocales

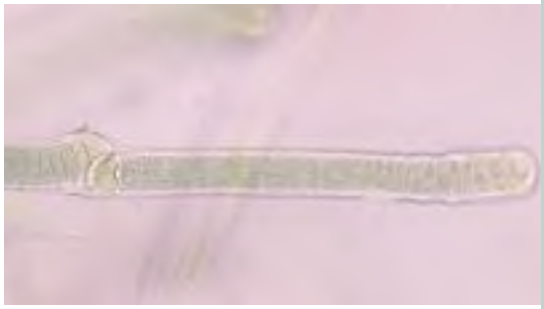
How to recognise the genus:

Macroscopic: Forms tangled, brown to yellow-brown clumps/tufts often mixed with other periphyton, or attached to rocks or aquatic plants.

Microscopic: Long, straight (not tapered) blue-green trichomes in a thin, firm sheath. Cells typically barrel-shaped and rounded at the edges forming a slight constriction between successive cells. *Tolypothrix* has false branches that always arise just below a heterocyst.

Habitat and distribution: *Tolypothrix* is more often encountered in lakes than rivers but we have found it in a range of rivers and streams. The genus seems to be associated with clean conditions and can be very abundant in hard-water streams.

Possible confusion: A distinctive genus. Possible confusion with *Dichothrix* (page 200). However, the latter has a thick brown sheath and tapering trichomes.



from top:

Tolypothrix *in a mixed periphyton community, Waiau River. x 270*

Tolypothrix*, the beginnings of a new "branch". x 400*

Chamaesiphon

BG: single cells

Division: Cyanobacteria, **Order:** Chamaesiphonales

How to recognise the genus:

Microscopic: Single cells forming polarized "pseudofilaments". The cells, encased in an open-ended sheath, are attached to the substrate at the narrow end. Division of cells occurs rapidly at the terminal end. Often you will see the new young cells separating off from the mother cell as round "exospores".

Habitat and distribution:

Chamaesiphon grows as an epiphyte on other periphyton, attached to larger substrates, e.g., wood. It is fairly widespread in fast-flowing waters throughout New Zealand, but not common. It seems more likely to occur in cleaner waters.

Possible confusion:

A distinctive genus, unlikely to be confused with any others.

from top:

Chamaesiphon *colonising* Tolypothrix*, from a foothills stream, Canterbury. x 350*

Long Chamaesiphon *cells on* Cladophora*, Moawhango River, central North Island. x 600*

Aphanocapsa

BG: colonial

Division: Cyanobacteria, **Order:** Chroococcales

How to recognise the genus:

Macroscopic: Gelatinous masses.

Microscopic: Usually pale blue-green globular cells, around 2 to 4 µm across, loosely distributed through a gelatinous mass.

Habitat and distribution: Most often in tarns and lakes, but does occur in outflow streams.

Possible confusion: *Aphanothece* and *Gloeothece* (not in this manual) also form colonies of cells embedded in mucilage. In both of these the cells are more oval or elongated than round and in *Gloeothece* there are sheaths around individual cells.

Aphanocapsa. *x 1400*

Merismopedia

BG: colonial

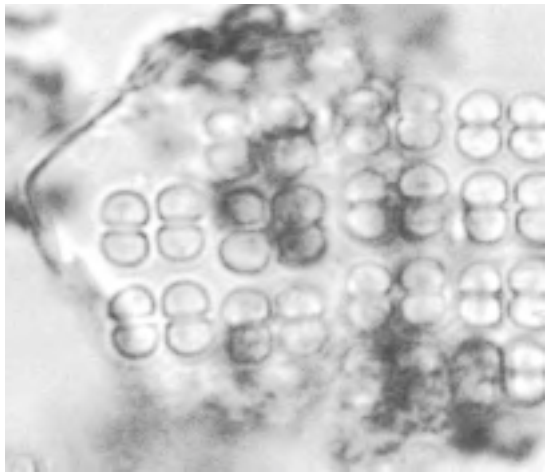
Division: Cyanobacteria, **Order:** Chroococcales

How to recognise the genus:

Microscopic: Flat colonies of round or oval blue-green cells arranged in mucilage (not always visible) in a regular right-angled criss-cross pattern. Species are determined from the number of cells in each colony (from 4 to hundreds) and the shape and size of individual cells. Cells are typically 3 to 6 µm across.

Habitat and distribution: Although usually found in lakes, *Merismopedia* occasionally appears in periphyton samples from a range of river types. The genus is normally rare.

Possible confusion: None. A distinctive genus.



Merismopedia. *x 700 (photo: Phillippe Gerbeaux)*

10.5 Glossary of terms used in taxonomic descriptions

akinete in cyanobacteria, specialised reproductive cell within the trichome.

araphid term for diatoms that have no raphe.

areolae in diatoms, holes or depressions on the valve surface that make up the striae (see puncta, which often means small holes).

axial area in diatoms, a break in the striae that runs along the middle of the valve; may contain the raphe system.

biraphid term for diatoms that have a raphe system on both valves.

capitate ending in a knob or head.

central area in diatoms, the part of the axial area at the centre of the valve.

chloroplast structure within a cell which holds the photosynthetic pigments.

convergent in diatoms, striae that are inclined from the valve margin towards the valve ends (plural: costae).

costa in diatoms, ribs of silica that run across the valve and appear under LM as heavy lines.

dorsal top or upper.

eukaryote refers to organisms in which cell inclusions are enclosed within membranes (includes all the algae except for cyanobacteria).

false in cyanobacteria, branches that form when a new trichome grows within the

branching same sheath as an existing trichome, rather than branches that form when cells divide in a different direction.

frustule the entire silica cell wall of a diatom.

genus the unit of classification next most detailed after species. Subsequent units are family, order, class, division.

girdle (also called the cingulum) bands of silica that join together the two parts (valves) of a diatom frustule.

girdle view diatoms seen from the side (i.e., you see the side of each valve, plus the girdle in between).

heterocsyt in cyanobacteria, cells within the trichome that are different from the normal vegetative cells; the site of nitrogen fixation.

hormogonium in cyanobacteria, part of the trichome that detaches as a reproductive body (plural hormogonia).

intercalary in cyanobacteria, in amongst the vegetative cells.

keel in diatom species whose valve is sharply angled at the raphe, the edge of the ridge where the raphe is situated.

keel puncta openings through the keel that look like a series of dots or lines under the light microscope

lamellated in layers.

monoraphid term for diatoms that have a raphe system on one valve only.

motile capable of moving.

naviculoid in diatoms, shaped like *Navicula*: symmetrical, lens-shaped.

parietal referring to chloroplasts: lying against the inside of the cell wall.

prokaryote refers to organisms that do not have cell inclusions (such as chloroplasts) contained in membranes, e.g., cyanobacteria.

pseudoraphe in araphid diatoms, a break in the striae as if a raphe was present.

puncta in diatoms, small openings or depressions that make up the striae.

pyrenoid structure within a cell where starch is stored.

radiate in diatoms, striae that are inclined from the valve margin towards the centre of the valve.

raphe in diatoms, a pair of slits in the valve face which appears as lines under the light microscope.

reticulate referring to chloroplasts, with holes, like lace or netting.

rimoportula in diatoms, an isolated hole lying at the edge of the valve face.

septum in diatoms, plates of silica within the girdle bands, lying parallel to the valve face (plural: septa) .

setae hair-like extensions from cells.

species usually the most detailed unit of biological classification (though some species are further split into varieties).

stigma in diatoms, isolated hole(s) on the valve face (plural stigmata).

stria in diatoms, a row of areolae, under low power LM appearing like a line (plural: striae).

taxon any taxonomic unit (species, genus, family, etc.)

thallus the overall form of an alga.

trichome in cyanobacteria, a multi-celled filament.

true branching branches that form by cells dividing in a different direction (compare with false branching in cyanobacteria).

valve in diatoms, one of the pair of silica structures that fit together to form the frustule.

valve view diatoms seen from the top (i.e., the face of the valve).

vegetative cells the ordinary cells of a filament (or a trichome in cyanobacteria), i.e., not reproductive cells, holdfast cells, etc.

ventral bottom or lower.

10.6 Recently re-named diatom genera, with their equivalent traditional names

The following table lists diatom genera mentioned in this manual which have new names as a result of re-classification of exisiting genera, or new descriptions. These changes are just a small proportion of the many revisions currently being applied in diatom taxonomy.

Revised classification	Traditional classification
Achnanthidium	*Achnanthes minutissima* group
Aulacoseira	some freshwater *Melosira* species
Brachysira	*Anomoeoneis vitrea* group
Cyclostephnos	*Stephanodiscus dubius* group
Encyonema	*Cymbella cesatii* group
Eunophora	newly described
Fragilariforma	*Fragilaria viriscens* group
Placoneis	*Navicula gastrum* group
Planothidium	*Achnanthes lanceolata* group
Pseudostaurosira	*Fragilaria brevistriata* group
Reimeria	*Cymbella sinuata*
Rossithidium	*Achnanthes linearis group*
Sellaphora	*Navicula pupula* group
Staurosira	*Fragilaria construens* group
Staurosirella	*Fragilaria leptostauron* group

10.7 Selection of texts that may be useful in stream periphyton identifications

Bourelly, P. 1981: Les Algues d'eau douce: Initiation à la systématique. Tome II: Les Algues jaunes at brunes. Boubee, Paris.

Bourelly, P. 1985: Les Algues d'eau douce: Initiation à la systématique. Tome III: Les Algues bleues et rouge. Boubee, Paris.

Bourelly, P. 1988: Les Algues d'eau douce: Initiation à a systématique. Compléments Tome I: Algues vertes. Boubee, Paris.

Bourelly, P. 1990: Les Algues d'eau douce: Initiation à la systématique. Tome I: Les Algues vertes. Boubee, Paris.

Cox, E.J. 1996. Identification of freshwater diatoms from live material. Chapman & Hall.

Croasdale, H. and Flint, E.A. 1986. Flora of New Zealand: freshwater algae, chlorophyta, desmids. Volume I. Christchurch: DSIR Botany Division.

Croasdale, H. and Flint, E.A. 1988. Flora of New Zealand: freshwater algae, chlorophyta, desmids. Volume II. Christchurch: DSIR Botany Division.

Croasdale, H., Flint, E.A. and Racine, M.M. 1994. Flora of New Zealand: freshwater algae, chlorophyta, desmids. Volume III. Christchurch: DSIR Botany Division.

Drouet, F. 1981: Revision of the Stigonemataceae with a summary of the classification of the blue-green algae. Nova Hedwigia 66: 1–221.

Entwistle, T.J.; Kraft, G.T. 1984. Survey of freshwater red algae (Rhodophyta) of south-eastern Australia. *Australian Journal of Marine and Freshwater Research 35*: 213–259.

Entwistle, T.J., Sonneman, J.A.; Lewis, S.H. 1998: Freshwater algae of Australia. a guide to the conspicuous genera. Sainty and Associates, NSW, Australia.

Etheredge, M.K.; Pridmore, R.D. 1987. The Freshwater Planktonic Blue-greens (Cyanophyta / Cyanobacteria) of New Zealand. A taxonomic guide. Water & Soil Miscellaneous Publication No. 111. Ministry of Works and Development, Wellington.

Foged, N. 1979: Diatoms in New Zealand. The North Island. *Bibliotheca Phycologica. Band 47*. J. Cramer, Vaduz.

Krammer, K.; Lange-Bertalot, H. 1991–1997. Bacillariophyceae. Vols 1–4. (Susswasserflora von Mitteleuropa; Bd. 2). Fischer, Stuttgart.

Patrick, R.; Reimer, C. W. 1966: The diatoms of the United States. Vol 1. Academy of Natural Sciences, Philadelphia.

Patrick, R.; Reimer, C. W. 1975: The diatoms of the United States. Vol. 2. Academy of Natural Sciences, Philadelphia.

Prescott, G. W. 1973: Algae of the Western Great Lakes area. Wm C. Brown, Iowa.

Prescott, G. W. 1954: How to know the freshwater algae. Wm C. Brown, Iowa.

Whitford, L. A.; Schumacher, G. J. 1973: A manual of freshwater algae. Spars Press, Raleigh, North Carolina.

10.8 List of diatom species with authorities and selected references for identification

Abbreviations used for references:

P&R Patrick and Reimer

KLB Krammer and Lange-Bertalot

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Achnanthes exigua Grunow 158
P&R 1966. p. 257. pl 16, f. 21-22.

Achnanthes inflata (Kutzing) Grunow 156
P&R 1966. p. 279, pl. 19, figs 15-16

Achnanthes oblongella Oestrup 158
KLB 1991-1997. vol. 2/4, p. 29, pl 16/1-14

Achnanthidium lanceolatum Brebisson 157
P&R 1966. p. 269, Pl 18, f 1-10 (as *Achnanthes lanceolata*; now *Planothidium lanceolatum* (see Round and Bukhityarova 1996)

Achanthidium linearis W. Smith 158
P&R 1966. p. 251, pl.16, f3-4 (as *Achnanthes linearis*; now *Rossithidium linearis* (see Round and Bukhityarova 1996).

Achnanthidium minutissimum (Kutzing) Czarnecki 157
P&R 1966. p. 253, pl. 16 figs 9-10 (as *Achnanthes minutissima*).

Amphora ovalis (Kutzing) Kutzing 160
KLB 1991-1997. vol. 2/1, p. 345, pl.149.

Amphora veneta var *capitata* (Haworth)
KLB 1991-1997. vol. 2/1, p. 349, pl.151/8. 160

Asterionella formosa Hassall
KLB 1991-1997. vol. 2/3, p. 103, pl. 103-104

Aulacoseira granulata (Ehrenberg) Simonsen 1979 148
KLB 1991-1997. vol. 2/3, p.22, pl 16/10); Foged 1979, pl. I (as *Melosira granulata*).

Brachysira serians (Brebisson in Kutzing) Round & Mann 161
Cox 1996. p. 84, fig. 27b, P&R 1966, p. 378, pl 33/1,2 (var acuta) (as *Anomoeoneis*).

Brachysira vitrea (Grunow) Ross 161
KLB 1991-1997. vol. 2/1, p. 256, pl 94/21-28 (as *Anomoeoneis*). (also listed in Foged 1979 as *Anomoeoneis exilis* var. *lanceolata*).

Cocconeis pediculus Ehrenberg 162
P&R 1966. p. 240, pl 15, figs 3-4.

Cocconeis placentula Ehrenberg 162
P&R 1966. p. 240, pl 15, fig 7

Cyclotella meneghiniana Kutzing1 163
KLB 1991-1997. vol. 2/3, p. 44, pl 44/1-10; Foged 1979, pl. III, fig. 6.

Cyclotella stelligera Cleve & Grunow 163
KLB 1991-1997. vol. 2/3 p. 50, pl 49/1-4; Foged 1979, p. 36, pl III, figs 8,9,11

Cymbella aspera (Ehrenberg) Peragallo 164
KLB 1991-1997. vol. 2/1, p. 319, pl 131/1; Foged, pl. 35, fig. 14

Cymbella cistula (Hempr.) Grunow var *gracilis* Hustedt 165
Foged 1979, p. 37, pl 35, fig. 12

Cymbella kappii Cholnoky 164
Foged 1979. p. 39, pl. 34, figs 8-10

Cymbella tumida (Brebisson) Van Heurck 165
KLB 1991-1997. vol. 2/1 p. 318, pl. 130/4-6; P&R 1975. p. 58, pl 10, f. 8

Diatoma heimale var. *mesodon* (Ehrenberg) Grunow 149
P&R 1966. p. 108

Diatoma tenuis Agardh 149
KLB 1991-1997. vol. 2/3. p. 97, pl 96, figs 8-10

Diatomella balfouriana Greville 165
P&R 1966. p. 297, pl. 20, figs 6-7

Diatomella parva Manguin 165
Foged 1979. p 43, pl XVI, figs 8,9

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Diploneis elliptica (Kutzing) Cleve 166
P&R 1966. p. 414. pl 38, fig 10;
KLB 1991-1997. vol 2/1, p. 285, pl. 16/4

Encyonema caespitosum Kutzing 167
P&R 1975, p. 41, pl 6, f 5-6 (as *Cymbella prostrata* v. *auerswaldii*); Cox 1996 p. 57, fig 18c; KLB 1991-1997. vol. 2/1 p. 310, pl. 121/12-16, 122/1-2 (as *Cymbella caespitosa*)

Encyonema gracile Rabenhorst 167
KLB 1991-1997. vol. 2/1, p. 308, pl. 120/1-16 (as *Cymbella gracilis*). Also in P&R 1966. p. 46, pl 7, figs 11-14 as *Cymbella lunata.*

Encyonema minutum (Hilse in Rabenhorst) D.G. Mann 166
P&R 1975, p. 47, pl 8, figs 3, 4 (as *Cymbella minuta*)

Encyonema prostratum (Berkeley) Kutzing 167
P&R 1975, p. 40, pl 6 f 4. (as *Cymbella prostrata*)

Epithemia adnata (Kutzing) Brebisson 168
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P&R 1975. pl 24 figs 3-4
(also in Foged 1979, pl XXXVIII as *E. zebra*)

Epithemia sorex Kutzing 168
P&R 1975. p. 188, pl 27, f. 4
Foged 1975. pl XXXVIII, f 2,6,10,11.
KLB 1991-1997. vol. 2/2, p. 154, pl. 106/1-14.

Eunotia serpentina Ehrenberg 150
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Fragilaria vaucheriae (Kutzing) Petersen 151
Foged 1979. p. 143, pl. 7
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Fragilaria capucina var *distans* (Grunow) Lange-Bertalot 151
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Fragilariforma viriscens (Ralfs) Williams & Round 152
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P&R 1966 p. 119, pl 3, fig. 9 as *Fragilaria*
(genus initially named *Neofragilaria*, renamed *Fragilariforma* (Williams and Round 1988)).

Frustulia rhomboides (Ehrenberg) De Toni 169
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P&R 1966. p. 306, pl 21, f. 5

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Gomphoneis minuta var. *cassieae* Kociolek & Stoermer 170
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Gomphonema acuminatum Ehrenberg 172
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Gomphonema angustatum (Kutzing) Rahenhorst 171
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(see note, page 171 of this guide)

Gomphonema angustum Agardh 172
KLB 1991-1997. vol. 2/1. p. 370, pl 164/1-6
P&R 1975, p. 134, pl. 18, fig. 1

Gomphonema clavatum Ehrenberg 172
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Gomphonema minutum (C. Agardh) C. Agardh 172
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Gomphonema parvulum Kutzing 171
P&R 1975. p. 123. pl 17 fig. 8
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Gomphonema truncatum Ehrenberg 172
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Gyrosigma scalproides (Rabenhorst) Cleve 173
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Meridion circulare (Greville) Agardh 174
P&R 1966. p. 113, pl 2, fig 15

Navicula capitoradiata Germain 175
KLB 1991-1997. vol. 2 / 1, p. 105, pl 32 / 12-15 (= *N. cryptocephala* v. *intermedia*, see Foged 1979, pl 29, f.12)

Navicula cryptocephala Kutzing 175
P&R 1966. p. 503, pl 48 f. 3;
KLB 1991-1997. vol. 2 / 1, p. 102, pl 31 / 8-11

Navicula lanceolata (Agardh) Ehrenberg 174
KLB 1991-1997. vol. 2 / 1, p. 100, pl. 29 / 5-7
(In Foged 1979, p. 68, pl XXIX f. 2-4 as *Navicula avenacea* (Brebisson) Cleve)

Navicula margalithi Lange-Bertalot 176
KLB 1991-1997. vol. 2 / 1, p. 95, pl 27 / 4-6
(n.b. uncertain identification. Compare with *N. tripunctata* (= *N. gracilis* in Foged 1979).

Navicula radiosa Kutzing 175
KLB 1991-1997. vol. 2 / 1, p. 99, pl. 29 / 1-4.
P&R 1966. p. 509, pl 48, f. 15 (also a smaller version, var. *parva*, f. 16)

Navicula rhynchocephala Kutzing 175
P&R 1966. p. 505, pl 48, fig. 6

Neidium affine (Ehrenberg) Pfitzer 176
P&R 1966. p. 390, pl 35 f.2
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Nitzschia gracilis Hantzsch 179
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Nitzschia inconspicua Grunow 178
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P&R 1966 p. 600, pl. 55 / 17-18. See also: Krammer 1992 (pl. 44)

Pinnularia microstauron (Ehrenberg) Cleve 180
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Pinnularia subcapitata Gregory 180
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Pinnularia viridis (Nitzsch) Ehrenberg 179
P&R 1966. p. 639, pl 64, f. 5;
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Placoneis placentula (Ehrenberg) Heinzerling 181
KLB 1991-1997. vol. 2 / 1, p. 145, pl 50 / 1-4
(as *Navicula placentula*, see Cox 1987)

Planothidium lanceolatum (see *Achnanthidium lanceolatum*)

Reimeria sinuata (Gregory) Kociolek & Stoermer) 181
P&R 1966. p. 51, pl 9, fig. 3-4 (as *Cymbella sinuata*, see Kociolek & Stoermer 1987).

Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot 182
KLB 1991-1997. vol. 2 / 1, p. 381, pl. 91 / 20-28
(usually recorded as *R. curvata*; *R. abbreviata* seems to be now accepted as the correct name as it precedes the species name *curvata*.)

Rhopalodia novae-zelandiae Hustedt 182
Foged 1979, p. 103, pl XXXIX, fig 1.

Rhopalodia operculata (Agardh) Hakansson 183
KLB 1991-1997. vol. 2 / 2. p. 165, pl 115 (note: see *R. musculus* in Foged 1979, pl XL, f. 1-3)

Rossithidium linearis (see *Achnanthidium linearis*)

Sellaphora bacillum (Ehernberg) Mann 183
KLB 1991-1997. vol. 2 / 1, p. 187, pl 67 / 3 (as *Navicula bacillum*, see Mann 1989)

Sellaphora pupula (Kutzing) Mann 183
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P&R 1966. p. 495, pl 68, figs 4,9. (as *Navicula pupula*)

Stauroneis anceps Ehrenberg 184
P&R 1966. p. 360, pl 30, fig. 1.

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KLB 1991-1997, vol. 2 / 3, p. 159, pl. 133 / 33-41 (as *Fragilaria leptostauron*)

Stenopterobia curvula (W. Smith) Krammer 185
KLB 1991-1997. vol. 2 / 2, p. 209, pl 170 – 172. (in Foged as *Stenopterobia intermedia* Lewis)

Stenopterobia delicatissima (Lewis) Brebisson 185
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(in Foged 1979 as *Surirella delicatissima* Lewis)

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P&R 1966. p. 148, pl. 7 fig 11
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P&R 1966. p. 150. pl 7 fig. 3

Synedra ulna var. *ramesi* (Herib.) Hustedt 188
P&R 1966, p. 153, pl 6 fig. 1.

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KLB 1991-1997. vol. 2 / 3, p. 146, pl 121 / 1-5;
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P&R 1966. p. 143, pl .5, f. 19

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P&R 1966. p. 57, pl. 4, fig. 5

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Appendix 1

Nonparametric methods and equivalence tests

Graham McBride, NIWA, Hamilton, May 2000

This manual has discussed a number of statistical techniques that may be thought of as "standard"; certainly they are in wide use. However there are two items about which some elaboration has been thought desirable. The first bears on the increasing use of "nonparametric" techniques. These are of wide applicability because they require fewer assumptions than the more traditional methods and can be fruitful in some cases. The second bears on more fundamental questions of how we may, or may not, make useful inferences using the standard null hypothesis testing procedure. An alternative procedure – equivalence tests – is outlined.

Nonparametric methods

Many statistical procedures are built on the idea of sampling from a known distribution-commonly the normal distribution. This distribution has two "parameters" – the mean and the standard deviation. It is completely defined once these two parameters are specified. Such "parametric" procedures are always the best to use when we have strong grounds to believe that we are indeed sampling from distributions that are reasonably normal.

But we often encounter datasets that suggest that we are sampling from a distinctly non-normal distribution. Most usually they are right-skewed, i.e., having a small number of very high values with the rest being much smaller. Such a pattern may compromise the behaviour of the normal methods and other more appropriate methods may give better results. Two alternative approaches may be taken to this problem.

The first uses transformations. Most typically we may take logarithms of the data, adding 1 to each datum first if any zeroes are present (the logarithm of zero is undefined). This is appropriate when the data are right-skewed and so the logarithms' distribution becomes much more normal-looking (logarithms have the effect of reducing large numbers to much smaller numbers, but small number are reduced much less, e.g., $\log_{10}(100) = 2$ and $\log_{10}(10) = 1$). Parametric methods are then applied to these transformed data, and, by taking the antilog of the result, we get the final answer in the original measurement scale of the data. Some (at times overlooked) features of this approach should be noted:

- the parameter about which hypotheses are made is typically not the arithmetic mean but the geometric mean;
- when transforming data back to the original scale some statistical bias is introduced (Gilbert 1987: 149), for which correction procedures are available (Gilbert 1987: 165).

The second alternative notes that we may have data that look neither reasonably normal nor lognormal. While in some cases other transformations could possibly be found, it can be wiser to dispense with the need to invoke any distribution at all. This is done by replacing each datum by its rank, i.e., the largest datum is assigned rank 1, the next rank is assigned rank 2, etc. This approach, while preserving the relative order of the data, replaces the actual differences between adjacent data by 1 ranking unit. It therefore loses information, but has the advantage of not requiring a particular distribution to be assumed. Methods based on this approach are called nonparametric (parameters are not required, because a distribution is not assumed), or, equivalently, distribution-free. They require fewer assumptions than do

normal methods, but they do invoke some (for which the interested reader may refer to Conover 1980 or Johnson 1995).

If sampling is from the normal distribution, parametric tests will always be more powerful than nonparametric tests. But if sampling is from distinctly non-normal distributions, nonparametric tests tend to have more powe – sometimes much more (e.g., in the case of highly skewed distributions, typical of microbiological concentrations). It is worth noting that the hypotheses tested by nonparametric procedures are often in terms of some form of rank statistic, usually the median. Since the true median and geometric mean of a lognormal distribution are identical (Gilbert 1987) there seems good reason to prefer the more generally-applicable nonparametric approaches as we may often be dealing with distributions that are neither particularly normal nor lognormal.

Details of how to perform these tests are available in good texts (Conover 1980; Sokal & Rohlf 1981; Iman & Conover 1983; Zar 1984, 1996; Gilbert 1987). A brief summary for commonly encountered tests on means and medians is given below.

Type of data	Parametric test	Equivalent nonparametric test
Two sets of samples	t-test	Wilcoxon Rank Sum test*
A set of paired samples	Paired t-test	Wilcoxon Signed Rank test
Several sets of samples	Anova	Kruskal-Wallis test

*This is essentially the same as the Mann-Whitney test. It is sometimes called the Wilcoxon–Mann-Whitney test.

Note also that there are nonparametric statistics that measure correlation. The commonly-quoted correlation coefficient (r") is actually Pearson's (parametric) correlation coefficient which measures the degree of linear correlation; if x,y pairs of data fall exactly on an upward-sloping straight line then $r = 1$ (if the line is downward-sloping then $r = -1$). If the y data tend to increase as x increases, but in a jagged or curvilinear fashion, r may be considerably lower than 1, yet the data are strongly correlated. In this situation it is appropriate to use the nonparametric analogue of Pearson's r. This is Spearman's rho (sometimes denoted by rS). It measures the degree of monotonicity in the relationship between x and y, whereas r measures the degree of linearity. One can obtain a rather higher value of rS than of r, showing that relying on r alone as the "correlation coefficient" can be misleading.

Because most modern software includes some nonparametric options it is a good idea to use both parametric and nonparametric procedures on the same dataset and compare the results obtained. This has the desirable effect of forcing the investigator to explain why any differences arise and which result is to be relied upon.

Equivalence tests

There has been a long tradition of using two-sided "null hypothesis" tests in many of the sciences. The tested hypothesis posits that there is no difference whatsoever between tested parameters. These tests are usually presented (e.g., by Zar 1996) as clear-cut, well-established procedures that adequately answer many questions relating to inferences about our environment.

Some question this view (e.g., Johnson 1999, Germano 1999). In particular, what relationship is there between the null hypothesis and a research hypothesis? Let's take the case of a study

of the impact of gold mining operations on stream invertebrates (McBride 1998, 1999) – it could equally well apply to stream periphyton. The former hypothesis, being "null", posits that the difference in species richness between upstream and downstream sites is exactly zero. But a research hypothesis is not "null". It will be along the lines of "whatever difference is present, it is not ecologically important, and so we will not infer that an impact has occurred". Procedures for such hypotheses do exist, and are often called "equivalence tests". They require the investigator to state the minimum value of the difference that would be considered to be environmentally important. This must be done before the test can be performed. Contrast this with the situation in performing a null hypothesis test (e.g., a *t*-test) using standard software-the investigator is not required to state the null test value (it is assumed to be zero).

Fundamental nature of the hypotheses

A two-sided null hypothesis cannot be true: the probability that two parameters (e.g., mean periphyton densities) are exactly equal is vanishingly small. In contrast, either a one-sided hypothesis or a two-sided interval hypothesis can be true. That is, the difference could be either less than or greater than a critical value, or it may be within or beyond a critical interval.

Interpreting the test result

If the null hypothesis is rejected "impact" will be inferred, with the investigator using the phrase that a "statistically significant difference" has been found. That hypothesis may not be rejected of course, in which case it is often mistakenly inferred that there is "no difference".

If the research hypothesis is rejected one might use the language of being "confident that an ecologically important impact has occurred". If it is not rejected one may regard the upstream and downstream sites as "equivalent" (yet recognising that they are in fact different), so saying there probably was not an impact. Accordingly, one can argue that this procedure, and not the null hypothesis testing procedure, is relevant to examination of the research hypothesis.

Performing equivalence tests

Procedures for testing equivalence hypotheses do exist.

If one is concerned about only increases (or decreases) in periphyton then one-sided tests (parametric or nonparametric) can be performed. These tests are well explained in current texts.

If there is interest in either increases or decreases then two-sided tests of an interval are appropriate. Procedures for performing these tests have been developed in the drugs-testing agencies (Chow & Liu 1992) but are only just becoming available for environmental scientists (McBride 1998, 1999). A reliable spreadsheet calculator (with accompanying commentary) for the stream invertebrate species richness case described in these two articles is available from the author (g.mcbride@niwa.cri.nz), and will be available at our website later (http://www.niwa.cri.nz/_private/pgsf/stats/index.html). It is easily extensible to other data. Nonparametric equivalence tests procedures also exist, though are not embedded in the above software.

Why the fuss?

At the risk of being repetitious, the two-sided null hypothesis procedure tests a barren hypothesis. This posits that there is no impact whatsoever, i.e., mean upstream and downstream invertebrate densities or periphyton densities are exactly equal. It is barren because it cannot be true. There will always be some impact, however small. Yet the procedure assumes the hypothesis to be true and only rejects it if data are in some way convincing to the

contrary. That "convincing" is encapsulated in the test's *p*-value, which is the probability of getting data at least as extreme as was obtained if the hypothesis were true. This *p*-value has the unfortunate property of tending to get ever smaller as the number of samples is increased, so that with a very large number of samples one would almost always reject the null hypothesis – precisely because it isn't true. As a consequence we have to note these important features:

- null hypotheses may be rejected but should never be "accepted", merely "not rejected";
- the "minimum detectable difference" tends to become ever-smaller as the number of samples is increased, and as a consequence;
- comparisons of the "statistical significance" attained in various studies is only valid if the numbers of samples are the same (or nearly so) in each case.

If one tests an interval (equivalence) hypothesis these difficulties are very much diminished, precisely because we are then testing a hypothesis that can be true. We do not invoke a barren hypothesis and attempt to shoehorn it into a meaningful scientific research programme; either hypothesis (i.e., the tested hypothesis or its alternative) is potentially fruitful and this can be argued to be more in conformity with actual science practice (Chalmers 1978, Veiland & Hodge 1998).

Isn't all this a bit contorted?

One can argue that it is indeed so. Questions that may arise to an enquiring mind include:

- Why is the calculation of the p-value based on all data at least as extreme as was obtained? (it is based on a consideration of data that was not obtained?!)
- How then does the *p*-value actually constitute "evidence"?
- Why can't the procedures directly address the real question: the probability of there having been an environmentally important impact given the actual data obtained (and not more extreme data that were not obtained).

Such matters can be addressed using different statistical methods, known as likelihood and Bayesian methods (Hilborn & Mangel 1997, Royall 1998, Lee 1999). These can be applied to equivalence testing. While not appropriate to give all details here, readers should be aware of the potential for new and fruitful statistical methods becoming more readily available for environmental studies in the coming years. A Bayesian method is available in the above-noted spreadsheet.

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Predominant periphyton community types commonly found in New Zealand streams during summer low flows and their habitats
(secondary and filamentous taxa listed in decreasing order of abundance that they are usually found in communities)

Macroscopic appearance	Dominant taxon/taxa	Secondary filamentous taxa / Understorey taxa	Range in peak AFDM (g/m^2)	Range in peak chl. a (mg/m^2)	Typical habitat	Conductivity (µS/cm)	Comment
Oligotrophic habitats							
O1: Moderate to thin mats of light green to violet coloured filaments	*Audouinella hermanii*	*Cymbella kappii, Synedra ulna, Fragilaria vaucheriae*	< 15	< 40	Widespread over unenriched to moderately enriched streams in hard rock catchments with no to extensive pastoral agriculture.	50 - 200	*Audouinella*-dominated communities require a very stable substrata. Often found mixed with bryophytes and willow roots submerged in the streams. Can form a violet-red mat on bedrock and boulders. Usually firmly attached.
O2: Forms a light brown-green film on rocks.	*Lyngbya* sp.	*Synedra ulna, Gomphoneis, Navicula avenacea*	< 35	< 150	Moderate to unenriched foothills-fed streams draining tussock or beech forest catchments, generally with hard rock geology. Predominantly low biomass on cobbles in swifter waters.	< 100	Appears highly resistant to removal by small floods and grazing. Strongly attached basal pad of cells. Can be found quite abundantly in mesotrophic – eutrophic streams subject to high grazing pressure. Taxonomic designation of this representative of *Lyngbya* a little uncertain. Looks somewhat like *Amphithrix*.
O3: Small dark brown-black patches	*Schizothrix / Calothrix / Lyngbya*		< 5	< 10	Unenriched, stable bed foothills streams in forested catchments with hard rock geology.	< 80	These communities appear to be highly grazer resistant and may also dominate mesotrophic or eutrophic streams subject to high grazing pressure.
O4: Short to long mats of green filaments	*Ulothrix zonata*	*Spirogyra* spp., *Oedogonium* spp. / *Synedra ulna, Cymbella kappii, Gomphoneis, Gomphonema parvulum, Fragilaria vaucheriae*	< 40	< 200	Common in unenriched streams draining bush and alpine catchments. May dominated more enriched streams in winter.	< 100	Tolerant of cold water and often forms large green filamentous mats along the periphery of high country streams. May occasionally form high biomass where groundwater discharges into streams or in winter in enriched streams.

Macroscopic appearance	Dominant taxon/taxa	Secondary filamentous taxa / Understorey taxa	Range in peak AFDM (g/m^2)	Range in peak chl. a (mg/m^2)	Typical habitat	Conductivity ($\mu S/cm$)	Comment
O5: Mucilaginous olive green or dark	Nostoc *sp.*	*Microspora* sp., , *Phormidium* spp., *Audouinella hermanii* / *Gomphoneis minuta* var. *cassieae*, *Synedra* spp., *Navicula* spp.	< 20	< 100	Unenriched streams, predominantly flowing form foothills areas (more commonly with tussock landuse and hard rock geology such as schist).	< 100	Forms conspicuous dark green mucilaginous balls on rocks in low velocity areas. May also proliferate in very damp / partially inundated grassy areas where it often becomes dark green / black as the out mucilage of the ball dries.
Mesotrophic habitats							
M1: Mats of yellow-green filaments	*Cladophora* sp.	*Oedogonium* spp., *Melosira varians* / *Gomphonema parvulum, Cymbella kappii, Synedra ulna, Cocconeis placentula, Navicula rhyncocephala*	25 - 35	100 - 300	Mixed scrub / pastoral tussock and or exotic forest with some Tertiary sediments in catchment. Mod. - low vel. runs, entangled on rocks / projections in v. shallow cobbly streams	100 - 250	Conspicuous in some unshaded high country streams of Hawkes Bay, Marlborough, and Canterbury in mid-late summer. Often collects near surface of stream wrapped around projections; coarse feel.
M2: Thin yellow-green film	*Fragilaria* spp. / *Gomphonema tenellum Synedra ulna* / *S. rumpens* / *Encyonema minutum* / *Gomphoneis*		< 35	< 200	Foothills or spring-fed streams that are moderately enriched. Catchments of unimproved tussock, scrub, or bush with hard rock geology.	< 80	Often these communities are maintained at a low biomass through intense invertebrate grazing. Overall these are the most commonly observed throughout the year in moderate to unenriched streams (thin films often dominated by *G. tenellum* in the unenriched streams).
M3: Thick white-brown mucilages (with olive-green surface)	*Gomphoneis* / *Cymbella kappii* / *Synedra ulna*	*Ulothrix zonata, Stigeoclonium lubricum* / *Cymbella kappii, C. minuta, Synedra ulna, Fragilaria vaucheriae*	< 50	50 - 200	Ubiquitous, but most common in foothills, mountain and spring-fed streams draining improved tussock catchments. Proliferates in riffles with localised enrichment from groundwater.	50 - 120	Often prolific in riffles. Also commonly attaches to macrophytes in swift springs and submerged willow tree roots. This community may also dominate highly disturbed systems regardless of degree of enrichment (e.g. glacier fed rivers), but at a very low biomass.

Macroscopic appearance	Dominant taxon/taxa	Secondary filamentous taxa / Understorey taxa	Range in peak AFDM (g/m^2)	Range in peak chl. a (mg/m^2)	Typical habitat	Conductivity ($\mu S/cm$)	Comment
M4: Small - large mats of olive green filaments	*Oedogonium* / *Microspora* / *Zygnema* spp.	*Spirogyra* spp., *Melosira varians, Microspora* sp. / *Synedra ulna, Cocconeis placentula, Navicula rhyncocephala*	10 - 40		Wide range of habitats. Conspicuous in moderately enriched foothills and lowland-fed gravel / cobble bed streams (with greywacke and / or basaltic geology) throughout New Zealand.	80 - 250	*Oedogonium* tends to be more dominant in the North Island in these habitats, whereas *Zygnema* and *Microspora* tend to be more dominant in the South Island. Difficult to discern dominant taxon without a microscope, though small *Oedogonium* mats may occur as small tufts of filaments (like small patches of green cotton wool) to forming huge mats with filaments metres long.
M5: Skin of dark brown - black on mucilage	*Phormidium* spp.	*Synedra ulna, Cymbella kappii, Gomphoneis, Cocconeis placentula, Gomphonema parvulum, Cymbella minuta*	< ? 20		Wide range, but more commonly in foothills streams with low - moderate enrichment from pastoral agriculture (greywacke and / or basaltic geology).	50 - 120	Conspicuous in generally low velocity areas on stable cobbles to silt throughout New Zealand after long periods (several months) without floods. Forms a dark brown skin over a mucilage base that usually has abundant diatoms and may be up to 1 cm thick.
M6: Loosely entwined mat of light to dark slimy green filaments	*Spirogyra* spp.	*Oedogonium* spp., *Cladophora* sp., *Phormidium* spp, *Stigeoclonium lubricum* / *Gomphoneis, S. ulna, Cymbella kappii, Gomphonema parvulum, A. lanceolatum, Cocconeis placentula*	< 20		Ubiquitous community, most commonly dominates moderately enriched to unenriched habitats	50 - 200	Most conspicuous as mats along the periphery of stream channels / braids during flow recessions. May form clouds of bright green filaments in pools or backwater areas. Filaments have a "slimy" feel.
M7: Bright green tufts of filaments	*Stigeoclonium lubricum*	*Gomphonema parvulum, Gomphoneis, Cymbella minuta, Cymbella kappii, Synedra ulna*	< 20	< 70	Often in moderate - unenriched foothills-fed cobble bed rivers, in moderately developed catchments generally with hard rock geology.	50 - 120	Forms bright green tree-like tufts on cobbles, particularly late in summer. Often associated with diatom mucilage.
M8: Light to dark green fibrous mats	*Vaucheria* spp.		20 - 80	200 - 1500	Wide distribution. Silty banks in oligotrophic streams to gravels in eutrophic habitats. Mostly mesotrophic habitats.	100 - 400	This taxon is easily identified in the field by its macroscopic, fibrous, matted growth form. The mats are generally attached by "rhizoid-like" structures. While some diatoms may be deposited in the mats, they are generally monospecific.

Macroscopic appearance	Dominant taxon/taxa	Secondary filamentous taxa / Understorey taxa	Range in peak AFDM (g/m^2)	Range in peak chl. a (mg/m^2)	Typical habitat	Conductivity ($\mu S/cm$)	Comment
Enriched habitats							
E1: Fluffy-brown filaments	*Melosira varians*	*Oedogonium* spp. / *Synedra ulna, Cocconeis placentula, Navicula cryptocephala, N. rhynchocephala*	20 - 35		Intensively developed pastoral and / or exotic forestry catchments with greywacke / hard sediment alluvium geology. Also common in some areas with andesitic volcanics and pastoral agriculture.	100 - 250	Often forms "sludgy brown" mats covering the sediments (sometimes as mats of very fragile blue / brown filaments). Common throughout N.Z., but particularly in Taranaki, Rangitikei / Wanganui, Wairarapa, Wellington, Nelson and Canterbury.
E2: Mats of yellow-green filaments	*Cladophora glomerata*	*Rhizoclonium* sp. / *Epithemia sorex, Cocconeis placentula, Synedra ulna, Cymbella kappii, Gomphoneis*	40 - 80	400 - 1000	Intensively developed pastoral catchments and / or catchments with a high proportion of Tertiary sediments.	200 - 450	Conspicuous in unshaded gravel-bed and lowland streams of Hawkes Bay / Gisborne, Manawatu / Wanganui and the Wairarapa in mid-late summer. Can form large floating mats and beds, as well as extensive mats along the periphery of channels. Blooms in low velocity runs and pools, particularly where temp. > 15 oC
E3: Mats of coarse green filaments	*Rhizoclonium* spp.	*Melosira varians* / *Cocconeis placentula, Synedra ulna, Cymbella kappii, Navicula avenacea, Rhoicosphenia curvata*	> 50	> 400	Intensively developed pastoral catchments with a high proportion of Tertiary sediments.	200 - 450	Conspicuous in unshaded lowland streams of Hawkes Bay / Gisborne, Manawatu / Wanganui and the Wairarapa in mid-late summer. Can form large floating mats and beds, as well as extensive mats along the periphery of channels. Mats have a very coarse wiry feel. Blooms in low velocity runs and pools, particularly where temp. > 15 oC
E4: Mats of whitish, fluffy, often branch slime	*Sphaerotilus natans, Zoogloea*	*Stigeoclonium tenue, Melosira varians* / *Cocconeis placentula, Cymbella* spp., *Fragilaria* spp., *Gomphonema parvulum, Nitzschia palea*	10 - 20	10 - 80	Associated with waste discharges high in low molecular weight BOD. Most likely to be found in lowland and spring-fed streams.		Commonly called "sewage fungus". Dominant organisms are actually filamentous bacteria. A rare occurrence now as most discharges are regulated to prevent proliferations of these communities.

Periphyton field identification chart. Part 1

(from the New Zealand Stream Health Monitoring and Assessment Kit)

For more information on each periphyton type, see page 45 in this Manual.

Thin mat or film (less than 0.5 mm thick)

Green

Light brown

Black/dark brown

Medium mat (0.5 to 3 mm thick)

Green

Light brown

Black/dark brown

Thick mat (more than 3 mm thick)

Green

Light brown

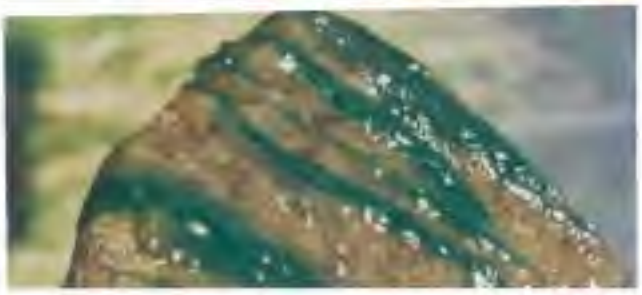
Black/dark brown

Periphyton field identification chart. Part 2

(from the New Zealand Stream Health Monitoring and Assessment Kit)

Short filaments (less than about 2 cm long)

Green



Brown/reddish

Long filaments (more than about 2 cm long)

Green

Brown/reddish

Method Summary: Rapid Assessment Method 1 (RAM-1)

Percentage cover of a site by filamentous green/brown algae for assessing compliance with aesthetic/recreational guidelines for proliferations

Equipment:

1. 2 tape measures (50 m and 20 m long).
2. 4 pegs (>20 cm long) and mallet.
3. Sampling quadrat.
4. Glass or clear plastic bowl.
5. Field data sheet.

Procedure:

- Select a reference point at the downstream end of your site and mark with a peg.
- Attach the 50 m tape to the peg and lay taut for its full distance (or 5 times the stream width).
- Mark 10 equally spaced intervals along the tape.
- Attach the end of the 20 m tape to a third peg and stretch across the stream and anchor.
- Divide the width of the stream (water's edge to water's edge) into 10 equally spaced intervals
- Place the sampling quadrat on the stream bed centred on the selected interval
- Hold the glass bowl on the surface of the water to obtain a clear view of the stream bed.
- Estimate the percentage cover within the quadrat of filamentous green/brown algae which have filaments >3 cm long and record on your field sheet. Move to the next point.
- Complete the transect then move the transect upstream for the pre-selected interval and repeat the recordings.
- When complete, enter the data onto a spreadsheet and calculate the mean percentage cover of the site by filamentous algae >3 cm long.

For a full version of this method, see Section 6.4.2 in: Biggs, B.J.F.: Kilroy, C. 2000. Stream Periphyton Monitoring Manual. Published by NIWA for Ministry for the Environment.

Method Summary: Rapid Assessment Method 2 (RAM-2)

Percentage cover of substrates by different categories of periphyton for general resource surveys and assessing broadscale effects of perturbations

Equipment:

1. 2 tape measures (10 m and 20 m long).
2. 4 pegs (>20 cm long) and mallet.
3. Small tea strainer (approximately 8 cm in diameter).
4. Field data sheet (preferably of water proof paper).
5. Periphyton field identification chart.

Procedure:

- Select a reference point at the downstream end of your site and mark with a peg.
- Attach the tape measure to the peg and lay it out taut for a distance of 10 metres (or 5 times the stream width, whichever is the smaller).
- Divide the distance along the tape into thirds and mark the tape.
- Attach the 20 m tape measure to a third peg at the location of the reference peg stretch the tape across the stream.
- Divide the width of the stream (water's edge to water's edge) into 5 equally spaced points.
- At the first point close your eyes and pick up the first stone that you touch.
- If the stream bottom is gravel, sand or silt, take a scooped sample with the tea strainer.
- Estimate the percentage cover of the stone in by periphyton in each category (± 5%) according to the field identification chart and enter this on the field data sheet.
- Complete the transect then move the tape upstream for the second transect at one-third interval and repeat the recordings.
- When complete, calculate the mean percentage cover of sampling points for each category of periphyton.

For a full version of this method, see Section 6.4.3 in: Biggs, B.J.F.: Kilroy, C. 2000. Stream Periphyton Monitoring Manual. Published by NIWA for Ministry for the Environment.

Method Summary: Quantitative Method 1a (QM-1a)

Whole cobble/gravel sampling

Equipment:

1. 20–30 m tape.
2. 2 pegs (>20 cm long) and mallet.
3. Deep sided laboratory tray or container.
4. Brushes.
5. Scalpel.
6. Squirt bottle.
7. Sample containers.
8. Calipers.

Procedure:

- Select a reference point and drive a peg into the ground.
- Attach the tape measure to the peg and lay it out taut across the stream.
- Divide the width of the stream (water's edge to water's edge) into 10 equally spaced intervals.
- Move out to the first point and with closed eyes pick up the first retrievable stone touched.
- Return it to the stream bank.
- Scrape off thick periphyton with scalpel and rinse stone and place in sample container.
- Then scrub the stone thoroughly with the brush and rinse.
- Transfer the contents of the white tray into your sample container.
- Finally, rinse the tray into the sample container until no trace of periphyton remains.
- Store the labelled container on ice in a chilli-bin (cooler) for transport to the laboratory.
- Measure the *x*, *y* and *z* dimensions of the stone with the calipers.
- Proceed to the next sampling point and repeat the above procedures.

For a full version of this method, see Section 6.5.4 in: Biggs, B.J.F.: Kilroy, C. 2000. Stream Periphyton Monitoring Manual. Published by NIWA for Ministry for the Environment.

Method Summary: Quantitative Method 1b (QM-1b)

Scraping or brushing a sample from a defined area on the top of a stone

Equipment:

1. 20–30 m tape measure.
2. 2 pegs (>20 cm long) and mallet.
3. Deep sided laboratory tray or container.
4. Scalpel.
5. Small scrubbing brushes.
6. Sample containers.
7. Pipettes / 'eye-dropper'.
8. Squirt bottle.
9. Sampling ring.

Procedure:

- Select a reference point in the middle of your site and on one bank drive a peg into the ground.
- Attach the tape measure to the peg and lay it out taut across the stream.
- Divide the width of the stream into 10 equally spaced intervals.
- Move out to the first point across the transect and with eyes closed pick up the first retrievable stone you touch.
- Return the stone to the stream bank.
- Define a circle on the top of the stone.
- Scrape away all the surrounding periphyton from the outside of the ring.
- Remove the ring and then scrape off periphyton with scalpel and rinse into a container.
- Scrub the sample area with a toothbrush then remove the slurry with the small pipette and some additional drops of water.
- Store the labelled container on ice for transport to the laboratory.
- For a mat of filaments slide your hand underneath the filaments, lift and press the ring down through the mat onto your palm to cut out a circle of the mat for the sample.

For a full version of this method, see Section 6.5.5 in: Biggs, B.J.F.: Kilroy, C. 2000. Stream Periphyton Monitoring Manual. Published by NIWA for Ministry for the Environment.

Method Summary: Live Algal Biomass

Chlorophyll *a*: ethanol – spectrophotometer method

Equipment:

1. Data sheets.
2. 90% Ethanol.
3. Filter papers.
4. Centrifuge tubes.
5. Forceps.
6. Vacuum filter.
7. Pipettes (5 ml and 0.1 ml).
8. Squirt bottle.
9. 0.36 molar HCl.
10. Water bath.
11. Spectrophotometer.
12. Pipettes set to 4 ml and 0.1ml.
13. Tissues.
14. 0.36-M hydrochloric acid

Procedure (filtering):

- Place 5 ml of 90% ethanol in tubes.
- Place fresh filter papers in each filtering chamber.
- Blend sample, shake the bottle and filter ~3 ↔ 5 ml aliquots (shake bottle between each).
- Remove fragments of leaves, mosses, invertebrates etc from filter paper with forceps.
- Apply suction.
- Record sub-sample volume.
- Rinse pipette.
- After filtering fold the paper in half, loosely roll up and place in centrifuge tube with ethanol.

Procedure (spectrophotometer analysis):

- Pre-heat water bath to 78°C (boiling point of ethanol).
- Immerse the racks of tubes in the bath for exactly five minutes.
- Place the racks in the refrigerator overnight.
- Warm up spectrophotometer.
- Set the wavelengths at peak for chlorophyll a for your spectrophotometer and 750 nm.
- Clean cuvette and take blank readings using 90% ethanol.
- Compress filter papers to the bottom of the centrifuge tubes, and re-close firmly.
- Centrifuge at 6000 rpm for 10 minutes.
- Pipette 4 ml of extract of first sample into the cuvette.
- Read absorbances at 665 (or wherever the chlorophyll *a* peak is on your spectrophotomer) and at 750 nm.
- Insert 0.1 ml of 0.3-M HCl in the cuvette, shake and analyse again at 665 and 750 nm.

Calculations:

Chlorophyll *a* (mg / sample) =

[(abs.665 before – abs.665 after) * 28.66 * sample vol. * extractant vol.]
/ (filtered sub-sample vol.)

(Subtract the respective turbidity blanks read at 750 nm from each reading first.)

For a full version of this method, see Section 7.1 in: Biggs, B.J.F.: Kilroy, C. 2000. Stream Periphyton Monitoring Manual. Published by NIWA for Ministry for the Environment.

Method Summary: Total Biomass

Ash-free dry mass

Equipment:

1. Data sheets.
2. Filter papers.
3. Crucibles, with number/ID mark, on metal trays.
4. Forceps.
5. Vacuum filter.
6. Glass beakers, stoppered bottles (marked at 50 ml intervals).
7. Blender.
8. Automatic pipettes (5 ml).
9. Squirt bottle.
10. Desiccator.
11. Muffle furnace
12. Drying oven
13. Precision balance.

Procedure (filtering and weighing):

- Place a filter paper in each crucible.
- Pre-ash in the muffle furnace at 400°C for two hours.
- If dry weight is required cool in desiccator and weigh.
- Place fresh filter papers in each filtering chamber.
- Blend sample, shake the bottle and filter ~3 ↔ 5 ml aliquots (shake bottle between each).
- Remove fragments of leaves, mosses, invertebrates etc from filter paper with forceps.
- Apply suction.
- Record sub-sample volume.
- Rinse pipette.
- After filtering fold the paper in half and place crucible.
- Dry the sub-samples for 24 hours at 105°C.
- Weigh each crucible after cooling in a desiccator.
- Ash for 4 hours at 400°C.
- Cool in desiccator and weigh.

Calculations:

Ash-free dry mass (g/sample) = [{(weight of crucible + filter + sample after drying) – (weight of crucible + filter + sample after ashing)} * sample volume] / [volume of filtered sub-sample]

Dry mass (g per sample) = [{(weight of crucible + filter after drying) – (weight of crucible + filter + sample after drying)} * sample volume] / [volume of filtered sub-sample]

For a full version of this method, see Section 7.2 in: Biggs, B.J.F.: Kilroy, C. 2000. Stream Periphyton Monitoring Manual. Published by NIWA for Ministry for the Environment.

Periphyton – the slimy coating that grows on the beds of streams and rivers – is an essential component of stream ecosystems, but can also be a management issue.

This *Stream Periphyton Monitoring Manual* is a follow-up to the *New Zealand Periphyton Guideline: Detecting, Monitoring and Managing Enrichment of Streams*. The latter publication reviews periphyton communities and their use as environmental indicators, and presents guidelines to prevent proliferations. This manual describes a standard set of methods to enable water managers and researchers to collect and analyse data on periphyton. Included are field sampling systems, laboratory procedures, microscope studies, data analysis techniques and an illustrated guide to the common types of periphyton found in New Zealand. The methods – based on internationally accepted protocols – have been used within NIWA for many years and are suitable for most streams and rivers in New Zealand.

NIWA, Christchurch

A STREAM VISUAL ASSESSMENT PROTOCOL (SVAP) FOR RIPARIAN LANDOWNERS

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Abstract. A user-friendly Stream Visual Assessment Protocol (SVAP) was recently developed in a joint effort by the Natural Resources Conservation Service (NRCS) of the U.S. Department of Agriculture and the University of Georgia. SVAP was designed to be an introductory screening–level assessment method for people unfamiliar with stream assessments. It was designed for use by NRCS field staff who work with agricultural landowners. NRCS is in a key position to influence conservation practices since the organization works with private stakeholders, maintaining more than 2000 field offices throughout the U.S. with a central office in each state. The SVAP measures a maximum of 15 elements and is based on visual inspection of the physical and biological characteristics of instream and riparian environments. Each element is assigned a numerical score relative to reference conditions and an overall score for the stream reach is calculated. A qualitative description of the stream reach is made based on overall numerical score. While SVAP is not intended to replace more robust stream assessment protocols, it provides quick and reliable information for use in NRCS farm assistance programs. It is also an educational tool through which landowners can learn about conservation of aquatic resources. An abridged copy of SVAP is attached as an appendix to this article and the complete document can be found on the web at http://www.ncg.nrcs.usda.gov/tech_notes.html.

Keywords: screening, stream assessment protocol, SVAP, water quality

1. Introduction

Stream assessments are carried out for many different reasons including: (1) detection of changes in stream conditions following a disturbance (natural or anthropogenic) or project implementation (e.g., Best Management Plans); (2) characterization of stream conditions for resource utilization (e.g., impoundments); (3) development of status reports as part of resource inventories, and (4) establishment of reference sites (USEPA, 1996; Yoder, 1995). Assessments provide a 'score' on environmental conditions of streams. Additionally, assessments may also provide diagnostic information helpful in identifying sources and causes of stream degradation.

There are many government and voluntary stream assessment and monitoring programs in the U.S.. Information resulting from these programs is used in land management, stream protection and restoration activities. There has been signific-

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ant development in stream assessment methodology over the past 20 yr, and the recent trend has been to move away from strictly quantitative approaches toward qualitative evaluations (Resh, 1991). Traditional biological assessment methods that incorporate in-depth sampling and analysis of numerous metrics have been replaced, in part, by rapid assessment protocols (Taylor, 1997; Resh *et al*., 1995; Resh and Jackson, 1993). These procedures have become very popular (Taylor, 1997) and are widely used by state and federal agencies in the U.S. (Plafkin *et al*., 1989; Barbour *et al*., 1992, 1996; Resh *et al*., 1995) and in other parts of the world (Wright *et al*., 1988; Chessman, 1995; Gowns *et al*., 1995). Despite criticisms of their effectiveness (Taylor, 1997), they provide useful information for many agencies and institutions. However, many of these protocols are region specific and/or require more resources (money, personnel, time, equipment) than are routinely available; consequently, the number of stream assessments conducted is severely limited.

Moreover, simple user-friendly stream assessment protocols have not been available to riparian landholders so that they can assess the environmental status of streams that drain their lands. This is unfortunate given that private lands constitute more than 70% of the entire landmass of the conterminous U.S. and Hawaii (NRCS, 1996). Low-order streams draining private lands represent a disproportionately large share of the fluvial system. Small, first- and second- order streams constitute almost 95% of all identified streams and rivers in the U.S. and account for about 75% of their collective length of 3.2 million miles (Leopold *et al*., 1964). However, sampling points for many of the monitoring and assessment programs (e.g., the U.S. Geological Survey's National Water Quality Assessment Program and Hydrologic Benchmark Network) are generally located on larger streams in order to broadly represent many of the cultural factors influencing water quality (USGS, 1996). Consequently, low-order streams are not included in routine assessments or monitoring programs. Additionally, stream assessments and long-term monitoring programs have been limited to a relatively few representative sites nationally because of budgetary, personnel, and other resource constraints. While these programs monitor many water quality constituents (e.g., fecal coliform, dissolved oxygen, biological oxygen demand, heavy metals, toxins) in large streams, there are few programs which assess ecological conditions of low-order streams. Nevertheless, the influence of these low-order streams on health and integrity of the entire aquatic ecosystem is well known (Burt, 1992).

The goal of this article is to introduce a simple and user-friendly stream assessment tool developed for riparian landowners to assess the environmental status of low-order streams draining their land. This Stream Visual Assessment Protocol (SVAP) was developed by the NRCS (NRCS, 1998a). We will discuss technical aspects of the protocol and identify applications for its use.

2. Development of the SVAP: Background Information

NRCS, a non-regulatory agency of the U.S. Department of Agriculture, is the successor to the Soil Conservation Service (SCS). Formed in 1935 to help the nation's farmers and ranchers implement more efficient and environmentally sound agricultural practices, the initial focus of the SCS was addressing soil erosion problems of crisis proportions. The current NRCS mandate has expanded to include all natural resource concerns on private lands (NRCS, 1996).

NRCS is in a key position to influence conservation practices over a large part of the U.S. It works extensively with agricultural producers and local communities and maintains a central office in each state and more than 2000 field offices (NRCS, 1996). Additionally, it has more than 60 yr of direct field experience working with landowners in a wide variety of environments.

A 1996 survey of NRCS state biologists indicated that less than a third of the state units were active in supporting stream assessments within their states. Most respondents said they would like their field staff to be more active in stream assessments and requested additional support from the NRCS national office. In response, an NRCS Aquatic Assessment Workgroup was formed. Workgroup members identified the need for a simple assessment protocol that could be used as a programmatic and educational tool. Development of the *Stream Visual Assessment Protocol* (SVAP) began in 1997 as a joint effort of the NRCS National Water and Climate Center, seven State NRCS offices, three NRCS Institutes, the US Environmental Protection Agency, and the University of Georgia. After field testing, it was issued in December 1998 (NRCS, 1998a). The SVAP is an introductory screening-level assessment method for people who are unfamiliar with stream assessments. It is not intended to replace more robust protocols. The protocol was developed as a tool to qualitatively characterize stream ecological condition and to help facilitate the work of NRCS personnel who work with riparian landowners. Participation by the landowner in making assessments is encouraged. By participating, the landowner learns about stream processes, signs of impairment, and effects of land use activities on ecological health and integrity[1]. The primary uses of SVAP by NRCS field staff include: 1) inventory and analysis steps in developing a conservation plan; 2) priority setting; and 3) pre- and post-assessments to evaluate the effectiveness of cost-share contracts and conservation plans.

[1] Ecological integrity implies the capacity to support and maintain a balanced, adaptive system (Karr, 1996) whereas ecological health also includes the notion of what society values in the ecosystem (Meyer, 1997).

TABLE I

Stream characteristics considered by SVAP

For all streams	For streams only where applicable
Channel condition	Canopy cover
Hydrologic condition	Manure presence
Riparian zone	Salinity
Bank stability	Riffle embeddedness
Water appearance	Macroinvertebrates
Nutrient enrichment	
Barriers to fish movement	
Instream fish cover	
Pools	
Invertebrate habitat	

3. Technical Aspects of SVAP

The SVAP is designed to be a basic assessment guide for non-scientists. It is a 'first-tier' assessment in a multi-tiered assessment framework. It is not intended to replace a biological survey or habitat inventory.

The SVAP was developed by drawing on existing visually-based assessment procedures (e.g., Georgia DNR, 1996; USEPA, 1997a, b). It is based on visual inspection of the physical and biological characteristics of instream and riparian environments and entails evaluation of up to 15 stream and riparian elements (Table I). Only those elements that are applicable for a given stream reach are evaluated. The user matches observed conditions to 4 or 5 narrative descriptions provided in the assessment protocol (see Appendix 1). A scoring sheet is used to record evaluations and other site descriptors. A score is assigned to each element based on the narrative descriptions. With the exception of the macroinvertebrate component, each element is rated from 1 to 10; the range of values for the macroinvertebrate element is –3 to 15. Highest scores represent a close match with reference site conditions and low scores represent a poor match. The overall score is the mean of the individual element scores. A qualitative description of 'excellent', 'good', 'fair' and 'poor' for each stream reach is assigned based on the overall numerical score. A copy of SVAP is included with this article as Appendix 1.

TABLE II

Summary of field study trials (Note FO refers to ‘field office’)

Location	No. of sites	No. of replicates	Assessment protocol that SVAP was compared to	Reference	SVAP conducted by	Source of data
CO	1	3	Professional judgment	–	FO personnel	T. Skadeland, NRCS, CO, pers. comm.
GA	9	4–5	Macroinvertebrates, EPT	a,b	FO personnel	S. Davis, Univ. of Georgia, pers. comm.
GA	1	12	Macroinvertebrates	a	FO personnel	L. Justice, NRCS, GA, pers. comm.
GA	1	None	Mussel taxa	c	FO personnel	J. Brim Box, USGS, UT, pers. comm.
GA	10	None	None	–	Engineer	R. Fuller, Univ. of Georgia, pers. comm.
MI	5	None	Professional judgment	–	State biologist	L. Sampson, NRCS, MI, pers. comm.
MI	24	2	GLEAS procedure # 51	d	Students	J. Lessard, Michigan State University, pers. comm.
NJ	3	3, 5, 8	NJIS rating	e	FO personnel	T. Dunne, NRCS, NJ, pers. comm.
NC/SC	90	None	IBI, EPT	f,b	Soil scientist	B. McQuaid, NRCS, NC, pers. comm.
OR	3	None	IBI	f	Scientist	B. Newton, NRCS, OR, pers. comm.
OR	2	3	None	–	FO personnel	B. Newton, NRCS, OR, pers. comm.
VA	56	3	IBI (fish), Ohio QHEI	f,g	FO personnel	B. Teels, NRCS, VA, pers. comm.
WA	3	None	Professional judgment	–	State biologist	B. Streif, NRCS, OR, pers. comm.

[a] Macroinvertebrates, Kellogg, L., 1992.
[b] EPT (Ephemeroptera, Plecoptera, Trichoptera). Plafkin, J. *et al*., 1989.
[c] Mussel taxa, Brim Box and Williams (in press).
[d] GLEAS Procedure # 51 (Great Lakes and Environmental Assessment Section), Michigan Dept. of Environmental Quality, 1997.
[e] NJIS (New Jersey Impairment Score), Kurtenbach, J., 1991.
[f] IBI (Index of Biological Integrity), Karr, J. *et al*., 1986.
[g] QHEI (Quality Habitat Evaluation Index), Rankin, E., 1989.

TABLE III

Summary table of agreement between SVAP and other assessment procedure scores (See Table II for references of the assessment protocols that SVAP was compared to)

Location	No. sites	Assessment protocol that SVAP was compared to	SVAP score[a]	Other procedure score	Correlation coefficient	Procedure conformity
GA	1	Mussel taxa	Good	Good-excellent	na.[b]	Good
GA	9	EPT	na.	na.	0.82	Good
GA	9	Chemicals[c]	na.	na.	0.42–0.05	Poor
MI	11	MIDEQ	na.	na.	na.	Poor
NJ	2	NJIS (macro)	Poor	Moderately impaired	na.	Good
NJ	1	NJIS (macro)	Poor	Not impaired	na.	Poor
NC/SC	90	IBI (macro)	na.	na.	0.19	Poor
NC/SC	90	EPT	na.	na.	0.25	Poor
OR	1	IBI (fish)	Poor	Poor	na.	Good
OR	1	IBI (fish)	Poor	Poor	na.	Good
OR	1	IBI (fish)	Fair	Good	na.	Fair
VA	56	IBI (fish)	na.	na.	0.63	Good
VA	56	Ohio QHEI	na.	na.	0.91	Good

[a] SVAP scores are overall average scores for stream reach.
[b] na. = Not applicable.
[c] Chemicals tested include NH_4-N, NO_3-N and PO_4-P.

4. Evaluation of SVAP

The SVAP has been extensively reviewed and tested in the field. All state NRCS offices were sent draft copies of the SVAP and asked to comment on the protocol. Ten states and the Bureau of Land Management responded. All comments were supportive and they were incorporated into the final version. Field tests to evaluate accuracy, precision, utility and ease of use involved more than 200 sites in the U.S. and 70 individuals (Table II). Sites included: low and high gradient, warm and cold water, perennial and annual streams. NRCS staff, other agency personnel and student volunteers conducted the testing. The majority of participants had little or no training or experience with aquatic resource assessment procedures.

In order to determine accuracy of the protocol, we compared stream assessment ratings from the SVAP with results obtained using other assessment procedures. These included macroinvertebrate indices, fish indices, and other protocols and procedures (e.g., the Ohio Quality Habitat Evaluation Index (QHEI) (Rankinx, 1989), the New Jersey Impairment Score (NJIS) (Kurtenbach, 1991) and the Michigan Great Lakes Environmental Assessment Section (GLEAS) Procedure #51 (Michigan Dept. of Environmental Quality, 1997)), chemical tests, and pro-

fessional judgment. Table III, which summarizes test results, shows a range of comparison values between SVAP and other assessment procedures. Comparisons were based on the overall numerical index rating or the qualitative description for a stream reach rather than on individual assessment elements. Factors that explain differences in assessment values or scores include: 1) variability in the participant's level of training and experience in stream assessments; 2) use of different drafts of the protocol during field testing; and 3) regional differences in stream types. Lack of training or unfamiliarity with the SVAP protocol generally resulted in higher scores for the SVAP elements. This observation is in keeping with other studies of assessment protocols, especially those based on visual cues (Resh *et al.*, 1995). The narrative scoring descriptions were less clear in earlier SVAP drafts than in later versions and contributed to misinterpretation and/or confusion when elements were scored. Notwithstanding scoring differences, results show that SVAP provided a 'reasonably good' characterization of stream ecological conditions.

Precision was determined by comparing stream assessment results obtained by trained individuals who independently assessed the same stream reach. There was only one test case where there were adequate replicates to provide statistically significant results: eleven replicates were compared at a test site in Americus, Georgia. The coefficient of variation was 8.8% for the overall stream score. The largest standard deviations were reported for the hydrologic alteration (2.3), canopy cover (2.4) and observed macroinvertebrate (2.8) elements while the mean standard deviation was 0.5.

5. Application of SVAP

The 1996 Farm Bill incorporates a number of incentive-oriented conservation programs such as the Environmental Quality Incentives Program, the Wildlife Habitat Incentives Program and the Wetlands Reserve Program (NRCS, 1996). These programs are targeted for privately held lands and offer technical and cost-sharing assistance to improve the health and integrity of streams and rivers. The SVAP can be used as a tool to assess stream conditions during the development and implementation phases of these programs. The protocol has also been introduced to other government agencies (e.g., USEPA, U.S. Forest Service, State Environmental Protection and Forestry Units), non-governmental organizations such as volunteer stream monitoring groups (e.g., Adopt-a-Stream) and private environmental businesses (e.g., environmental restoration and engineering businesses) through presentations and field demonstrations. Additionally, with minimal training, the landowner himself/herself can use SVAP to periodically check on changes in stream conditions.

Research shows that training improves the precision and accuracy results of visually-based protocols (Hannaford *et al.*, 1997; Diliey, 1992). Despite the user-friendly quality of SVAP, users should be trained in its use. Critical elements of training can include technical aspects of the SVAP, familiarization with the range

of conditions within the study area, identification of reference site characteristics, and basic stream ecology. In order to facilitate training in the use of SVAP, we developed the *Introduction to Steam Ecological Assessment Course* (NRCS, 1998b). This multi-media training course covers an introduction to stream ecology, how to use the SVAP, more advanced assessment protocols, stream classification, reference site selection, and technical support for stream assessments. It also includes field exercises. Materials to conduct the training course were provided to each NRCS state office.

While the national version of SVAP may be used for a wide range of low-order stream types, it can be modified to better reflect local geographic and environmental conditions. Modifying the protocol may result in better precision and accuracy, easier use, and a rating scale that is calibrated to regional criteria for qualitative assessments. Modifications may be made to individual elements and their narrative descriptions and/or to the rating scale for assigning overall qualitative assessments of excellent, good, fair and poor. The simplest approach to modify the protocol is based on professional experience and the judgment of an interdisciplinary team and includes developing, testing and evaluating proposed revisions. A second, more scientifically rigorous approach is an iterative process and involves a series of eight sequential steps. These steps include developing a stream classification system, assessing a range of sites that represent a gradient of environmental conditions, and evaluating the responsiveness of the revised SVAP to a range of stream conditions. Guidance on refining the protocol is included in the complete SVAP document. Despite the benefits of a modified SVAP, substantial revisions may complicate comparisons of SVAP scores obtained using versions based on different criteria and descriptions.

6. Conclusions

Field trials have demonstrated that SVAP is an effective introductory screening-level assessment method of ecological and 'health' conditions on most types of wadeable, low-order streams. While certain parts of this protocol may need to be modified to 'fit' specific local conditions, it can be used as a template for a preliminary assessment of streams throughout the U.S. NRCS field offices are using this protocol while working with landowners to implement conservation and management plans. Additionally, SVAP is being used at long-term ecological research sites (e.g., Coweeta, North Carolina) and is being adapted for use on tropical streams in Beliz (P. Esselman, University of Georgia, *pers. comm.*) and Costa Rica (C. Charpertier, Universidad Nacional de Costa Rica, *pers. comm.*).

The SVAP is intended to be an introductory screening–level assessment method for people unfamiliar with stream assessments and not a replacement for more advanced assessment procedures when they are needed. However, it is a suitable tool for many of the initial stream assessments identified by NRCS, and it also

serves as a 'hands-on' educational tool when working with the landowner. The principal strengths of SVAP include: 1) user-friendly; 2) low cost; 3) quick turn-around of results; 4) assessment information is easy to understand; 5) minimal training; and 6) procedure is environmentally benign. The complete 36 page NRCS Technical Note 99-1 (which includes the SVAP protocol, supporting documentation and background information) is on the NRCS website at http://www.ncg.nrcs.usda.gov/tech_notes.html. Please note the underscore between *tech* and *notes*.

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Appendix 1. Abridged version of SVAP

NRCS Stream Visual Assessment Protocol

Owner's Name _____ Evaluator's Name _____ Date _____

Stream Name _____ Waterbody ID Number _____

Reach Location _____

_____

Ecoregion _____ Drainage Area _____ Gradient _____

Applicable Reference Site _____

Land Use within Drainage (%): Row crop _____ Hayland _____ Grazing/pasture _____

Forest _____ Residential _____

Confined Animal Feeding Operations _____ Cons. Reserve _____

Industrial _____ Other _____

Weather Conditions – Today _____ Past 2-5 Days _____

Active Channel Width _____

Dominant Substrate: boulder _____ graven _____ sand _____ silt _____ mud _____

Site Diagram

Assessment Scores

Channel Condition	☐	Pools	☐
Hydrologic Alteration	☐	Invertebrate Habitat	☐
Riparian Zone	☐	*Score only if applicable*	
Bank Stability	☐	Canopy Cover	☐
Water Appearance	☐	Manure Presence	☐
Nutrient Enrichment	☐	Salinity	☐
Barriers to Fish Movement	☐	Riffle Embeddedness	☐
Instream Fish Cover	☐	Macroinvertebrates Observed (optional)	☐

Overall Score	< 6.0	POOR
(Total divided by number scored)	6.1–7.4	FAIR
_____	7.5–8.9	GOOD
	> 9.0	EXCELLENT

Suspected Causes of Observed Problems _____

_____

_____

_____

Recommendations _____

_____

_____

_____

_____

_____

_____

_____

Scoring Descriptions

Each assessment element is rated with a value of 1 to 10. Rate only those elements appropriate to the stream. Record the score that best fits the observations you make based on the narrative descriptions provided. Unless otherwise directed, assign the lowest score that applies. For example, if a reach has aspects of several narrative descriptions, assign a score based on the lowest scoring description that contains indicators present within the reach. You may record values intermediate to those listed. Some background information is provided for each assessment element, as well as a description of what to look for. The length of the assessment reach should be 12 times the active channel width.

Channel Condition

Natural channel; no structures, dikes. No evidence of downcutting or excessive lateral cutting.	Evidence of past channel alteration, but with significant recovery of channel and banks. Any dikes or levies are set back to provide access to an adequate floodplain.	Altered channel; <50% of the reach with riprap and/or channelization. Excess aggradation; braided channel. Dikes or levees restrict floodplain width.	Channel is actively downcutting or widening. >50% of the reach with riprap or channelization. Dikes or levees prevent access to the floodplain.
10	7	3	1

Stream meandering generally increases as the gradient of the surrounding valley decreases. Often, development in the area results in changes to this meandering pattern and the flow of a stream. These changes in turn may affect the way a stream naturally does its work, such as the transport of sediment and the development and maintenance of habitat for fish, aquatic insects, and aquatic plants. Some modifications to stream channels have more impact on stream health than others. For example, channelization and dams affect a stream more than the presence of pilings or other supports for road crossings.

Active downcutting and excessive lateral cutting are serious impairments to stream function. Both conditions are indicative of an unstable stream channel. Usually, this instability must be addressed before committing time and money toward improving other stream problems. For example, restoring the woody vegetation within the riparian zone becomes increasingly difficult when a channel is downcutting – banks continue to be undermined and the water table drops below the root zone of the plants during their growing season. In this situation, or when a channel is fairly stable but already incised from previous downcutting or mechanical dredging, it is usually necessary to plant upland, rather than hydrophytic, species and/or apply irrigation for several growing seasons. Extensive bank-armoring of channels to stop lateral cutting usually leads to more problems (especially downstream). Often stability can be obtained by using a series of structures (barbs, groins, jetties, deflectors, weirs, vortex weirs, etc.) that reduce water velocity, deflect currents, or act as gradient controls. These structures are used in conjunctions with large woody debris and woody vegetation plantings.

What to Look For

Signs of channelization or straightening of the stream may include an unnaturally straight section of the stream, high banks, dikes or berms, lack of flow diversity (e.g., few point bars and deep pools), and uniform-sized bed materials (e.g., all cobbles where there should be mixes of gravel and cobble). In newly channelized reaches, vegetation may be missing or appear very different (different species, not as well developed) from the bank vegetation of areas that were not channelized. Older

channelized reaches may also little or no vegetation or have grasses instead of woody vegetation. Drop structures (such as check dams), irrigation diversions, culverts, bridge abutments, and riprap also indicate changes to the stream channel.

Indicators of downcutting in the stream channel include nickpoints associated with headcuts in the stream bottom and exposure of cultural features, such as pipelines that were initially buried under the stream. Exposed footings in bridges and culvert outlets that are higher than the water surface during low flows are other examples. A lack of sediment depositional features, such as regularly-spaced point bars, is normally an indicator of incision. A low vertical scarp at the toe of the stream bank may indicate downcutting, especially if the scarp occurs on the inside of a meander. Another visual indicator of current or past downcutting is high stream banks with woody vegetation growing well below the top of the bank (as a channel incises the bankfull flow line moves downward within the former bankfull channel). Excessive bank erosion is indicated by raw banks in areas of the stream where they are not normally found, such as straight sections between meanders or on the inside of curves.

Hydrologic Alteration

Flooding every 1.5–2 yr. No dams, no water withdrawals, no dikes or other structures limiting the stream's access to the floodplain. Channel is not incised.	Flooding occurs only once every 3–5 yr; limited channel incision, **or** Withdrawals, although present, do not affect available habitat for biota.	Flooding only once every 6–10 yr; channel deeply, **or** Withdrawals significantly affect available low flow habitat for biota.	No flooding; channel deeply incised or structures prevent access to floodplain or dam operations prevent flood flows, **or** Withdrawals have caused severe loss of low flow, **or** Flooding occurs on a 1-year rain event or less.
10	7	3	1

Bankfull flows, as well as flooding, are important to maintaining channel shape and function (e.g. sediment transport) and maintaining the physical habitat for animals and plants. High flows scour fine sediments to keep gravel areas clean for fish and other aquatic organisms. These flows also redistribute larger sediments such as gravel, cobbles, and boulders, as well as large woody debris, to form pool and riffle habitat important to stream biota. The river channel and floodplain exist in dynamic equilibrium, having evolved in the present climatic regime and geomorphic setting. The relationship of water and sediment is the basis for the dynamic equilibrium that maintains the form and function of the river channel. The energy of the river (water velocity and depth) should be in balance with the bedload (volume and particle size of the sediment). Any change in the flow regime alters this balance. If a river is not incised and has access to its flood plain, decreases in the frequency of bankfull and out-of-bank flows decrease the river's ability to transport sediment. This can result in excess sediment deposition, channel widening and 'shallowing', and, ultimately, in braiding of the channel. Rosgen (1996) defines braiding as a stream with three or more smaller channels. These smaller channels are extremely unstable, rarely have woody vegetation along their banks, and provide poor habitat for stream biota. A 'split channel', however, has two or more smaller channels (called side channels) which are usually very stable, with woody vegetation along their banks, and provide excellent habitat. Conversely, an increase in flood flows or the confinement of the river away from its floodplain (from either incision or levees) increases the energy available to transport sediment and can result in bank and channel erosion.

The low flow or baseflow during the dry periods of summer or fall usually comes from groundwater entering the stream through the stream banks and bottom. A decrease in the low-flow rate will result in a smaller portion of the channel suitable for aquatic organisms. The withdrawal of water from streams for irrigation or industry and the placement of dams often change the normal low-flow pattern. Baseflow can also be affected by management and land use within the watershed – less infiltration of precipitation reduces baseflow and increases the frequency and severity of high flow events. For example, urbanization increases runoff and can increase the frequency of flooding to every year or more often and also reduce low flows. Overgrazing and clearcutting can have similar, although typically less severe, effects. The last description in the last box refers to the increased flood frequency that occurs with the above watershed changes.

What to Look For

Ask the landowner about the frequency of flooding and about summer low-flow conditions. A floodplain should be inundated during flows that equal or exceed the 1.5–2.0-year flow event. Evidence of flooding includes high water marks (such as water lines), sediment deposits, or stream debris. Look for these on the banks, on the bankside trees or rocks, or on other structures (such as road pilings or culverts).

Excess sediment deposits and wide, shallow channels could indicate a loss of sediment transport capacity. The loss of transport capacity can result in a stream with three or more channels (braiding).

Riparian Zone

Natural vegetation extends at least two active channel widths on each side.	Natural vegetation extends one active channel width on each side, **or** If less than one width, covers entire flood plain.	Natural vegetation extends 1/2 of the active channel width on each side.	Natural vegetation extends 1/3 of active channel width on each side, **or** Filtering function moderately compromised.	Natural vegetation less than 1/3 of active channel width on each side, **or** Lack of regeneration, **or** Filtering function severely compromised.
10	8	5	3	1

This element is the width of the natural vegetation zone from the edge of the active channel out onto the floodplain. For this element, the word natural means plant communities with (1) all appropriate structural components and (2) species native to the site or introduced species that function similar to native species at reference sites.

A healthy riparian vegetation zone is the most important element for a healthy stream ecosystem. The quality of the riparian zone increases with the width and the complexity of the woody vegetation within it. This zone (1) reduces the amount of pollutants that reach the stream in surface runoff, (2) helps control erosion, (3) provides a micro-climate that is cooler during the summer providing cooler water for aquatic organisms, (4) provides large woody debris from fallen trees and limbs that form instream cover, create pools, stabilize the streambed, and provide habitat for stream biota, (5) provides fish habitat in the form of undercut banks with the ‘ceiling’ held together by roots of woody vegetation, (6) provides organic material for stream biota that, among other functions, is the base of the food chain in lower order streams, (7) provides habitat for terrestrial insects that drop in the stream and become food for fish and habitat and travel corridors for terrestrial animals,

(8) dissipates energy during flood events, and (9) often provides the only refuge areas for fish during out-of-bank flows (behind trees, stumps, and logs).

The type, timing, intensity, and extent of activity in riparian zones are critical in determining the impact on these areas. Narrow riparian zones and/or riparian zones that have roads, agricultural activities, residential or commercial structures, or significant areas of bare soils have reduced functional value for the stream. The filtering function of riparian zones can be compromised by concentrated flows. There should be no evidence of concentrated flows through the zone or, if there are concentrated flows, they should be from land areas appropriately buffered with vegetated strips.

What to Look For

Compare the width of the riparian zone to the active channel width. In steep, V-shaped valleys there may not be enough room for a floodplain riparian zone to extend as far as one or two active channel widths. In this case, observe how much of the floodplain is covered by riparian zone. The vegetation must be natural and consist of all of the structural components (aquatic plants, sedges/rushes, grasses, forbs, shrubs, understory trees, and overstory trees) appropriate for the area. A common problem is lack of shrubs and understory trees. Another common problem is lack of regeneration. The presence of only mature vegetation and few seedlings indicate lack of regeneration. Do not consider incomplete plant communities as natural. Healthy riparian zones on both sides of the stream are important for the health of the entire system. If one side is lacking the protective vegetative cover, the entire reach of the stream will be affected. In doing the assessment, be certain that you examine both sides of the stream and note on the diagram which side of the stream has problems. There should be no evidence of concentrated flows through the riparian zone that are not adequately buffered before entering the riparian zone.

Bank Stability

Banks are stable; banks are low (at elevation of active flood plain); 33% or more of eroding surface area of banks in outside bends is protected by roots that extend to the base-flow elevation.	Moderately stable; banks are low (at elevation of active flood plain); less than 33% of eroding surface area of banks in outside bends is protected by roots that extend to the base-flow elevation.	Moderately unstable; banks may be low, but typically are high (flooding occurs 1 yr out of 5 or less frequently); outside bends are actively eroding (overhanging vegetation at top of bank, some mature trees falling into stream annually, some slope failures apparent).	Unstable; banks may be low, but typically are high; some straight reaches and inside edges of bends are actively eroding as well as outside bends (overhanging vegetaion at top of bare bank, numerous mature trees falling into stream annually, numerous slope failures apparent).
10	7	3	1

This element is the existence of or the potential for detachment of soil from the upper and lower stream banks and its movement into the stream. Some bank erosion is normal in a healthy stream. Excessive bank erosion occurs where riparian zones are degraded or where the stream is unstable due to changes in hydrology, sediment load, or isolation from the flood plain. High and steep banks are more susceptible to erosion and collapse. All outside bends of streams erode, so even a stable stream may have 50% of its banks bare and eroding. A healthy riparian corridor with a vegetated

flood plain contributes to bank stability. The roots of perennial grasses or woody vegetation typically extend to the baseflow elevation of water in streams that have bank heights of 6 feet or less. The root mass helps hold the bank soils together and physically protect the bank from scour during bankfull and flood events. Vegetation seldom becomes established below the elevation of the bankfull surface because of the frequency of inundation and the unstable bottom conditions as the stream moves its bedload.

The type of vegetation is important. For example, trees, shrubs, sedges, and rushes have the type of root masses capable of withstanding high streamflow events, while Kentucky bluegrass does not. Soil type at the surface and below the surface also influences bank stability. For example, banks with a thin soil cover over gravel or sand are more prone to collapse than are banks with a deep soil layer.

What to Look For

Signs of erosion include unvegetated stretches, exposed tree roots, or scalloped edges. Evidence of construction, vehicular, or animal paths near the banks or grazing areas that lead directly to the water's edge suggest conditions that may lead to the collapse of banks. Estimate the size or area of the bank affected relative to the total bank area. This element may be difficult to score during high water.

Water Appearance

Very clear, or clear but tea-colored; objects visible at depth 3–6 ft (less if slightly colored); no oil sheen or foaming on surface; no noticeable film on submerged objects or rocks.	Occasionally cloudy, especially after storm event, but clears rapidly; objects visible at depth 1.5–3 ft; may have slightly green color; no oil sheen on water surface.	Considerable cloudiness most of the time; objects visible to depth 0.5–1.5 ft; slow sections may appear pea-green; bottom rocks or submerged objects covered with heavy green or olive-green film, **or** Moderate odor of ammonia or rotten eggs.	Very turbid or muddy appearance most of the time; objects visible to depth <1/2 ft; slow moving water may be bright-green; other obvious water pollutants; floating algal mats, surface scum, sheen or heavy coat of foam on surface, **or** Strong odor of chemicals, oil, sewage, other pollutants
10	7	3	1

This element compares turbidity, color, and other visual characteristics with a healthy or reference stream. The depth to which an object can be clearly seen is a measure of turbidity. Turbidity is caused mostly by particles of soil and organic matter suspended in the water column. Water often shows some turbidity after a storm event because of soil and organic particles carried by runoff into the stream or suspended by turbulence. The water in some streams may be naturally tea-colored. This is particularly true in watersheds with extensive bog and wetland areas. Water that has slight nutrient enrichment may support communities of algae, which will provide a greenish color to the water. Streams with heavy loads of nutrients will have thick coatings of algae attached to the rocks and other submerged objects. In very degraded streams, floating algal mats, surface scum, or pollutants (such as dyes and oil) may be visible.

What to Look For

Clarity of the water is an obvious and easy feature to assess. The deeper an object in the water can be seen, the lower the amount of turbidity. Use the depth that objects are visible only if the stream is deep enough to evaluate turbidity using this approach. For example, if the water is clear but only 1 foot deep, do not rate it as if an object became obscured at a depth of 1 foot. This measure should be taken after a stream has had the opportunity to 'settle down' following a storm event. A pea-green color indicates nutrient enrichment beyond what the stream can naturally absorb.

Nutrient Enrichment

Clear water along entire reach; diverse aquatic plant community includes low quantities of many species of macrophytes; little algal growth present.	Fairly clear or slightly greenish water color along entire reach; moderate algal growth on stream substrates.	Greenish water color along entire reach; overabundance of lush green macrophytes; abundant algal growth, especially during warmer months.	Pea green, gray, brown water along entire reach; dense stands of macrophytes clog stream; severe algal blooms create thick algal mats in stream.
10	7	3	1

Nutrient enrichment is often reflected by the types and amounts of aquatic vegetation in the water. High levels of nutrients (especially phosphorus and nitrogen) promote an over-abundance of algae and floating and rooted macrophytes. The presence of some aquatic vegetation is normal in streams. Algae and macrophytes provide habitat and food for all stream animals. However, an excessive amount of aquatic vegetation is not beneficial to most stream life. Plant respiration and decomposition of dead vegetation consume dissolved oxygen in the water. Lack of dissolved oxygen creates stress for all aquatic organisms and can cause fish kills. A landowner may have seen fish gulping for air at the water surface during warm weather indicating a lack of dissolved oxygen.

What to Look For

Some aquatic vegetation (rooted macrophytes, floating plants and algae attached to substrates) is normal and indicates a healthy stream. Excess nutrients cause excess growth of algae and macrophytes, which can create greenish color to the water. As nutrient loads increase the green becomes more intense and macrophytes become more lush and deep green. Intense algal blooms, thick mats of algae, or dense stands of macrophytes degrade water quality and habitat. Clear water and a diverse aquatic plant community without dense plant populations are optimal for this characteristic.

Barriers to Fish Movement

No barriers	Seasonal water withdrawals inhibit movement within the reach.	Drop structures, culverts, dams, or diversions (<1 foot drop) within the reach.	Drop structures, culverts, dams, or diversions (>1 foot drop) within 3 miles of the reach.	Drop structures, culverts, dams, or diversions (>1 foot drop) within the reach.
10	8	5	3	1

Barriers that block the movement of fish or other aquatic organisms, such as fresh water mussels, must be considered as part of the overall stream assessment. If sufficiently high, these barriers may prevent the movement or migration of fish, deny access to important breeding and foraging habitats, and isolate populations of fish and other aquatic animals.

What to Look For

Some barriers are natural, such as waterfalls and boulder dams, and some are developed by humans. Note the presence of such barriers along the reach of the stream you are assessing, their size, and whether provisions have been made for the passage of fish. Ask the landowner about any dams or other barriers that may be present 3 to 5 miles upstream or downstream. Larger dams are often noted on maps, so you may find some information even before going out into the field. Beaver dams generally do not prevent fish migration. Look for structures that may not involve a drop, but still present a hydraulic barrier. Single, large culverts with no slope and sufficient water depth usually do not constitute a barrier. Small culverts or culverts with slopes may cause high water velocities that prevent passage.

Instream Fish Cover

>7 cover types available	6 to 7 cover types available	4 to 5 cover types available	2 to 3 cover types available	None to 1 cover type available
10	8	5	3	1

Cover Types: Logs/large woody debris; deep pools; overhanging vegetation;

boulders/cobble; riffles; undercut banks; thick root mats; dense macrophyte beds;

isolated/backwater pools; other: . . . . . .

This assessment element measures availability of physical habitat for fish. The potential for the maintenance of a healthy fish community and its ability to recover from disturbance is dependent on the variety and abundance of suitable habitat and cover available.

What to Look For

Observe the number of different habitat and cover types *within a representative subsection of the assessment reach* that is equivalent in length to *five times* the active channel width. Each cover type must be present in appreciable amounts to score. Cover types are described below.

Logs/large woody debris – Fallen trees or parts of trees that provide structure and attachment for aquatic macroinvertebrates and hiding places for fish.

Deep pools – Areas characterized by a smooth undisturbed surface, generally slow current, and deep enough to provide protective cover for fish (75–100% deeper than the prevailing stream depth).

Overhanging vegetation – Trees, shrubs, vines, or perennial herbaceous vegetation that hangs immediately over the stream surface, providing shade and cover.

Boulders/cobble – Boulders are rounded stones over 10 inches in diameter or large 'slabs' more than 10 inches in length; cobbles are stones between 2.5 and 10 inches in diameter.

Undercut banks – Eroded areas extending horizontally beneath the surface of the bank forming underwater pockets used by fish for hiding and protection.

Thick root – Dense mats of roots and rootlets (generally from trees) at or beneath the water surface forming structure for invertebrate attachment and fish cover.

Dense macrophyte beds – Beds of emergent (e.g., water willow), floating leaf (e.g., water lily), or submerged (e.g., riverweed) aquatic vegetation thick enough to provide invertebrate attachment and fish cover.

Riffles – Area characterized by broken water surface, rocky or firm substrate, moderate or swift current, and relatively shallow depth (usually less than 18 inches).

Isolated/backwater pools – Areas disconnected from the main channel or connected as a 'blind' side channel, characterized by a lack of flow except in periods of high water.

Pools

Deep and shallow pools abundant; greater than 30% of the pool bottom is obscure due to depth, or the pools are at least 5 feet deep.	Pools present but not abundant; between 10–30% of the pool bottom is obscure due to depth, or the pools are at least 3 feet deep.	Pools present but shallow; between 5–10% of the pool bottom is obscure due to depth, or the pools are less than 3 feet deep.	Pools absent or the entire bottom is discernible.
10	7	3	1

Pools are important resting and feeding sites for fish. A healthy stream has a mix of shallow and deep pools. A 'deep' pool is 1.6 to 2 times deeper than the prevailing depth, while a 'shallow' pool is less than 1.5 times deeper than the prevailing depth. Pools are abundant if a deep pool is in each of the meander bends in the reach being assessed. To determine if pools are abundant, look at a longer sample length than one that is 12 active channel widths in length. Generally 1 or 2 pools typically form within a reach as long as 12 active channel widths. In low order, high gradient streams, pools are abundant if there is more than one pool every 4 channel widths.

What to Look For

Pool diversity and abundance are estimated based on walking the stream or probing from the streambank with a stick or length of rebar. You should find deep pools on the outsides of meander bends. In shallow, clear streams a visual inspection may provide an accurate estimate. In deep streams or streams with low visibility, this assessment characteristic may be difficult to determine and should not be scored.

Insect/Invertebrate Habitat

At least 5 types of habitat available. Habitat is at a stage to allow full insect colonization (woody debris and logs not freshly fallen).	3–4 types of habitat. Some potential habitat exists, such as overhanging trees, which will provide habitat but have not yet entered the stream.	1–2 types habitat. The substrate is often disturbed, covered, or removed by high stream velocities and scour or by sediment deposition.	None to 1 type of habitat.
10	7	3	1

Cover types: Fine woody debris; submerged logs; leaf packs; undercut banks;

cobbles; boulders; coarse gravel; other: . . . . . .

Stable substrate is important for insect/invertebrate colonization. Substrate refers to the stream bottom, woody debris, or other surfaces on which invertebrates can live. Optimal conditions include a variety of substrate types within a relatively small area of the stream (5× the active channel width). Stream and substrate stability are also important. High stream velocities, high sediment loads, and frequent flooding may cause substrate instability even if substrate is present.

What to Look For

Observe the number of different types of habitat and cover within a representative subsection of the assessment reach that is equivalent in length to five times the active channel width. Each cover type must be present in appreciable amounts to score.

Score the Following Assessment Elements only if Applicable

Canopy Cover (if applicable)

Coldwater Fishery

>75% of water surface shaded and upstream. 2–3 miles generally well shaded.	>50% shaded in reach, **or** >75% in reach, but upstream 2–3 miles poorly shaded.	20–50% shaded.	<20% of water surface in reach shaded.
10	7	3	1

Warmwater Fishery

25–90% of water surface shaded; mixture of conditions.	>90% shaded; full canopy; same shading condition throughout the reach.	(intentionally blank)	<25% water surface shaded in reach.
10	7	3	1

Do not assess this element if active channel width is greater than 50 feet. Do not assess this element if woody vegetation is naturally absent (e.g., wet meadows).

Shading of the stream is important because it keeps water cool and limits algal growth. Cool water has a greater oxygen holding capacity than does warm water. When streamside trees are removed, the stream is exposed to the warming effects of the sun causing the water temperatures to increase for longer periods of time during the daylight hours and for more days during the year. This shift in light intensity and temperature will cause a decline in the numbers of certain species of fish, insects, and other invertebrates and some aquatic plants. They may be replaced altogether by other species that are more tolerant of increased light intensity, low dissolved oxygen, and warmer water temperatures. For example, trout and salmon require cool, oxygen-rich water. Loss of streamside vegetation (and also channel widening) that cause increased water temperature and decreased oxygen levels have been cited as major contributing factors to the decrease in abundance of trout and salmon from many streams that historically supported these species. Increased light and the warmer water also promote excessive growth of submerged macrophytes and algae that compromises the biotic community of the stream. The temperature at the reach you are assessing will be affected by the amount of shading 2 to 3 miles upstream.

What to Look For

Try to estimate the portion of the water surface area for the whole reach that is shaded by estimating areas with no shade, poor shade, and shade. Time of the year, time of the day, and weather can affect your observation of shading. Therefore, the relative amount of shade is estimated by assuming that the sun is directly overhead and the vegetation is in full leaf-out. First evaluate the shading conditions for the reach; then determine (by talking with the landowner) shading conditions 2 to 3 miles upstream. Alternatively, use aerial photographs taken during full leaf out. The following rough guidelines for percent shade may be used: stream surface not visible – >90; surface slightly visible or visible only in patches – 70–90; surface visible but banks not visible – 40–70; surface visible and banks visible at times – 40; surface and banks visible – <20.

Manure Presence (if applicable)

Intentionally blank	Evidence of livestock access to riparian zone.	Occasional manure in stream or waste storage structure located in the flood plain.	Extensive amount of manure on banks or in stream, **or** Untreated human waste discharge pipes present.
	5	3	1

Do not score this element unless livestock operations or human waste discharges are present.

Manure from livestock may enter the water if livestock have access to the stream or from runoff of grazing land adjacent to the stream. In some communities, untreated human waste may also empty directly into streams. Manure and human waste increase biochemical oxygen demand, increase the loading of nutrients, and alter the trophic state of the aquatic biological community. Untreated human waste is a health risk.

What to Look For

Do not score this element unless livestock operations or human waste discharges are present. Look for evidence of animal droppings in or around streams, on the streambank, or in the adjacent riparian zone. Well-worn livestock paths leading to or near streams also suggest the probability of manure in the stream. Areas with stagnant or slow-moving water may have moderate to dense amounts of vegetation or algal blooms, indicating localized enrichment from manure.

Salinity (if applicable)

Intentionally blank	Minimal wilting, bleaching, leaf burn, or stunting of aquatic vegetation; some salt-tolerant streamside vegetation.	Aquatic vegetation may show significant wilting, bleaching, leaf burn, or stunting; dominance of salt- tolerant streamside vegetation.	Severe wilting, bleaching, leaf burn, or stunting; presence of only salt-tolerant aquatic vegetation; most streamside vegetation salt tolerant.
	5	3	1

Do not assess this element unless elevated salinity due to anthropogenic sources is known to occur in the stream.

High salinity levels most often occur in arid areas and in areas that have high irrigation requirements. High salinity can also result from oil and gas well operations. Salt accumulation in soil causes a breakdown of soil structure, decreased infiltration of water, and potential toxicity. High salinity in streams affects aquatic vegetation, macroinvertebrates, and fish. Salts are a product of natural weathering processes of soil and geologic material.

What to Look For

High salinity levels cause a ‘burning’ or ‘bleaching’ of aquatic vegetation. Wilting, loss of plant color, decreased productivity, and stunted growth are readily visible signs. Other indicators include whitish salt encrustments on the stream banks and the displacement of native vegetation by salt-tolerant aquatic plants and riparian vegetation (such as tamarix or salt cedar).

Riffle Embeddedness (if applicable)

Gravel or cobble particles are <20% embedded.	Gravel or cobble particles are 20–30% embedded.	Gravel or cobble particles are 30–40% embedded.	Gravel or cobble particles >40% embedded.	Riffle is completely embedded.
10	8	5	3	1

Do not assess this element unless riffles are present or are a natural feature that should be present.

Riffles are areas, often downstream of a pool, where the water is breaking over rocks or other debris causing surface agitation. In coastal areas riffles can be created by shoals and submerged objects. (This element is sensitive to regional differences and should be related to reference conditions.) Riffles are critical for maintaining high species diversity and abundance of insects for most streams and for serving as spawning and feeding grounds for some fish species. Embeddedness measures the degree to which gravel and cobble substrate are surrounded by fine sediment. Embeddedness relates directly to the suitability of the stream substrate as habitat for macroinvertebrates, fish spawning, and egg incubation.

What to Look For

This assessment characteristic should be used only in riffle areas and in streams where this is a natural feature. The measure is the depth to which objects are 'buried' by sediment. This assessment is made by picking up particles of gravel or cobble with your fingertips at the fine sediment layer. Pull the particle out of the bed and estimate what percent of the particle was buried. Some streams have been so smothered by fine sediment that the original stream bottom is not visible. Test for complete burial of a stream bed by probing with a length of rebar.

Macroinvertebrates Observed (Optional)

Community dominated by Class 1 or intolerant species with good species diversity. Examples include: caddisflies, mayflies, stoneflies, hellgrammites.	Community dominated by Class 2 or facultative species such as damselflies, dragonflies, aquatic sowbugs, blackflies, crayfish.	Community dominated by Class 3 or tolerant species such as midges, craneflies, horseflies, leeches, aquatic earthworms, tubificid worms.	Very reduced number of species or near absence of all macroinvertebrates.
15	6	2	-3

This important characteristic reflects the ability of the stream to support aquatic invertebrate animals. However, successful assessment requires knowledge of the life cycles of some aquatic insects and other macroinvertebrates and the ability to identify them. For this reason, this is an optional element. The presence of intolerant insect species (cannot survive in polluted water) indicates healthy stream conditions. Some kinds of macroinvertebrates, such as stoneflies, mayflies, and caddisflies, are sensitive to pollution and do not live in polluted water; they are considered Class I. Another group of macroinvertebrates, known as Class II or faculative macroinvertebrates, can tolerate limited pollution and includes damselflies, aquatic sowbugs, and crayfish. The presence of Class III macroin-

vertebrates, including midges, craneflies and leeches, suggests the water is significantly polluted. The presence of a single Class I species in a community does not constitute good diversity and should generally not be given a score of 15.

What to Look For

You can collect macroinvertebrates by picking up cobbles and other submerged objects in the water. Look carefully for the insects; they are often well camouflaged and may appear as part of the stone or object. Note the kinds of insects, number of species, and relative abundance of each Class of insects/macroinvertebrates. *Note that the scoring values for this element range from –3 to 15.*

Glossary

Active channel width: The width of the stream at the bankfull discharge. Permanent vegetation generally does not become established in the active channel.

Aggradation: Geologic process by which a stream bottom or floodplain is raised in elevation by the deposition of material.

Bankfull discharge: The stream discharge (flow rate such as cubic feet per sec) that forms and controls the shape and size of the active channel and creates the floodplain. This discharge generally occurs once every 1.5 yr on average.

Bankfull stage: The stage at which water starts to flow over the floodplain; the elevation of the water surface at bankfull discharge.

Base flow: The portion of stream flow that is derived from natural storage; average stream discharge during low flow conditions.

Benthos: Bottom-dwelling or substrate-oriented organisms.

Boulders: Large rocks measuring more than 10 inches across.

Channel: A natural or artificial waterway of perceptible extent that periodically or continuously contains moving water. It has a definite bed and banks that serve to confine the water.

Channel roughness: Physical elements of a stream channel upon which flow energy is expended including coarseness and texture of bed material, the curvature of the channel, and variation in the longitudinal profile.

Channelization: Straightening of a stream channel to make water move faster.

Cobbles: Medium-sized rocks which measure 2.5–10 inches across.

Confined channel: A channel that does not have access to a floodplain.

Degradation: Geologic process by which a stream bottom is lowered in elevation due to the net loss of substrate material. Often called downcutting.

Downcutting: See degradation.

Ecoregion: A geographic area defined by similarity of climate, landform, soil, potential natural vegetation, hydrology, or other ecologically relevant variables.

Embeddedness: The degree to which an object is buried in steam sediment.

Emergent plants: Aquatic plants that extend out of the water.

Floodplain: The flat area of land adjacent to a stream that is formed by current flood processes.

Forb: Any broad-leaved herbaceous plant other than those in the Gramineae (Poceae), Cyperacea, and Juncaceae families (Society for Range Management, 1989)

Gabions: A wire basket filled with rocks. Used to stabilize streambanks and to control erosion.

Geomorphology: The study of the evolution and configuration of landforms.

Glide: A fast water habitat type that has low to moderate velocities, no surface agitation, no defined thalweg, and a U-shaped, smooth, wide bottom.

Gradient: Slope calculated as the amount of vertical rise over horizontal run expressed as ft/ft or as percent (ft/100 ft).

Grass: An annual to perennial herb, generally with round erect stems and swollen nodes; leaves are alternate and two-ranked; flowers are in spikelets each subtended by two bracts.

Gravel: Small rocks measuring 0.25–2.5 inches across.

Habitat: The area or environment in which an organism lives.

Herbaceous: Plants with non-woody stems.

Hydrology: The study of the properties, distribution, and effects of water on the Earth's surface, soil, and atmosphere.

Incised channel: A channel with a stream bed lower in elevation that its historic elevation in relation to the floodplain.

Intermittent stream: A stream in contact with the ground water table that flows only certain times of the year, such as when the ground water table is high or when it receives water from surface sources.

Macrophyte bed: A section of stream covered by a dense mat of aquatic plants.

Meander: A winding section of stream with many bends that is at least 1.2 times longer, following the channel, than its straight-line distance. A single meander generally comprises two complete opposing bends, starting from the relatively straight section of the channel just before the first bend to the relatively straight section just after the second bend.

Macroinvertebrate: A spineless animal visible to the naked eye or larger than 0.5 mm.

Nick point: The point where a stream is actively eroding (downcutting) to a new base elevation. Nick points migrate upstream (through a process called headcutting).

Perennial stream: A steam that flows continuously throughout the year.

Point bar: A gravel or sand deposit on the inside of a meander; an actively mobile river feature.

Pool: Deeper area of a stream with slow-moving water.

Reach: A section of stream (defined in a variety of ways, such as the section between tributaries or a section with consistent characteristics).

Riffle: A shallow section in a stream where water is breaking over rocks, wood, or other partly submerged debris and producing surface agitation.

Riparian: The zone adjacent to a stream or any other waterbody (from the Latin word ripa, pertaining to the bank of a river, pond, or lake).

Riprap: Rock material of varying size used to stabilize streambanks and other slopes.

Run: A fast-moving section of a stream with a defined thalweg and little surface agitation.

Scouring: The erosive removal of material from the stream bottom and banks.

Sedge: A grasslike, fibrous-rooted herb with a triangular to round stem, and leaves that are mostly three-ranked and with close sheaths; flowers are in spikes or spikelets, axillary to single bracts.

Substrate: The mineral or organic material that forms the bed of the stream; the surface on which aquatic organisms live.

Surface fines: That portion of streambed surface consisting of sand/silt (less than 6 mm).

Thalweg: The line followed by the majority of the streamflow. The line connecting the lowest or deepest points along the streambed.

Turbidity: Murkiness or cloudiness of water caused by particles, such as fine sediments (silts, clays) and algae.

Watershed: A ridge of high land dividing two areas that are drained by different river systems. The land area draining to a waterbody or point in a river system; catchment area, drainage basin, drainage area.

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Wastewater nitrogen is linked to changes in submerged aquatic vegetation and invertebrates in Oyster Pond

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Abstract

In order to assess the affect of wastewater nitrogen on submerged aquatic vegetation (SAV) and its associated invertebrates we took six random core samples from established stands in each of six recharge zones in Oyster Pond, a coastal estuary in Cape Cod, Massachusetts. We measured the biomass (g m^{-2}) of the plants and abundance (# m^{-2}) of invertebrates, as well as the $\delta^{15}N$ values of plant tissues. The dominant plant *Ceratophyllum demersum* showed an increased $\delta^{15}N$ signature in areas with higher rates of wastewater N loading. *C. demersum* and other plant species changed their contribution to total biomass with higher wastewater N input, with *C. demersum* comprising up to 80% in the areas of highest N loading. Midges and phantom midge larvae increased in abundance with increasing wastewater input and this was not a function of increasing plant biomass. Sensitive taxa, mayflies and caddisflies, had higher abundances in the macrophyte *Chara vulgaris* and not *C. demersum*. We estimate that *C. demersum*, found at depths of up to 3 m could be holding 32% of the annual N load of Oyster Pond.

Introduction

Submerged aquatic vegetation (SAV) has an important structuring role in the dynamics of shallow eutrophic lakes. Aquatic macrophytes may out-compete phytoplankton (a major component of turbidity) for nutrients directly, as well producing allelopathic substances that affect phytoplankton growth (Sheffer et. al. 1993). Additionally, SAV canopies create suitable conditions for denitrification, and supply shelter for zooplankton which may graze on phytoplankton (van Donk and van de Bund. 2002).

Oyster Pond is a shallow, estuarine kettle pond in the southwestern portion of Cape Cod, Massachusetts. In recent years, local residents have become concerned because of increases in

SAV, which have become a nuisance to boaters. Over the last year alone the standing crop of SAV has increased (Sara Grady, personal comm.).

Increased nutrient loads may be one change promoting blooms of SAV. In the case of Oyster Pond, land derived nitrogen loads (S. Good, this volume) in 2002 are more than 10 times the amount that entered the Pond in the eighteenth century (Emery, 1997). Wastewater nitrogen from septic tanks now comprises between 60% and 80% of the nitrogen loading into the pond (S. Good, this volume). To understand the role of SAV in the pond it is important to investigate the connection between nutrient loading and submerged vegetation.

There are a variety of methods to examine this connection. Stable isotope analysis reliably tracks nitrogen from a given external source (Robinson, 2001). Watershed derived nitrogen that enters estuaries typically has high $\delta^{15}N$ values (McClelland and Valiela 1998), and this signature may be detected in the tissues of any organism which takes up this nitrogen.

Other variables may also demonstrate a wastewater nitrogen linkage to the SAV. The biomass of a single species of submerged macrophyte may not be a reliable indicator of increased nitrogen input (Best 1979), but recent studies have shown that nitrogen input can affect the proportion that different species contribute to total biomass (Engelhardt & Ritchie 2001; Lehmann & Lachavanne 1999). It is also important to know the amount of nitrogen held in the total biomass of SAV to understand its importance in the nitrogen cycling of the Pond.

The SAV canopies proliferating in Oyster Pond also harbor a suite of freshwater macroinvertebrates that may also be sensitive to N loads. Macroinvertebrates such as mayflies and caddisflies are affected by nutrient influx (Scarsbrook et al. 2000). Aquatic communities with more mayfly nymphs can shift to those with more midge larvae with higher nitrogen input (Lenat & Crawford 1994). These shifts in fauna that may be associated with plant biomass

changes could then extend their influence, for example by altering the cover and food supply for consumers such as fish.

The goal of this study is to examine the effect of total N and wastewater loading on the biomass of submerged macrophytes in Oyster Pond, as well as on the invertebrates that they harbor. First we used $\delta^{15}N$ signatures for SAV to explicitly link wastewater nitrogen load to the organisms in the pond. Second, we sampled SAV biomass to examine the relationships to different nitrogen loads entering different areas of Oyster Pond. Third, we evaluated the relationship of invertebrate species to wastewater nitrogen loading as well as to the biomass of different species of SAV.

Materials and Methods

Stable Isotope Analysis

We took samples of one or two representative plant species from across each recharge zone for isotope analysis. The samples were washed with distilled water, and dried for 24 hours at 60°C. We then ground each sample to a powder with a mortar and pestle and weighed out 5 mg to be sent to the University of California at Davis Stable Isotopes Facility to be analyzed on a mass spectrometer.

Macrophyte Sampling

To ascertain the effect of nitrogen loading on plant biomass we first chose sampling sites from around Oyster Pond that receive N loads from each of six recharge zones that had been delineated by Sarah Good (this volume), based on water table contours (Fig. 1). The N loads vary from 7 kg yr^{-1} in zone 7 to 340 kg yr^{-1} in zone 3 (table 1). In each zone, we took 6 samples from established stands of vegetation at depths of less than 2 meters. We used a plastic cylinder

(26 cm diameter) as a coring device; an attached 0.5 mm plankton net retained all macrophytes and invertebrates.

Plants were sorted to species, blotted dry, and weighed. An aliquot of each species was dried for 24 hours at 60°C and weighed to obtain dry weight.

Invertebrate Abundance

We sieved the plant samples for macroinvertebrates over a 1mm sieve, and sorted the invertebrates to order or family and counted them.

Results and Discussion

We identified 6 genera of submerged aquatic vegetation in Oyster Pond (Table 1). *Ceratophyllum demersum* (Coontail) was the dominant plant species by biomass (g dry weight m^{-2}) in all recharge zones except for zone 2. *C. demersum* formed dense monotypic stands at depths between 2 and 3 meters, but was mixed with other species, particularly *Najas flexilis* (Bushy Pondweed), above 2 m (the littoral zone).

Stable Isotope Analysis

$\delta^{15}N$ values in floating plants (Table 2) increased significantly as wastewater nitrogen load increased (Fig. 2). Floating plants take up nutrients from the water and are likely to reflect the signature of the N in the water. Submerged plants that have well developed roots showed no relationship to wastewater N (Fig. 2). Rooted plants derive most of their nutrients from the substrate (where constant recycling of N leads to higher $\delta^{15}N$ values) while floating plants must take up nutrients from the surrounding water. Floating plants such as *C. demersum* could be used to monitor wastewater input by measuring their $\delta^{15}N$ values.

Macrophytes

We found no correlation between nitrogen loading from wastewater and mean plant biomass, or the mean biomass of the dominant plant, *C. demersum* (Fig. 3). Individual species therefore showed no evident response to N loads. If, however, we considered the assemblage of major plant species (Fig. 4), there were notable shifts in the species that contributed biomass to the macrophyte canopies. The average proportion of *C. demersum* biomass in the littoral zone increased significantly with increasing wastewater nitrogen load ($F = 16.68$, $p < 0.05$), while the proportion of both *Najas flexilis* and of *Potamogeton* biomass correspondingly decreased (Fig. 4). Similar shifts in plants species composition have been reported elsewhere (Chambers and Prepas 1990; Lehmann & Lachavanne, 1999).

Using a bathymetry map of Oyster Pond (Emery, 1997) in Arcview 8.2, we calculated the total area of each recharge zone that is shallower than 3 m (the deepest extent to which we observed *C. demersum* growing)(Fig.1). Using this value and the percent nitrogen in *C. demersum* tissue (derived from our stable isotope analysis), we estimated that throughout the pond, *C. demersum* is storing about 279 kg of N.

The increase in aquatic plants in Oyster Pond may have considerable effect on water quality of the Pond. Plant surveys done in 2001 (Grady and Muto 2001) showed much less developed plant canopies. In one year then, perhaps 279 kg of N may have become stored in plant tissues. This one year increase is comparable to 32 % of the annual N load received by Oyster Pond from its watershed (S. Good, this volume). This suggests that the plants are holding a considerable part of the annual N input. Moreover, our data from 2002 show a 37% decrease in dissolved inorganic nitrogen ($NO_3 + NH_4$) (Dixon et al., this volume) compared to similar measurements done in 2001 (Al-Qatami et al. 2001). It may be that the decrease is related to the

uptake and storage of DIN by plants. This implies that the aquatic plants, while a nuisance to boaters, may be also improving water quality. If the plants were not there, the nutrient concentrations in the water would be substantially higher. This, in addition, suggests that a management option might be to rake floating aquatic plants out of the pond and export the biomass away from the pond, perhaps for use as green manure or compost elsewhere.

Invertebrates

Our sampling collected 17 different groups of invertebrates with large ranges in abundance among replicates. The representative taxa at each site were similar, so to test invertebrate variables with wastewater N load, and macrophytes of Oyster Pond, all 33 replicates were combined.

The abundance of midges and phantom midges increased in the areas receiving higher N load (Fig. 5). Midges are an important food for fish, but unmanaged populations can cause increases in the swarms of the flying adult form. The midge abundance did not increase with increasing plant biomass, but seemed to reach a peak at intermediate biomass (200 g m-2), before lowering at the highest biomasses (Fig. 6). The pattern, although not statistically analyzed, was similar for midges at the sites of the highest and lowest wastewater N loading. More detailed sampling is necessary, but this suggests a preferred level of plant biomass by the midges.

Samples with a higher proportion of *Chara vulgaris* had higher abundances of mayfly nymphs and caddisfly larvae (Fig. 7). Charophytes have a strong ability to compete with phytoplankton and reduce turbidity (van Donk and van de Bund. 2002, Blindow 2002). It could be that *Chara vulgaris* is providing less turbid waters for these sensitive taxa (Harding et. al.,

1999). There was also an increase in overall number of invertebrate taxa in samples that had a higher percentage of *Chara vulgaris* (Fig. 7).

In conclusion, changes in the species composition of SAV and its associated invertebrates in Oyster Pond can be used to infer the wastewater nitrogen load in a given area of Oyster Pond. Additionally, the large biomass of *C. demersum* this year has sponged up about one-third of the annual nitrogen load from the Oyster Pond watershed. In the future it will be important to account for this factor when making management decisions about the Pond.

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Table 1. Mean ± SD for plant biomass (dry weight m^{-2}) in the 6 recharge zones in Oyster Pond. The sign (+) stands for trace amounts

	Recharge zone					
	1	**2**	**3**	**4**	**5**	**7**
Ceratophyllum demursum	79±54	0	146±103	169±161	104±181	96±151
Najas flexilis	67±87	30±59	29±41	172±204	88±115	0
Potamogeton spp.	2±3	10±16	0	0	0	0
Chara aspera	0	37±48	0	0	0	11±27
Ruppia maritima	0	0	0	0	0	58±65
Lemna minor	(+)	0	(+)	(+)	0	0
Total biomass	148	77	175	341	192	165
Wastewater N load (kg N yr^{-1})	96	106	242	75	60	4
Total N load (kg N yr-1)	136	152	340	116	74	7

Table 2. Stable Isotope $\delta^{15}N$ and plant functional type.

Recharge Zone	Species	Type	$\delta^{15}N$
1	*Ceratophyllum demersum*	floating	1.27
3	*Ceratophyllum demersum*	floating	7.30
3	*Najas flexilis*	rooted	4.42
3	*Lemna minor*	floating	7.49
3	*Ceratophyllum demersum*	floating	6.41
3	Algal Epiphyte	floating	7.30
4	*Ceratophyllum demersum*	floating	5.57
4	*Najas flexilis*	rooted	4.27
4	*Lemna minor*	floating	5.91
5	*Ceratophyllum demersum*	floating	1.74
5	*Najas flexilis*	rooted	3.28
7	Algal epiphyte	floating	4.24
7	*Ceratophyllum demersum*	floating	0.07

Figure Legends

Fig. 1. Map of Oyster Pond. Inset shows area of recharge zones. Sampling sites (dots) are numbered by recharge area. Bathymetry is at 1 m intervals (3 m line bold). Shaded area shows estimated extent of beds of *Ceratophyllum demersum*.

Fig. 2. $\delta^{15}N$ values of floating (*Lemna minor*, *Ceratophyllum demersum*, algal epiphytes) and rooted (*Potamogeton* spp., *Najas flexilis*) plants versus wastewater N load (data from S. Good, this volume) received by the recharge areas.

Fig. 3. Mean biomass of *Ceratophyllum demersum*, other plant taxa, and all plant taxa versus wastewater N load.

Fig. 4. Percent of SAV biomass contributed by different species versus wastewater N load.

Fig. 5. Abundances of Chironomids and Chaoborids versus wastewater N load.

Fig. 6. Abundance of Chironomids and Chaoborids versus plant biomass.

Fig. 7. Abundance of Ephemeroptera and Trichoptera as well as total # of taxa versus percentage of *Chara vulgaris* and *Ceratophyllum demersum*.

Fig. 1

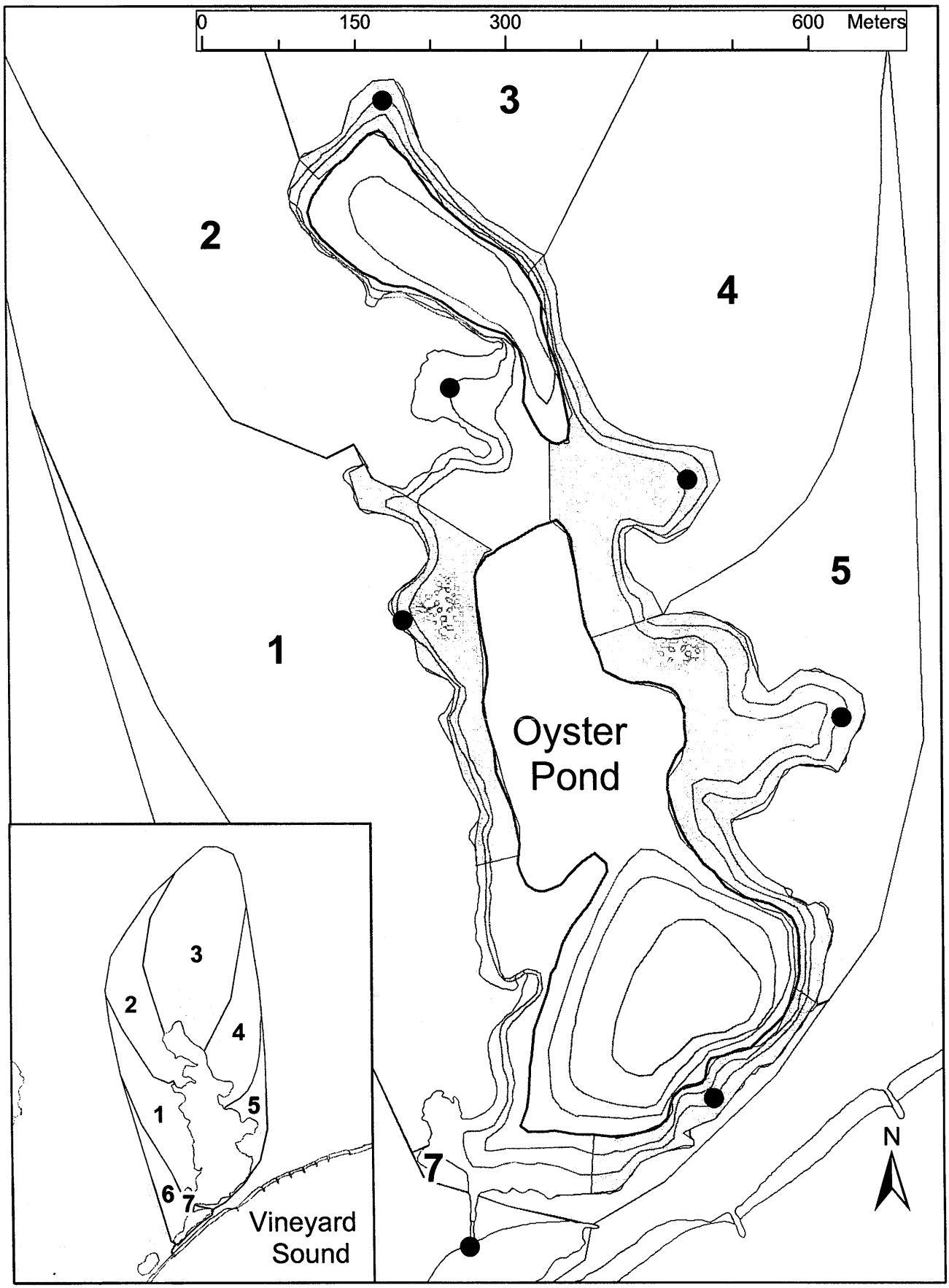

0
150
300
600
Meters
3
2
4
5
1
Oyster
Pond
7
N
3
2
4
1
5
6
7
Vineyard
Sound

Fig. 2

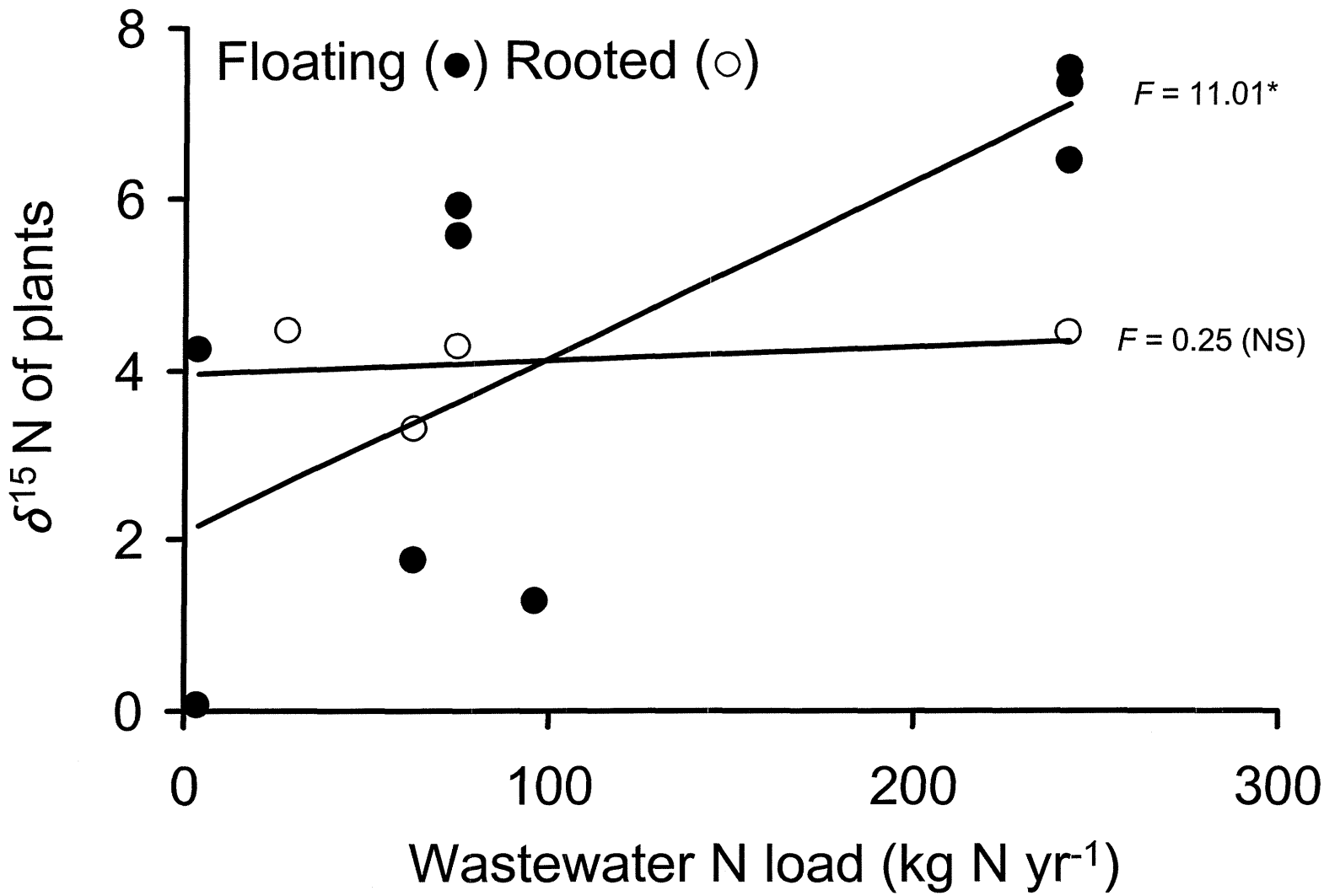

Floating (●) Rooted (○)
F = 11.01*
F = 0.25 (NS)
δ15N of plants
0
2
4
6
8
0
100
200
300
Wastewater N load (kg N yr-1)

Fig. 3

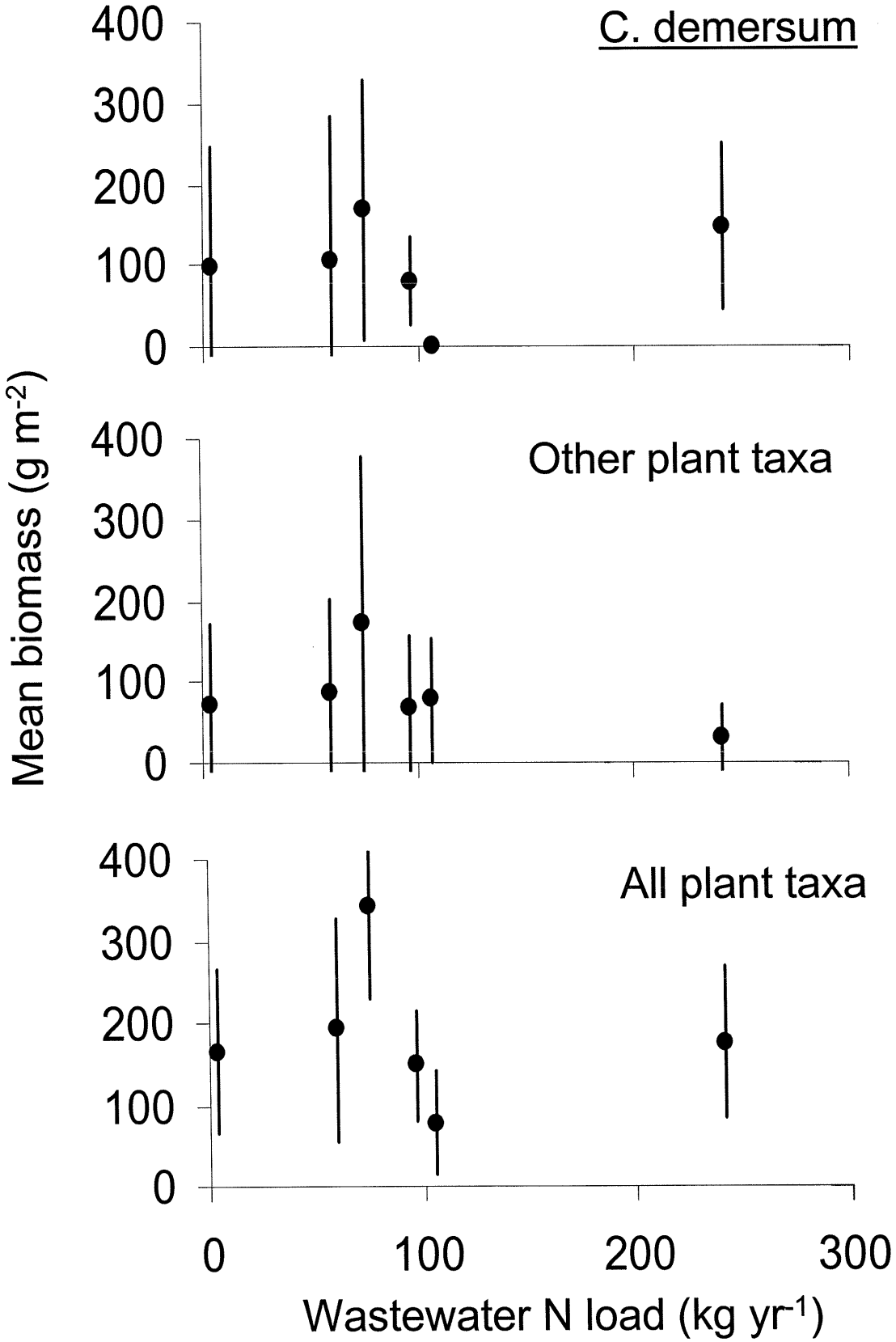

C. demersum
Other plant taxa
All plant taxa
Mean biomass (g m-2)
Wastewater N load (kg yr-1)
400
300
200
100
0
0
100
200
300

Fig. 4

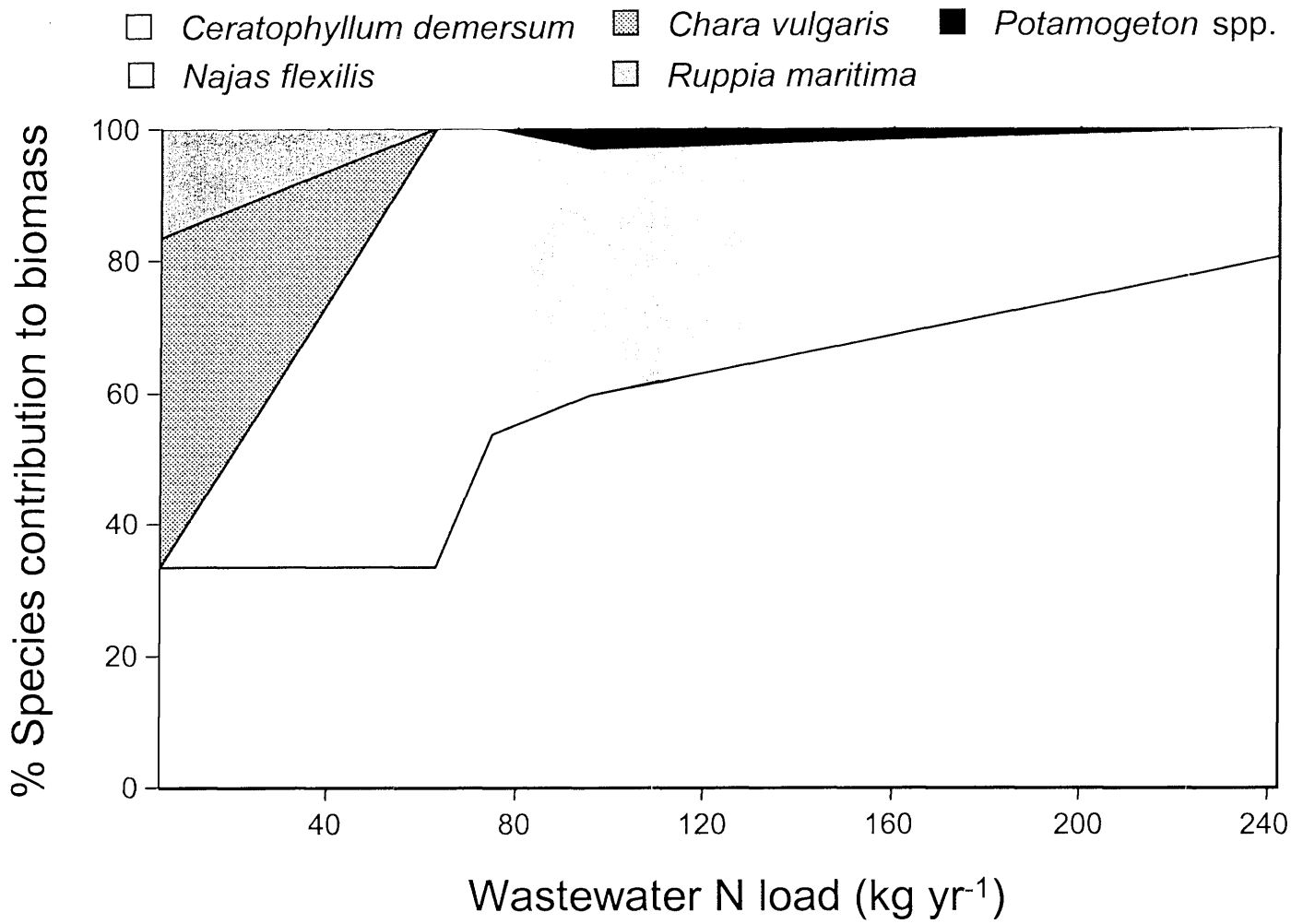

Ceratophyllum demersum
Najas flexilis
Chara vulgaris
Ruppia maritima
Potamogeton spp.
% Species contribution to biomass
100
80
60
40
20
0
40
80
120
160
200
240
Wastewater N load (kg yr-1)

Fig. 5

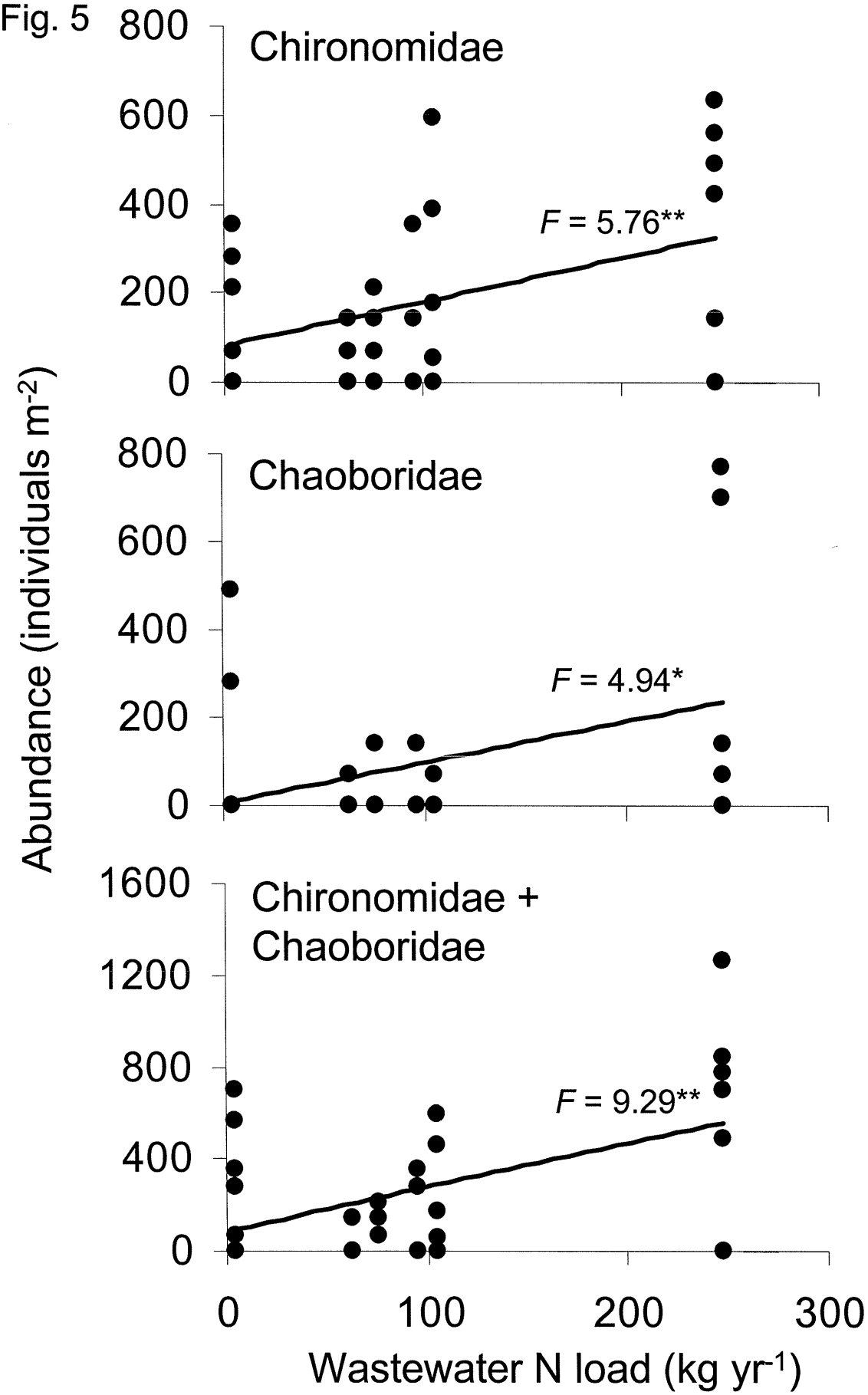
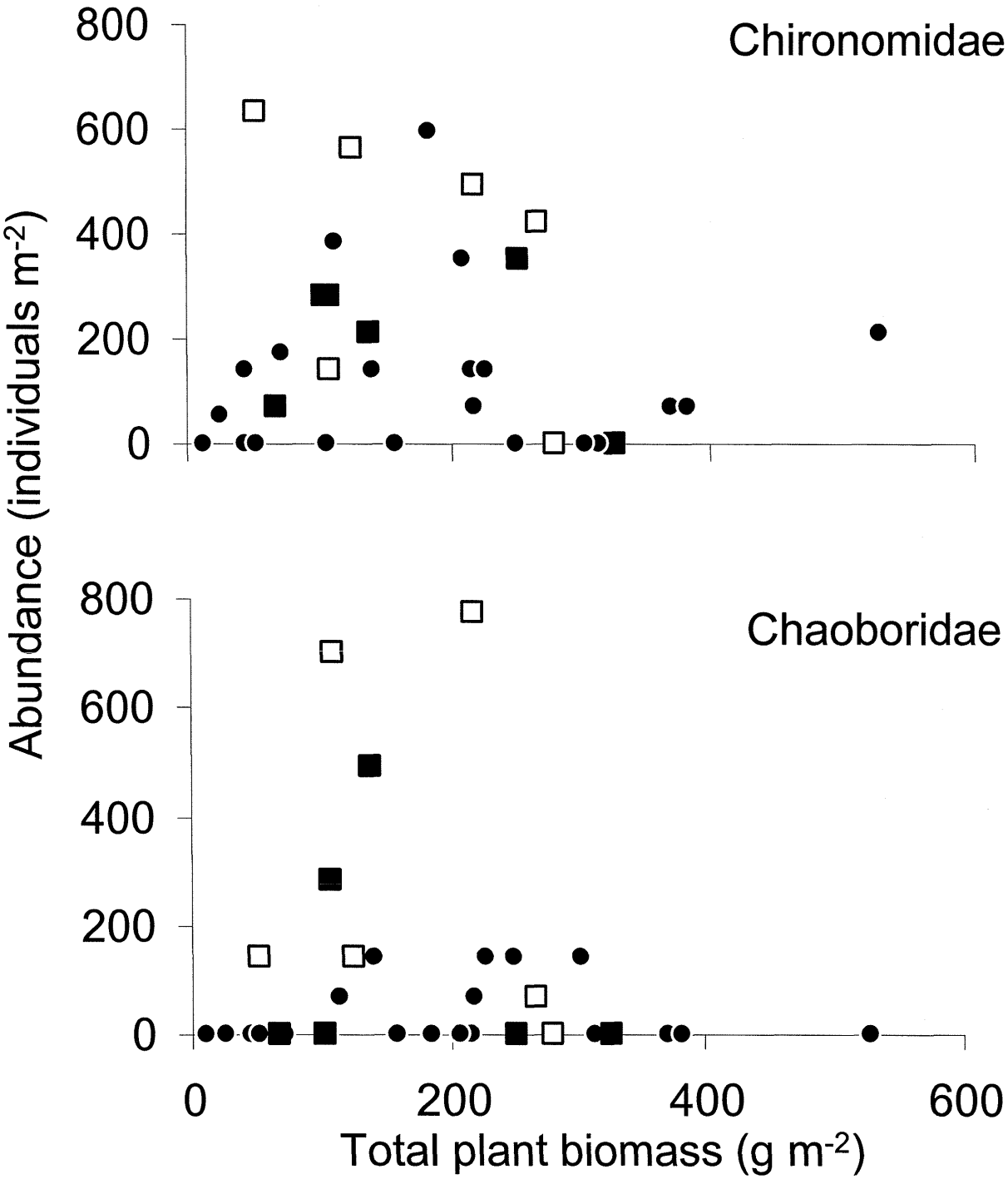
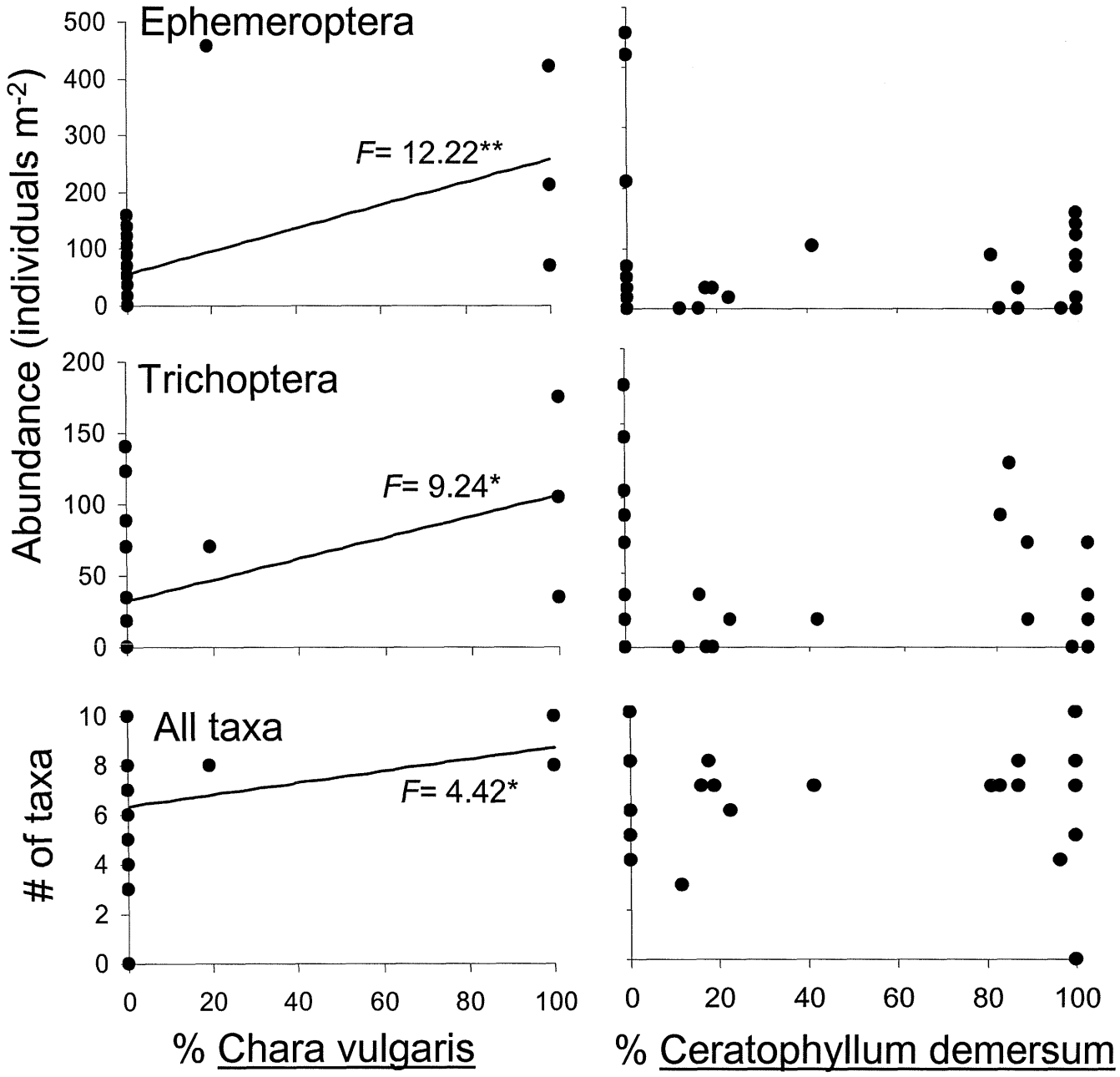

Chironomidae
F = 5.76**
Chaoboridae
F = 4.94*
Chironomidae +
Chaoboridae
F = 9.29**
Abundance (individuals m-2)
Wastewater N load (kg yr-1)
0
200
400
600
800
1200
1600
100
300

Fig. 6



Wastewater N load: Maximum (□)
Minimum (■)
Other (●)

Fig. 7


Ephemeroptera
F= 12.22**
Trichoptera
F= 9.24*
All taxa
F= 4.42*
Abundance (individuals m-2)
# of taxa
% Chara vulgaris
% Ceratophyllum demersum

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BRIDGES

BRIDGES is a recurring feature of J-NABS intended to provide a forum for the interchange of ideas and information between basic and applied researchers in benthic science. Articles in this series will focus on topical research areas and linkages between basic and applied aspects of research, monitoring policy, and education. Readers with ideas for topics should contact Associate Editors, Nick Aumen and Marty Gurtz.

Criteria for setting nutrient levels in lotic ecosystems are relevant to US states and other countries in the process of setting water-quality regulations. There are few articles in the peer-reviewed literature on this topic, and policy makers have had little information from which to base their decisions for streams. This lack of information is particularly troublesome because of the large number of streams and rivers that have impaired water quality, and the ever-increasing pace of urban and agricultural development. In addition to the effects of high nutrient concentrations on stream ecosystem structure and function, high nutrient concentrations, particularly nitrate, may have adverse effects on human health.

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Co-editors

Establishing nutrient criteria in streams

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"Every child deserves to grow up with water that is pure to drink, lakes that are safe for swimming, rivers that are teeming with fish. We have to act now to combat these pollution challenges with new protections to give our children the gift of clean, safe water in the 21st century."

—President Clinton, 23 February 1999, Baltimore

The US Environmental Protection Agency (USEPA) and the US Department of Agriculture (USDA) recently have been directed to set criteria for nutrients in rivers, lakes, and estuaries. State reports compiled by the USEPA (National Water Quality Inventory: 1996 Report to Congress) claim that 40% of streams or rivers surveyed were impaired because of the nutrients N and P, but no well-defined standards have been proposed to determine if nutrients impair flowing waters (USEPA 1998). A rational framework for determining criteria is necessary because the USEPA has been charged with establishing maximum acceptable levels of nutrients in streams and rivers by 2001 as part of the Clean Water Action Plan. State and tribal governments will use these criteria to set total maximum daily loads (TMDLs) for nutrients and adopt their own standards by 2003. The USEPA, the USDA, and other national governmental agencies (e.g., US Geological Survey [USGS], US Army Corps of Engineers), state and tribal officials, and private parties will set these criteria.

Data analyses are needed to explain the relationships between stream algae and nutrients, which previously have received attention from researchers. Given the potential economic impacts of nutrient control, the process by which nutrient levels are set likely will engender controversy. Basing the criteria on the best scientific data available will minimize conflict and maximize the potential benefits related to controlling nutrients in streams.

Although the question of how to set nutrient criteria is framed above in terms of US politics and policies, other countries also are interested in nutrient criteria for streams, particularly developed countries where industrialization, urbanization, and modern agriculture have resulted in extensive nutrient discharge into water courses. The following discussion will be based

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primarily on examples from the US, but the general principles could apply to any watershed.

We 1st will address why nutrient criteria are needed. Next, we will discuss the scientific basis behind possible criteria. Last, we discuss ways to account for variability in streams (i.e., factors that may decouple nutrient concentrations from biomass). The main thrust of this paper is to outline what scientific methods currently are available to managers for setting nutrient criteria, given specific reasons for setting the criteria.

Why do we need nutrient criteria?

Reasons for nutrient criteria include: 1) adverse effects on humans and domestic animals, 2) aesthetic impairment, 3) interference with human use, 4) negative impacts on aquatic life, and 5) excessive nutrient input into downstream systems. Each of these will be discussed in sequence; they share several characteristics, but they also have facets that may result in criteria being set at different levels.

High levels of some nutrients may have adverse effects on human health. Control of NO_3^- levels particularly is important to avoid metheamoglobemia. Furthermore, NO_3^- consumption has been correlated with stomach cancer (Hartman 1983). Although correlation does not guarantee causation, NO_3^- could be regulated as a carcinogen in the future.

Eutrophication from N and P causes proliferation of algal masses, some of which may be toxic. In one of the worst cases, eutrophication caused Cyanobacteria to bloom in the stagnant Murray-Darling River system (Australia) during a drought, leading to livestock deaths and concerns about impacts on humans (Bowling and Baker 1996). Such toxic blooms are most likely to occur in very enriched, slow-moving, and nonturbid rivers.

Eutrophication causes taste and odor problems in lakes (Arruda and Fromm 1989, Wnorowski 1992), but these negative effects have not been linked directly to trophic state of streams and rivers. Such problems often can be traced to production of odorous metabolites by Cyanobacteria (e.g., geosmin) and other algae and their subsequent leakage into surrounding waters. Algae that cause taste and odor problems can reach high biomass in eutrophic streams and rivers, both in the phytoplankton of slow-moving rivers and the periphyton of shallow streams.

Aesthetic impairment is more difficult to quantify, but usually is associated with filamentous algal forms. Nuisance levels may be reached somewhere between 100 and 200 mg/m^2 chlorophyll (Horner et al. 1983, Nordin 1985, Welch et al. 1988, Quinn 1991). Enriched waters often have benthic chlorophyll concentrations >150 mg/m^2, and many stream users find high levels of algal growth objectionable (Welch et al. 1989, V. Watson, University of Montana, personal communication). A link also may exist between property values and trophic state in lotic waters, as has been documented for lakes (Michael et al. 1996). However, to our knowledge, such analyses have not been conducted for rivers and streams.

Excessive growth of algae and macrophytes can interfere with human uses of flowing waters. Such interference is exemplified by problems caused by the filamentous green alga *Cladophora.* Exorbitant amounts of this alga can slow water flow in canals (decreasing delivery rates and increasing water losses), interfere with swimming, and snag fishing lures (Dodds and Gudder 1992). Furthermore, excessive algal growth may clog screens on water intakes for water treatment plants and industries.

High concentrations of NH_3 in the water column clearly are toxic to aquatic animals (Russo 1985). For example, levels of >1 mg/L NH_3-N in Ohio streams have negative impacts on the fish communities (Miltner and Rankin 1998). Negative impacts on aquatic life related to stimulation of algal biomass by increased nutrients are subtler. As a system becomes more productive, different species of algae may become more competitive and species composition can shift (Kelly and Whitton 1995, Pan et. al. 1996, Kelly 1998). However, unless such species shifts cause specific water-quality symptoms (e.g., toxic algae) or aesthetic problems (e.g., very long streamers of filamentous algae), the public is unlikely to be concerned.

Nutrient enrichment may adversely affect stream animal communities. Enriched streams have increased invertebrate biomass and altered invertebrate communities (Bourassa and Cattaneo 1998). Community structure has been correlated directly with P concentration (Miltner and Rankin 1998). Excessive levels of algae were damaging to invertebrates (Nordin 1985).

Changes in community structure may be viewed as problematic, particularly if game fish are affected. In extreme cases, levels of primary production can be stimulated by nutrients; organic C will build up in the system and cause a subsequent low dissolved O_2 (DO) and high pH event. Fish and invertebrates will grow poorly and even die if the O_2 depletion and pH increases are severe (Welch 1992).

Because streams drain into lakes and oceans, eutrophication caused by influx of nutrients from flowing waters is a concern for downstream lake and coastal areas. Examples of marine eutrophication are the zone of hypoxia that develops in the Gulf of Mexico (Rabalais et al. 1998) and the production of toxic estuarine dinoflagellate blooms (Burkholder and Glasgow 1997). Eutrophication problems in lakes are well documented, and the control of external and internal nutrient loading necessary to minimize eutrophication can be calculated (Cooke et al. 1993). Requirements for control of nutrient loading to lakes and coastal marine systems may lead to more stringent nutrient criteria in rivers than those required for controlling instream eutrophication, especially in localities where stream algae are limited by factors other than nutrients.

What are the scientific bases for criteria?

In this section we discuss what nutrients and what forms of those nutrients should be used to set criteria. We also describe some models and approaches that can be used to set criteria. Last, we offer some discussion on how criteria may vary depending upon the reason for the criteria.

What nutrients and forms should be used to set criteria?

The traditional view is that P limits primary production in fresh waters (e.g., Correll 1998), and N limits it in the ocean. However, nutrient bioassays and correlation analyses do not substantiate this point of view. Data were compiled from 158 bioassays reported in the literature in which the response of stream periphyton to nutrient fertilization was measured. Of the studies, 13% showed stimulation by N alone, 18% by P alone, 44% by simultaneous N and P additions, and 25% by neither nutrient (W. K. Dodds, unpublished data). The absolute proportions associated with each type of limitation should not be viewed as a general guide to nutrient limitation in streams. However, bioassay results do suggest that both N and P can limit primary producers in streams.

Correlation analyses also do not support the idea of P as the sole limiting nutrient in rivers and streams. Mean and maximum benthic chlorophyll correlated better with total N (TN) than total P (TP) in the water column in several hundred streams. Nitrogen and P occur in several forms in rivers and streams, including dissolved organic and inorganic forms and in particulate material. All of these forms together are referred to as TN and TP. Total N does not include dissolved N_2 gas. A regression model using both nutrients explained the highest proportion of the variance in biomass (Dodds et al. 1997). Thus, both N and P can control primary production in at least some streams and rivers.

Control of P alone may cause P to limit and lower algal biomass, as has occurred in many lakes (Sas 1989, Cooke et al. 1993). However, if pulses of P occur, they can be taken up in excess of requirements and stored inside algal cells in a process called *luxury consumption*. This stored P can allow algae to grow even if P concentrations are low in the water column. If controlling such P pulses is impossible (e.g., pulses associated with high runoff events in spring), control of N could become necessary. For example, Lohman and Priscu (1992) demonstrated that intracellular P concentrations in *Cladophora* increased, while P availability in the surrounding water decreased. Thus, *Cladophora* was N limited, even though analyses of available N and P in the river water column suggested P limitation. Given the bioassay and correlation data, and that periphyton can consume P in excess of immediate needs, setting nutrient criteria for both N and P makes sense.

Unless clear limitation by other nutrients has been demonstrated in a particular system, N and P should be assumed to be the dominant nutrients controlling the trophic states of streams and rivers. Fortunately, nuisance and some toxic heterocystous Cyanobacteria that can use N_2 gas as a N source generally are not part of eutrophic stream periphyton, but may occur in the plankton of slowly flowing rivers (Bowling and Baker 1996). The decreased dominance of heterocystous Cyanobacteria in streams leads to some situations where N control alone may

lead to decreases in algal biomass. Controlling N and not P inputs in lakes can encourage blooms of nuisance Cyanobacteria (Stockner and Shortread 1988). However, the strategy of controlling N alone should be viewed with caution, especially in plankton-dominated rivers. More data on cyanobacterial problems in eutrophic streams are necessary before we can be certain that N control will not lead to cyanobacterial dominance.

Control based on measured levels of dissolved inorganic N and P may not be effective because these pools are replenished rapidly by remineralization in surface waters (Dodds 1993). Correlation of algal biomass with dissolved inorganic nutrients was poor in some studies (Dodds et al. 1997), but not all (Biggs and Close 1989). Also, lake managers are aware of problems with using dissolved inorganic nutrient concentrations to set nutrient criteria. Last, most of the data linking land-use practices to N and P loading have been reported in TN and TP (Loehr 1974), so basing criteria on total nutrients for calculating TMDLs is more practical than using dissolved inorganic nutrients.

Two caveats are necessary to the generalization that TN and TP should be emphasized. First, if nutrients are released directly into streams in dissolved inorganic form, their influence may be more intense and localized near the point source of release. Dissolved inorganic nutrients will be taken up rapidly, which can lead to a very high, localized concentration of biomass (Hynes 1969). Second, some models using seasonal means of dissolved inorganic nutrients to predict algal biomass have been very successful (Biggs 1995, 2000), and some sites have considerably more data on dissolved than total nutrients on which to base decisions.

At what concentrations should criteria be set?

One difficulty in setting criteria involves assessment of the trophic state of a stream or river. Stated another way, how can we declare that a river or stream is in an unacceptable trophic state if there is no basis for scaling the trophic state relative to other rivers? A generally accepted system for classifying the trophic states of streams and rivers is lacking (Dodds et al. 1998). In general, trophic state is classified by nutrients and algal biomass. System metabolism may be more relevant to ecosystem function, but difficulties with methods and limited data have precluded use of production and respiration to classify trophic states of lakes and streams. One classification system proposed for streams relies upon the cumulative frequency distributions of chlorophyll and nutrients. The lower 1/3 of the distribution sets the range for oligotrophic streams, and the upper 1/3 for eutrophic streams. This approach is consistent with the convention of classifying trophic state into 3 categories, while basing classification on the actual distribution of biomass and nutrient levels found in streams (Dodds et al. 1998). The published classification was based on only 286 temperate streams. More data are necessary to determine how well this classification scheme applies to rivers from different ecoregions, how distributions of nutrients correlate to algal biomass, and how well such classification represents pristine conditions. Analyses of existing databases may provide a valuable tool in extending this approach to trophic classification.

A few models directly link TN and TP to benthic algal biomass in streams (e.g., Lohman et al. 1992, Dodds et al. 1997, Bourassa and Cattaneo 1998, Chételat et al. 1999). Such models can be applied to estimate algal biomass as a function of water column nutrients. A similar correlation approach has been very successful in managing eutrophication in lakes and reservoirs. Extension of these models to link instream nutrient concentrations to known sources of nutrient loading also has been described (Dodds et al. 1997).

Models describing the correlation between nutrients and chlorophyll in lakes differ from those for streams because benthic chlorophyll may be much more variable in streams as a result of the effects of floods, turbidity, and grazing. This difference is exemplified by the ratios of maximum to mean chlorophyll. This ratio describes the variance in level of chlorophyll, with high numbers denoting a high variance. The maximum/mean chlorophyll ratio is 4.5 for stream benthos compared to 1.7–2.6 for lake phytoplankton (Dodds et al. 1998). Furthermore, total water column nutrients usually are correlated strongly with chlorophyll because phytoplankton contain chlorophyll, N, and P. This linkage leads to high correlation coefficients between total nutrients and algal biomass in lakes. This relationship is not as highly coupled in streams when *benthic* chlorophyll and *water col-*

umn TN and TP are considered. Thus, the correlation models developed for stream benthic algae contain a much greater degree of uncertainty than those for lakes.

Biggs (2000) proposes a correlation method that considers hydrodynamic disturbance and inorganic nutrients in New Zealand streams, that is pertinent for predicting benthic algal biomass. Such an approach may prove useful within an ecoregion, and could be used to provide a sliding scale of nutrient criteria, with higher nutrient content allowed in more hydrodynamically unstable rivers (i.e., criteria may be more lenient because of regular scouring of algal biomass in rivers that flood frequently).

An alternative approach to correlation models also has been developed. This method consists of sampling nutrients in reference stream reaches where chlorophyll levels are deemed acceptable. Gary Ingman (Montana Department of Environmental Quality) and Vicki Watson (University of Montana) proposed this technique for use in the Clark Fork River in Montana (Dodds et al. 1997). General regional criteria have yet to be established using this method. In systems where the entire stream receives nutrient loading, or regions where all watersheds are enriched, locating suitable reference reaches may be impossible. Data from other similar streams should be used to identify the obtainable baseline nutrient concentrations in those cases.

A regression model linking TP to river phytoplankton is available (Van Niewenhuyse and Jones 1996). This model can be used to set TP criteria. The TP levels can be used to calculate corresponding TN concentrations with the Redfield ratio (Harris 1986). This model captures additional variance when watershed area is considered.

Setting nutrient criteria is difficult based on subjective impressions of what constitutes excessive levels of benthic algae. However, $>$200 mg/L of benthic chlorophyll generally produces a very green stream bottom (Welch et al. 1988). To further complicate matters, filamentous green algae have a less desirable appearance than brown-colored diatoms, even when the biomass of the 2 is similar. Moreover, a large amount of the variance in benthic chlorophyll levels in streams is not related to nutrient levels. We simply do not have the data in the US to predict when benthic algal community structure will shift to more nuisance forms with changes in nutrients. Preliminary data from Canada indicate that rhodophytes make up a large portion of the algal community when biomass is low, and *Cladophora* and *Melosira* prefer high nutrient water (Chételat et al. 1999). More research clearly is needed in this area, both original research and analysis of existing data. Thus, criteria based on current data will need to be set based on what amount of chlorophyll is acceptable, not on how nutrient amounts and ratios will influence algal communities.

Dissolved O_2 deficit and high pH are perhaps the most severe algal-related problems affecting the aquatic life-support characteristics of a river or stream. Deficits of DO can occur when respiration of organic C produced by photosynthetic processes in the stream exceeds the ability of reaeration to supply DO. Depletion of DO in streams was described years ago (Odum 1956). However, the severity of the deficit is difficult to predict in specific situations. Deficits of DO are most likely to occur in rivers with laminar flow (slow, non-turbulent flow), when a large algal biomass is present, with high water temperature, and during times of low light (early morning or after protracted cloudy periods). Given that such events rarely are recorded (though they may occur frequently), and that so many factors are related to DO depletion rates, existing data for most streams are insufficient to develop nutrient criteria for avoiding DO deficits. Such models probably will be developed in the future and development will be facilitated by recent improvements in tools for measuring and storing temporal data on instream DO concentrations. As more data become available, it will be possible to directly link frequency and severity of low DO events with nutrient loading.

Similar problems exist for predicting pH excursions. High pH is promoted by laminar flow and sunny conditions that, respectively, minimize atmosphere-to-water transport of CO_2 and maximize photosynthetic uptake of CO_2. Again, limited data for most streams hamper prediction of the degree of pH excursions as a function of TMDLs of N and P.

Nutrient criteria also could be set relative to other streams on a regional or national basis. Dodds et al. (1998) combined data from the EPA eutrophication survey (Omernik 1977) and several hundred streams and rivers in the US and analyzed the resulting cumulative frequency distributions. Half of the systems had TP $>$0.04

mg/L, and ½ had TN >0.9 mg/L. If the target is to bring streams and rivers to nutrient levels at or below current means, then using frequency distributions would be a viable approach to setting nutrient criteria. Problems with using such frequency distributions are discussed below.

Nutrient criteria may be more stringent when potential eutrophication of systems fed by rivers is a factor driving adoption of criteria. A common classification system suggests that 35 μg/L TP and a mean of 8 μg/L chlorophyll constitutes the dividing line between eutrophic and mesotrophic lakes (Organization for Economic Cooperation and Development [OECD] as cited in Rast et al. 1989). In contrast, data from Dodds et al. (1997) suggest that maximum benthic chlorophyll values are likely to exceed 200 mg/m^2 at 90 μg/L TP, and mean values of chlorophyll of 50 mg/m^2 are likely with 55 μg/L TP. Thus, unacceptable levels of chlorophyll may occur at much lower nutrient concentrations in lakes than streams.

Streams and rivers are less likely to accumulate as much algal biomass as lakes, given the same TP, because the lentic planktonic habitat is considerably more benign. Thus, there is fairly low chlorophyll yield per unit nutrient in streams. Comparing streams that flood at moderate frequency to more hydrodynamically stable artificial and spring-fed streams substantiates this view. Much higher benthic chlorophyll yield per unit TP than predicted by Dodds et al. (1997) is possible in controlled laboratory streams, outdoor artificial streams, or spring-fed rivers (Welch et al. 1992, Walton et al. 1995, Anderson et al. 1999, Welch et al., in press).

Likewise, planktonic chlorophyll yield is less in flowing waters than in lakes. A river with 8 μg/L chlorophyll would have ~48 μg/L TP, using the relationship proposed by Van Niewenhuyse and Jones (1996) for suspended chlorophyll in rivers as a function of TP. This value is ~1.4 times greater than the proposed mesotrophic/eutrophic boundary value for lakes and reservoirs (OECD as cited in Rast et al. 1989).

Last, a missing link in the above discussion is how to relate instream TN and TP concentrations to nonpoint and point sources of nutrients (i.e., to set TMDLs). Models predicting nutrient loading in streams need to be developed if mitigation strategies based on water column nutrients are to be successful. A method for determining instream TN and TP concentrations based on loading from point sources has been developed for use in the Clark Fork River (Dodds et al. 1997). Simple correlation techniques using data available in various regions may yield a relationship that can be used to predict what management strategies are necessary to bring nutrients from point sources, and consequently algal biomass, to target levels.

What factors may alter responses to nutrient control?

Variation of benthic algal biomass occurs among areas with different geology, land-use practices, and as a function of other biotic and abiotic factors. In this section, we discuss how regional differences (ecoregions) may play a role in setting nutrient criteria. In general, the relationships described above that can be used to set criteria based on algal biomass response, represent average responses.

Nutrient criteria should be set after considering the natural state of streams and rivers in an ecoregion. For example, in watersheds with high-PO_4^{3-} rock that is weathering at significant rates, low P concentrations may never occur. Large rivers will have higher TP, and yield of suspended algae will be different than in smaller streams (Van Niewenhuyse and Jones 1996). Furthermore, some watersheds have very high natural NO_3^- weathering rates (Halloway et al. 1998). Such areas naturally high in nutrients occur in several places in the US (Omernik 1977). Clearly, if nutrient levels naturally are high in a watershed, restrictive nutrient criteria cannot be met. Furthermore, when pristine systems are absent, determining natural baselines could be impossible.

Considerably greater levels of accuracy for prediction of benthic algal biomass with regression models are possible if region-specific data are available. For example, the general data sets used in regression models relating water column nutrients to benthic algae developed by Dodds et al. (1997) have a maximum r^2 of 0.43. Data from Missouri streams alone have r^2 values ranging from 0.47–0.60, depending upon year and whether TN or TP is used to predict algal biomass (Lohman et al. 1992). Biggs (1995) was able to construct a model for algal biomass with an r^2 of 0.89 in a region of New Zealand by normalizing for the effect of floods and using conductivity as a surrogate for nutrients. Fur-

TABLE 1. Various potential nutrient criteria set using different outcomes of concern related to instream nutrient concentrations. TN = total N, DIN = dissolved inorganic N.

Outcome	N (mg/L)	Total P (mg/L)	Comments
Toxicity, human	10 NO_3		US national standard
Toxicity, aquatic life, acute	0.03–5 NH_3		Fish and invertebrate data (Russo 1985)
Toxicity, aquatic life, chronic	0.005–1 NH_3		Fish data (Russo 1985, Miltner and Rankin 1998)
Oxygen deficit, pH excursion	?	?	Probably greater than levels presented below
Mean benthic chlorophyll <50 mg/m^2	0.47 TN	0.055	Large data set (Dodds et al. 1997)
Mean benthic chlorophyll <50 mg/m^2	0.25 TN	0.021	Lohman et al. (1992)
Maximum benthic chlorophyll <200 mg/m^2	3.0 TN	0.415	Calculated form Dodds et al. (1997)
Significant effect on biotic integrity index using invertebrates and fish	1.37 inorganic N	0.17	Headwater streams, Ohio (Miltner and Rankin 1998); effects less apparent in larger rivers
Systems with nutrient concentrations in upper ½	0.9 TN	0.04	Dodds et al. (1998)
Planktonic stream chlorophyll <8 μg/L	0.29 TN	0.042	Calculated from Van Nieuwenhuyse and Jones (1996); chlorophyll level from Organization for Economic Cooperation and Development (OECD, as cited in Rast et al. 1989); TN set by Redfield ratio (Harris 1986)
Lake mesotrophic/eutrophic boundary (planktonic chlorophyll <8 μg/L)	0.25 TN	0.035	OECD (as cited in Rast et al. 1989); TN set by Redfield ratio
Values set by State of Montana and cooperators	0.30 TN	0.020	Tri-State Implementation Council, Clark Fork Voluntary Nutrient Reduction Program
Levels leading to periphyton and macrophyte control	1.0 DIN	<0.020 (total dissolved)	Bow River, Alberta (A. Sosiak, Alberta Environmental Protection, personal communication)
Levels set to control summer phytoplankton		0.07	Tualatin River, Oregon (R. Burkhart, Oregon Department of Environmental Quality, personal communication).
Levels recommended to control maximum periphyton below 200 mg/m^2 for 50 d accrual	0.019 DIN	0.002 (soluble reactive)	(Biggs 2000)

thermore, all relationships that have been developed to date are from temperate regions, with most data from North America and New Zealand. Subtropical or polar regions could have quite different relationships. Thus, if data are available for an ecoregion, they should be used to set criteria for that region. Extant data such as state and tribal water quality records, USGS National Water Quality Assessment Program data, and Environmental Monitoring and Assessment Program results may serve as sources for such analyses.

Streams in a local region also may exhibit different relationships between TN or TP and benthic chlorophyll than those observed with larger-scale data sets. Thus, large, generalized data sets should not be the 1st choice for setting criteria, if local data are available. For example, the TN and TP values that yield a mean benthic chlorophyll of 50 mg/m^2, were lower for the detailed data set from Missouri than those from a larger data set (Table 1).

Nevertheless, one should not expect that the nutrient concentration yielding a given peri-

phytic biomass will be markedly different among regions if other factors (i.e., light, grazing, etc.) are similar. For example, most regression relationships for chlorophyll-TP in lakes show slopes or chlorophyll:TP ranging from 0.5–1.0 (Ahlgren et al. 1988). Invertebrate grazing may result in low chlorophyll yield per unit nutrient in streams regardless of ecoregion (Bourassa and Cattaneo 1998), as is the case in lakes.

One potential problem with the ecoregion approach is that variation over time and space within a small area may be as great as the variation among ecoregions. The nutrient bioassays of Wold and Hershey (1999) demonstrate high variation of responses to N or P additions in 6 watersheds within 100 km of each other. The responses also were variable across season. Similar seasonal responses have been documented in New Zealand streams (Francoeur et al. 1999).

All the data sets that have been published linking algal biomass to water column nutrients in rivers and streams have a potential statistical problem (Lohman et al. 1992, Biggs 1995, Van Niewenhuyse and Jones 1996, Dodds et al. 1997, Chételat et al. 1999). Investigators may have introduced bias in site selection because sites were not selected randomly. In many cases, study sites are selected specifically to represent the broadest possible range of site types. Thus, extremely eutrophic and oligotrophic systems may be overrepresented. Such models may work well for the streams used to construct the models, but their application should be viewed with caution. For example, Dodds et al. (1997) reported relationships among nutrients and chlorophyll derived from literature values. The investigators who conducted this literature analysis had no way of knowing why investigators choose to investigate particular sites or if all data were reported. Streams with low amounts of periphyton may have been excluded, or researchers may have preferred to work in pristine systems. Last, much ecological investigation has concentrated on temperate, forested streams, which may have low levels of nutrients and where canopy cover may have restricted algal growth. Temperate forested streams may not be globally representative of all streams because they provide $<1/3$ of the runoff from the earth's continents (Dodds 1997). Thus, future sampling strategies to generate data that will be used to link stream eutrophication with nutrients should attempt to avoid investigator-specific biases. The models for setting criteria should be based on representative streams with data taken from the full population of streams and with each type of stream sampled in proportion to its relative occurrence. Such an approach has been taken in lakes (Peterson et al. 1999). Large data sets such as those collected by the USGS water quality monitoring network of the National Water Quality Assessment Program may be useful because sites could be selected from the databases to provide data specific to individual ecoregions.

If streams and rivers are turbid as a result of suspended particles, nutrient enrichment will have less influence on trophic status of the entire system. Sediments attenuate light, which becomes the factor limiting ecosystem production. However, even in turbid systems, enrichment may increase periphyton and macrophyte production in shallow portions of the river. Similarly, extensive shading by a riparian canopy will inhibit algal growth. Both conditions reduce chlorophyll yield per unit nutrient.

If macrophyte production predominates in streams and rivers, setting nutrient criteria will be difficult. We are not aware of any general published relationships between water column nutrients and macrophyte biomass. Such relationships may be very difficult to establish for macrophytes that are able to acquire nutrients from sediments through their root systems. However, nutrient control resulted in lowered macrophyte biomass in the Bow River, Alberta (A. Sosiak, Alberta Environmental Protection, personal communication), so future work on macrophyte-nutrient relationships could yield useful predictive models.

Conclusions

Many factors can regulate primary producers in streams, including nutrient availability, hydrodynamics, grazing, turbidity, riparian shading, and human impacts (e.g., addition of toxic compounds, global change, introduced species, watershed development). However, nutrient inputs are usually the most effectively managed factor. Factors in addition to nutrients need to be considered mainly because they can lead to cases of low algal biomass with high nutrients. Although these additional factors may decouple nutrient enrichment from algal biomass, most of these (e.g., flooding, grazing, turbidity) are not

easily controlled at most sites. Thus, we are left with setting nutrient criteria as the primary way to mitigate problems of excessive algae.

Developing a single value that can be used for nutrient criteria in streams and rivers will be difficult, given the variety of reasons for setting the criteria (Table 1). To protect human health, no more than 10 mg/L NO_3^--N should be present. To avoid chronic toxicity by NH_3, no more than 0.02 mg/L NH_3^--N should be present. If the concern is eutrophication, then setting criteria for TN and TP is most reasonable.

If streams are not turbid, preventing maximum benthic chlorophyll levels from exceeding 200 mg/m^2 is reasonable because streams with higher levels are not aesthetically pleasing, and their recreational uses may be compromised. For benthic chlorophyll to remain below 200 mg/m^2 at the very least, TN should remain below 3 mg/L, and TP below 0.4 mg/L. Based on cumulative frequency distributions of nutrients, and assuming that ~½ the systems in the US have been impaired by excessive nutrients, levels of TN and TP would be set at 0.9 and 0.4 mg/L, respectively. If a mean of 50 mg/m^2 chlorophyll is the target (thus ensuring chlorophyll is <100 mg/m^2 most of the time), TN should be 0.47 and TP 0.06 mg/L. Lower levels for nutrient criteria should be considered for regions with more pristine systems (e.g., TN and TP levels of 0.3 and 0.02 mg/L, respectively, were chosen for the Clark Fork River in Montana, Table 1). If systems downstream are to be protected, even lower stream nutrient concentrations will be necessary in some situations.

A significant amount of monitoring data are necessary to refine recommendations for nutrient criteria. Some regions and agencies have data that can be used for this purpose. Data that would be useful to collect or glean from existing sources for many more systems include seasonal means and maxima for benthic and planktonic chlorophyll, associated water column nutrients, and diurnal DO concentrations for a variety of stream types. Such data should be collected in a way that avoids sampling bias. Data on macrophyte abundance related to nutrients, reference streams with acceptable algal and macrophyte biomass, and factors related to dominance by nuisance algal and macrophyte species also are sorely lacking for many regions.

Establishing rational criteria will require bridging the gap between managers and scientists. The managers will provide the realistic assessment of what needs to be accomplished, whereas the scientists can suggest the best available means to reach the management goals. Continued interplay between applied and basic approaches will be necessary if eutrophication in streams is to be controlled in an efficient manner.

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Walter Dodds is an Associate Professor at Kansas State University. He specializes in algal ecology with emphasis on nutrients and algae in streams. Gene Welch is a Professor at the University of Washington. He has spent many years working on eutrophication control in lakes and streams. Both authors are involved in developing a manual with the USEPA that can be used by state, tribal, and regional officials to guide establishment of nutrient criteria.

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Eutrophication and trophic state in rivers and streams

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Abstract

Many natural streams are net heterotrophic, so I propose that trophic state be divided into autotrophic and heterotrophic state. This division allows consideration of the influence of external carbon sources as well as nutrients such as nitrogen and phosphorus. Empirical results suggest that phosphorus and nitrogen are the most important nutrients regulating autotrophic state in flowing waters and that benthic algal biomass is positively correlated to gross primary production in streams. Reference (minimally influenced by human activities) nutrient concentrations and correlations of nutrients with algal biomass are used to characterize reference distributions of stream autotrophic state. Only when reference nutrient concentrations are in the upper one third of those expected in the United States, is maximum benthic chlorophyll projected to exceed 100 mg m^{-2} (a concentration commonly used to indicate nuisance levels) >30% of the time. Average reference nutrient concentrations lead to sestonic chlorophyll concentrations above those considered typical of eutrophic lakes (>8 mg m^{-3}) less than half the time. Preliminary analysis suggests that autotrophic state is variable in small pristine streams because it is influenced by canopy cover (light), but heterotrophic state is less variable because it can be based on allochthonous or autochthonous production. Nitrogen and phosphorus enrichment can influence both heterotrophic and autotrophic state, and these effects could cascade to animal communities. Stoichiometry should be considered because carbon, nitrogen, and phosphorus are all involved in trophic state. The proposed definition of trophic state offers a starting conceptual framework for such considerations.

The evolution of concepts regarding enrichment in streams

In its course from the source to the sea, the progressive eutrophication of a river water by drainage from cultivated and inhabited districts is an almost inevitable natural process.
—Butcher 1947

Although current concerns about stream eutrophication mainly focus on nitrogen (N) and phosphorus (P) enrichment (e.g., Smith 2003), early water quality and nutrient enrichment studies in lotic systems focused on carbon (C) enrichment from untreated sewage. Excessive loading of biochemical oxygen demand (BOD) made rivers completely anoxic downstream of sewage treatment plants. Hynes (1960) considered the physical, biological, and chemical effects of sewage loading to create a general conceptual model on the basis of the research of Butcher (1946) and others. The conceptual model of Hynes in part considered the influence of increased organic C on dissolved oxygen (O_2) and subsequently on hypoxia- and anoxia-sensitive animals. He noted that most animals immediately downstream from a sewage outfall disappear under anoxic conditions and that, as O_2 enters the stream via aeration, high densities of pollution-tolerant fauna could be found. Eventually, as the influence of the sewage diminished downstream, Hynes predicted a return to the clean water animal communities found upstream of the sewage outfall.

Enrichment by N and P were also considered in the Hynes model. He noted a substantial increase in ammonium, phosphate, and nitrate immediately downstream from the sewage outfall that diminished farther downstream. In the anoxic zone, the prevalence of cyanobacteria (*Oscillatoria* and *Phormidium*) and *Euglena*, and further downstream substantial biomass of *Cladophora*, were predicted. A decade later, Hynes (1970) specifically noted that nutrient enrichment could occur in rivers and streams as a direct result of human alteration of land use (in addition to sewage input). He described the amounts of increase in fertilizer use and made preliminary calculations of how much fertilizer might reach rivers and streams. At that time, however, Hynes documented a paucity of studies on the effects of nutrient enrichment alone but predicted that planktonic algae in large rivers would be stimulated.

There has been conceptual progress related to defining trophic state and characterizing lotic eutrophication on several fronts in the last three decades. Omernik (1977) proposed that various regions are expected to have distinct baseline amounts of nutrients related to geology, topography, and land uses. He described areas of the United States that would be expected to have relatively greater concentrations of nutrients in streams, and he defined the concept of nutrient ecoregions. The idea that a reference baseline trophic level occurs naturally in a region forms the basis of many current efforts to regulate stream nutrients. Over the last three decades, numerous research programs were designed to link nutrient enrichment to increases in autotrophic biomass in rivers and streams by methods that included the ''clay pot'' nutrient–diffusing substrata experiments, experimental

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stream channel enrichment experiments, whole-stream enrichments, and a definition of nuisance amounts of algae (Welch et al. 1988).

At a more fundamental level, there have been few attempts to define the trophic state of lotic ecosystems and provide a comprehensive definition of eutrophication applicable to rivers and streams. Thus, I initially attempt to provide such a definition, and under this framework, I describe how prior research can be viewed given my definition.

Defining trophic state and eutrophication in streams

The definition of tropic state I develop here is designed to include both autotrophic and heterotrophic components; thus, there is a ''heterotrophic state'' and an ''autotrophic state'' of a stream or river. Heterotrophic state can be defined as the metabolic activity of the stream (typically measured as average O_2 demand [respiration, R] during dark periods and scaled to 24 h). Autotrophic state is the gross primary production (GPP) during lighted periods (typically measured as production and scaled to 24 h). The delineation of heterotrophic and autotrophic state in flowing waters was pioneered by Odum (1956). I propose that eutrophication in lotic habitats be defined as an increase in a nutritive factor or factors that leads to greater whole-system heterotrophic or autotrophic metabolism.

Heterotrophic state and autotrophic state are not mutually exclusive; a system with substantial autotrophic activity will likely have high heterotrophic activity and certainly have high respiration. This link between autotrophy and respiration can lead to a positive correlation between respiration and GPP (Fig. 1). But, a system with more heterotrophic activity does not necessarily have more autotrophic activity (e.g., the condition obtained with high BOD loading). Thus, GPP : R can indicate the balance between heterotrophic and autotrophic state. Considering both autotrophic and heterotrophic components accounts for enrichment by organic C in addition to N and P, and accounts for the observation that lotic food webs can be based on consumption of autotrophic or heterotrophic organisms.

My proposed definition of lotic trophic state is based on total heterotrophic and autotrophic production and influenced by emerging research on lakes. Although production of lakes has often been linked to planktonic biomass (usually expressed as chlorophyll concentrations), lakes can be net heterotrophic and highly influenced by terrestrial C inputs (e.g., Cole et al. 1994). Thus, solely emphasizing autotrophic biomass might not accurately describe trophic structure in lentic ecosystems. Rivers and streams are likely to be more dominated by heterotrophic processes than lakes given their stronger linkage to terrestrial systems as a source of organic C and the greater likelihood that light is intercepted. In small streams, the riparian canopy often shades the stream bottom, turbidity greatly attenuates light in many large, well-mixed rivers, and in some streams (blackwater streams), dissolved organic C colors the water and retards primary production. In many rivers and streams, much allochthonous organic matter enters seasonally and through storm water runoff. The net production of most streams is negative (i.e., GPP : R < 1), even in open-canopy, shallow, clear-water streams (Mulholland et al. 2001). Thus, any definition of eutrophication in streams should consider heterotrophic activity.

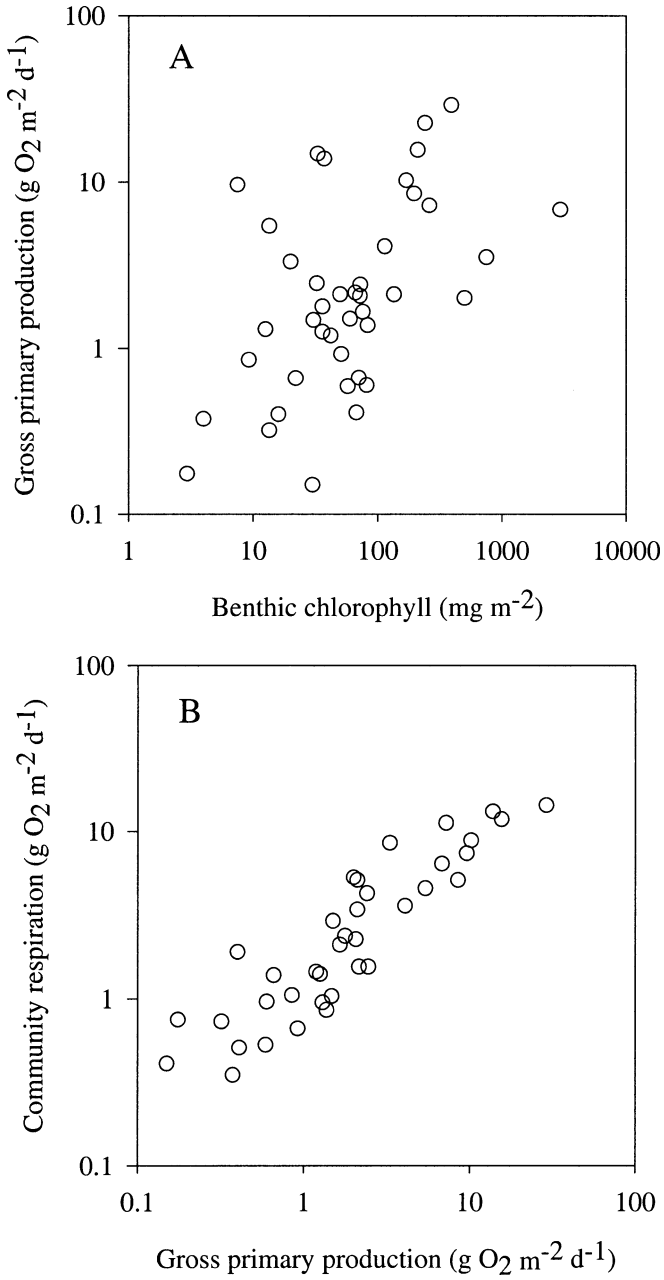

Fig. 1. (A) Relationships between benthic chlorophyll and gross primary production and (B) between gross primary production and community respiration. Data are taken from literature compiled by Bott et al. (1985); ranges were reported for values at one site, and the mean of the minimum and maximum is plotted. The relationship in (A) is significant by linear regression ($p < 0.05$, $r^2 = 0.24$) and in (B) ($p < 0.05$, $r^2 = 0.80$).

Autotrophic activity can also be important in rivers and streams. Some streams with open canopies are net autotrophic (Mulholland et al. 2001). Phytoplankton production can supply a significant portion of the productivity in medium to large rivers that are not highly turbid and do not com-

pletely mix because they have zones with limited water replacement (e.g., Thorp et al. 1998; Wehr and Descy 1998). Thus, allochthonous and autochthonous sources of C both should be considered, as well as inorganic and organic forms of nutrients such as N and P, when defining trophic status of lotic ecosystems.

Historically, trophic state in lakes was defined on the basis of clear delineation between anoxic hypolimnia and oxygenated waters (i.e., the difference between a mesotrophic and a eutrophic lake) and subsequent increases in the prevalence of cyanobacterial blooms, eutrophication- resistant animals, decreased water clarity, and taste and odor problems. Foremost, biogeochemical processes favor increased internal loading of P, leading to a positive feedback that stabilizes the eutrophic state with an anoxic hypolimnion (Dodds 2002). Such clear delineation of eutrophic conditions does not occur in shallow lakes, wetlands, and lotic systems for a variety of reasons.

Rivers and streams are relatively shallow and have considerably greater rates of atmospheric exchange compared with lentic systems, except under very low flow conditions when they become similar to small, shallow lentic systems. Thus, it is difficult for biota to consume all the O_2 in the water column without substantial inputs of BOD and adequate nutrients to support very rapid rates of heterotrophic activity. Anoxia is rare in the water column of natural rivers and streams, even in forested streams under deciduous canopies immediately after leaf fall. In most lotic systems, internal loading of P and N tends to be dominated by remineralization, groundwater inputs, and erosion. Subsequently, alternative methods are required for describing trophic distributions in lotic ecosystems.

An approach that uses statistical distribution of benthic chlorophyll and water column nutrients was proposed to classify trophic state in streams given a lack of breakpoints (Dodds et al. 1998). Trophic categories by statistical distributions signify the probabilities of each trophic state. However, Dodds et al. (1998) used distributions from data sets that included affected sites; thus, the proposed categories do not represent natural trophic distributions. Many regions of developed countries completely lack such reference sites. However, a broad definition of stream trophic state requires consideration of the historical condition of streams before substantial modification that might influence heterotrophic or autotrophic state.

Which nutrients might be expected to control trophic state in lotic systems?

Before I propose trophic categories, it is important to justify which nutrients need to be considered to classify trophic state (i.e., if respiration and production are the response variables, what are the driver variables?). The most influential limnologist in modern times, G. E. Hutchinson (1957), stated ''Phosphorus is in many ways the element most important to the ecologist, since it is more likely to be deficient, and therefore to limit the biological productivity of any region of the earth's surface, than are the other major biological elements.'' This has led to the view that ''Excessive concentrations of P is [sic] the most common cause of eutrophication in freshwater lakes, reservoirs, streams, and in the headwaters of estuarine systems'' (Correll 1999). These assertions are not based on specific experimental and empirical observations of lotic ecosystems. How well do the data support the statement that P is the primary agent of autotrophic eutrophication in rivers and streams?

One of the common methods for assessing nutrient limitation of benthic algae in flowing waters is measuring chlorophyll accumulation on nutrient-diffusing substrata (e.g., Pringle et al. 1986; Winterbourn 1990). Francoeur (2001) performed a meta-analysis of 237 nutrient enrichment studies in temperate streams and found that 16.5% indicated an N response, 18.1% indicated a P response, 23.2% required N and P be added together for a response, 5% had N or P inhibition, and 43% had no response to N or P.

Tank and Dodds (2003) tested for autotrophic nutrient limitation across 10 North American streams with the use of silica (glass fiber filters) or wood (thin layers of wood veneer) substrata in nutrient-diffusing agar devices. Algae responded differently to nutrients, depending on the substrata on which they were growing, and there was no primary positive response of algae to P enrichment alone at any site. No response to nutrient enrichment was a common result of these experiments, but N or N and P stimulated algal biomass at unshaded sites. The lack of nutrient response was closely linked to sites with limited light and a large amount of canopy cover. Tank and Dodds (2003) also presented a literature review that closely mirrored that of Francoeur (2001) in the percent responses to N and N+P treatments.

Few nutrient-releasing substrata experiments have tested for nutrient responses other than N and P. Chessman et al. (1992) tested for trace nutrient concentrations in eight streams during two seasons in Australia. They found no evidence for trace nutrients (Ca, Mg, K, S, Fe, Mn, Co Cu, Zn, thiamine, biotin, or B_{12}) stimulating algal growth in any stream during any season. The most common response was to N addition alone, with secondary P limitation occurring frequently.

Whole-stream fertilization experiments are rarely conducted under natural conditions, but Stockner and Shortreed (1978) used streamside enrichment troughs in British Columbia and demonstrated a strong P enrichment effect on algal biomass, with a secondary N enrichment effect. Enrichment of the Nechako River in British Columbia indicated N limitation (Perrin and Richardson 1997). Enrichment of a tundra river with P for four consecutive summers first stimulated algal biomass and productivity and eventually stimulated fish production (Peterson et al. 1993).

An alternative, empirical approach for determining the response of algal biomass to nutrients is to establish the statistical relationship between in-stream nutrients and algal biomass. This approach was applied across Missouri streams of varied nutrient enrichment, and positive relationships between water column total N and benthic chlorophyll were found, with a similar relationship between total P and mean benthic chlorophyll (Lohman et al. 1992). Nutrient-enriched sites in this study had more rapid chlorophyll accrual after a scouring flood than nutrient-poor sites. Lohman et al. (1992) speculated that N was more important in these

Table 1. Some studies of flowing waters reporting nitrogen and phosphorus enhancement of heterotrophic activities.

Location	Response variables	Response	Reference
Shaded New Zealand stream	^{14}C glucose incorporation, endocellulase activity	N and P stimulated endocellulase activity, but not glucose uptake	Tank and Winterbourn 1996
Small Appalachian streams	Microbial respiration, fungal biomass, extracellular enzyme activity	N and P colimitation	Tank and Webster 1998
Laboratory with stream assemblages	Leaf degradation by bacteria and fungi	N and P stimulated degradation	Gulis and Suberkropp 2002
10 North American streams	Fungal biomass (ergosterol)	2 streams, no effect; 2, P effect; 4, N stimulation; 2, P stimulation	Tank and Dodds 2003
Laboratory experiment	Leaf mass loss	Both N and P effects found	Bärlocher and Corkum 2003
Coastal wetland soil	Bacterial activity	P stimulated bacteria, whereas N stimulated macrophytes	Sundareshwar et al. 2003
Laboratory experiments on plankton from blackwater streams of North Carolina	Bacterial counts	Both N and P stimulated bacteria	Mallin et al. 2004
Carolina streams	Fungal biomass (ergosterol)	High N- and P-sites had greater ergosterol accumulation	Padgett et al. 2000

streams than P. Similar relationships were subsequently established for 13 rivers in southern Ontario (Chételat et al. 1999).

A cross-system analysis of temperate streams established that total N and total P in the water column were significantly related to benthic algal biomass (Dodds et al. 1997). This relationship is relevant to trophic state because production is positively correlated with algal biomass (Fig. 1). Subsequent analysis of an expanded data set suggested that mean and maximum algal biomass were significantly correlated with total N and, to a lesser extent, with total P in the water column and that the best predictive model for algal biomass included both N and P. This analysis also suggested that in excess of a threshold value of total N and total P, there are no increases in mean benthic chlorophyll, thus indicating that nutrient limitation is overcome when water column nutrient concentrations are great enough (Dodds et al. 2002).

Positive correlations also exist between planktonic chlorophyll and water column nutrients in lotic systems. An analysis of suspended chlorophyll in temperate rivers and streams showed a positive relationship between water column total P and suspended chlorophyll (Van Nieuwenhuyse and Jones 1996), with an apparent decrease in planktonic chlorophyll yield per unit P when total P was in excess of approximately 300 mg m^{-3}. These authors did not consider total N, so the relative importance of N and P could not be assessed from their data. However, Basu and Pick (1996) studied 31 Canadian Shield rivers and found positive correlations of sestonic chlorophyll with total N in addition to total P, but did not demonstrate any decrease in chlorophyll yield at high nutrient concentrations.

Nutrient enrichment experiments on heterotrophic activity are less numerous. However, the existing laboratory and field experiments suggest that nutrients can limit heterotrophic activity (N, P, or both can be important, Table 1). A survey of stream metabolism across eight streams from various North American biomes indicated that soluble reactive P concentrations were positively correlated with both GPP and respiration (Mulholland et al. 2001).

The forms that N and P are in might not be extremely important determinants of heterotrophic or autotrophic state. Algae can use organic N as a primary N source (Antia et al. 1991). In addition, heterotrophic bacteria can be strong competitors for dissolved inorganic nutrients as well as nutrients in dissolved organic compounds (Dodds 2002).

Both N and P (in organic and inorganic forms) could be important determinants of autotrophic and heterotrophic activity in rivers and streams. This is borne out by manipulative experimental approaches and empirical analyses. There is little experimental support for minor nutrients stimulating heterotrophic or autotrophic microbial activity. As discussed in the introductory section, it is clear that C additions will have a strong influence on system heterotrophic activity (O_2 consumption). It is probably unwise to assume a priori that P is the limiting nutrient of the autotrophic state in any particular stream.

Determining boundaries of trophic state

Data presented in the previous section suggest that water column N and P should be considered when characterizing the autotrophic state of rivers and streams and perhaps when determining heterotrophic state. The relative trophic state should be based on the frequency distribution of relatively pristine lotic waters because anthropogenic inputs change over time, as will trophic boundaries. Whereas Dodds et al. (1998) considered total N, total P, and benthic chlorophyll across a wide variety of streams, they did not account for streams that are naturally heterotrophic and did not attempt to use only reference streams to create an expected distribution in the absence of anthropogenic effects.

Reference nutrient data can be used to establish rough limits on the autotrophic state of streams with regard to nutrients; I present one possible approach. Reference nutrient

Table 2. Lower one-third and upper one-third of the distribution of stream total N and total P pooled across 14 ecoregions according to reference values determined for each individual ecoregion by Smith et al. (2003), 13 ecoregions for total P, and 12 ecoregions for total N from Dodds and Oakes (2004) and the relationship of the boundary numbers from Smith et al. (2003) data to cumulative frequency distribution of benthic chlorophyll (Chl) as a function of total N or total P (Fig. 1) expressed as the percentage of benthic chlorophyll mean or maximum values exceeding 100 mg m^{-2} when nutrient values were less than the boundary value. For example, when seasonal mean of total N was <714 mg m^{-3}, then 10% of the streams had mean benthic chlorophyll values exceeding 100 mg m^{-2} and 29% had maximum values exceeding that amount.

		Concentration (mg m^{-3})		Cases exceeding 100 mg m^{-2} (%)	
Nutrient	Autotrophic state boundary	Smith et al. 2003	Dodds and Oakes 2004	Mean Chl	Maximum Chl
Total N	Lower one-third	285	370	7	27
	Upper one-third	714	659	10	29
Total P	Lower one-third	29	23	5	17
	Upper one-third	71	48	13	25

concentrations from modeling, including a correction for atmospheric N deposition, have been proposed for 14 nutrient ecoregions across the United States (Smith et al. 2003). I ranked the median values (one for each ecoregion), and the distribution was divided into the lower, middle, and upper one third (oligotrophic, mesotrophic, and eutrophic, respectively, following limnological convention) of the reference nutrient values (Table 2). The distribution of reference nutrient values roughly agreed with those provided by Dodds and Oakes (2004), who corrected for anthropogenic influences (as represented by human population density and land use characteristics) on stream nutrient concentrations with analysis of covariance across the same ecoregions (Table 2).

There is a positive correlation between autotrophic activity and benthic chlorophyll concentrations in rivers and streams (Fig. 1). Therefore, I initially base autotrophic boundaries on standing stocks of algal biomass, as is the convention in lakes. To accomplish this, the reference nutrient values from Smith et al. 2003 are applied to observed frequency distributions of seasonal mean and maximum benthic chlorophyll, plotted against water column nutrients (Fig. 2). These frequency distributions are used to calculate the probability that a stream will have a given amount of chlorophyll at a specific level of nutrients (Table 2). Relationships derived from those developed by Dodds et al. (2002, corrected for errors Dodds made when entering data from Lohman) also can be used to calculate expected mean and maximum values for benthic chlorophyll on the basis of the nutrient boundaries presented in Table 2 (Table 3).

Benthic chlorophyll values >100 mg m^{-2} previously have been considered a nuisance (Welch et al. 1988). This analysis suggests that a mean value of 100 mg m^{-2} of chlorophyll is attained in <7% of oligotrophic streams and in 10–13% of eutrophic systems. The regression analyses also suggest that oligotrophic systems should exhibit maximum benthic chlorophyll values >100 mg m^{-2} only 27% of the time. Other approaches are possible (e.g., Dodds et al. 1998), but the method presented in this paper considers the dynamic nature of chlorophyll in streams and is reference based.

A similar approach to determining reference trophic state can be taken with regard to planktonic chlorophyll in rivers and streams. A large data set ($n = 292$) of lotic planktonic chlorophyll and water column total P was assembled for temperate rivers and streams, and associated regression equations can be used to link nutrients and phytoplankton biomass (Van Nieuwenhuyse and Jones 1996). A smaller data set from 31 rivers in southern Ontario and western Quebec related total N (mg m^{-3}) and total P to planktonic chlorophyll (mg m^{-3}; Basu and Pick 1996). This paper presented a regression equation for total P, but regression of their raw data yielded the following relationship.

$$\log_{10}(\text{planktonic chlorophyll}) = -1.247 + 0.676 \log_{10}(\text{total N}) \qquad r^2 = 0.65$$

The distribution of reference values from Smith et al. (2003) can then be used to calculate autotrophic categories from these equations (Table 4). These data agree roughly with both the Van Nieuwenhuyse and Jones (1996) and the Basu and Pick (1996) equations for total P, but the total N boundaries derived from the Basu and Pick chlorophyll–total N relationship were substantially lower than those derived for total P from the same data set. The data suggest that planktonic chlorophyll only exceeds values considered typical of eutrophic lakes (8 mg m^{-3}; Dodds 2002) when nutrients are abundant relative to the reference condition. The data also are consistent with the idea that the amount of planktonic chlorophyll per unit total N or total P is less in lotic waters than in lentic waters (Søballe and Kimmel 1987).

More limited data are available for whole-stream estimates of autotrophic and heterotrophic state, but some idea of the ranges expected for the trophic states can be gleaned from analysis of the results of a cross-system study (Mulholland et al. 2001). Although this study and an additional data point (P. Mulholland pers. comm.) only covers nine streams, it has three important characteristics. First, all the measurements were done the same way at each site with methods likely to give the best results (two-station diel O_2 method, corrected for groundwater influences). Second, all the sites studied but one were relatively pristine small streams, so the data can be used to determine trophic boundaries mostly in the absence of human effects. Third, the streams were located in a variety of biomes, including one desert, one prairie, one tropical, one arid montane, one mesic montane, and four temperate deciduous biomes (Mulholland et al. 2001). Whole-stream autotrophic state varied over 150-fold in this data set (very high rates of GPP were associated with the lighted desert stream), with the central one third of the distribution falling between 0.4 and 1.8 g O_2 m^{-2} d^{-1} (Table 5). Heterotrophic state was considerably less variable, ranging about 10-fold with the central one third of the distribution falling between 6.7 and 8.3 g O_2 m^{-2} d^{-1} (Table 5).

Bott et al. (1985) reviewed studies of ~70 streams with

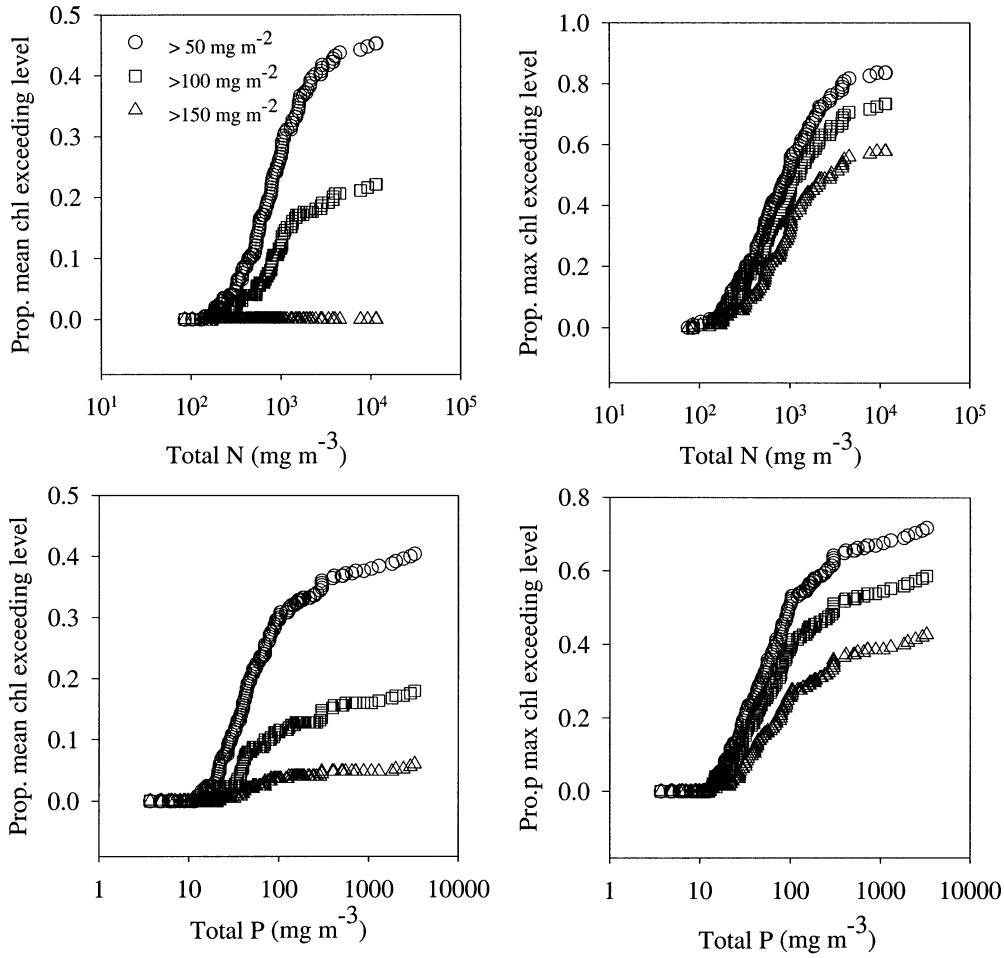

Fig. 2. Relationships between seasonal mean water column nutrients (total N and total P) and proportion of instances in which seasonal mean and maximum chlorophyll exceed 50, 100, or 150 mg m^{-3}. Data are from literature sources compiled in Dodds et al. (2002), mostly for shallow rivers and streams. This compilation previously had incorrect values for data reported by Lohman et al. (1992). Those values now match the original source. $n = 250$ for total P and $n = 199$ for total N.

maximum rates of 48 and 50 g O_2 m^{-2} d^{-1} for GPP and respiration, respectively. These rates were from streams with human effects and were several-fold higher than the maximum from more pristine streams. This indicates that both autotrophic state and heterotrophic state can be influenced by eutrophication. Maximum rates of GPP are probably limited by light under nutrient-replete conditions, whereas respiration is probably limited by O_2 aeration rate in streams with high loading of biochemical oxygen demand.

I speculate that light limits autotrophic state of streams (interception by the canopy), but not heterotrophic state, because although light is intercepted by riparian vegetation, it

Table 3. Corrected regression equations for data presented in Dodds et al. (2002) and expected autotrophic state mean and maximum benthic chlorophyll (Chl) values calculated from nutrient concentrations in Table 1 with these equations. Equations are of the form $\log_{10}$(mg chlorophyll m^{-2}) = Intercept + B1 $\log_{10}$(mg m^{-3} total N or total P) + B2 [$\log_{10}$(mg m^{-3} total N or total P)]^2.

Relationship	Intercept	B_1	B_2	R^2	Expected chlorophyll (mg m^2) Lower ⅓	Upper ⅓
Mean Chl versus total N	−2.638	2.460	−0.320	0.401	30	60
Maximum Chl versus total N	0.438	0.613		0.295	88	154
Mean Chl versus total P	−0.608	1.486	−0.255	0.402	36	65
Maximum Chl versus total P	0.216	1.680	−0.297	0.371	109	204

Table 4. Autotrophic state boundaries for suspended chlorophyll in temperate rivers and streams as calculated from the reference nutrient concentrations from Smith et al. (2003) and regression equations based on Van Nieuwenhuyse and Jones (1996) and Basu and Pick (1996).

			Planktonic chlorophyll (mg m^{-3})	
Nutrient	Autotrophic state boundary	Nutrient conc. (mg m^{-3})	Van Nieuwenhuyse and Jones (1996)	Basu and Pick (1996)
Total N	Lower one-third	285		2.4
	Upper one-third	714		4.5
Total P	Lower one-third	29	4.6	6.4
	Upper one-third	71	11.9	12.3

Table 5. Distribution of whole-stream metabolism rates from nine small, relatively pristine streams (data from Mullholland et al. [2001] plus one point from Ball Creek, North Carolina [Mulholland pers. comm.]). Respiration rates are corrected for groundwater input.

	Metabolism (g O_2 m^{-2} d^{-1})		
Distribution	Gross primary production	Respiration	Net primary production
Upper one-third	1.8	8.3	−4.2
Lower one-third	0.4	6.7	−6.7
Minimum	0.06	2.4	−29
Maximum	15	29	6.7

does not substantially influence rates of C input. I predict that the amount of C fixed by the riparian canopy that enters the streams to fuel heterotrophic activity is approximately equal to what would enter by autochthonous production in a lighted stream without canopy cover.

Small streams in forested biomes are shaded, have substantial amounts of organic C input from nearby riparian areas fueling heterotrophic activity, and have minimal autotrophic production (except in deciduous seasonal forests in which light can penetrate the canopy when leaves are not present). Prairie, tundra, or desert streams have limited riparian canopy and substantial autotrophic production fueling heterotrophic activity. An independent measure of total metabolic activity, N uptake rates, also varied little across the range of biomes studied by Mulholland et al. (2001), supporting the concept of relatively constant heterotrophic activity in small pristine streams (Webster et al. 2003). Heterotrophic state might be more variable in rivers; canopy has less of an influence, and turbidity could substantially interfere with riverine C production.

Although the approach taken here might provide useful in setting boundaries for autotrophic and heterotrophic state, more comprehensive measurements of stream metabolism are required. Until such comprehensive measurements are made, the values for boundaries presented here should be used with caution. In addition, whole-river metabolism rates are difficult to measure, and data are difficult to come by for such rivers. Very few large rivers remain in temperate regions that are relatively weakly influenced by humans, so it might not be possible to set definitive autotrophic and heterotrophic state boundaries for larger lotic systems in some regions.

Although determining trophic boundaries could be useful in describing fundamental ecosystem processes, changes in trophic state must be linked to other aspects of stream ecosystems for such boundaries to be relevant. Furthermore, it is important to explore how stream eutrophication is propagated through the food web to influence biotic integrity and community structure.

Effects of eutrophication

Producers—Stevenson and Pan (1999) reviewed the uses of diatoms for assessing environmental conditions in rivers and streams. They traced the use of species compositions of algae to infer amount of pollution to work by Kolkwitz and Marsson in the early 1900s, with substantial contributions by Ruth Patrick in the 1940s and 1950s (as cited by Stevenson and Pan 1999). Studies that use algal assemblages as indicators of the extent of pollution rely on the concept that predictable species shifts occur with set amounts of enrichment (e.g., Kelly 2002). Detailed work has been carried out relating nutrients to diatom and other algal assemblages in several places, mostly in temperate, developed countries.

The green alga *Cladophora* has often been associated with eutrophication events (Hynes 1960) and is ubiquitous in nutrient-rich flowing waters (Dodds and Gudder 1992). Large streamers of *Cladophora* develop under nutrient-rich conditions. These streamers potentially lead to low O_2 events at night, alter the community structure, snag fish lures, slow water flow in canals, and clog industrial and domestic water intakes (Dodds and Gudder 1992).

One of the problems with predicting eutrophication effects in streams is that variability caused by flooding can influence autotrophic state. At one extreme, algal biomass might not accrue with ample light and nutrients if floods always scour biomass. On the other end of the spectrum, attached algae might be able to attain impressive biomass in nutrient-poor water because periphyton can use the small amounts of nutrients that continuously flow by. Biggs (2000) developed a comprehensive model linking hydrologic regime and nutrients to accrual of algal biomass. This model was developed with a database from New Zealand rivers and streams across a wide range of land use practices and hydrologic patterns. Regressions considering only dissolved inorganic nutrients could predict algal biomass with r^2 values of approximately 30%. Consideration of the time of accrual (time since the last scouring flood) increased r^2 values to about 70%. The work of Biggs (2000) supports the proposition that eutrophication effects will be stronger under stable flow regimes.

The effects of eutrophication on macrophytes in flowing waters have been poorly studied, and the effects of nutrient reductions on macrophyte biomass are difficult to predict (Chambers et al. 1999). Biomass of macrophytes declined in the Bow River (Alberta) in response to nutrient control (particularly N) from municipal wastewater sources (Sosiak 2002). Sewage effluent led to substantially greater macrophyte biomass in the Saskatchewan River (Saskatchewan),

and this was correlated with somewhat decreased dissolved O_2 concentration (Chambers and Prepas 1994).

In some rivers and streams with reduced water replacement times, phytoplankton blooms can become problematic, with cyanobacterial blooms more likely in excess-nutrient conditions (Smith 2003). Shorter water turnover time (hydraulic residence time) leads to a decreased amount of suspended chlorophyll per unit concentration of P (Søballe and Kimmel 1987). Problems occur with phytoplankton blooms in European and other rivers around the world (Wehr and Descy 1998). In the Murray Darling river system in South Australia, water withdrawals reduce flow to a near standstill in the river, and excess amounts of nutrients, stratification, and warm temperature stimulate algal blooms (Maier et al. 2001). These blooms are commonly dominated by the hepatotoxic *Microcystis.* Other slow-flowing rivers in the world suffer a similar fate, particularly those with limited quantities of light-intercepting fine sediments.

Microbial heterotrophs—Although enrichment experiments have documented that rates of microbial heterotrophic processing of organic materials can be stimulated by nutrients (as previously discussed), less is known about influences on the heterotrophic microbial community. If the primary source of organic C to a stream or river is leaf material, N and P need to be obtained from the water column, and nutrient enrichment will increase C utilization rates. One study documented that nutrient enrichment causes shifts in fungal taxa associated with decomposing leaf litter (Gulis and Suberkropp 2002). Presumably, some bacteria that decompose organic matter are better competitors for organic nutrients than others, leading to shifts in community structure in response to nutrient enrichment. Future studies are likely to document this effect, given the recent expansion of molecular techniques. Clear increases in the rates of heterotrophic microbial biogeochemical cycling (denitrification) related to nutrient enrichment by agricultural practices have been demonstrated (Kemp and Dodds 2002).

Food web effects—Effects of C and, particularly, N and P loading on animals in streams are less clear. The effects of C on the animal community are obvious, with greater rates of organic C loading leading to dominance by pollution-tolerant invertebrates (such as *Tubifex, Limnodrilus, Chironomus*), decreases in diversity, and increases in raw abundance (Hynes 1960). With the advent of BOD treatment in sewage and industrial effluents in developed nations, less attention has been paid to the effects of BOD loading.

Enrichment effects related to N and P are less well established. Macroinvertebrate assemblage structure has been correlated statistically with P concentration (Miltner and Rankin 1998). Nutrient enrichment can cause increases in invertebrate abundance and alters assemblage structure (Bourassa and Cattaneo 1998). The clearest study to date on the importance of sustained nutrient loading to the food web occurred on the Lawrence River downstream of Montreal, Quebec. This study used the distinctive isotopic signal of ^{15}N to establish that nutrients from the sewage outfall significantly enriched macroinvertebrates and production of both macroinvertebrates and fishes (deBruyn et al. 2003). The sewage was treated for BOD, but stimulated secondary production over fivefold in spite of the small amount of N and P that entered the food web in the sewage plume 10 km down from the sewage outfall.

Control of cultural eutrophication—Given the definition of the trophic state proposed, and the potential effects of autotrophic and heterotrophic eutrophication, what considerations are important in controlling eutrophication? Mechanistic methods are only beginning to be established for linking in-stream nutrient concentrations to watershed activities. Empirical methods have prevailed (e.g., Dodds et al. 1997) until recently. Modeling efforts are beginning to refine nutrient concentration and loading estimates for rivers, but there still is some difficulty in linking models created for small streams with larger river systems (e.g., Alexander et al. 2002). Ultimately, linking land use practices, including both point and nonpoint sources of nutrients, to instream nutrient concentrations will be necessary to control cultural eutrophication that influences autotrophic state, and potentially influences heterotrophic state.

Nutrient control is, on one level, simple. Agricultural practices, atmospheric loading, and human sewage outfall increase inorganic and organic nutrients in rivers and streams. Technology is available to decrease that input (but nonpoint sources of nutrients such as atmospheric deposition and runoff from cropland remain difficult to control). Best management practices of cropland include riparian buffer strips, cropland terracing, and the use of only the necessary amounts of fertilizer. Effluent from human sewage and livestock-handling facilities can be treated with existing tertiary treatment methods (e.g., denitrification facilities, P precipitation) to reduce N and P loads to lotic waters. The effective reduction of BOD into the waters of most developed countries exemplifies the technical ability of water treatment engineers and managers to remove potentially harmful pollutants at acceptable costs. The challenge now is to determine what lengths are necessary to control point and nonpoint source pollution, and to what degree the benefits of nutrient control justify the costs. Determining the reference trophic state provides a starting point for cost–benefit and feasibility analyses of eutrophication control schemes.

Nutrient cycles do not occur in isolation, and colimitation of algal and heterotrophic activity is commonly seen in bioassays (Tank and Dodds 2003). We are only beginning to understand the implications of the effects of humans on the stoichiometry of nutrient loading (Turner 2002). Stoichiometric changes could alter algal assemblages and relative rates of material flux (e.g., Woodruff et al. 1999). Changes in stoichiometry could then cascade to higher trophic levels (Frost et al. 2002).

Given the broad definition of eutrophication presented herein, organic C enrichment should be considered, as well as anthropogenic processes causing shifts in the relative heterotrophic and autotrophic states. For example, increased BOD from sewage has definite influences on stream heterotrophic state. In addition, shifts in riparian vegetation, such as loss of riparian forests, might increase the autotrophic state and decrease the heterotrophic state. In systems such as tallgrass prairies, historically dominated by little riparian

vegetation, increases in riparian vegetation could alter the fundamental ecosystem and community structure (Dodds et al. 2004). Finally, organic C enrichment might interact with N and P enrichment. The highest rates of C consumption and the greatest biomass of heterotrophic organisms are expected when loading of N, P, and C are simultaneously high.

Water retention times might alter nutrient stoichiometry and heterotrophic and autotrophic states by influencing deposition and nutrient processing rates. Small and large impoundments that were not historically present are now a ubiquitous feature on many river networks. Such impoundments could also alter the balance between heterotrophic and autotrophic states because many recalcitrant C-rich particulate organic materials can settle in the reservoir, and plankton with relatively low values of C : N and C : P could dominate reservoir tail waters (Whiles and Dodds 2002).

Humans will affect ever more river miles with hydrologic modification, alter the inputs of organic C and its form to lotic waters through alteration of riparian vegetation and input of BOD in sewage from humans and livestock. Increased fertilizer to grow the crops necessary to feed an expanding human population and increases in industrial livestock operations resulting in vast production of animal waste will cause further eutrophication of already affected rivers and streams. These effects will continue to spread into the few relatively pristine watersheds that remain on earth, altering water quality and influencing the biotic integrity of these waters. Understanding the full implications of these effects will require further knowledge of the native trophic state of streams as a baseline. More complete comprehension of how nutrient interactions influence trophic state, and determination of trophic states of medium to large rivers will improve the scientific basis for managing eutrophication of lotic waters.

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Ambient Water Quality Criteria Recommendations

Information Supporting the Development of State and Tribal Nutrient Criteria

Rivers and Streams in Nutrient Ecoregion IX

EPA -822-B-00-019

AMBIENT WATER QUALITY CRITERIA RECOMMENDATIONS

INFORMATION SUPPORTING THE DEVELOPMENT OF STATE AND TRIBAL NUTRIENT CRITERIA

FOR

RIVERS AND STREAMS IN NUTRIENT ECOREGION IX

Southeastern Temperate Forested Plains and Hills

including all or parts of the States of

Maryland, Pennsylvania, Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Tennessee, Kentucky, Indiana, Illinois, Iowa, Missouri, Kansas, Oklahoma, Arkansas, Louisiana, Texas

and the authorized Tribes within the Ecoregion

U.S. ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF WATER
OFFICE OF SCIENCE AND TECHNOLOGY
HEALTH AND ECOLOGICAL CRITERIA DIVISION
WASHINGTON, D.C.

DECEMBER 2000

FOREWORD

This document presents EPA's nutrient criteria for **Rivers and Streams in Nutrient Ecoregion IX.** These criteria provide EPA's recommendations to States and authorized Tribes for use in establishing their water quality standards consistent with section 303(c) of CWA. Under section 303(c) of the CWA, States and authorized Tribes have the primary responsibility for adopting water quality standards as State or Tribal law or regulation. The standards must contain scientifically defensible water quality criteria that are protective of designated uses. EPA's recommended section 304(a) criteria are not laws or regulations – they are guidance that States and Tribes may use as a starting point for the criteria for their water quality standards.

The term "water quality criteria" is used in two sections of the Clean Water Act, Section 304(a)(1) and Section 303(c)(2). The term has a different impact in each section. In Section 304, the term represents a scientific assessment of ecological and human health effects that EPA recommends to States and authorized Tribes for establishing water quality standards that ultimately provide a basis for controlling discharges or releases of pollutants or related parameters. Ambient water quality criteria associated with specific waterbody uses when adopted as State or Tribal water quality standards under Section 303 define the level of a pollutant (or, in the case of nutrients, a condition) necessary to protect designated uses in ambient waters. Quantified water quality criteria contained within State or Tribal water quality standards are essential to a water quality-based approach to pollution control. Whether expressed as numeric criteria or quantified translations of narrative criteria within State or Tribal water quality standards, quantified criteria serve as a critical basis for assessing attainment of designated uses and measuring progress toward meeting the water quality goals of the Clean Water Act.

EPA is developing section 304(a) water quality criteria for nutrients because States and Tribes consistently identify excessive levels of nutrients as a major reason why as much as half of the surface waters surveyed in this country do not meet water quality objectives, such as full support of aquatic life. EPA expects to develop nutrient criteria that cover four major types of waterbodies – lakes and reservoirs, rivers and streams, estuarine and coastal areas, and wetlands – across fourteen major ecoregions of the United States. EPA's section 304(a) criteria are intended to provide for the protection and propagation of aquatic life and recreation. To support the development of nutrient criteria, EPA is publishing Technical Guidance Manuals that describe a process for assessing nutrient conditions in the four waterbody types.

EPA's section 304(a) water quality criteria for nutrients provide numeric water quality criteria, as well as procedures by which to translate narrative criteria within State or Tribal water quality standards. In the case of nutrients, EPA section 304(a) criteria establish values for causal variables (e.g., total nitrogen and total phosphorus) and response variables (e.g., turbidity and chlorophyll *a*). EPA believes that State and Tribal water quality standards need to include quantified endpoints for causal and response variables to provide sufficient protection of uses and to maintain downstream uses. These quantified endpoints will most often be expressed as numeric water quality criteria or as procedures to translate a State or Tribal narrative criterion into a quantified endpoint.

EPA will work with States and authorized Tribes as they adopt water quality criteria for nutrients into their water quality standards. EPA recognizes that States and authorized Tribes require flexibility in adopting numeric nutrient criteria into State and Tribal water quality standards. States and authorized Tribes have several options available to them. EPA recommends the following approaches, in order of preference:

(1) Wherever possible, develop nutrient criteria that fully reflect localized conditions and protect specific designated uses using the process described in EPA's Technical Guidance Manuals for nutrient criteria development. Such criteria may be expressed either as numeric criteria or as procedures to translate a State or Tribal narrative criterion into a quantified endpoint in State or Tribal water quality standards.

(2) Adopt EPA's section 304(a) water quality criteria for nutrients, either as numeric criteria or as procedures to translate a State or Tribal narrative nutrient criterion into a quantified endpoint.

(3) Develop nutrient criteria protective of designated uses using other scientifically defensible methods and appropriate water quality data.

Geoffrey H. Grubbs, Director
Office of Science and Technology

DISCLAIMER

This document provides technical guidance and recommendations to States, authorized Tribes, and other authorized jurisdictions to develop water quality criteria and water quality standards under the Clean Water Act (CWA) to protect against the adverse effects of nutrient overenrichment. Under the CWA, States and authorized Tribes are to establish water quality criteria to protect designated uses. State and Tribal decision-makers retain the discretion to adopt approaches on a case-by-case basis that differ from this guidance when appropriate and scientifically defensible. While this document contains EPA's scientific recommendations regarding ambient concentrations of nutrients that protect aquatic resource quality, it does not substitute for the CWA or EPA regulations; nor is it a regulation itself. Thus it cannot impose legally binding requirements on EPA, States, authorized Tribes, or the regulated community, and it might not apply to a particular situation or circumstance. EPA may change this guidance in the future.

EXECUTIVE SUMMARY

Nutrient Program Goals

EPA developed the National Strategy for the Development of Regional Nutrient Criteria (National Strategy) in June 1998. The strategy presents EPA's intentions to develop technical guidance manuals for four types of waters (lakes and reservoirs, rivers and streams, estuaries and coastal waters, and wetlands) and produce section 304(a) criteria for specific nutrient ecoregions by 2000. In addition, the Agency formed Regional Technical Assistance Groups (RTAGs) which include State and Tribal representatives working to develop more refined and more localized nutrient criteria based on approaches described in the waterbody guidance manuals. This document presents EPA's current recommended criteria for total phosphorus, total nitrogen, chlorophyll *a*, and turbidity for rivers and streams in Nutrient Ecoregion IX (Southeastern Temperate Forested Plains and Hills) which were derived using the procedures described in the Rivers and Streams Nutrient Criteria Technical Guidance Manual (2000b).

EPA's ecoregional nutrient criteria are intended to address cultural eutrophication-- the adverse effects of excess nutrient inputs. The criteria are empirically derived to represent conditions of surface waters that are minimally impacted by human activities and protective of aquatic life and recreational uses. The information contained in this document represent starting points for States and Tribes to develop (with assistance from EPA) more refined nutrient criteria.

In developing these criteria recommendations, EPA followed a process which included, to the extent they were readily available, the following elements critical to criterion derivation:

- **Historical and recent nutrient data in Nutrient Ecoregion IX.**
  Data sets from Legacy STORET, NASQAN, NAWQA, Auburn University, and EPA Regions 3, 5 and 7 were used to assess nutrient conditions from 1990 to 1998.

- **Reference sites/reference conditions in Nutrient Ecoregion IX.**
  Reference conditions presented are based on 25th percentiles of all nutrient data including a comparison of reference condition for the aggregate ecoregion versus the subecoregions. States and Tribes are urged to determine their own reference sites for rivers and streams within the ecoregion at different geographic scales and to compare them to EPA's reference conditions.

- **Models employed for prediction or validation.**
  EPA did not identify any specific models used in the ecoregion to develop nutrient criteria. States and Tribes are encouraged to identify and apply appropriate models to support nutrient criteria development.

- **RTAG expert review and consensus**.
  EPA recommends that when States and Tribes prepare their nutrient criteria, they obtain the expert review and consent of the RTAG.

- **Downstream effects of criteria.**
  EPA encourages the RTAG to assess the potential effects of the proposed criteria on downstream water quality and uses.

In addition, EPA followed specific **QA/QC procedures** during data collection and analysis: All data were reviewed for duplications. All data are from ambient waters that were not located directly outside a permitted discharger. The following States indicated that their data were sampled and analyzed using either Standard methods or EPA approved methods: Florida, Georgia, Illinois, Indiana, Iowa, Maryland, Missouri, North Carolina, South Carolina, and Tennessee.

The following tables contain a summary of Aggregate and level III ecoregion values for TN, TP, water column chl *a*, and turbidity:

BASED ON 25th PERCENTILE ONLY

Nutrient Parameters	Aggregate Nutrient Ecoregion IX Reference Conditions
Total phosphorus (μg/L)	36.56
Total nitrogen (mg/L)	0.69
Chlorophyll *a* (μg/L) (Spectrophotometric method)	0.93
Periphyton Chlorophyll *a* (mg/m^2)	20.35
Turbidity (FTU)	5.7

For subecoregions, 29,33, 35, 37, 40, 45, 64, 65, 71, 72, and 74, the ranges of nutrient parameter reference conditions are:

BASED ON 25th PERCENTILE ONLY

Nutrient Parameters	Range of Level III Subecoregions Reference Conditions
Total phosphorus (μg/L)	22.5 - 100.00
Total nitrogen (mg/L)	0.07 - 1.0
Chlorophyll *a* (μg/L)	0.05 - 5.74
Periphyton Chlorophyll *a* (mg/m^2)	3.13 - 20.35
Turbidity (FTU)	3.15 - 13.5

NOTICE OF DOCUMENT AVAILABILITY

This document is available electronically to the public through the INTERNET at: (http://www.epa.gov/OST/standards/nutrient.html). Requests for hard copies of the document should be made to EPA's National Service Center for Environmental Publications (NSCEP), 11029 Kenwood Road, Cincinnati, OH 45242 or (513) 489-8190, or toll free (800) 490-9198. Please refer to EPA document number **EPA-822-B-00-019**.

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1.0 INTRODUCTION

Background

Nutrients are essential to the health and diversity of our surface waters. However, in excessive amounts, nutrients cause hypereutrophication, which results in overgrowth of plant life and decline of the biological community. Excessive nutrients can also result in potential human health risks, such as the growth of harmful algal blooms - most recently manifested in the *Pfiesteria* outbreaks of the Gulf and East Coasts. Chronic nutrient overenrichment of a waterbody can lead to the following consequences: low dissolved oxygen, fish kills, algal blooms, overabundance of macrophytes, likely increased sediment accumulation rates, and species shifts of both flora and fauna.

Historically, National Water Quality Inventories have repeatedly shown that nutrients are a major cause of ambient water quality use impairments. EPA's 1996 National Water Quality Inventory report identifies excessive nutrients as the leading cause of impairment in lakes and the second leading cause of impairment in rivers (behind siltation). In addition, nutrients were the second leading cause of impairments reported by the States in their 1998 lists of impaired waters. Where use impairment is documented, nutrients contribute roughly 25-50% of the impairment nationally. The Clean Water Act establishes a national goal to achieve, wherever attainable, water quality which provides for the protection and propagation of fish, shellfish, and wildlife and recreation in and on the water. In adopting water quality standards, States and Tribes designate uses for their waters in consideration of the Clean Water Act goals, and establish water quality criteria that contain sufficient parameters to protect those uses. To date, EPA has not published information and recommendations under section 304(a) for nutrients to assist States and Tribes in establishing numeric nutrient criteria to protect uses when adopting water quality standards.

In 1995, EPA gathered a set of national experts and asked the experts how to best deal with the national nutrient problem. The experts recommended that the Agency not develop single criteria values for phosphorus or nitrogen applicable to all water bodies and regions of the country. Rather, the experts recommended that EPA put a premium on regionalization, develop guidance (assessment tools and control measures) for specific waterbodies and ecological regions across the country, and use reference conditions (conditions that reflect pristine or minimally impacted waters) as a basis for developing nutrient criteria.

With these suggestions as starting points, EPA developed the National Strategy for the Development of Regional Nutrient Criteria (National Strategy), published in June 1998. This strategy presented EPA's intentions to develop technical guidance manuals for four types of waters (lakes and reservoirs, rivers and streams, estuaries and coastal waters, and wetlands) and, thereafter, to publish section 304(a) criteria recommendations for specific nutrient ecoregions. Technical guidance manuals for lakes/reservoirs and rivers/streams were published in April 2000 and July 2000, respectively. The technical guidance manual for estuaries/coastal waters will be published in spring 2000 and the draft wetlands technical guidance manual will be published by December 2001. Each manual presents EPA's recommended approach for developing nutrient criteria values for a specific waterbody type. In addition, EPA is committed to working with

States and Tribes to develop more refined and more localized nutrient criteria based on approaches described in the waterbody guidance manuals and this document.

Overview of the Nutrient Criteria Development Process

For each Nutrient Ecoregion, EPA developed a set of recommendations for two causal variables (total nitrogen and total phosphorus) and two early indicator response variables (chlorophyll *a* and some measure of turbidity). Other indicators such as dissolved oxygen and macrophyte growth or speciation, and other fauna and flora changes are also deemed useful. However, the first four are considered to be the best suited for protecting designated uses.

The technical guidance manuals describe a process for developing nutrient criteria that involves consideration of five factors. The first of these is the Regional Technical Assistance Group (RTAG), which is a body of qualified regional specialists able to objectively evaluate all of the available evidence and select the value(s) appropriate to nutrient control in the water bodies of concern. These specialists may come from such disciplines as limnology, biology, natural resources management-- especially water resource management, chemistry, and ecology. The RTAG evaluates and recommends appropriate classification techniques for criteria determination, usually physical within an ecoregional construct.

The second factor is the historical information available to establish a perspective of the resource base. This is usually data and anecdotal information available within the past ten-twenty five years. This information gives evidence about the background and enrichment trend of the resource.

The third factor is the present reference condition. A selection of reference sites chosen to represent the least culturally impacted waters of the class existing at the present time. The data from these sites is combined and a value from the distribution of these observations is selected to represent the reference condition, or best attainable, most natural condition of the resource base at this time.

A fourth factor often employed is theoretical or empirical models of the historical and reference condition data to better understand the condition of the resource.

The RTAG comprehensively evaluates the other three elements to propose a candidate criterion (initially one each for TP, TN, chl *a*, and some measure of turbidity).

The last and final element of the criteria development process is the assessment by the RTAG of the likely downstream effects of the criterion. Will there be a negative, positive, or neutral effect on the downstream waterbody? If the RTAG judges that a negative effect is likely, then the proposed State/Tribal water quality criteria should be revised to ameliorate the potential for any adverse downstream effects.

While States and authorized Tribes would not necessarily need to incorporate all five elements into their water quality criteria setting process (e.g., modeling may be significant in only some instances), the best assurance of a representative and effective criterion for nutrient management decision making is the balanced incorporation of all five elements, or at least all elements except modeling.

Because some parts of the country have naturally higher soil and parent material enrichment, and different precipitation regimes, the application of the criterion development process has to be adjusted by region. Therefore, an ecoregional approach was chosen to develop nutrient criteria appropriate to each of the different geographical and climatological areas of the country. Initially, the continental U.S. was divided into 14 separate ecoregions of similar geographical characteristics. Ecoregions are defined as regions of relative homogeneity in ecological systems; they depict areas within which the mosaic of ecosystem components (biotic and abiotic as well as terrestrial and aquatic) is different than adjacent areas in a holistic sense. Geographic phenomena such as soils, vegetation, climate, geology, land cover, and physiology that are associated with spatial differences in the quantity and quality of ecosystem components are relatively similar within each ecoregion.

The Nutrient ecoregions are aggregates of U.S. EPA's hierarchal level III ecoregions. As such, they are more generalized and less defined than level III ecoregions. EPA determined that setting ecoregional criteria for the large scale aggregates is not without its drawbacks - variability is high due to the lumping of many waterbody classes, seasons, and years worth of multipurpose data over a large geographic area. For these reasons, the Agency recommends that States and Tribes develop nutrient criteria at the level III ecoregional scale and at the waterbody class scale where those data are readily available. Data analyses and recommendations on both the large aggregate ecoregion scale as well as more refined scales (level III ecoregions and waterbody classes), where data were available to make such assessments, are presented for comparison purposes and completeness of analysis.

Relationship of Nutrient Criteria to Biological Criteria

Biological criteria are quantitative expressions of the desired condition of the aquatic community. Such criteria can be based on an aggregation of data from sites that represent the least-impacted and attainable condition for a particular waterbody type in an ecoregion, subecoregion, or watershed. EPA's nutrient criteria recommendations and biological criteria recommendations have many similarities in the basic approach to their development and data requirements. Both are empirically derived from statistical analysis of field collected data and expert evaluation of current reference conditions and historical information. Both utilize direct measurements from the environment to integrate the effects of complex processes that vary according to type and location of waterbody. The resulting criteria recommendations, in both cases, are efficient and holistic indicators of water quality necessary to protect uses.

States and authorized Tribes can develop and apply nutrient criteria and biological criteria in tandem, with each providing important and useful information to interpret both the nutrient enrichment levels and the biological condition of sampled waterbodies. For example, using the

same reference sites for both types of criteria can lead to efficiencies in both sample design and data analysis. In one effort, environmental managers can obtain information to support assessment of biological and nutrient condition, either through evaluating existing data sets or through designing and conducting a common sampling program. The traditional biological criteria variables of benthic invertebrate and fish sampling can be readily incorporated to supplement a nutrient assessment. To demonstrate the effectiveness of this tandem approach, EPA has initiated pilot projects in both freshwater and marine environments to investigate the relationship between nutrient overenrichment and apparent declines in diversity indices of benthic invertebrates and fish.

2.0 BEST USE OF THIS INFORMATION

EPA recommendations published under section 304(a) of the CWA serve several purposes, including providing guidance to States and Tribes in adopting water quality standards for nutrients that ultimately provide a basis for controlling discharges or releases of pollutants. The recommendations also provide guidance to EPA when promulgating Federal water quality standards under section 303(c) when such action is necessary. Other uses include identification of overenrichment problems, management planning, project evaluation, and determination of status and trends of water resources.

State water quality inventories and listings of impaired waters consistently rank nutrient overenrichment as a top contributor to use impairments. EPA's water quality standards regulations at 40 CFR §131.11(a) require States and Tribes to adopt criteria that contain sufficient parameters and constituents to protect the designated uses of their waters. In addition, States and Tribes need quantifiable targets for nutrients in their standards to assess attainment of uses, develop water quality-based permit limits and source control plans, and establish targets for total maximum daily loads (TMDLs).

EPA expects States and Tribes to address nutrient overenrichment in their water quality standards, and to build on existing State and Tribal initiated efforts where possible. States and Tribes can address nutrient overenrichment through establishment of numerical criteria or through use of new or existing narrative criteria statements (e.g., free from excess nutrients that cause or contribute to undesirable or nuisance aquatic life or produce adverse physiological response in humans, animals, or plants). In the case of narrative criteria, EPA expects that States and Tribes establish procedures to quantitatively translate these statements for both assessment and source control purposes.

The intent of developing ecoregional nutrient criteria is to represent conditions of surface waters that are minimally impacted by human activities and thus protect against the adverse effects of nutrient overenrichment from cultural eutrophication. EPA's recommended process for developing such criteria includes physical classification of waterbodies, determination of current reference conditions, evaluation of historical data and other information (such as published literature), use of models to simulate physical and ecological processes or determine empirical relationships among causal and response variables (if necessary), expert judgement, and evaluation of downstream effects. To the extent allowed by the information available, EPA has

used elements of this process to produce the information contained in this document. The values for both causal (total nitrogen, total phosphorus) and biological and physical response (chlorophyll *a*, turbidity) variables represent a set of starting points for States and Tribes to use in establishing their own criteria in standards to protect uses.

In its water quality standards regulations, EPA recommends that States and Tribes establish numerical criteria based on section 304(a) guidance, section 304(a) guidance modified to reflect site-specific conditions, or other scientifically defensible methods. For many pollutants, such as toxic chemicals, EPA expects that section 304(a) guidance will provide an appropriate level of protection without further modification in most cases. EPA has also published methods for modifying 304(a) criteria on a site-specific basis, such as the water effect ratio, where site-specific conditions warrant modification to achieve the intended level of protection. For nutrients, however, EPA expects that, in most cases, it will be necessary for States and authorized Tribes to identify with greater precision the nutrient levels that protect aquatic life and recreational uses. This can be achieved through development of criteria modified to reflect conditions at a smaller geographic scale than an ecoregion such as a subecoregion, the State or Tribe level, or specific class of waterbodies. Criteria refinement can occur by grouping data or performing data analyses at these smaller geographic scales. Refinement can also occur through further consideration of other elements of criteria development, such as published literature or models.

The values presented in this document generally represent nutrient levels that protect against the adverse effects of nutrient overenrichment and are based on information available to the Agency at the time of this publication. However, States and Tribes should critically evaluate this information in light of the specific designated uses that need to be protected. For example, more sensitive uses may require more stringent values as criteria to ensure adequate protection. On the other hand, overly stringent levels of protection against the adverse effects of cultural eutrophication may actually fall below levels that represent the natural load of nutrients for certain waterbodies. In cases such as these, the level of nutrients specified may not be sufficient to support a productive fishery. In the criteria derivation process, it is important to distinguish between the natural load associated with a specific waterbody and current reference conditions, using historical data and expert judgement. These elements of the nutrient criteria derivation process are best addressed by States and Tribes with access to information and local expertise. Therefore, EPA strongly encourages States and Tribes to use the information contained in this document and to develop more refined criteria according to the methods described in EPA's technical guidance manuals for specific waterbody types.

To assist in the process of further refinement of nutrient criteria, EPA has established ten Regional Technical Advisory Groups (experts from EPA Regional Offices and States/Tribes). In the process of refining criteria, States and authorized Tribes need to provide documentation of data and analyses, along with a defensible rationale, for any new or revised nutrient criteria they submit to EPA for review and approval. As part of EPA's review of State and Tribal standards, EPA intends to seek assurance from the RTAG that proposed criteria are sufficient to protect uses.

In the process of using the information and recommendations contained in this document, as well as additional information, to develop numerical criteria or procedures to translate narrative criteria, EPA encourages States and Tribes to:

- Address both chemical causal variables and early indicator response variables. Causal variables are necessary to provide sufficient protection of uses before impairment occurs and to maintain downstream uses. Early response variables are necessary to provide warning signs of possible impairment and to integrate the effects of variable and potentially unmeasured nutrient loads.
- Include variables that can be measured to determine if standards are met, and variables that can be related to the ultimate sources of excess nutrients.
- Identify appropriate periods of duration (i.e., how long) and frequency (i.e., how often) of occurrence in addition to magnitude (i.e., how much). EPA does not recommend identifying nutrient concentrations that must be met at all times, rather a seasonal or annual averaging period (e.g., based on weekly measurements) is considered appropriate. However, these seasonal or annual central tendency measures should apply each season or each year, except under the most extraordinary of conditions (e.g., a 100 year flood).

3.0 AREA COVERED BY THIS DOCUMENT

The following sections provide a general description of the aggregate ecoregion and its geographical boundaries. Descriptions of the level III ecoregions contained within the aggregate ecoregion are also provided.

3.1 Description of Aggregate Ecoregion IX - Southeastern Temperate Forested Plains and Hills

Region IX is composed of irregular plains and hills. Originally, the Southeastern Temperate Forested Plains and Hills (IX) was mostly forested in contrast to the South Central Cultivated Great Plains (V); areas of savannah and grassland also occurred. Today, Region IX is a mosaic of forest, cropland, and pasture. The Southeastern Temperate Forested Plains and Hills (IX) is not as arable as the South Central Cultivated Great Plains (V) or the Corn Belt and Northern Great Plains (VI). However, there is much more cropland than in the more rugged Central and Eastern Forested Uplands (XI). Lateritic soils are common and are a contrast to the soils of the surrounding regions. Areas of depleted soils are found in Region IX. Major poultry and aquaculture operations occur locally in the Southeastern Temperate Forested Plains and Hills (IX). Stream quality in the Southeastern Temperate Forested Plains and Hills (IX) has been significantly affected by urban, suburban, and industrial development as well as by poultry, livestock, silviculture, and aquaculture operations. Downstream of sewage treatment plants, poultry farms, and hog operations, nutrient levels and fecal coliform bacteria concentrations can be very high. There are a large number of intensive chicken, turkey, and hog operations in Region IX; effluent from intensive livestock production poses a substantial eutrophication threat to surface waters. In contrast, streams draining relatively undisturbed and forested watersheds have low median concentrations of fecal coliform bacteria, sulfate, dissolved solids, and phosphorus. Silviculture, agriculture, and urban development have impacted suspended sediment levels in

streams especially where soils are highly erodible. Coal mining has degraded water quality and affected aquatic biota in several areas including southern Iowa, northern Missouri, and eastern Pennsylvania. Excessive PCB and DDT concentrations have been detected in the Schuylkill River of Pennsylvania and have led to advisories against local fish consumption.

3.2 Geographical Boundaries of Aggregate Ecoregion IX

Ecoregion IX is an expansive region encompassing parts of twenty States (Figure 1). The region's northeastern border is the southeastern corner of Pennsylvania. The region runs southward through the States of Maryland, Virginia, North Carolina, South Carolina, Georgia and Florida. Only the northwestern corner of Florida is included in the region. West of Georgia, the region includes parts of Alabama, Mississippi, Louisiana and Texas. The region runs north up through the middle of the country to include parts of Oklahoma, Arkansas, Kansas, Missouri, Tennessee, Kentucky, Iowa, Illinois and Indiana. The northwestern boundary of the region is approximately described by the southeastern corner of Iowa, the southern half of Illinois and the southwestern third of Indiana.

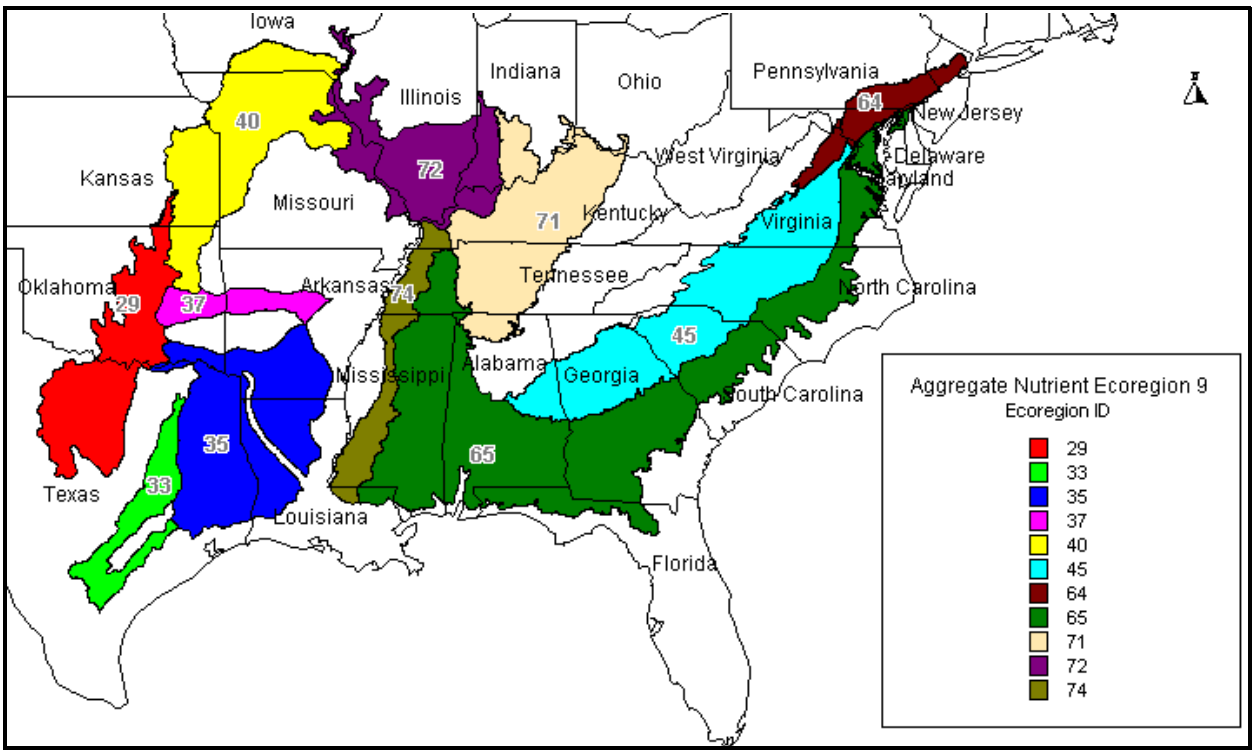

Figure 1. Aggregate Ecoregion IX

3.3 Level III Ecoregions Within Aggregate Ecoregion IX

There are eleven Level III ecoregions contained within Aggregate Ecoregion IX (Figure 2). The following provides brief descriptions of the climate, vegetation cover, topography, and other ecological information pertaining to these subecoregions**.**

29. Central Oklahoma/Texas Plains

The Central Oklahoma/Texas Plains ecoregion is a transition area between the once prairie, now winter wheat growing regions to the west, and the forested low mountains of eastern Oklahoma. The region does not possess the arability and suitability for crops such as corn and soybeans that are common in the Central Irregular Plains to the northeast. Transitional "cross-timbers" (little bluestem grassland with scattered blackjack oak and post oak trees) is the native vegetation, and presently rangeland and pastureland comprise the predominant land cover. Oil extraction has been a major activity in this region for over eighty years.

33. East Central Texas Plains

Also called the Claypan Area, this region of irregular plains was originally covered by a post oak savanna vegetation, in contrast to the more open prairie-type regions to the north, south and west and the piney woods to the east. The bulk of this region is now used for pasture and range.

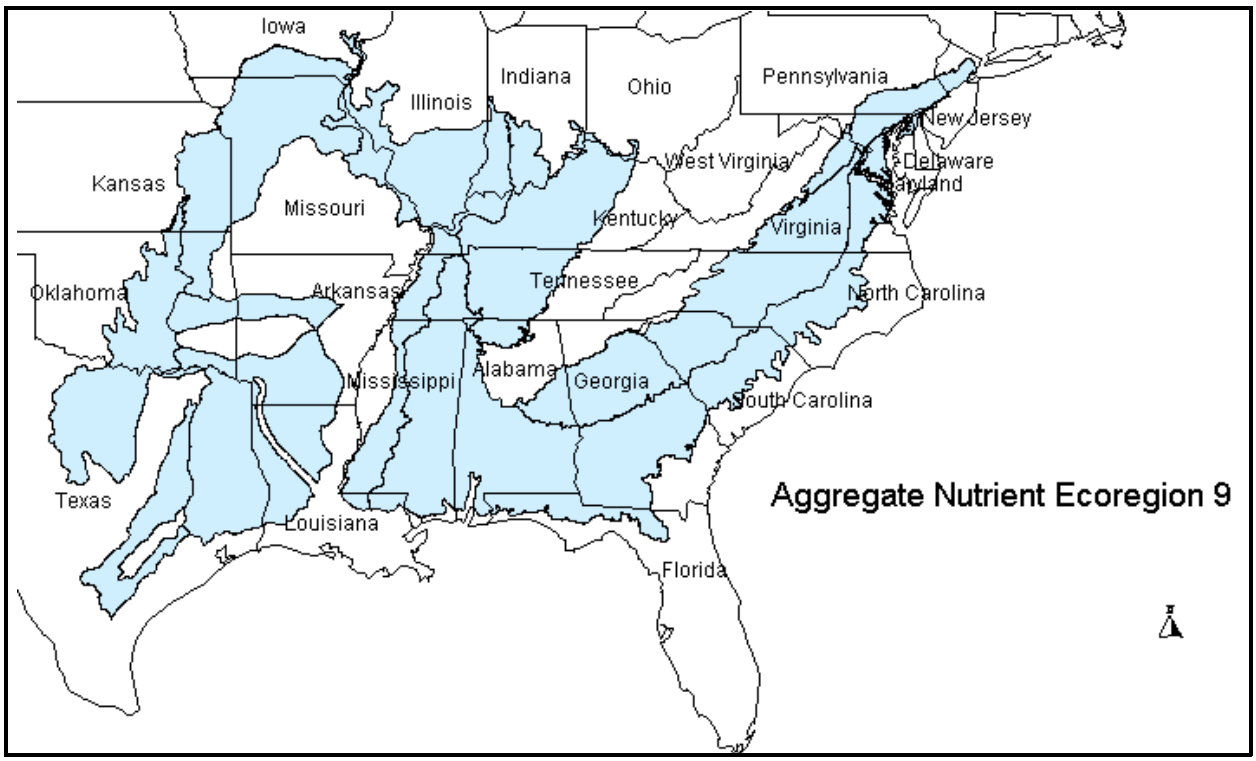

Figure 2. Aggregate Ecoregion IX with level III ecoregions shown.

35. South Central Plains

Locally termed the "piney woods", this region of mostly irregular plains was once blanketed by oak-hickory-pine forests, but is now predominantly in loblolly and shortleaf pine. Only about one sixth of the region is in cropland, whereas about two thirds is in forests and woodland. Lumber and pulpwood production are major economic activities

37. Arkansas Valley

A region of mostly forested valleys and ridges, the physiography of the Arkansas Valley is much less irregular than that of the Boston Mountains to the north and the Ouachita Mountains to the south, but is more irregular than the ecological regions to the west and east. About one fourth of the region is grazed and roughly one tenth is cropland. In the Arkansas Valley, even streams that have been relatively unimpacted by human activities have considerably lower dissolved oxygen levels, and hence support different biological communities, than those of most of the adjacent regions.

40. Central Irregular Plains

The Central Irregular Plains has a mix of land use types and tends to be topographically more irregular than the Western Corn Belt Plains to the north, where most of the land is in crops; however, the region is less irregular and less forest covered than the ecoregions to the south and east. The potential natural vegetation of this ecological region is a grassland/forest mosaic with wider forested strips along the streams compared to the region to the north. The mix of land use activities in the Central Irregular Plains also includes mining operations of high-sulfur bituminous coal. The disturbance of these coal strata in southern Iowa and northern Missouri has degraded water quality and affected aquatic biota.

45. Piedmont

Considered the nonmountainous portion of the old Appalachians Highland by physiographers, the northeast-southwest trending Piedmont ecoregion comprises a transitional area between the mostly mountainous ecoregions of the Appalachians to the northwest and the flat coastal plain to the southeast. Once largely cultivated, much of this region has reverted to pine and hardwood woodlands.

64. Northern Piedmont

The Northern Piedmont is transitional region of low rounded hills, irregular plains, and open valleys in contrast to the low mountains of ecoregions to the north and west and the flat coastal plains of the ecoregion to the east. Potential natural vegetation here was predominantly Appalachian oak forest as compared to the mostly oak-hickory-pine forests of the Piedmont ecoregion to the southwest.

65. Southeastern Plains

These irregular plains have a mosaic of cropland, pasture, woodland, and forest. Natural vegetation is mostly oak-hickory-pine and Southern mixed forest. The Cretaceous or Tertiary-age sands, silts, and clays of the region contrast geologically to the older igneous and metamorphic rocks of the Piedmont, and the older limestone, chert, and shale found in the Interior Plateau. Streams in this area are relatively low-gradient and sandy-bottomed.

71. Interior Plateau
The Interior Plateau is a diverse ecoregion extending from southern Indiana and Ohio to northern Alabama. Rock types are distinctly different from the coastal plain sands and alluvial deposits to the west, and elevations are lower than the Appalachian ecoregions to the east. Mississippian to Ordovician-age limestone, chert, sandstone, siltstone and shale compose the landforms of open hills, irregular plains, and tablelands. The natural vegetation is primarily oak-hickory forest, with some areas of bluestem prairie and cedar glades. The region has a diverse fish fauna.

72. Interior River Lowland
The Interior River Lowland is made up of many wide, flat-bottomed terraced valleys, forested valley walls, and dissected glacial till plains. In contrast to the generally rolling to slightly irregular plains in adjacent ecological regions to the north, east and west, where most of the land is cultivated for corn and soybeans, a little less than half of this area is in cropland, about 30 percent is in pasture, and the remainder is in pasture.

74. Mississippi Valley Loess Plains
This ecoregion stretches from near the Ohio River in western Kentucky to Louisiana. It consists primarily of irregular plains, with oak-hickory and oak-hickory-pine natural vegetation. Thick loess tends to be the distinguishing characteristic. With flatter topography than the Southeastern Plains ecoregion to the east, streams tend to have less gradient and more silty substrates. Agriculture is the dominant land use in the Kentucky and Tennessee portion of the region, while in Mississippi there is a mosaic of forest and cropland.

Suggested ecoregional subdivisions or adjustments.

EPA recommends that the RTAG evaluate the adequacy of EPA nutrient ecoregional and subecoregional boundaries and refine them as needed to reflect local conditions.

4.0 DATA REVIEW FOR RIVERS AND STREAMS IN AGGREGATE ECOREGION IX

The following section describes the nutrient data EPA has collected and analyzed for this Ecoregion, including an assessment of data quantity and quality. The data tables present the data for each causal parameter-- total phosphorus and total nitrogen (both reported and calculated from TKN and nitrite/nitrate), and the primary response variables-- some measure of turbidity and chlorophyll *a*. These are the parameters which EPA considers essential to nutrient assessment because the first two are the main causative agents of enrichment and the two response variables are the early indicators of system enrichment for most of the surface waters (see Chapter 3 of the Rivers and Streams Nutrient Criteria Technical Guidance Manual [U.S. EPA, 2000b] for a complete discussion on choosing causal and response variables.)

4.1 Data Sources

Data sets from Legacy STORET, NASQAN, NAWQA, Auburn University, and EPA Regions 3, 5, and were used to assess nutrient conditions from1990 to1999. EPA recommends that the RTAGs identify additional data sources that can be used to supplement the data sets listed above. In addition, the RTAGs may utilize published literature values to support quantitative and qualitative analyses.

4.2 Historical Data from Aggregate Ecoregion IX (TP, TN, Chl *a* and Turbidity)

EPA recommends that States/Tribes assess long-term trends observed over the past 50 years. This information may be obtained from scientific literature or documentation of historical trends. To gain additional perspective on more recent trends, it is recommended that States and Tribes assess nutrient trends over the last 10 years (e.g., what do seasonal trends indicate?)

4.3 QA/QC of Data Sources

An initial quality screen of data was conducted using the rules presented in Appendix C. Data remaining after screening for duplications and other QA measures (.e.g., poor or unreported analytical records, sampling errors or omissions, stations associated with outfalls, storm water sewers, hazardous waste sites) is the data used in statistical analyses.

The following States indicated that their data were sampled and analyzed using either Standard methods or EPA approved methods: Florida, Georgia, Illinois, Iowa, Indiana, Maryland, Missouri, North Carolina, South Carolina, Tennessee. Other States in Ecoregion IX did not provide information at this time.

4.4 Data for All Rivers and Streams Within Aggregate Ecoregion IX

Figure 3 shows the location of the sampling stations within each subecoregion. Table 1 presents all data records for all parameters for Aggregate Ecoregion IX and subecoregions within the Aggregate Ecoregion.

4.5 Statistical Analysis of Data

EPA's Technical Guidance Manual for Developing Nutrient Criteria for Rivers and Streams describes two ways of establishing a reference condition. One method is to choose the upper 25th percentile (75th percentile) of a reference population of streams. This is the preferred method to establish a reference condition. The 75th percentile was chosen by EPA since it is likely associated with minimally impacted conditions, will be protective of designated uses, and provides management flexibility. When reference streams are not identified, the second method is to determine the lower 25th percentile of the population of all streams within a region. The 25th percentile of the entire population was chosen by EPA to represent a surrogate for an actual reference population. Data analyses to date indicate that the lower 25th percentile from an entire population roughly approximates the 75th percentile for a reference population (see case studies

for Minnesota lakes in the Lakes and Reservoirs Nutrient Criteria Technical Guidance Document [U.S. EPA, 2000a], the case study for Tennessee streams in the Rivers and Streams Nutrient Criteria Technical Guidance Document [U.S. EPA, 2000b], and the letter from Tennessee Department of Environment and Conservation to Geoffrey Grubbs [TNDEC, 2000]). New York State has also presented evidence that the 25th percentile and the 75th percentile compare well based on user perceptions of water resources (NYSDEC, 2000).

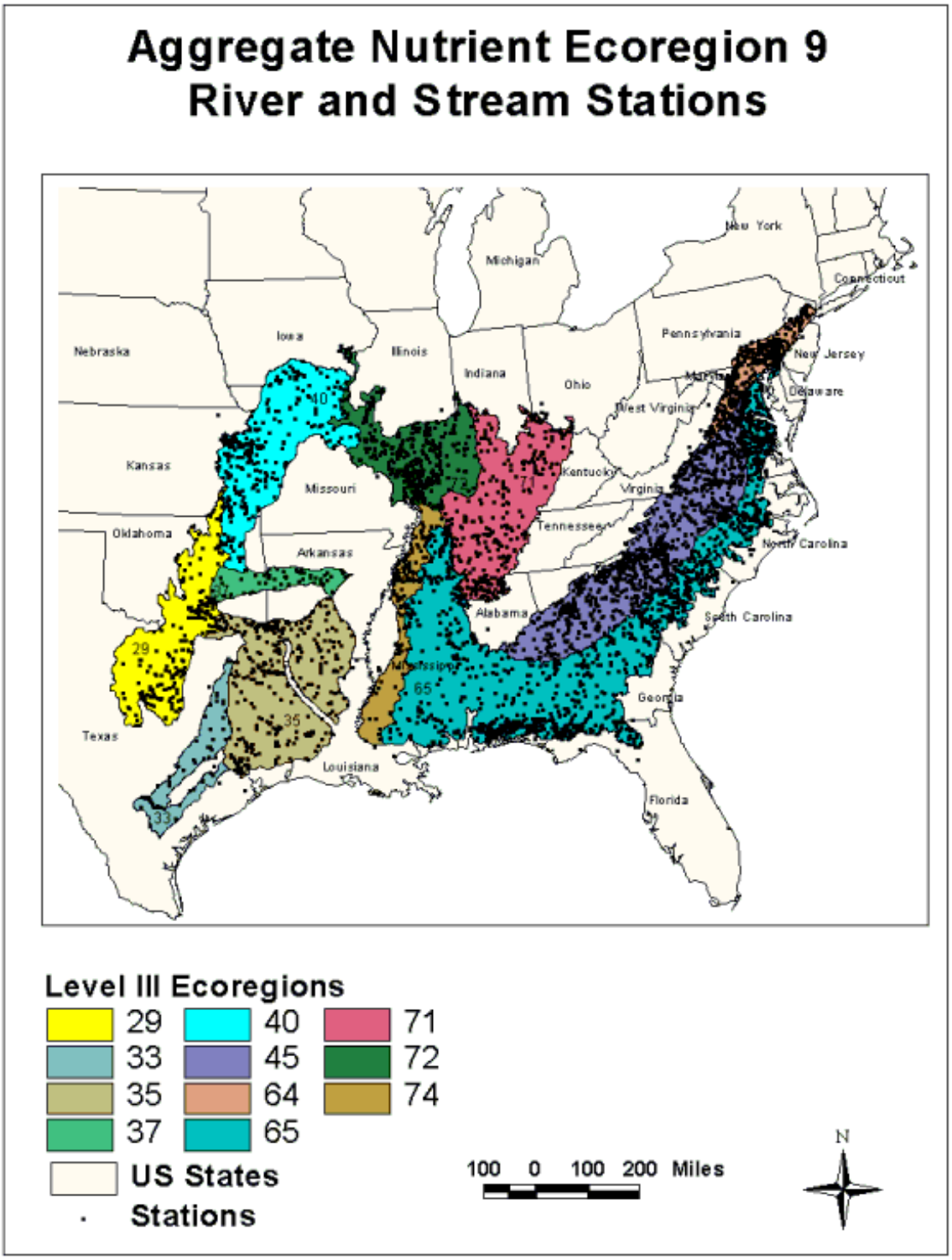

Figure 3 **Map of sampling locations within each level III ecoregion.**

Table 1. Rivers and Streams records for Aggregate Ecoregion IX - Southeastern Temperate Forested Plains and Hills

	Aggregate Ecoregion IX	Sub ecoR 29	Sub ecoR 33	Sub ecoR 35	Sub ecoR 37	Sub ecoR 40	Sub ecoR 45
# of Stream names	3,278	160	44	286	56	220	639
# of Stream Stations		256	73	465	93	445	1,298
Key Nutrient Parameters (listed below)							
- # of records for Turbidity (all methods)	115,125	1,631	203	8,137	2,226	3,569	36,404
- # of records for Chlorophyll *a* (all methods) + Periphyton	16,756	698	521	889	2	229	858
- # of records for Total Kjeldahl Nitrogen (TKN)	116,104	2,173	856	6,808	1,215	2,522	31,205
- # of records for Nitrate + Nitrite (NO_2 + NO_3)	117,925	1,334	469	9,184	2,469	3,015	29,898
- # of records for Total Nitrogen (TN)	13,749	351	80	317	123	390	1,014
- # of records for Total Phosphorus (TP)	164,145	2,412	981	10,173	2,421	5,305	42,948
Total # of records for key nutrient parameters	543,804	8,599	3,110	35,508	8,456	15,030	142,327

Table 1(continued). Rivers and Streams records for Aggregate Ecoregion IX - Southeastern Temperate Forested Plains and Hills

	Sub ecoR 64	Sub ecoR 65	Sub ecoR 71	Sub ecoR 72	Sub ecoR 74
# of Stream names	284	1,001	213	309	103
# of Stream Stations	880	1,870	429	550	162
Key Nutrient Parameters (listed below)					
- # of records for Turbidity (all methods)	8,325	40,234	3,574	9,772	1,050
- # of records for Chlorophyll *a* (all methods) + Periphyton	3,397	9,336	606	194	24
- # of records for Total Kjeldahl Nitrogen (TKN)	14,572	41,353	3,709	7,801	3,890
- # of records for Nitrate + Nitrite (NO_2 + NO_3)	6,407	37,963	6,640	13,823	6,723
- # of records for Total Nitrogen (TN)	2,396	6,382	206	2,424	66
- # of records for Total Phosphorus (TP)	17,541	52,744	6,454	16,108	7,058
Total # of records for key nutrient parameters	52,638	180,012	21,189	50,122	18,811

Definitions used in filling Table 1

1. # of records refers to the total count of observations for that parameter over the entire decade (1990-1999) for that particular aggregate or subecoregion. These are counts for all seasons over that decade.

2. # of stream stations refers to the total number of river and stream stations within the aggregate or subecoregion from which nutrient data was collected. Since streams and rivers can cross ecoregional boundaries, it is important to note that only those portions of a river or stream (and data associated with those stations) that exist within the ecoregion are included within this table.

Tables 2 and 3a-k present potential reference conditions for both the aggregate ecoregion and the subecoregions using both methods. However, the reference stream column is left blank because EPA does not have reference data and anticipates that States/Tribes will provide information on reference streams. Appendix A provides a complete presentation of all descriptive statistics for both the aggregate ecoregion and the level III subecoregion.

4.6. Classification of River/Stream Type

It is anticipated that assessing the data by stream type will further reduce the variability in the data analysis. There were no readily available classification data in the National datasets used to develop these criteria. States and Tribes are strongly encouraged to classify their streams before developing a final criterion.

4.7. Summary of Data Reduction Methods

All descriptive statistics were calculated using the medians for each stream within ecoregion IX, for which data existed. For example, if one stream had 300 observations for phosphorus over the decade or one year's time, one median resulted. Each median from each stream was then used in calculating the percentiles for phosphorus for the aggregate nutrient ecoregion/subecoregion (level III ecoregion) by season and year (Figure 4a & b).

Table 2. Reference conditions for aggregate ecoregion IX streams.

Parameter	No. of Streams N ++	Reported values		25th Percentiles based on all seasons data for the Decade	Reference Streams **
		Min	Max	P25-all seasons+	P75 - all seasons
TKN (mg/L)	1,609	0	4.825	0.3	
$NO_2 + NO_3$ (mg/L)	1,671	0	9.78	0.125	
TN (mg/L) - calculated	NA	0	15.60	0.425	
TN (mg/L) - reported	274	0.24	12.4	0.692	
TP (*u*g/L)	2,104	0	2,400	36.56	
Turbidity (NTU)	476	0.175	162.5	7.02	
Turbidity (FTU)	1,143	0.475	148	5.7	
Turbidity (JCU)	97	0.713	164.5	3.53	
Chlorophyll *a* (*u*g/L) -F	71	0.225	36.73	2.25	
Chlorophyll *a* (*u*g/L) -S	235	0	78.9	0.93	
Chlorophyll *a* (*u*g/L) -T	70	0	93.92	0.53	
Periphyton Chl *a* (mg/m^2)	6	11	62	20.35	

P25:	25th percentile of all data
P75:	75th percentile of all data
**	as determined by the Regional Technical Assistance Groups (RTAGs)
+	Median for all seasons' 25th percentiles. E.g. this value was calculated from four seasons' 25th percentiles. If the seasonal 25th percentile (P25) TP values are - spring 10*u*g/L, summer 15*u*g/L, fall 12*u*g/L, and winter 5*u*g/L, the median value of all seasons P25 will be 11*u*g/L.
++	N = largest value reported for a decade / Season. TN calculated is based on the sum of TKN + NO_2+NO_3. TN reported is actual TN value reported in the database for one sample.
F	Chlorophyll *a* measured by Fluorometric method with acid correction.
S	Chlorophyll *a* measured by Spectrophotometric method with acid correction.
T	Chlorophyll *a b c* measured by Trichromatic method.
NA	Not Applicable
zz	calculated medians from less than 3 seasons' data.

Table(s) 3a.-k. present the potential reference conditions for rivers and streams in the Level III subecoregions within the Aggregate Ecoregion. The footnotes for Table 2 apply to tables 3a-k.

Table 3a. Reference conditions for level III ecoregion 29 streams.

Parameter	No. of Streams N ++	Reported values		25th Percentiles based on all seasons data for the Decade	Reference Streams **
		Min	Max	P25-all seasons+	P75 - all seasons
TKN (mg/L)	**93 W**	**0.05**	**2.058**	**0.4**	
$NO_2 + NO_3$ (mg/L)	**78 W**	**0.01**	**4.7**	**0.078**	
TN (mg/L) - calculated	**NA**	**0.06**	**6.758**	**0.478**	
TN (mg/L) - reported	**20**	**0.39**	**3.228**	**0.68**	
TP (*u*g/L)	**100 W**	**2.5**	**1,332.5**	**37.5**	
Turbidity (NTU)	**6**	**2.738**	**27.4**	**3.713**	
Turbidity (FTU)	**53 F**	**0.9**	**95.5**	**8.825**	
Turbidity (JCU)	**17**	**2**	**164.5**	**9.125**	
Chlorophyll *a* (*u*g/L) -F	**1 z**	**13**	**13**	**13 zz**	
Chlorophyll *a* (*u*g/L) -S	**32**	**0.25**	**33.8**	**1.238**	
Chlorophyll *a* (*u*g/L) -T	**--**	**–**	**–**	**–**	
Periphyton Chl *a* (mg/m^2)	**--**	**–**	**–**	**–**	

Table 3b. Reference conditions for level III ecoregion 33 streams.

Parameter	No. of Streams N ++	Reported values		25th Percentiles based on all seasons data for the Decade	Reference Streams **
		Min	Max	P25-all seasons+	P75 - all seasons
TKN (mg/L)	30	0.285	2.48	**0.543**	
$NO_2 + NO_3$ (mg/L)	21	0.04	7.582	**0.138**	
TN (mg/L) - calculated	NA	0.325	10.062	**0.681**	
TN (mg/L) - reported	3	0.935	5.688	**0.935**	
TP (*u*g/L)	28	45	1,880	**100**	
Turbidity (NTU)	1 z	0.5	0.5	**0.5 zz**	
Turbidity (FTU)	7	4.3	90.25	**10.9**	
Turbidity (JCU)	0	–	–	**–**	
Chlorophyll *a* (*u*g/L) -F	0	–	–	**–**	
Chlorophyll *a* (*u*g/L) -S	24	0.25	21.15	**0.733**	
Chlorophyll *a* (*u*g/L) -T	--	–	–	**–**	
Periphyton Chl *a* (mg/m^2)	--	–	–	**–**	

Table 3c. Reference conditions for level III ecoregion 35 streams.

Parameter	No. of Streams N ++	Reported values		25th Percentiles based on all seasons data for the Decade	Reference Streams **
		Min	Max	P25-all seasons+	P75 - all seasons
TKN (mg/L)	124	0.05	3.213	**0.44**	
$NO_2 + NO_3$ (mg/L)	140	0.005	6.245	**0.067**	
TN (mg/L) - calculated	NA	0.055	9.458	**0.507**	
TN (mg/L) - reported	19	0.33	3.738	**0.385**	
TP (*u*g/L)	164	2.5	1900	**50**	
Turbidity (NTU)	82	2.863	106.5	**9.513**	
Turbidity (FTU)	57 S/W	2	69.375	**6.938**	
Turbidity (JCU)	8 z	3.5	127	**13**	
Chlorophyll *a* (*u*g/L) -F	0	–	–	**–**	
Chlorophyll *a* (*u*g/L) -S	44	0.25	34.213	**0.566**	
Chlorophyll *a* (*u*g/L) -T	--	–	–	**–**	
Periphyton Chl *a* (mg/m^2)	2 z	3.13	5.85	**3.13 zz**	

Table 3d. Reference conditions for level III ecoregion 37 streams.

Parameter	No. of Streams N ++	Reported values		25th Percentiles based on all seasons data for the Decade	Reference Streams **
		Min	Max	P25-all seasons+	P75 - all seasons
TKN (mg/L)	26	0.23	2.315	**0.53**	
$NO_2 + NO_3$ (mg/L)	37	0.015	6.479	**0.075**	
TN (mg/L) - calculated	NA	0.245	8.794	**0.605**	
TN (mg/L) - reported	8	0.55	1.75	**0.683**	
TP (*u*g/L)	39	5	1410	**42.5**	
Turbidity (NTU)	23	3.15	71.5	**6.95**	
Turbidity (FTU)	17 S/W	5.15	53.5	**13.5**	
Turbidity (JCU)	7	2	61.5	**15.25**	
Chlorophyll *a* (*u*g/L) -F	0	–	–	**--**	
Chlorophyll *a* (*u*g/L) -S	1 z	4.5	4.5	**4.5 zz**	
Chlorophyll *a* (*u*g/L) -T	--	–	–	**–**	
Periphyton Chl *a* (mg/m^2)	--	–	–	**–**	

Table 3e. Reference conditions for level III ecoregion 40 streams.

Parameter	No. of Streams N ++	Reported values		25th Percentiles based on all seasons data for the Decade	Reference Streams **
		Min	Max	P25-all seasons+	P75 - all seasons
TKN (mg/L)	81	0.184	4.175	**0.625**	
$NO_2 + NO_3$ (mg/L)	137	0.003	9.203	**0.23**	
TN (mg/L) - calculated	NA	0.187	13.378	**0.855**	
TN (mg/L) - reported	31	0.28	6.225	**0.712**	
TP (*u*g/L)	146	10	2090	**92.5**	
Turbidity (NTU)	53	7.825	96.575	**15.5**	
Turbidity (FTU)	78	2.2	73.625	**12.25**	
Turbidity (JCU)	19	4.9	115.25	**10.5**	
Chlorophyll *a* (*u*g/L) -F	12	0.65	24.8	**2.75 zz**	
Chlorophyll *a* (*u*g/L) -S	16	2.025	22.55	**5.488**	
Chlorophyll *a* (*u*g/L) -T	--	–	–	**–**	
Periphyton Chl *a* (mg/m^2)	--	–	–	**–**	

Table 3f. Reference conditions for level III ecoregion 45 streams.

Parameter	No. of Streams N ++	Reported values		25th Percentiles based on all seasons data for the Decade	Reference Streams **
		Min	Max	P25-all seasons+	P75 - all seasons
TKN (mg/L)	338	0.025	3.1	**0.234**	
$NO_2 + NO_3$ (mg/L)	327	0.003	8.813	**0.177**	
TN (mg/L) - calculated	NA	0.028	11.913	**0.411**	
TN (mg/L) - reported	18	0.238	2.57	**0.615**	
TP (*u*g/L)	436	0	1425	**30**	
Turbidity (NTU)	35	2.25	35.45	**5.713**	
Turbidity (FTU)	356	1.125	108	**7.488**	
Turbidity (JCU)	10	1.9	26.05	**5.95**	
Chlorophyll *a* (*u*g/L) -F	33	1	36.725	**3.3**	
Chlorophyll *a* (*u*g/L) -S	11	1.8	25	**3.493**	
Chlorophyll *a* (*u*g/L) -T	--	–	–	**–**	
Periphyton Chl *a* (mg/m^2)	--	–	–	**–**	

Table 3g. Reference conditions for level III ecoregion 64 streams.

Parameter	No. of Streams N ++	Reported values		25th Percentiles based on all seasons data for the Decade	Reference Streams **
		Min	Max	P25-all seasons+	P75 - all seasons
TKN (mg/L)	125	0.05	2.843	**0.3**	
$NO_2 + NO_3$ (mg/L)	77	0.225	8.362	**0.995**	
TN (mg/L) - calculated	NA	0.275	11.205	**1.295**	
TN (mg/L) - reported	76	0.56	12.4	**2.225**	
TP (*u*g/L)	181	1.25	1545	**40**	
Turbidity (NTU)	33	1.05	23	**2.825**	
Turbidity (FTU)	46 F	0.625	13.088	**3.15**	
Turbidity (JCU)	2	4.4	5.425	**4.4**	
Chlorophyll *a* (*u*g/L) -F	0	–	–	**–**	
Chlorophyll *a* (*u*g/L) -S	18	0.443	8.41	**1.205**	
Chlorophyll *a* (*u*g/L) -T	--	–	–	**–**	
Periphyton Chl *a* (mg/m^2)	6 F	17	62	**20.35**	

Table 3h. Reference conditions for level III ecoregion 65 streams.

Parameter	No. of Streams N ++	Reported values		25th Percentiles based on all seasons data for the Decade	Reference Streams **
		Min	Max	P25-all seasons+	P75 - all seasons
TKN (mg/L)	554	0	4.138	**0.3**	
$NO_2 + NO_3$ (mg/L)	518	0	5.077	**0.095**	
TN (mg/L) - calculated	NA	0	9.215	**0.395**	
TN (mg/L) - reported	65	0.33	2.938	**0.618**	
TP (*u*g/L)	650	0	1735	**22.5**	
Turbidity (NTU)	173	0.25	100	**6.2**	
Turbidity (FTU)	426	0.475	88.75	**4.338**	
Turbidity (JCU)	21 S	1.875	88	**6.55**	
Chlorophyll *a* (*u*g/L) -F	14	0.25	8.8	**1.438**	
Chlorophyll *a* (*u*g/L) -S	74	0	65.552	**0.049**	
Chlorophyll *a* (*u*g/L) -T	--	–	–	**–**	
Periphyton Chl *a* (mg/m^2)	--	–	–	**–**	

Table 3i. Reference conditions for level III ecoregion 71 streams.

Parameter	No. of Streams N ++	Reported values		25th Percentiles based on all seasons data for the Decade	Reference Streams **
		Min	Max	P25-all seasons+	P75 - all seasons
TKN (mg/L)	65	0.05	2.045	**0.284**	
$NO_2 + NO_3$ (mg/L)	109	0.008	5.373	**0.345**	
TN (mg/L) - calculated	NA	0.058	7.418	**0.629**	
TN (mg/L) - reported	10	0.625	4.35	**0.8**	
TP (*u*g/L)	117	2.5	1280	**30**	
Turbidity (NTU)	47	0.875	104.138	**6.975**	
Turbidity (FTU)	22	1.8	44.075	**7.3**	
Turbidity (JCU)	21 S	0.813	15.75	**1.325**	
Chlorophyll *a* (*u*g/L) -F	9	2.6	15.4	**3.85**	
Chlorophyll *a* (*u*g/L) -S	14	0.25	7.75	**1.5**	
Chlorophyll *a* (*u*g/L) -T	--	–	–	**–**	
Periphyton Chl *a* (mg/m^2)	--	–	–	**–**	

Table 3j. Reference conditions for level III ecoregion 72 streams.

Parameter	No. of Streams N ++	Reported values		25th Percentiles based on all seasons data for the Decade	Reference Streams **
		Min	Max	P25-all seasons+	P75 - all seasons
TKN (mg/L)	154	0.025	4.318	**0.539**	
$NO_2 + NO_3$ (mg/L)	173	0.003	8.625	**0.215**	
TN (mg/L) - calculated	NA	0.028	12.943	**0.754**	
TN (mg/L) - reported	21	0.47	7.088	**1.669**	
TP (*u*g/L)	183	1.25	1,600	**83.125**	
Turbidity (NTU)	5	13.5	39	**15**	
Turbidity (FTU)	118	1.15	126.75	**6.263**	
Turbidity (JCU)	3	29.75	37	**29.75**	
Chlorophyll *a* (*u*g/L) -F	2 **z**	1.5	6.55	**1.5 zz**	
Chlorophyll *a* (*u*g/L) -S	1	5.74	5.74	**5.74**	
Chlorophyll *a* (*u*g/L) -T	--	–	–	**–**	
Periphyton Chl *a* (mg/m^2)	--	–	–	**–**	

Table 3k. Reference conditions for level III ecoregion 74 streams.

Parameter	No. of Streams N ++	Reported values		25th Percentiles based on all seasons data for the Decade	Reference Streams **
		Min	Max	P25-all seasons+	P75 - all seasons
TKN (mg/L)	31	0.137	2.875	**0.364**	
$NO_2 + NO_3$ (mg/L)	65	0.022	2.515	**0.14**	
TN (mg/L) - calculated	NA	0.159	5.39	**0.504**	
TN (mg/L) - reported	3	0.6	1.25	**0.6**	
TP (*u*g/L)	70	2.5	1,162.5	**75**	
Turbidity (NTU)	18	6.3	91.75	**16.25**	
Turbidity (FTU)	11	3.25	78.375	**13.5**	
Turbidity (JCU)	5	1.775	18.25	**7.55**	
Chlorophyll *a* (*u*g/L) -F	2 F z	2	2.1	**2 zz**	
Chlorophyll *a* (*u*g/L) -S	0	–	–	**–**	
Chlorophyll *a* (*u*g/L) -T	--	–	–	**–**	
Periphyton Chl *a* (mg/m^2)	--	–	–	**–**	

Definitions used in filling Tables 2 and 3 - Reference Condition tables

Definitions used in filling Tables 2 and 3 - Reference Condition tables

1. **Number of Streams in Table 2** refers to the largest number of streams and rivers for which data existed for a given season within an aggregate nutrient ecoregion.

2. **Number of Streams in Table 3** refers to the number of streams and rivers for which data existed for the summer months since summer is generally when the greatest amount of nutrient sampling is conducted. If another season greatly predominates, notification is made (s=spring, f=fall, w=winter).

3. **Medians. All values** (min, max, and 25th percentiles) included in the table are based on waterbody medians. All data for a particular parameter within a stream for the decade were reduced to one median for that stream. This prevents over-representation of individual waterbodies with a great deal of data versus those with fewer data points within the statistical analysis.

4. **25th percentile for all seasons** is calculated by taking the median of the 4 seasonal 25th percentiles. If a season is missing, the median was calculated with 3 seasons of data. If less than 3 seasons were used to derive the median, the entry is flagged **(z).**

5. **A 25th percentile for a season** is best derived with data from a minimum of 4 streams/season. However, this table provides 25th percentiles that were derived with less than 4 streams/season in order to retain all information for all seasons. In calculating the 25th percentile for a season with less than 4 stream medians, the statistical program automatically used the minimum value within the less-than-4 population. If less than 4 streams were used in developing a seasonal quartile and or all-seasons median, the entry is flagged **(zz).**

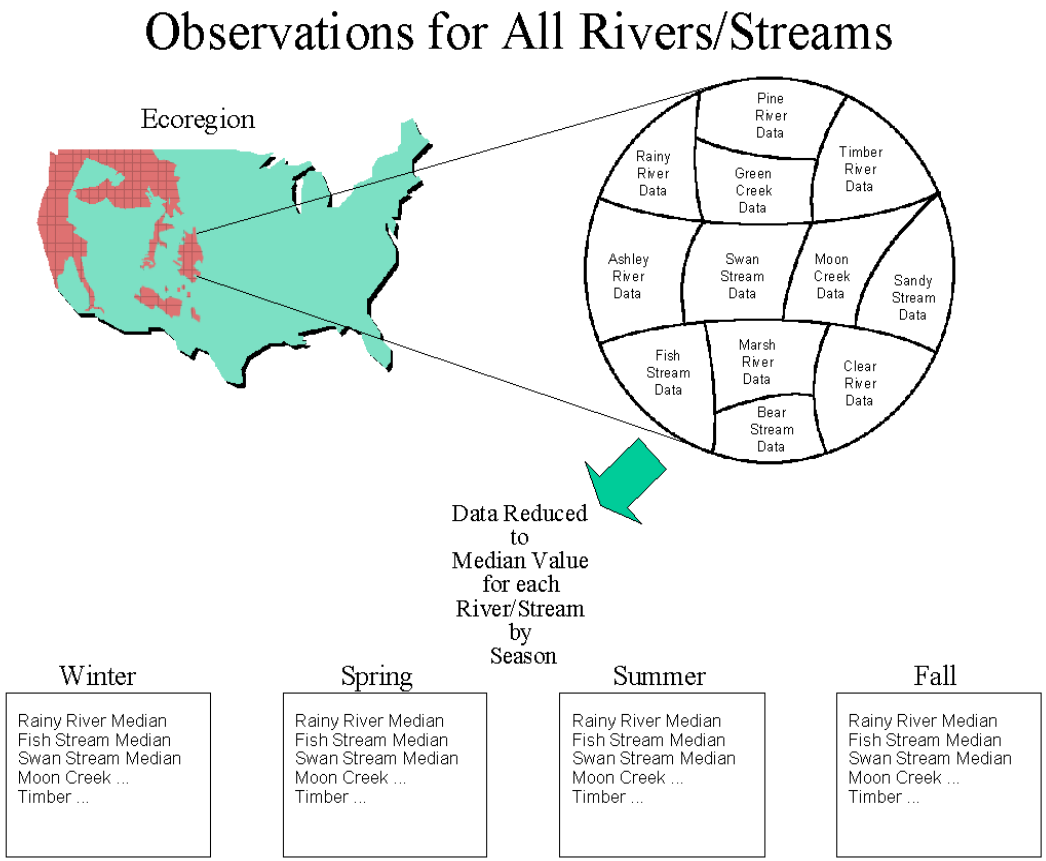

Figure 4a. Illustration of data reduction process for stream data.

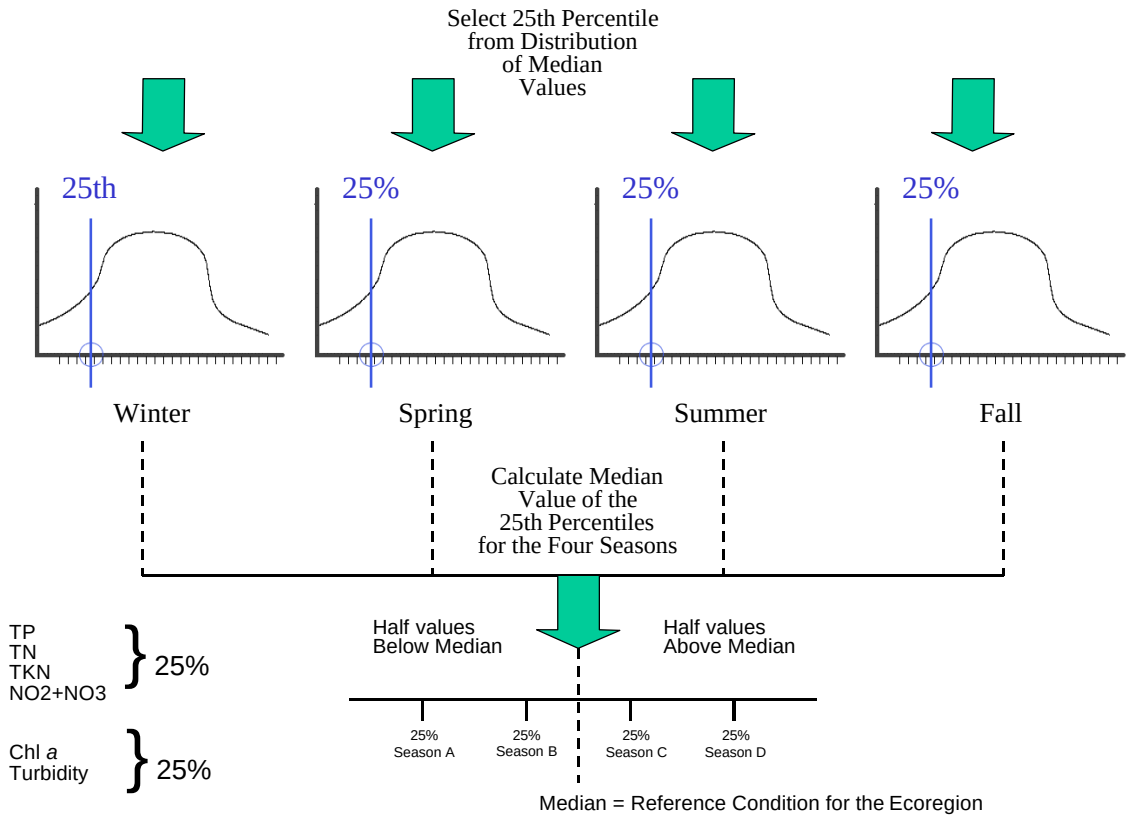

Figure 4b. Illustration of reference condition calculation.

Preferred Data Choices and Recommendations When Data Are Missing

1. Where data are missing or are very low in total records for a given parameter, use 25th percentiles for parameters within an adjacent, similar subecoregion within the same aggregate nutrient ecoregion **or** when a similar subecoregion can not be determined, use the the 25th percentile for the Aggregate ecoregion or consider the **lowest** 25th percentile from a subecoregion (level III) within the aggregate nutrient ecoregion. The rationale being that without data, one may assume that the subecoregion in question may be as sensitive as the most sensitive subecoregion within the aggregate.

2. TN calculated: When reported Total Nitrogen (TN) median values are lacking or very low in comparison to TKN and Nitrate/Nitrite-N values, the medians for TKN and nitrite/nitrate-N were added, resulting in a calculated TN value. The number of samples (N) for calculated TN is not filled in since it is represented by two subsamples of data: TKN and nitrite/nitrate-N. Therefore, N/A is placed in this box.

3. TN reported: This is the median based on reported values for TN from the database.

4. Chlorophyll *a*: Medians based on all methods are reported, however, the acid corrected medians are preferred to the uncorrected medians. In developing a reference condition from a particular method, it is recommended that the method with the most observations be used. Fluorometric and Spectrophotometric are preferred over all other methods. However, when no data exist for Fluorometric and Spectrophotometric methods, Trichromatic values may be used. Data from the variance techniques are not interchangeable.

5. Periphyton: Where periphyton data exist, record them separately For periphyton-dominated streams, a measure of periphyton chlorophyll is a more appropriate response variable than planktonic chlorophyll *a*. See Table 4, p. 101 of the Rivers and Streams Nutrient Technical Guidance Manual (U. S. EPA, 2000b) for values of periphyton and planktonic chlorophyll *a* related to eutrophy in streams.

6. Secchi depth: The 75th percentile is reported for Secchi depth since this is the only variable for which the value of the parameter **increases** with greater clarity. (For lakes and reservoirs only.)

7. Turbidity units: All turbidity units from all methods are reported. FTUs and NTUs are preferred over JCUs. If FTUs and NTUs do not exist, use JCUs. These units are not interchangeable. Turbidity is chosen as a response variable in streams since it can be an indicator of increasing algal biomass due to nutrient enrichment. See pages 32 -33 of the Rivers and Streams Nutrient Technical Guidance Manual for a discussion of turbidity and correlations with algal growth.

8. Lack of data: A dash (-) represents missing, inadequate, or inconclusive data. A zero (0) is reported if the reported median for a parameter is 0 or if the component value is below detection.

5.0 REFERENCE SITES AND CONDITIONS IN AGGREGATE ECOREGION IX

Reference conditions represent the natural, least impacted conditions or what is considered to be the most attainable conditions. This section compares the different reference conditions determined from the two methods and establishes which reference condition is most appropriate.

A priori determination of reference sites. The preferred method for establishing reference condition is to choose the upper percentile of an *a priori* population of reference streams. States and Tribes are encouraged to identify reference conditions based on this method.

Statistical determination of reference conditions (25th percentile of entire database.) See Tables 2 and 3a-k in section 4.0.

RTAG discussion and rationale for selection of reference sites and conditions in Ecoregion IX. The RTAG should compare the results derived from the two methods described above and present a rationale for the final selection of reference sites.

6.0 MODELS USED TO PREDICT OR VERIFY RESPONSE PARAMETERS

The RTAG is encouraged to identify and apply relevant models to support nutrient criteria development. The following are three scenarios under which models may be used to derive criteria or support criteria development.

- Models for predicting correlations between causal and response variables
- Models used to verify reference conditions based on percentiles
- Regression models used to predict reference conditions in impacted areas

7.0 FRAMEWORK FOR REFINING RECOMMENDED NUTRIENT CRITERIA FOR RIVERS AND STREAMS IN AGGREGATE ECOREGION IX

Information on each of the following six weight of evidence factors is important to refine the criteria presented in this document. All elements should be addressed in developing criteria, as is expressed in our nutrient criteria technical guidance manuals. It is our expectation that EPA Regions, States, and Tribes (as RTAGs) will consider these elements as States/Tribes develop their criteria. This section should be viewed as a work sheet (sections are left blank for this purpose) to assist in the refinement of nutrient criteria. If many of these elements are ultimately unaddressed, EPA may rely on the proposed reference conditions presented in Tables 3a-k and other literature and information readily available to the HQ nutrient team to develop nutrient water quality recommendations for this ecoregion.

7.1 Example Worksheet for Developing Aggregate Ecoregion and Subecoregion Nutrient Criteria

- *Literature sources*

- *Historical data and trends*

- *Reference condition*

- *Models*

- *RTAG expert review and consensus*

- *Downstream effects*

7.2 Tables of Refined Nutrient Water Quality Criteria for Aggregate Ecoregion IX and Level III Subecoregions for TP, TN, Chl *a*, Turbidity (where sufficient data exist)

Aggregate Ecoregion IX- Southeastern Temperate Forested Hills and Plains	**Proposed Criterion**
Total Phosphorus (µg/L)	
Total Nitrogen (mg/L)	
Chlorophyll *a* (µg/L or mg/m^2))	
Turbidity (NTU or other units)	
Other (Index; other parameter such as DO)	

- *Literature sources*

- *Historical data and trends*

- *Reference condition*

- *Models*

- *RTAG expert review and consensus*

- *Downstream effects*

Ecoregion #29 Central Oklahoma/Texas Plains	**Proposed Criterion**
Total Phosphorus (µg/L)	
Total Nitrogen (mg/L)	
Chlorophyll *a* (µg/L or mg/m^2))	
Turbidity (NTU or other units)	
Other (Index; other parameter such as DO)	

7.3 Setting Seasonal Criteria

The recommendations presented in this document are based in part on medians of all the 25th percentile seasonal data (decadal), and as such are reflective of all seasons and not one particular season or year. It is recommended that States and Tribes monitor in all seasons to best assess compliance with the resulting criterion. States/Tribes may choose to develop criteria which reflect **each** particular season or a **given year** when there is significant variability between seasons/years or designated uses that are specifically tied to one or more seasons of the year (e.g., recreation, fishing). Using the tables in Appendix A and B, one can set reference conditions based on a particular season or year and then develop a criterion based on each individual season. Obviously, this option is season-specific and would also require increased monitoring within each season to assess compliance.

7.4 When Data/Reference Conditions are Lacking

When data are unavailable to develop a reference condition for a particular parameter(s) within a subecoregion, EPA recommends one of three options: 1. Use data from a similar neighboring subecoregion. E.g., If data are few or nonexistent for the northern cascades, consider using the data and reference condition developed for the cascades; or 2. Use the 25th perecentiles for the Aggregate ecoregion or 3. Consider using the lowest of the yearly medians for that parameter calculated for all the subecoregions within the Aggregate Ecoregion.

7.5 Site-specific Criteria Development

Criteria may be refined in a number of ways. The best way to refine criteria is to follow the critical elements of criteria development as well as to refer to the Rivers and Streams Nutrient Criteria Technical Guidance Manual (U.S. EPA, 2000b).

The Technical Guidance Manual presents sections on each of the following factors to consider in setting criteria

- refinements to ecoregions (Section 2.3)
- classification of waterbodies (Chapter 2)
- setting seasonal criteria to reflect major seasonal climate differences and accounting for significant or cyclical precipitation events (high flow/low flow conditions) (Chapter 4).

8.0 LITERATURE CITED

NYSDEC (New York State Department of Environment and Conservation). 2000. Memorandum from Scott Kishbaugh to Jay Bloomfield, September 26, 2000, regarding reference lakes for nutrient criteria.

TNDEC (Tennessee Department of Environment and Conservation). 2000. Letter to Geoff Grubbs, October 5, 2000, containing comments on draft nutrient criteria recommendations.

U.S. EPA. 2000a. Nutrient Criteria Technical Guidance Manual: Lakes and Reservoirs, U.S. Environmental Protection Agency, Washington, DC. EPA-822-B00-001.

U.S. EPA. 2000b. Nutrient Criteria Technical Guidance Manual: Rivers and Streams, U.S. Environmental Protection Agency, Washington, DC. EPA-822-B00-002.

9.0 APPENDICES

A. Descriptive Statistics Data Tables for Aggregate Ecoregion

B. Descriptive Statistics Data Tables for Level III Subecoregions within Aggregate Ecoregion

C. Quality Control/Quality Assurance Rules

APPENDIX A

Descriptive Statistics Data Tables for Aggregate Ecoregion

Aggregate Nutrient Ecoregion: IX 1
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chla_Fluo_ug_L_Median

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	54	8.03	.250	36.35	9.19	1.25	114	0.40	1.80	4.10	8.80	29.8
SPRING	37	8.27	.000	37.10	8.19	1.35	99	0.25	2.70	4.50	11.7	24.5
SUMMER	71	10.7	.625	95.10	12.9	1.53	120	1.00	3.65	7.00	12.8	26.8
WINTER	18	4.40	.200	13.90	3.92	0.92	89	0.20	1.10	3.40	7.90	13.9

Aggregate Nutrient Ecoregion: IX 2
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chla_Phyto_C_F_ug_L_Med

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	1	3.40	3.40	3.40	.	.	.	3.40	3.40	3.40	3.40	3.40
SPRING	2	6.68	1.90	11.45	6.75	4.78	101	1.90	1.90	6.68	11.5	11.5
SUMMER	1	9.60	9.60	9.60	.	.	.	9.60	9.60	9.60	9.60	9.60
WINTER	1	0.78	.775	0.78	.	.	.	0.78	0.78	0.78	0.78	0.78

Aggregate Nutrient Ecoregion: IX 3
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chla_Phyto_Spec_A_ug_L_Median

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	187	6.03	.000	78.60	9.79	0.72	162	0.25	0.90	2.85	7.50	19.8
SPRING	206	6.54	.000	98.52	11.1	0.77	170	0.00	0.97	3.38	7.95	25.0
SUMMER	235	7.44	.000	79.20	11.5	0.75	154	0.00	1.03	3.48	9.00	25.4
WINTER	178	3.13	.000	34.80	4.80	0.36	153	0.00	0.25	1.65	3.48	12.0

Aggregate Nutrient Ecoregion: IX 4
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chla_Phyto_Spec_U_ug_L_Median

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	1	0.03	.025	0.03	.	.	.	0.03	0.03	0.03	0.03	0.03
SPRING	1	4.37	4.37	4.37	.	.	.	4.37	4.37	4.37	4.37	4.37
SUMMER	1	0.02	.021	0.02	.	.	.	0.02	0.02	0.02	0.02	0.02
WINTER	0	.	.	.	.	.	.	.	.	.	.	.

Aggregate Nutrient Ecoregion: IX 5
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chla_Tric_U_ug_L_Median

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	48	9.75	.000	97.78	18.2	2.63	187	0.00	1.06	1.91	10.5	43.4
SPRING	44	9.22	.000	95.06	17.2	2.59	186	0.00	1.60	3.47	7.11	40.8

SUMMER	70	12.0	.000	92.78	19.9	2.38	166	0.00	0.00	3.94	13.0	63.9
WINTER	50	2.09	.000	16.90	3.76	0.53	180	0.00	0.00	1.08	2.34	12.5

Aggregate Nutrient Ecoregion: IX 6
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chlb_Phyto_C_F_ug_L_Med

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	1	0.40	.400	0.40	.	.	.	0.40	0.40	0.40	0.40	0.40
SPRING	2	0.31	.200	0.43	0.16	0.11	51	0.20	0.20	0.31	0.43	0.43
SUMMER	1	0.70	.700	0.70	.	.	.	0.70	0.70	0.70	0.70	0.70
WINTER	1	0.05	.050	0.05	.	.	.	0.05	0.05	0.05	0.05	0.05

Aggregate Nutrient Ecoregion: IX 7
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chlb_Phyto_Spec_ug_L_Median

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	0	.	.	.	.	.	.	.	.	.	.	.
SPRING	1	0.24	.236	0.24	.	.	.	0.24	0.24	0.24	0.24	0.24
SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
WINTER	0	.	.	.	.	.	.	.	.	.	.	.

Aggregate Nutrient Ecoregion: IX 8
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter DIP_ug_L_Median

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	217	175	.000	9600.00	742	50.4	423	5.00	16.3	50.0	115	568
SPRING	180	64.1	.000	812.00	115	8.58	180	5.00	10.0	30.0	70.0	224
SUMMER	230	65.9	.000	930.00	102	6.70	154	5.00	12.5	37.3	81.5	183
WINTER	170	63.3	.000	1000.00	108	8.29	171	5.00	13.8	35.0	75.0	200

Aggregate Nutrient Ecoregion: IX 9
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter DO_mg_L_Median

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	1437	7.73	.000	17.88	1.87	0.05	24	4.30	6.75	8.00	8.75	10.4
SPRING	1417	8.57	1.80	13.80	1.61	0.04	19	5.83	7.60	8.60	9.50	11.2
SUMMER	1579	6.83	.000	13.50	1.67	0.04	24	3.50	6.00	7.10	7.83	9.10
WINTER	1240	10.5	2.90	14.70	1.60	0.05	15	7.73	9.64	10.5	11.6	13.0

Aggregate Nutrient Ecoregion: IX 10
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter NO2_NO3_mg_L_Median

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	1527	0.74	.000	10.00	1.30	0.03	175	0.01	0.09	0.27	0.74	3.20
SPRING	1529	0.71	.000	9.80	1.15	0.03	161	0.03	0.14	0.32	0.73	2.95
SUMMER	1671	0.73	.000	9.03	1.20	0.03	165	0.01	0.12	0.29	0.74	3.11
WINTER	1378	0.83	.000	9.76	1.28	0.03	155	0.03	0.14	0.36	0.90	3.41

Aggregate Nutrient Ecoregion: IX 11
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Org_P_ug_L_Median

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	15	70.4	22.5	188.30	50.2	13.0	71	22.5	37.6	51.1	84.1	188
SPRING	0	.	.	.	.	.	.	.	.	.	.	.
SUMMER	15	52.4	6.77	115.44	28.5	7.37	54	6.77	27.4	48.7	66.3	115
WINTER	0	.	.	.	.	.	.	.	.	.	.	.

Aggregate Nutrient Ecoregion: IX 12
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Orthophosphate_T_as_P_ug_L_Med

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	100	117	5.00	1600.00	261	26.1	223	5.00	17.5	40.0	98.8	490
SPRING	103	82.9	5.00	1095.00	162	15.9	195	5.00	17.5	37.5	80.0	238
SUMMER	103	104	5.00	1307.50	202	19.9	194	5.00	16.3	41.3	103	310
WINTER	101	77.6	5.00	815.00	113	11.3	146	5.00	20.0	40.0	100	225

Aggregate Nutrient Ecoregion: IX 13
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter TKN_mg_L_Median

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	1404	0.62	.000	5.40	0.55	0.01	89	0.13	0.30	0.50	0.78	1.50
SPRING	1452	0.60	.000	5.00	0.53	0.01	88	0.12	0.30	0.48	0.73	1.47
SUMMER	1609	0.64	.000	4.30	0.48	0.01	74	0.15	0.35	0.53	0.81	1.45
WINTER	1397	0.53	.000	4.65	0.43	0.01	80	0.12	0.30	0.44	0.65	1.20

Aggregate Nutrient Ecoregion: IX 14
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter TN_mg_L_Median

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	213	1.87	.180	12.50	2.06	0.14	110	0.35	0.67	1.17	2.20	6.03
SPRING	204	2.02	.300	11.00	1.98	0.14	98	0.51	0.71	1.26	2.27	6.55

SUMMER	274	2.31	.200	12.30	2.47	0.15	107	0.38	0.72	1.37	3.03	8.62
WINTER	211	1.95	.275	13.00	2.05	0.14	105	0.40	0.67	1.20	2.30	6.16

Aggregate Nutrient Ecoregion: IX 15
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter TP_ug_L_Median

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	1811	157	.000	2420.00	255	5.99	163	5.00	35.0	75.0	160	645
SPRING	1864	131	.000	2360.00	234	5.42	178	5.00	38.1	70.0	130	465
SUMMER	2104	148	.000	2400.00	226	4.92	152	7.50	40.0	80.0	160	530
WINTER	1685	122	.000	2400.00	199	4.86	163	5.00	30.0	65.0	125	430

Aggregate Nutrient Ecoregion: IX 16
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Turb_FTU_Median

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	1059	11.0	.000	180.00	13.4	0.41	122	2.00	4.55	7.70	12.8	29.5
SPRING	1113	14.1	.650	123.00	13.5	0.40	95	2.50	6.40	11.0	16.8	38.9
SUMMER	1143	14.6	.800	160.00	15.6	0.46	106	2.50	5.85	10.0	17.0	45.2
WINTER	991	14.5	.300	136.00	15.4	0.49	106	2.25	5.55	9.70	18.0	43.0

Aggregate Nutrient Ecoregion: IX 17
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Turb_JCU_Median

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	89	21.9	.600	171.00	31.9	3.38	145	1.10	3.25	11.0	24.0	90.0
SPRING	88	20.1	.700	158.00	28.9	3.08	144	1.50	3.46	10.7	23.5	88.8
SUMMER	97	25.8	.725	173.00	36.6	3.72	142	1.05	3.60	11.3	30.0	127
WINTER	92	18.9	1.10	96.00	18.8	1.96	100	1.50	6.70	13.5	21.5	60.8

Aggregate Nutrient Ecoregion: IX 18
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Turb_NTU_Median

SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
FALL	366	15.1	.250	113.00	16.4	0.86	109	2.00	5.50	9.55	19.0	55.0
SPRING	379	20.5	.100	175.00	20.4	1.05	99	2.25	8.70	15.0	25.0	57.0
SUMMER	476	20.2	.000	212.00	25.2	1.16	125	2.00	6.20	12.3	24.0	72.5
WINTER	323	19.6	.250	150.00	20.2	1.12	103	2.00	7.85	14.0	24.5	55.0

APPENDIX B

Descriptive Statistics Data Tables for Level III Subecoregions within Aggregate Ecoregion

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chla_Fluo_ug_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	1	15.6	15.6	15.60	.	.	.	15.6	15.6	15.6	15.6	15.6
29	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
29	SUMMER	1	10.4	10.4	10.40	.	.	.	10.4	10.4	10.4	10.4	10.4
29	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
33	FALL	0	.	.	.	.	.	.	.	.	.	.	.
33	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
33	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
33	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
35	FALL	0	.	.	.	.	.	.	.	.	.	.	.
35	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
35	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
35	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
37	FALL	0	.	.	.	.	.	.	.	.	.	.	.
37	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
37	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
37	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
40	FALL	11	4.44	.400	26.40	7.48	2.25	169	0.40	0.90	1.60	4.20	26.4
40	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
40	SUMMER	12	11.1	.900	23.20	7.19	2.07	65	0.90	4.60	12.4	16.9	23.2
40	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
45	FALL	26	11.4	1.00	36.35	10.9	2.14	96	1.10	3.00	8.20	16.5	32.0
45	SPRING	23	10.9	1.00	37.10	9.22	1.92	84	1.13	2.70	10.5	15.8	24.5
45	SUMMER	33	10.6	.625	41.00	9.61	1.67	91	1.00	3.60	9.00	11.5	30.9
45	WINTER	6	7.99	1.50	13.90	3.96	1.62	50	1.50	7.50	8.03	9.00	13.9
64	FALL	0	.	.	.	.	.	.	.	.	.	.	.
64	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
64	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
64	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
65	FALL	9	3.18	.250	8.00	3.10	1.03	98	0.25	0.63	2.20	6.30	8.00
65	SPRING	12	3.70	.250	8.80	2.33	0.67	63	0.25	2.25	4.06	4.70	8.80
65	SUMMER	14	13.0	1.00	95.10	24.0	6.42	184	1.00	4.00	6.59	8.00	95.1

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chla_Fluo_ug_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	12	2.61	.200	8.80	2.46	0.71	94	0.20	0.63	2.30	3.75	8.80
71	FALL	2	11.9	8.30	15.40	5.02	3.55	42	8.30	8.30	11.9	15.4	15.4
71	SPRING	2	4.95	.000	9.90	7.00	4.95	141	0.00	0.00	4.95	9.90	9.90
71	SUMMER	9	8.52	2.60	20.20	6.29	2.10	74	2.60	3.85	6.35	11.5	20.2
71	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
72	FALL	3	5.77	1.80	8.80	3.59	2.07	62	1.80	1.80	6.70	8.80	8.80

72	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
72	SUMMER	2	2.75	1.20	4.30	2.19	1.55	80	1.20	1.20	2.75	4.30	4.30
72	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
74	FALL	2	2.05	2.00	2.10	0.07	0.05	3	2.00	2.00	2.05	2.10	2.10
74	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
74	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
74	WINTER	0	.	.	.	.	.	.	.	.	.	.	.

Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chla_Phyto_C_F_ug_L_Med

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	0	.	.	.	.	.	.	.	.	.	.	.
29	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
29	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
29	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
33	FALL	0	.	.	.	.	.	.	.	.	.	.	.
33	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
33	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
33	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
35	FALL	0	.	.	.	.	.	.	.	.	.	.	.
35	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
35	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
35	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
37	FALL	0	.	.	.	.	.	.	.	.	.	.	.
37	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
37	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
37	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
40	FALL	0	.	.	.	.	.	.	.	.	.	.	.
40	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
40	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
40	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
45	FALL	0	.	.	.	.	.	.	.	.	.	.	.
45	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
45	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
45	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
64	FALL	0	.	.	.	.	.	.	.	.	.	.	.
64	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
64	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
64	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
65	FALL	0	.	.	.	.	.	.	.	.	.	.	.
65	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
65	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chla_Phyto_C_F_ug_L_Med

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
71	FALL	0	.	.	.	.	.	.	.	.	.	.	.
71	SPRING	1	1.90	1.90	1.90	.	.	.	1.90	1.90	1.90	1.90	1.90
71	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
71	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
72	FALL	1	3.40	3.40	3.40	.	.	.	3.40	3.40	3.40	3.40	3.40
72	SPRING	1	11.5	11.5	11.45	.	.	.	11.5	11.5	11.5	11.5	11.5
72	SUMMER	1	9.60	9.60	9.60	.	.	.	9.60	9.60	9.60	9.60	9.60
72	WINTER	1	0.78	.775	0.78	.	.	.	0.78	0.78	0.78	0.78	0.78
74	FALL	0	.	.	.	.	.	.	.	.	.	.	.
74	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
74	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
74	WINTER	0	.	.	.	.	.	.	.	.	.	.	.

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chla_Phyto_Spec_A_ug_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	25	4.84	.250	19.20	5.37	1.07	111	0.25	1.00	3.18	5.15	16.6
29	SPRING	29	8.68	.250	32.80	9.76	1.81	112	0.25	1.33	5.28	11.3	30.6
29	SUMMER	32	9.99	.250	54.50	11.5	2.03	115	0.25	3.73	7.36	12.4	44.3
29	WINTER	30	6.35	.250	34.80	8.71	1.59	137	0.25	1.15	3.33	7.80	27.8
33	FALL	22	6.62	.250	47.00	11.1	2.37	168	0.25	0.68	1.92	5.98	23.0
33	SPRING	24	4.37	.250	15.00	4.32	0.88	99	0.25	1.01	2.53	8.49	11.0
33	SUMMER	24	6.04	.250	27.30	7.49	1.53	124	0.25	0.44	3.19	10.8	20.1
33	WINTER	21	2.85	.250	9.38	2.68	0.58	94	0.25	0.79	1.78	5.28	7.11
35	FALL	41	5.59	.250	78.60	12.3	1.93	221	0.25	0.25	2.28	6.86	12.8
35	SPRING	41	4.42	.250	18.90	4.38	0.68	99	0.25	1.16	4.15	5.89	11.4
35	SUMMER	44	5.47	.250	49.53	8.24	1.24	151	0.25	0.88	2.73	7.10	17.4
35	WINTER	38	2.79	.250	13.60	3.40	0.55	122	0.25	0.25	1.92	3.19	12.8
37	FALL	1	6.00	6.00	6.00	.	.	.	6.00	6.00	6.00	6.00	6.00
37	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
37	SUMMER	1	3.00	3.00	3.00	.	.	.	3.00	3.00	3.00	3.00	3.00
37	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
40	FALL	11	10.1	2.45	18.90	5.58	1.68	55	2.45	5.45	9.95	15.4	18.9
40	SPRING	11	11.3	3.80	26.20	7.74	2.33	68	3.80	5.53	7.98	15.9	26.2
40	SUMMER	16	11.9	1.60	26.40	7.41	1.85	62	1.60	6.80	11.8	15.8	26.4
40	WINTER	6	3.50	1.40	10.10	3.29	1.34	94	1.40	1.50	2.55	2.90	10.1
45	FALL	4	7.90	2.60	19.80	7.99	4.00	101	2.60	3.49	4.60	12.3	19.8
45	SPRING	6	12.0	1.80	25.00	7.69	3.14	64	1.80	9.43	10.4	15.1	25.0
45	SUMMER	11	20.7	.800	67.93	22.4	6.75	108	0.80	3.00	15.7	22.0	67.9
45	WINTER	0	.	.	.	.	.	.	.	.	.	.	.

64	FALL	21	2.56	.635	9.43	2.43	0.53	95	0.67	0.94	1.50	3.00	7.63
64	SPRING	21	5.80	1.50	47.53	10.2	2.23	176	1.87	2.29	2.54	4.00	19.0
64	SUMMER	18	2.36	.250	7.39	1.68	0.40	71	0.25	1.41	1.79	3.00	7.39
64	WINTER	18	1.56	.250	4.13	0.92	0.22	59	0.25	1.00	1.20	1.89	4.13
65	FALL	49	7.67	.000	51.90	12.1	1.72	157	0.00	0.48	3.00	8.05	38.3
65	SPRING	64	7.30	.000	98.52	16.6	2.08	227	0.00	0.10	2.20	5.74	35.9
65	SUMMER	74	6.75	.000	79.20	13.3	1.55	198	0.00	0.00	2.01	7.00	31.0

Aggregate Nutrient Ecoregion: IX 6
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chla_Phyto_Spec_A_ug_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	59	2.45	.000	14.55	3.77	0.49	154	0.00	0.00	0.59	3.18	12.0
71	FALL	12	3.74	.250	10.00	3.16	0.91	84	0.25	1.50	2.50	5.31	10.0
71	SPRING	9	2.53	.250	5.50	1.55	0.52	61	0.25	1.50	3.00	3.00	5.50
71	SUMMER	14	5.35	.625	21.00	5.20	1.39	97	0.63	2.00	4.25	6.00	21.0
71	WINTER	6	1.13	.250	2.00	0.59	0.24	52	0.25	1.00	1.00	1.50	2.00
72	FALL	1	8.00	8.00	8.00	.	.	.	8.00	8.00	8.00	8.00	8.00
72	SPRING	1	0.50	.500	0.50	.	.	.	0.50	0.50	0.50	0.50	0.50
72	SUMMER	1	5.74	5.74	5.74	.	.	.	5.74	5.74	5.74	5.74	5.74
72	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
74	FALL	0	.	.	.	.	.	.	.	.	.	.	.
74	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
74	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
74	WINTER	0	.	.	.	.	.	.	.	.	.	.	.

Aggregate Nutrient Ecoregion: IX 7
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chla_Phyto_Spec_U_ug_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	0	.	.	.	.	.	.	.	.	.	.	.
29	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
29	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
29	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
33	FALL	0	.	.	.	.	.	.	.	.	.	.	.
33	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
33	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
33	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
35	FALL	0	.	.	.	.	.	.	.	.	.	.	.
35	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
35	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
35	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
37	FALL	0	.	.	.	.	.	.	.	.	.	.	.
37	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
37	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
37	WINTER	0	.	.	.	.	.	.	.	.	.	.	.

40	FALL	0	.	.	.	.	.	.	.	.	.	.	.
40	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
40	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
40	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
45	FALL	0	.	.	.	.	.	.	.	.	.	.	.
45	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
45	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
45	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
64	FALL	1	0.03	.025	0.03	.	.	.	0.03	0.03	0.03	0.03	0.03
64	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
64	SUMMER	1	0.02	.021	0.02	.	.	.	0.02	0.02	0.02	0.02	0.02
64	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
65	FALL	0	.	.	.	.	.	.	.	.	.	.	.
65	SPRING	1	4.37	4.37	4.37	.	.	.	4.37	4.37	4.37	4.37	4.37
65	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.

Aggregate Nutrient Ecoregion: IX 8
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chla_Phyto_Spec_U_ug_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
71	FALL	0	.	.	.	.	.	.	.	.	.	.	.
71	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
71	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
71	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
72	FALL	0	.	.	.	.	.	.	.	.	.	.	.
72	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
72	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
72	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
74	FALL	0	.	.	.	.	.	.	.	.	.	.	.
74	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
74	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
74	WINTER	0	.	.	.	.	.	.	.	.	.	.	.

Aggregate Nutrient Ecoregion: IX 9
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chla_Tric_U_ug_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	0	.	.	.	.	.	.	.	.	.	.	.
29	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
29	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
29	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
33	FALL	0	.	.	.	.	.	.	.	.	.	.	.
33	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
33	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
33	WINTER	0	.	.	.	.	.	.	.	.	.	.	.

35	FALL	0	.	.	.	.	.	.	.	.	.	.	.
35	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
35	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
35	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
37	FALL	0	.	.	.	.	.	.	.	.	.	.	.
37	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
37	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
37	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
40	FALL	3	4.17	1.25	10.00	5.05	2.92	121	1.25	1.25	1.25	10.0	10.0
40	SPRING	2	3.79	1.25	6.33	3.59	2.54	95	1.25	1.25	3.79	6.33	6.33
40	SUMMER	5	15.9	8.50	28.00	8.48	3.79	53	8.50	8.50	13.5	21.0	28.0
40	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
45	FALL	4	18.2	3.69	40.90	16.6	8.32	92	3.69	5.91	14.0	30.4	40.9
45	SPRING	5	19.9	2.50	37.50	14.2	6.36	71	2.50	13.7	14.7	31.4	37.5
45	SUMMER	10	31.8	3.09	92.78	31.3	9.89	98	3.09	5.90	26.8	34.1	92.8
45	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
64	FALL	14	1.83	1.00	4.19	0.82	0.22	45	1.00	1.35	1.60	2.03	4.19
64	SPRING	14	3.24	1.86	5.20	0.98	0.26	30	1.86	2.65	3.02	3.94	5.20
64	SUMMER	14	3.46	1.28	10.99	2.57	0.69	74	1.28	2.08	2.71	3.34	11.0
64	WINTER	14	1.53	.960	3.02	0.55	0.15	36	0.96	1.19	1.31	1.93	3.02
65	FALL	23	14.4	.000	97.78	23.8	4.96	166	0.00	0.00	4.07	14.8	58.4
65	SPRING	22	11.1	.000	95.06	22.5	4.80	203	0.00	0.00	3.47	7.65	45.4
65	SUMMER	37	9.21	.000	76.55	18.7	3.08	203	0.00	0.00	0.00	9.33	63.9

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	34	1.87	.000	16.68	3.75	0.64	200	0.00	0.00	0.00	2.53	12.5
71	FALL	0	.	.	.	.	.	.	.	.	.	.	.
71	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
71	SUMMER	2	8.27	7.62	8.92	0.92	0.65	11	7.62	7.62	8.27	8.92	8.92
71	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
72	FALL	1	27.1	27.1	27.10	.	.	.	27.1	27.1	27.1	27.1	27.1
72	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
72	SUMMER	1	25.1	25.1	25.10	.	.	.	25.1	25.1	25.1	25.1	25.1
72	WINTER	1	16.9	16.9	16.90	.	.	.	16.9	16.9	16.9	16.9	16.9
74	FALL	3	0.09	.065	0.12	0.03	0.01	27	0.07	0.07	0.10	0.12	0.12
74	SPRING	1	8.97	8.97	8.97	.	.	.	8.97	8.97	8.97	8.97	8.97
74	SUMMER	1	10.2	10.2	10.20	.	.	.	10.2	10.2	10.2	10.2	10.2
74	WINTER	1	2.41	2.41	2.41	.	.	.	2.41	2.41	2.41	2.41	2.41

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	0	.	.	.	.	.	.	.	.	.	.	.
29	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
29	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
29	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
33	FALL	0	.	.	.	.	.	.	.	.	.	.	.
33	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
33	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
33	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
35	FALL	0	.	.	.	.	.	.	.	.	.	.	.
35	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
35	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
35	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
37	FALL	0	.	.	.	.	.	.	.	.	.	.	.
37	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
37	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
37	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
40	FALL	0	.	.	.	.	.	.	.	.	.	.	.
40	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
40	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
40	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
45	FALL	0	.	.	.	.	.	.	.	.	.	.	.
45	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
45	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
45	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
64	FALL	0	.	.	.	.	.	.	.	.	.	.	.
64	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
64	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
64	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
65	FALL	0	.	.	.	.	.	.	.	.	.	.	.
65	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
65	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
71	FALL	0	.	.	.	.	.	.	.	.	.	.	.
71	SPRING	1	0.20	.200	0.20	.	.	.	0.20	0.20	0.20	0.20	0.20
71	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
71	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
72	FALL	1	0.40	.400	0.40	.	.	.	0.40	0.40	0.40	0.40	0.40

72	SPRING	1	0.43	.425	0.43	.	.	.	0.43	0.43	0.43	0.43	0.43
72	SUMMER	1	0.70	.700	0.70	.	.	.	0.70	0.70	0.70	0.70	0.70
72	WINTER	1	0.05	.050	0.05	.	.	.	0.05	0.05	0.05	0.05	0.05
74	FALL	0	.	.	.	.	.	.	.	.	.	.	.
74	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
74	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
74	WINTER	0	.	.	.	.	.	.	.	.	.	.	.

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chlb_Phyto_Spec_ug_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	0	.	.	.	.	.	.	.	.	.	.	.
29	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
29	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
29	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
33	FALL	0	.	.	.	.	.	.	.	.	.	.	.
33	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
33	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
33	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
35	FALL	0	.	.	.	.	.	.	.	.	.	.	.
35	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
35	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
35	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
37	FALL	0	.	.	.	.	.	.	.	.	.	.	.
37	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
37	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
37	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
40	FALL	0	.	.	.	.	.	.	.	.	.	.	.
40	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
40	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
40	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
45	FALL	0	.	.	.	.	.	.	.	.	.	.	.
45	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
45	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
45	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
64	FALL	0	.	.	.	.	.	.	.	.	.	.	.
64	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
64	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
64	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
65	FALL	0	.	.	.	.	.	.	.	.	.	.	.
65	SPRING	1	0.24	.236	0.24	.	.	.	0.24	0.24	0.24	0.24	0.24
65	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Chlb_Phyto_Spec_ug_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
71	FALL	0	.	.	.	.	.	.	.	.	.	.	.
71	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
71	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
71	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
72	FALL	0	.	.	.	.	.	.	.	.	.	.	.
72	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
72	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
72	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
74	FALL	0	.	.	.	.	.	.	.	.	.	.	.
74	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
74	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
74	WINTER	0	.	.	.	.	.	.	.	.	.	.	.

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter DIP_ug_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	11	45.3	.000	135.00	44.3	13.4	98	0.00	10.0	35.0	65.0	135
29	SPRING	9	30.1	5.00	80.00	24.5	8.16	81	5.00	10.0	25.0	35.0	80.0
29	SUMMER	10	27.8	5.00	92.50	28.1	8.89	101	5.00	6.25	18.8	40.0	92.5
29	WINTER	9	30.1	5.00	70.00	24.8	8.25	82	5.00	7.50	25.0	55.0	70.0
33	FALL	4	676	60.0	1412.50	683	342	101	60.0	95.0	615	1256	1413
33	SPRING	4	323	92.5	750.00	310	155	96	92.5	93.8	225	553	750
33	SUMMER	4	365	82.5	837.50	342	171	94	82.5	116	270	614	838
33	WINTER	4	483	52.5	1000.00	479	239	99	52.5	76.3	440	890	1000
35	FALL	11	146	5.00	790.00	281	84.6	192	5.00	5.00	20.0	65.0	790
35	SPRING	12	48.4	5.00	295.00	83.0	24.0	171	5.00	10.6	13.8	53.8	295
35	SUMMER	12	109	5.00	930.00	264	76.2	242	5.00	10.0	12.5	51.3	930
35	WINTER	12	60.4	6.25	360.00	102	29.6	170	6.25	13.8	20.0	47.5	360
37	FALL	3	45.0	20.0	70.00	25.0	14.4	56	20.0	20.0	45.0	70.0	70.0
37	SPRING	3	47.5	12.5	100.00	46.3	26.7	97	12.5	12.5	30.0	100	100
37	SUMMER	3	84.2	42.5	165.00	70.0	40.4	83	42.5	42.5	45.0	165	165
37	WINTER	3	98.3	25.0	202.50	92.7	53.5	94	25.0	25.0	67.5	203	203
40	FALL	22	58.0	18.7	293.02	56.3	12.0	97	18.9	26.1	47.6	65.0	87.5
40	SPRING	11	60.5	12.5	120.00	30.6	9.24	51	12.5	35.0	65.0	82.5	120
40	SUMMER	23	59.1	4.30	176.33	48.4	10.1	82	7.41	18.1	47.5	100	145
40	WINTER	11	81.5	23.8	215.00	62.0	18.7	76	23.8	25.0	70.0	125	215
45	FALL	16	44.0	5.00	275.00	68.0	17.0	155	5.00	8.75	15.0	62.5	275
45	SPRING	16	25.2	5.00	102.50	28.5	7.12	113	5.00	5.00	12.5	32.5	103
45	SUMMER	16	50.9	5.00	362.50	87.8	21.9	173	5.00	8.13	25.0	43.8	363

45	WINTER	16	25.7	5.00	125.00	29.6	7.39	115	5.00	7.50	18.1	34.8	125
64	FALL	48	516	5.00	9600.00	1512	218	293	15.0	70.0	143	285	1551
64	SPRING	25	171	5.00	812.00	221	44.2	129	5.00	37.0	75.0	204	752
64	SUMMER	62	68.3	4.25	412.50	68.2	8.66	100	7.00	21.0	50.0	96.0	183
64	WINTER	18	103	16.8	310.25	82.1	19.4	80	16.8	40.0	86.3	133	310
65	FALL	45	33.0	5.00	250.00	50.9	7.59	154	5.00	5.00	12.5	25.0	115
65	SPRING	45	24.8	5.00	205.00	35.8	5.33	144	5.00	7.50	10.0	25.0	80.0
65	SUMMER	45	34.6	5.00	360.00	60.1	8.96	174	5.00	7.50	12.5	30.0	120

Aggregate Nutrient Ecoregion: IX 16
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter DIP_ug_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	45	22.8	5.00	150.00	30.3	4.52	133	5.00	5.00	12.5	22.5	65.0
71	FALL	13	94.1	7.50	295.00	87.9	24.4	93	7.50	35.0	56.3	135	295
71	SPRING	13	48.2	7.50	162.50	46.9	13.0	97	7.50	15.0	30.0	55.0	163
71	SUMMER	13	80.6	10.0	322.50	85.7	23.8	106	10.0	20.0	60.0	80.0	323
71	WINTER	12	65.4	17.5	200.00	59.2	17.1	91	17.5	30.0	40.0	85.0	200
72	FALL	41	86.0	.000	240.00	66.6	10.4	78	5.00	35.2	70.0	128	220
72	SPRING	39	52.9	.000	160.00	35.6	5.69	67	0.00	30.0	50.0	70.0	125
72	SUMMER	39	70.0	.000	185.50	45.6	7.30	65	4.00	35.0	62.5	95.0	170
72	WINTER	37	66.9	.000	140.00	38.4	6.32	57	1.00	40.0	60.0	93.5	135
74	FALL	3	38.3	5.00	75.00	35.1	20.3	92	5.00	5.00	35.0	75.0	75.0
74	SPRING	3	33.3	15.0	47.50	16.6	9.61	50	15.0	15.0	37.5	47.5	47.5
74	SUMMER	3	38.8	11.3	60.00	25.0	14.4	64	11.3	11.3	45.0	60.0	60.0
74	WINTER	3	30.0	5.00	50.00	22.9	13.2	76	5.00	5.00	35.0	50.0	50.0

Aggregate Nutrient Ecoregion: IX 17
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter DO_mg_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	83	7.88	4.50	10.70	1.36	0.15	17	5.55	7.00	7.95	8.85	9.75
29	SPRING	82	8.19	4.00	11.45	1.31	0.14	16	6.10	7.60	8.20	8.80	10.4
29	SUMMER	87	6.91	3.60	9.45	1.21	0.13	17	4.85	6.00	7.00	7.80	8.40
29	WINTER	98	11.3	2.90	14.70	1.76	0.18	16	8.10	10.4	11.5	12.5	14.1
33	FALL	26	7.24	2.90	9.45	1.44	0.28	20	4.88	6.20	7.45	8.15	9.30
33	SPRING	31	7.54	5.00	9.90	1.15	0.21	15	5.20	6.88	7.65	8.35	9.40
33	SUMMER	26	6.55	3.40	10.60	1.69	0.33	26	3.80	5.70	6.60	7.40	9.75
33	WINTER	30	9.76	8.10	12.20	1.02	0.19	10	8.25	9.00	9.60	10.3	11.7
35	FALL	151	6.62	1.20	11.10	1.75	0.14	27	3.40	5.68	6.70	7.70	9.30
35	SPRING	144	7.56	2.80	11.80	1.61	0.13	21	5.40	6.69	7.40	8.48	10.6
35	SUMMER	146	5.58	1.20	9.60	1.66	0.14	30	2.40	4.55	5.75	6.80	7.95
35	WINTER	139	9.52	4.55	13.30	1.53	0.13	16	6.90	8.60	9.50	10.3	12.5
37	FALL	33	6.72	2.50	9.60	1.71	0.30	26	3.43	6.00	6.60	7.83	9.60
37	SPRING	27	8.15	6.15	10.53	1.22	0.24	15	6.63	7.35	7.90	9.25	10.3
37	SUMMER	32	5.94	2.20	8.45	1.53	0.27	26	2.60	5.18	6.08	6.85	8.40

37	WINTER	28	10.6	6.20	13.80	1.58	0.30	15	7.48	9.80	10.9	11.7	12.3
40	FALL	104	7.37	1.25	17.88	2.20	0.22	30	4.10	5.96	7.41	8.58	10.8
40	SPRING	90	9.34	4.25	13.80	1.38	0.15	15	7.20	8.75	9.43	10.1	11.5
40	SUMMER	115	6.74	.200	13.10	1.83	0.17	27	3.30	5.88	6.70	7.85	9.37
40	WINTER	75	11.8	6.30	13.80	1.33	0.15	11	9.21	11.4	12.0	12.7	13.5
45	FALL	344	8.24	1.70	10.95	1.02	0.06	12	6.40	7.90	8.30	8.80	9.70
45	SPRING	367	8.93	5.60	12.00	1.01	0.05	11	7.20	8.30	8.93	9.50	10.7
45	SUMMER	371	7.28	1.50	12.00	1.03	0.05	14	5.40	6.90	7.43	7.85	8.50
45	WINTER	264	10.7	8.80	14.10	0.83	0.05	8	9.50	10.2	10.6	11.2	12.1
64	FALL	115	10.2	6.50	13.85	1.26	0.12	12	8.00	9.40	10.3	10.9	12.2
64	SPRING	91	10.5	3.30	12.90	1.58	0.17	15	7.30	10.0	10.8	11.6	12.4
64	SUMMER	167	8.63	6.05	13.20	1.13	0.09	13	6.80	7.98	8.60	9.10	10.9
64	WINTER	69	12.4	9.13	14.10	1.07	0.13	9	10.3	11.9	12.8	13.1	13.7
65	FALL	352	7.08	.400	11.60	1.79	0.10	25	3.25	6.24	7.55	8.30	9.30
65	SPRING	371	7.73	1.80	11.90	1.64	0.09	21	4.70	6.83	7.90	8.80	10.1
65	SUMMER	402	6.20	.450	10.63	1.66	0.08	27	2.80	5.65	6.55	7.25	8.10

Aggregate Nutrient Ecoregion: IX 18
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter DO_mg_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	330	9.57	4.10	13.20	1.51	0.08	16	6.90	8.60	9.80	10.6	11.8
71	FALL	93	8.04	.000	12.50	2.09	0.22	26	4.00	7.45	8.20	9.20	11.0
71	SPRING	79	9.49	4.70	12.40	1.22	0.14	13	7.70	8.75	9.50	10.1	11.6
71	SUMMER	95	7.36	2.50	10.10	1.46	0.15	20	4.70	6.40	7.60	8.50	9.45
71	WINTER	78	11.0	7.68	14.60	1.28	0.15	12	8.80	10.2	10.9	11.8	13.0
72	FALL	84	7.55	2.33	11.80	2.04	0.22	27	4.00	6.30	7.63	8.95	11.0
72	SPRING	83	9.43	7.30	12.40	1.04	0.11	11	8.05	8.80	9.20	10.1	11.3
72	SUMMER	84	6.26	.000	8.85	1.52	0.17	24	3.60	5.38	6.40	7.30	8.40
72	WINTER	80	11.5	7.00	13.80	0.99	0.11	9	10.3	11.1	11.6	12.1	13.0
74	FALL	52	7.71	1.60	11.90	1.78	0.25	23	3.90	6.80	8.25	8.70	9.88
74	SPRING	52	8.55	5.85	12.15	1.31	0.18	15	6.60	7.55	8.55	9.39	10.5
74	SUMMER	54	6.93	2.35	13.50	1.74	0.24	25	3.35	6.15	7.20	7.70	9.30
74	WINTER	49	10.2	5.75	12.40	1.38	0.20	14	7.20	9.50	10.4	11.1	12.3

Aggregate Nutrient Ecoregion: IX 19
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter NO2_NO3_mg_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	74	0.57	.010	5.40	1.08	0.13	189	0.01	0.05	0.13	0.48	3.36
29	SPRING	65	0.37	.010	3.75	0.60	0.07	164	0.03	0.09	0.15	0.39	1.41
29	SUMMER	67	0.33	.000	6.20	0.79	0.10	235	0.01	0.07	0.13	0.35	0.92
29	WINTER	78	0.46	.025	4.00	0.66	0.07	143	0.05	0.12	0.21	0.49	1.70
33	FALL	24	2.31	.010	8.32	2.99	0.61	130	0.02	0.09	0.91	4.38	8.27
33	SPRING	22	1.33	.093	5.43	1.67	0.36	125	0.11	0.21	0.68	1.67	5.35
33	SUMMER	21	1.60	.010	6.84	2.30	0.50	144	0.02	0.11	0.35	2.04	6.60

33	WINTER	25	2.13	.070	8.45	2.51	0.50	118	0.10	0.16	0.77	3.42	6.51
35	FALL	142	0.56	.005	9.08	1.43	0.12	254	0.01	0.04	0.11	0.26	3.70
35	SPRING	134	0.37	.005	4.45	0.72	0.06	195	0.03	0.09	0.15	0.32	1.51
35	SUMMER	140	0.43	.005	6.15	0.92	0.08	212	0.01	0.06	0.14	0.29	2.65
35	WINTER	143	0.33	.005	6.34	0.72	0.06	220	0.02	0.07	0.14	0.25	1.19
37	FALL	35	0.57	.003	7.16	1.38	0.23	243	0.01	0.03	0.07	0.34	4.25
37	SPRING	30	0.41	.025	4.92	0.89	0.16	215	0.05	0.10	0.17	0.32	1.25
37	SUMMER	37	0.48	.005	6.84	1.27	0.21	265	0.01	0.05	0.13	0.23	4.05
37	WINTER	32	0.52	.093	6.12	1.05	0.19	205	0.10	0.15	0.27	0.45	1.28
40	FALL	125	0.82	.000	7.84	1.42	0.13	172	0.00	0.11	0.34	0.76	4.20
40	SPRING	111	1.03	.005	9.80	1.59	0.15	153	0.05	0.26	0.51	1.01	4.92
40	SUMMER	137	1.03	.000	9.03	1.72	0.15	167	0.00	0.20	0.50	0.83	5.63
40	WINTER	78	1.25	.025	9.38	1.71	0.19	137	0.13	0.40	0.67	1.24	6.32
45	FALL	315	0.73	.003	8.85	1.26	0.07	173	0.03	0.12	0.33	0.71	3.26
45	SPRING	332	0.64	.000	9.30	1.03	0.06	162	0.04	0.19	0.37	0.64	2.23
45	SUMMER	327	0.73	.003	7.00	1.14	0.06	157	0.01	0.17	0.37	0.75	3.11
45	WINTER	241	0.70	.020	8.78	1.03	0.07	148	0.06	0.21	0.38	0.73	2.40
64	FALL	62	2.24	.330	9.02	1.77	0.22	79	0.52	0.98	1.73	3.00	5.80
64	SPRING	61	2.20	.110	7.70	1.76	0.23	80	0.55	0.96	1.63	2.70	6.24
64	SUMMER	77	2.05	.120	6.27	1.47	0.17	72	0.23	1.01	1.69	2.80	5.57
64	WINTER	53	2.74	.660	9.76	1.97	0.27	72	0.80	1.57	2.16	3.10	8.28
65	FALL	439	0.45	.000	5.95	0.76	0.04	168	0.01	0.07	0.20	0.44	1.88
65	SPRING	496	0.39	.000	5.46	0.57	0.03	147	0.01	0.10	0.20	0.43	1.50
65	SUMMER	518	0.41	.000	4.70	0.62	0.03	152	0.01	0.09	0.21	0.45	1.66

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	433	0.44	.000	4.10	0.60	0.03	138	0.01	0.10	0.23	0.51	1.57
71	FALL	107	0.99	.003	10.00	1.50	0.14	151	0.03	0.20	0.44	1.35	2.72
71	SPRING	94	1.05	.025	5.41	1.06	0.11	101	0.13	0.40	0.60	1.17	3.38
71	SUMMER	109	0.98	.012	4.72	1.01	0.10	103	0.06	0.29	0.62	1.20	2.95
71	WINTER	91	1.36	.003	5.34	1.19	0.13	88	0.11	0.49	1.04	1.72	4.20
72	FALL	151	0.97	.000	5.70	1.24	0.10	127	0.01	0.14	0.44	1.40	3.75
72	SPRING	124	1.53	.023	8.45	1.81	0.16	118	0.07	0.29	0.86	1.90	5.50
72	SUMMER	173	1.13	.003	8.80	1.61	0.12	142	0.02	0.14	0.46	1.45	4.45
72	WINTER	151	1.58	.003	9.00	1.84	0.15	116	0.05	0.39	0.83	2.13	5.98
74	FALL	53	0.28	.005	1.05	0.23	0.03	81	0.04	0.14	0.21	0.33	0.69
74	SPRING	60	0.52	.020	3.30	0.67	0.09	129	0.03	0.14	0.27	0.63	2.11
74	SUMMER	65	0.48	.024	6.00	1.00	0.12	210	0.03	0.09	0.18	0.49	1.20
74	WINTER	53	0.43	.033	1.73	0.41	0.06	95	0.08	0.15	0.27	0.55	1.55

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Org_P_ug_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	1	37.6	37.6	37.64	.	.	.	37.6	37.6	37.6	37.6	37.6
29	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
29	SUMMER	1	36.5	36.5	36.48	.	.	.	36.5	36.5	36.5	36.5	36.5
29	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
33	FALL	0	.	.	.	.	.	.	.	.	.	.	.
33	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
33	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
33	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
35	FALL	0	.	.	.	.	.	.	.	.	.	.	.
35	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
35	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
35	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
37	FALL	0	.	.	.	.	.	.	.	.	.	.	.
37	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
37	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
37	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
40	FALL	11	73.3	22.5	188.30	52.1	15.7	71	22.5	37.6	58.6	84.1	188
40	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
40	SUMMER	12	53.7	23.1	115.44	25.3	7.30	47	23.1	36.4	49.8	64.3	115
40	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
45	FALL	0	.	.	.	.	.	.	.	.	.	.	.
45	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
45	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
45	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
64	FALL	0	.	.	.	.	.	.	.	.	.	.	.
64	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
64	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
64	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
65	FALL	0	.	.	.	.	.	.	.	.	.	.	.
65	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
65	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Org_P_ug_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
71	FALL	0	.	.	.	.	.	.	.	.	.	.	.
71	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
71	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
71	WINTER	0	.	.	.	.	.	.	.	.	.	.	.

72	FALL	3	70.6	34.6	138.53	58.9	34.0	83	34.6	34.6	38.7	139	139
72	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
72	SUMMER	2	52.0	6.77	97.22	64.0	45.2	123	6.77	6.77	52.0	97.2	97.2
72	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
74	FALL	0	.	.	.	.	.	.	.	.	.	.	.
74	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
74	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
74	WINTER	0	.	.	.	.	.	.	.	.	.	.	.

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Orthophosphate_T_as_P_ug_L_Med

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	5	45.0	20.0	60.00	15.8	7.07	35	20.0	40.0	50.0	55.0	60.0
29	SPRING	5	46.5	22.5	70.00	22.3	9.99	48	22.5	30.0	40.0	70.0	70.0
29	SUMMER	5	54.3	25.0	97.50	26.9	12.0	50	25.0	43.8	47.5	57.5	97.5
29	WINTER	4	90.9	31.3	225.00	91.0	45.5	100	31.3	34.4	53.8	148	225
33	FALL	3	920	60.0	1600.00	786	454	85	60.0	60.0	1100	1600	1600
33	SPRING	3	733	70.0	1095.00	575	332	78	70.0	70.0	1033	1095	1095
33	SUMMER	3	822	103	1307.50	635	367	77	103	103	1055	1308	1308
33	WINTER	3	522	70.0	815.00	397	229	76	70.0	70.0	680	815	815
35	FALL	10	265	5.00	1600.00	525	166	198	5.00	12.5	40.0	95.0	1600
35	SPRING	13	92.8	12.5	550.00	149	41.4	161	12.5	26.3	35.0	60.0	550
35	SUMMER	13	153	10.0	897.50	295	81.9	193	10.0	20.0	32.5	70.0	898
35	WINTER	13	50.4	12.5	130.00	36.4	10.1	72	12.5	30.0	37.5	70.0	130
37	FALL	3	90.0	30.0	200.00	95.4	55.1	106	30.0	30.0	40.0	200	200
37	SPRING	3	64.2	27.5	125.00	53.1	30.6	83	27.5	27.5	40.0	125	125
37	SUMMER	3	95.8	47.5	185.00	77.3	44.6	81	47.5	47.5	55.0	185	185
37	WINTER	3	102	30.0	225.00	107	61.9	106	30.0	30.0	50.0	225	225
40	FALL	11	87.7	30.0	130.00	33.6	10.1	38	30.0	50.0	95.0	118	130
40	SPRING	11	85.9	12.5	210.00	58.6	17.7	68	12.5	37.5	65.0	130	210
40	SUMMER	11	88.5	20.0	150.00	39.8	12.0	45	20.0	55.0	90.0	120	150
40	WINTER	11	104	30.0	240.00	68.9	20.8	66	30.0	40.0	95.0	170	240
45	FALL	8	63.8	5.00	255.00	82.8	29.3	130	5.00	10.0	40.0	75.0	255
45	SPRING	8	36.3	5.00	112.50	36.4	12.9	100	5.00	12.5	23.1	50.6	113
45	SUMMER	7	74.3	7.50	240.00	82.8	31.3	111	7.50	15.0	31.3	110	240
45	WINTER	8	38.0	5.00	115.00	38.4	13.6	101	5.00	9.38	22.5	60.0	115
64	FALL	6	257	20.0	595.00	219	89.3	85	20.0	40.0	250	385	595
64	SPRING	6	160	25.0	452.50	165	67.2	103	25.0	25.0	110	238	453
64	SUMMER	6	128	5.00	285.00	113	46.0	88	5.00	21.5	118	220	285
64	WINTER	6	96.3	20.0	180.00	65.8	26.9	68	20.0	27.5	95.0	160	180
65	FALL	34	32.6	5.00	150.00	41.1	7.05	126	5.00	5.00	20.0	30.0	150
65	SPRING	34	27.8	5.00	120.00	28.9	4.95	104	5.00	7.50	18.1	40.0	105
65	SUMMER	34	36.0	5.00	310.00	57.4	9.85	160	5.00	10.0	16.9	29.5	120

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Orthophosphate_T_as_P_ug_L_Med

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	34	29.5	5.00	155.00	31.4	5.39	107	5.00	7.50	15.6	35.0	90.0
71	FALL	10	90.0	5.00	270.00	84.1	26.6	93	5.00	20.0	80.0	120	270
71	SPRING	10	74.9	5.00	282.50	86.8	27.4	116	5.00	20.0	36.3	133	283
71	SUMMER	10	114	5.00	575.00	169	53.6	149	5.00	17.5	77.5	95.0	575
71	WINTER	9	111	21.3	260.00	83.4	27.8	75	21.3	50.0	90.0	130	260
72	FALL	7	92.9	15.0	175.00	58.7	22.2	63	15.0	30.0	120	130	175
72	SPRING	7	72.5	10.0	182.50	54.7	20.7	75	10.0	37.5	60.0	87.5	183
72	SUMMER	8	117	20.0	255.00	69.4	24.5	60	20.0	77.5	110	141	255
72	WINTER	7	103	20.0	157.50	50.1	18.9	49	20.0	50.0	120	140	158
74	FALL	3	48.3	5.00	75.00	37.9	21.9	78	5.00	5.00	65.0	75.0	75.0
74	SPRING	3	105	80.0	125.00	22.9	13.2	22	80.0	80.0	110	125	125
74	SUMMER	3	53.3	10.0	100.00	45.1	26.0	85	10.0	10.0	50.0	100	100
74	WINTER	3	66.7	17.5	102.50	44.0	25.4	66	17.5	17.5	80.0	103	103

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter TKN_mg_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	83	0.60	.050	1.75	0.33	0.04	54	0.17	0.40	0.55	0.79	1.20
29	SPRING	83	0.65	.125	1.84	0.34	0.04	52	0.25	0.40	0.60	0.73	1.35
29	SUMMER	83	0.64	.050	2.75	0.41	0.05	64	0.21	0.40	0.56	0.78	1.45
29	WINTER	93	0.58	.050	2.28	0.33	0.03	58	0.19	0.38	0.56	0.65	1.16
33	FALL	29	0.91	.240	5.40	0.91	0.17	100	0.35	0.57	0.71	0.97	1.60
33	SPRING	33	0.83	.390	1.63	0.25	0.04	30	0.46	0.70	0.80	0.93	1.33
33	SUMMER	30	0.83	.330	1.88	0.37	0.07	45	0.36	0.50	0.81	0.99	1.58
33	WINTER	32	0.77	.200	3.08	0.50	0.09	65	0.29	0.52	0.62	1.00	1.30
35	FALL	125	0.67	.010	2.61	0.40	0.04	59	0.23	0.43	0.60	0.80	1.45
35	SPRING	110	0.71	.050	2.63	0.45	0.04	63	0.05	0.45	0.63	0.84	1.59
35	SUMMER	124	0.78	.050	3.80	0.54	0.05	69	0.20	0.51	0.70	0.88	1.80
35	WINTER	137	0.68	.050	4.65	0.60	0.05	89	0.20	0.40	0.55	0.74	1.38
37	FALL	20	0.80	.200	1.80	0.47	0.10	58	0.22	0.50	0.67	1.19	1.68
37	SPRING	19	0.98	.345	2.83	0.68	0.15	69	0.35	0.60	0.70	1.20	2.83
37	SUMMER	24	0.82	.240	1.66	0.35	0.07	42	0.49	0.56	0.72	1.08	1.50
37	WINTER	26	0.82	.220	3.60	0.84	0.16	102	0.22	0.43	0.55	0.78	3.37
40	FALL	79	1.17	.175	5.00	0.95	0.11	81	0.45	0.60	0.90	1.27	4.55
40	SPRING	76	1.08	.315	4.02	0.68	0.08	63	0.33	0.65	0.96	1.28	1.98
40	SUMMER	81	1.11	.193	3.10	0.56	0.06	51	0.50	0.70	1.00	1.35	2.25
40	WINTER	49	0.87	.125	4.33	0.73	0.10	84	0.30	0.50	0.60	0.89	2.30
45	FALL	301	0.47	.025	3.20	0.42	0.02	90	0.10	0.24	0.31	0.56	1.23
45	SPRING	328	0.45	.025	3.95	0.48	0.03	108	0.10	0.23	0.33	0.50	1.10
45	SUMMER	338	0.49	.025	3.00	0.40	0.02	81	0.09	0.29	0.40	0.59	1.22
45	WINTER	279	0.40	.025	1.84	0.28	0.02	69	0.10	0.23	0.35	0.50	1.04

64	FALL	98	0.59	.050	3.01	0.51	0.05	87	0.10	0.30	0.43	0.80	1.85
64	SPRING	98	0.60	.050	2.68	0.52	0.05	87	0.05	0.31	0.46	0.65	1.60
64	SUMMER	125	0.59	.050	3.88	0.57	0.05	96	0.05	0.30	0.50	0.65	1.20
64	WINTER	78	0.55	.100	1.90	0.38	0.04	69	0.15	0.30	0.42	0.70	1.50
65	FALL	453	0.50	.000	4.10	0.38	0.02	75	0.10	0.30	0.42	0.60	1.10
65	SPRING	515	0.49	.000	3.93	0.34	0.02	70	0.13	0.30	0.42	0.60	1.00
65	SUMMER	554	0.58	.000	4.30	0.41	0.02	71	0.17	0.34	0.49	0.74	1.28

Aggregate Nutrient Ecoregion: IX 26
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter TKN_mg_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	492	0.45	.000	4.18	0.34	0.02	76	0.12	0.28	0.40	0.55	0.94
71	FALL	72	0.71	.050	3.60	0.68	0.08	97	0.10	0.29	0.46	0.90	1.82
71	SPRING	56	0.45	.035	1.58	0.31	0.04	68	0.05	0.27	0.40	0.54	1.00
71	SUMMER	65	0.56	.110	2.47	0.41	0.05	73	0.15	0.30	0.46	0.75	1.20
71	WINTER	48	0.47	.050	1.62	0.30	0.04	63	0.09	0.28	0.44	0.61	0.90
72	FALL	120	0.93	.025	4.80	0.75	0.07	81	0.28	0.53	0.79	0.99	2.70
72	SPRING	104	0.97	.025	4.80	0.75	0.07	77	0.30	0.60	0.80	1.07	2.30
72	SUMMER	154	0.85	.025	3.84	0.45	0.04	53	0.30	0.55	0.84	1.04	1.44
72	WINTER	134	0.68	.025	2.40	0.41	0.04	60	0.03	0.39	0.65	0.90	1.40
74	FALL	24	0.72	.195	1.65	0.41	0.08	57	0.25	0.38	0.70	0.96	1.40
74	SPRING	30	1.07	.110	5.00	1.30	0.24	122	0.16	0.35	0.58	1.25	4.85
74	SUMMER	31	0.88	.093	4.10	0.76	0.14	87	0.26	0.40	0.67	1.05	2.30
74	WINTER	29	0.67	.163	1.60	0.40	0.07	59	0.17	0.32	0.55	0.88	1.34

Aggregate Nutrient Ecoregion: IX 27
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter TN_mg_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	13	1.29	.450	2.80	0.81	0.22	63	0.45	0.80	1.00	1.17	2.80
29	SPRING	14	1.26	.330	4.30	1.04	0.28	83	0.33	0.56	1.13	1.45	4.30
29	SUMMER	20	0.80	.250	1.45	0.36	0.08	45	0.30	0.51	0.77	1.04	1.42
29	WINTER	21	1.24	.520	3.66	0.70	0.15	56	0.59	0.88	0.95	1.50	2.00
33	FALL	3	4.73	.600	7.70	3.69	2.13	78	0.60	0.60	5.90	7.70	7.70
33	SPRING	3	2.60	1.10	4.30	1.61	0.93	62	1.10	1.10	2.40	4.30	4.30
33	SUMMER	3	3.53	1.04	5.30	2.22	1.28	63	1.04	1.04	4.25	5.30	5.30
33	WINTER	3	3.94	.830	6.08	2.75	1.59	70	0.83	0.83	4.90	6.08	6.08
35	FALL	10	1.34	.300	4.90	1.71	0.54	128	0.30	0.35	0.68	0.81	4.90
35	SPRING	12	0.75	.400	1.60	0.38	0.11	51	0.40	0.51	0.55	1.03	1.60
35	SUMMER	19	1.49	.270	11.00	2.59	0.59	174	0.27	0.37	0.62	0.87	11.0
35	WINTER	18	0.69	.360	2.58	0.52	0.12	76	0.36	0.41	0.52	0.67	2.58
37	FALL	3	1.10	.600	1.90	0.70	0.40	64	0.60	0.60	0.80	1.90	1.90
37	SPRING	3	1.06	.760	1.60	0.47	0.27	44	0.76	0.76	0.82	1.60	1.60
37	SUMMER	8	0.94	.500	1.40	0.27	0.09	29	0.50	0.85	0.87	1.08	1.40
37	WINTER	8	1.02	.450	2.15	0.57	0.20	56	0.45	0.61	0.84	1.33	2.15

40	FALL	30	1.29	.180	5.10	1.04	0.19	81	0.25	0.72	0.98	1.45	3.83
40	SPRING	19	1.93	.340	7.90	1.83	0.42	95	0.34	0.67	1.48	2.43	7.90
40	SUMMER	31	1.38	.220	7.25	1.34	0.24	97	0.33	0.70	0.94	1.70	3.66
40	WINTER	19	1.54	.490	5.20	1.23	0.28	80	0.49	0.75	1.06	1.95	5.20
45	FALL	18	1.00	.200	2.98	0.68	0.16	69	0.20	0.56	0.70	1.30	2.98
45	SPRING	17	0.95	.300	1.80	0.38	0.09	41	0.30	0.70	0.89	1.23	1.80
45	SUMMER	18	0.93	.200	2.45	0.60	0.14	64	0.20	0.48	0.86	1.41	2.45
45	WINTER	16	1.03	.275	2.69	0.61	0.15	60	0.28	0.67	0.84	1.29	2.69
64	FALL	37	4.08	.567	12.50	3.30	0.54	81	0.63	1.65	3.12	4.02	11.0
64	SPRING	38	4.07	.547	11.00	2.50	0.41	61	0.65	2.10	3.63	5.25	10.0
64	SUMMER	76	4.84	.552	12.30	2.92	0.34	60	1.44	2.49	4.16	7.08	10.7
64	WINTER	30	4.48	1.85	13.00	3.04	0.55	68	1.88	2.35	3.60	4.57	12.0
65	FALL	64	1.00	.290	3.10	0.59	0.07	59	0.40	0.57	0.75	1.34	2.20
65	SPRING	65	1.06	.360	2.94	0.56	0.07	53	0.51	0.66	0.90	1.30	2.08
65	SUMMER	65	1.03	.385	2.75	0.54	0.07	52	0.46	0.65	0.83	1.36	2.06

Aggregate Nutrient Ecoregion: IX 28
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter TN_mg_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	65	1.10	.300	2.94	0.67	0.08	61	0.40	0.59	0.85	1.63	2.30
71	FALL	10	1.35	.370	3.60	1.14	0.36	84	0.37	0.51	0.97	1.65	3.60
71	SPRING	10	1.61	.550	4.65	1.43	0.45	89	0.55	0.72	0.94	1.70	4.65
71	SUMMER	10	1.73	.740	4.05	1.12	0.36	65	0.74	0.88	1.35	2.00	4.05
71	WINTER	9	2.28	.700	5.40	1.53	0.51	67	0.70	1.35	2.00	2.30	5.40
72	FALL	22	2.86	.230	6.30	1.73	0.37	61	0.46	1.32	2.86	4.26	5.03
72	SPRING	20	3.86	.943	7.88	2.29	0.51	59	1.09	1.81	3.72	5.62	7.76
72	SUMMER	21	2.74	.280	6.18	1.58	0.34	58	0.61	1.52	2.35	3.73	4.98
72	WINTER	19	4.10	.660	8.20	2.32	0.53	57	0.66	2.40	4.06	6.16	8.20
74	FALL	3	0.96	.570	1.20	0.34	0.20	35	0.57	0.57	1.10	1.20	1.20
74	SPRING	3	1.22	.935	1.45	0.26	0.15	21	0.94	0.94	1.28	1.45	1.45
74	SUMMER	3	0.81	.630	0.95	0.16	0.09	20	0.63	0.63	0.84	0.95	0.95
74	WINTER	3	0.99	.555	1.30	0.39	0.22	39	0.56	0.56	1.10	1.30	1.30

Aggregate Nutrient Ecoregion: IX 29
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter TP_ug_L_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	85	163	2.50	1500.00	265	28.7	163	20.0	40.0	75.0	155	445
29	SPRING	84	131	2.50	1235.00	188	20.5	143	16.3	35.0	70.0	135	475
29	SUMMER	90	106	2.50	690.00	125	13.2	118	10.0	30.0	50.0	140	385
29	WINTER	100	141	2.50	1430.00	201	20.1	142	8.13	43.8	87.5	170	380
33	FALL	29	486	40.0	2120.00	572	106	118	50.0	90.0	265	495	1800
33	SPRING	35	420	50.0	2360.00	493	83.3	117	50.0	140	218	480	1630
33	SUMMER	28	381	50.0	1640.00	420	79.4	110	50.0	110	213	450	1230
33	WINTER	31	352	20.0	1210.00	351	63.1	100	40.0	90.0	200	660	1100

35	FALL	158	173	2.50	2420.00	316	25.1	183	10.0	50.0	75.0	140	778
35	SPRING	153	151	2.50	2090.00	258	20.9	171	10.0	50.0	72.5	150	510
35	SUMMER	164	159	2.50	1510.00	247	19.3	155	10.0	50.0	80.0	147	700
35	WINTER	160	143	2.50	1710.00	251	19.8	175	18.8	45.0	70.0	130	450
37	FALL	34	176	5.00	1345.00	278	47.8	158	11.3	45.0	70.0	150	925
37	SPRING	31	188	5.00	1440.00	345	62.1	184	5.00	37.5	70.0	120	1215
37	SUMMER	39	229	17.5	1592.50	389	62.3	170	20.0	50.0	90.0	170	1515
37	WINTER	33	167	5.00	1380.00	316	55.0	189	5.00	40.0	60.0	100	1045
40	FALL	139	242	10.0	1880.00	299	25.3	124	41.2	95.0	150	240	930
40	SPRING	126	261	10.0	2140.00	377	33.6	144	40.0	90.0	135	255	1040
40	SUMMER	146	242	20.0	2040.00	284	23.5	117	49.3	100	156	260	940
40	WINTER	96	195	2.50	2140.00	320	32.7	164	10.0	43.8	87.5	198	975
45	FALL	395	130	.000	1650.00	221	11.1	170	10.0	30.0	56.3	115	565
45	SPRING	425	104	.000	1187.50	159	7.72	153	10.0	35.0	50.0	95.0	415
45	SUMMER	436	145	.000	2400.00	266	12.7	184	9.00	30.0	60.0	124	625
45	WINTER	322	93.0	.000	1200.00	137	7.64	147	6.25	30.0	51.3	100	310
64	FALL	110	182	2.50	1335.00	243	23.1	134	10.0	40.0	85.0	220	793
64	SPRING	108	144	.000	1755.00	233	22.4	162	20.0	42.5	70.0	130	590
64	SUMMER	181	150	2.50	1760.00	237	17.6	157	10.0	40.0	70.0	150	510
64	WINTER	86	114	.000	550.00	124	13.4	108	10.0	40.0	70.0	130	450
65	FALL	532	82.0	.000	1770.00	139	6.01	169	0.00	20.0	50.0	95.0	255
65	SPRING	603	72.8	.000	990.00	96.6	3.94	133	0.00	25.0	50.0	90.0	185
65	SUMMER	650	95.8	.000	1700.00	132	5.18	138	0.00	30.0	60.0	120	270

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	543	79.7	.000	2400.00	163	6.98	204	0.00	20.0	45.0	80.0	215
71	FALL	115	145	2.50	1900.00	250	23.3	173	4.50	30.0	75.0	140	435
71	SPRING	98	80.9	2.50	625.00	112	11.3	138	2.50	20.0	40.0	90.0	325
71	SUMMER	117	142	2.50	1150.00	203	18.8	143	2.50	30.0	60.0	163	615
71	WINTER	94	124	2.50	1410.00	216	22.2	174	5.00	30.0	60.0	97.5	475
72	FALL	156	275	2.50	1800.00	306	24.5	111	20.0	110	190	279	948
72	SPRING	136	231	10.0	2300.00	373	32.0	162	30.0	86.3	138	241	740
72	SUMMER	183	203	.000	1400.00	199	14.7	98	25.0	80.0	163	240	530
72	WINTER	160	174	.000	920.00	164	12.9	94	20.0	70.0	140	214	500
74	FALL	58	243	2.50	1125.00	279	36.7	115	25.0	50.0	110	370	970
74	SPRING	65	219	10.0	2170.00	338	42.0	154	25.0	70.0	123	205	760
74	SUMMER	70	212	.000	1200.00	228	27.3	107	20.0	80.0	140	250	660
74	WINTER	60	195	2.50	840.00	170	22.0	87	13.1	81.3	134	291	530

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Turb_FTU_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	53	23.0	2.10	95.00	25.1	3.45	109	2.90	5.95	11.8	29.0	89.5
29	SPRING	46	22.9	.900	96.00	22.2	3.27	97	3.10	7.10	13.0	32.5	66.0
29	SUMMER	46	26.1	.900	81.00	22.7	3.34	87	4.68	10.6	15.5	42.5	73.8
29	WINTER	52	30.1	.800	117.00	26.9	3.73	89	3.50	11.5	20.4	44.5	99.0
33	FALL	6	27.8	1.10	87.00	31.8	13.0	114	1.10	7.10	19.3	33.0	87.0
33	SPRING	8	42.2	1.00	64.00	23.6	8.36	56	1.00	21.9	55.0	59.3	64.0
33	SUMMER	7	44.4	8.50	110.00	39.1	14.8	88	8.50	11.8	29.7	81.8	110
33	WINTER	7	43.9	7.50	93.50	36.0	13.6	82	7.50	10.0	24.0	79.0	93.5
35	FALL	47	14.5	2.20	55.00	11.8	1.72	81	2.60	6.00	10.1	19.9	40.0
35	SPRING	57	18.3	1.80	75.00	12.9	1.71	71	3.50	9.00	15.3	25.0	47.0
35	SUMMER	42	14.8	1.30	77.00	15.2	2.34	103	2.95	6.00	8.69	21.5	47.0
35	WINTER	57	18.7	2.55	63.75	13.2	1.75	71	2.70	7.88	19.0	25.0	45.5
37	FALL	6	24.7	3.60	57.50	19.8	8.08	80	3.60	8.00	23.0	33.0	57.5
37	SPRING	17	24.8	5.50	57.00	15.6	3.79	63	5.50	15.0	20.0	32.0	57.0
37	SUMMER	7	28.8	12.0	50.00	15.1	5.70	52	12.0	17.5	22.5	49.4	50.0
37	WINTER	17	22.2	4.80	48.00	15.9	3.86	72	4.80	12.0	15.0	32.0	48.0
40	FALL	71	19.2	3.90	67.00	12.4	1.48	65	7.00	11.0	16.8	22.5	40.8
40	SPRING	73	30.3	3.00	123.00	25.9	3.03	86	7.30	13.5	20.7	38.7	92.0
40	SUMMER	78	31.5	1.40	74.50	17.2	1.94	55	8.00	19.0	27.3	41.5	67.0
40	WINTER	70	13.6	1.00	72.75	13.6	1.63	100	2.00	5.10	8.30	17.3	38.0
45	FALL	342	9.87	1.00	110.00	8.05	0.44	81	2.95	5.93	8.50	12.0	19.5
45	SPRING	357	13.1	1.00	106.00	8.98	0.48	68	2.95	7.65	12.0	16.0	25.5
45	SUMMER	356	13.1	1.25	102.50	9.64	0.51	74	2.65	7.33	11.8	16.0	27.0
45	WINTER	271	15.9	1.40	136.00	12.0	0.73	76	3.63	8.68	14.0	20.0	32.5
64	FALL	46	2.78	.450	8.75	1.90	0.28	68	0.70	1.20	2.25	3.80	6.40
64	SPRING	28	5.22	1.00	11.10	2.69	0.51	52	1.13	3.30	5.10	7.05	10.1
64	SUMMER	30	7.01	.800	16.75	4.16	0.76	59	1.00	4.05	6.08	9.40	16.5
64	WINTER	29	5.81	.400	15.08	3.65	0.68	63	1.00	3.00	5.25	7.30	15.0
65	FALL	358	6.86	.000	41.03	5.46	0.29	80	1.80	3.40	5.30	8.50	17.0
65	SPRING	418	9.61	.650	54.00	7.57	0.37	79	2.00	4.60	7.50	11.9	24.0
65	SUMMER	426	11.5	1.10	156.00	14.9	0.72	130	2.20	4.85	7.88	12.0	28.8

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Turb_FTU_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	350	10.4	.300	123.50	12.6	0.67	122	1.85	4.08	6.75	11.0	31.4
71	FALL	20	9.18	1.45	18.40	5.17	1.16	56	1.55	5.09	8.45	13.3	18.2
71	SPRING	21	13.4	1.80	41.00	10.5	2.30	78	2.40	6.40	10.1	17.0	31.0
71	SUMMER	22	15.3	1.95	47.15	10.8	2.31	71	2.25	8.20	13.7	19.4	34.5
71	WINTER	13	19.7	1.80	70.00	18.1	5.03	92	1.80	8.50	14.9	25.2	70.0
72	FALL	96	17.2	1.00	180.00	24.3	2.48	141	2.70	7.36	12.0	20.3	32.0

72	SPRING	77	15.8	2.80	61.50	11.5	1.31	73	4.60	9.00	13.0	17.8	48.0
72	SUMMER	118	13.5	1.00	160.00	16.6	1.53	123	2.70	5.10	9.45	16.9	35.0
72	WINTER	112	10.8	1.30	93.50	10.8	1.02	100	2.60	5.16	7.68	12.8	30.0
74	FALL	14	19.9	1.00	120.00	31.5	8.42	158	1.00	3.00	6.50	26.5	120
74	SPRING	11	29.9	4.00	64.00	18.2	5.49	61	4.00	22.3	28.3	34.0	64.0
74	SUMMER	11	24.6	2.50	62.50	23.0	6.92	93	2.50	7.00	9.50	49.0	62.5
74	WINTER	13	44.3	14.8	92.75	26.5	7.35	60	14.8	20.0	42.5	62.5	92.8

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Turb_JCU_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	11	50.2	2.00	171.00	51.0	15.4	102	2.00	3.80	35.0	79.5	171
29	SPRING	11	39.0	2.00	158.00	45.8	13.8	117	2.00	11.0	25.0	46.0	158
29	SUMMER	17	47.1	2.35	173.00	59.1	14.3	125	2.35	7.25	21.0	46.5	173
29	WINTER	15	32.3	2.00	79.10	23.4	6.04	72	2.00	13.0	31.3	44.0	79.1
33	FALL	0	.	.	.	.	.	.	.	.	.	.	.
33	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
33	SUMMER	0	.	.	.	.	.	.	.	.	.	.	.
33	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
35	FALL	5	56.2	3.50	140.50	57.4	25.7	102	3.50	22.0	25.0	90.0	141
35	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
35	SUMMER	8	46.4	2.00	127.00	48.7	17.2	105	2.00	5.00	25.9	90.0	127
35	WINTER	7	31.8	4.00	72.00	24.0	9.06	75	4.00	13.0	21.5	48.0	72.0
37	FALL	5	20.2	2.00	62.00	23.9	10.7	118	2.00	10.0	12.8	14.0	62.0
37	SPRING	3	62.3	19.0	115.00	48.7	28.1	78	19.0	19.0	53.0	115	115
37	SUMMER	7	31.9	1.00	61.00	20.3	7.66	64	1.00	16.5	27.0	47.0	61.0
37	WINTER	5	16.0	2.00	26.00	9.35	4.18	58	2.00	14.0	15.0	23.0	26.0
40	FALL	14	35.5	4.90	134.50	32.8	8.77	93	4.90	16.5	26.5	35.5	135
40	SPRING	13	37.6	.700	130.50	37.9	10.5	101	0.70	10.0	31.0	42.0	131
40	SUMMER	19	26.3	4.90	100.00	22.9	5.25	87	4.90	10.0	20.0	36.5	100
40	WINTER	11	20.4	7.00	57.00	13.4	4.04	66	7.00	11.0	17.5	23.0	57.0
45	FALL	11	8.04	1.70	30.00	8.03	2.42	100	1.70	3.05	5.70	11.0	30.0
45	SPRING	11	12.8	2.10	29.00	7.01	2.11	55	2.10	9.60	12.1	17.0	29.0
45	SUMMER	10	11.8	1.10	23.10	8.52	2.69	72	1.10	2.10	12.3	20.5	23.1
45	WINTER	12	11.6	3.60	19.50	4.87	1.40	42	3.60	8.85	10.5	15.4	19.5
64	FALL	1	3.73	3.73	3.73	.	.	.	3.73	3.73	3.73	3.73	3.73
64	SPRING	1	6.50	6.50	6.50	.	.	.	6.50	6.50	6.50	6.50	6.50
64	SUMMER	2	4.30	4.10	4.50	0.28	0.20	7	4.10	4.10	4.30	4.50	4.50
64	WINTER	2	5.53	4.70	6.35	1.17	0.83	21	4.70	4.70	5.53	6.35	6.35
65	FALL	17	16.5	2.30	80.00	18.8	4.55	114	2.30	7.50	11.1	18.0	80.0
65	SPRING	21	10.5	1.75	29.30	6.63	1.45	63	2.15	5.60	11.0	13.0	20.0
65	SUMMER	11	20.6	1.60	135.00	38.4	11.6	186	1.60	2.88	9.30	15.0	135

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Turb_JCU_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	18	22.9	2.00	96.00	24.0	5.65	105	2.00	10.7	15.0	21.7	96.0
71	FALL	18	2.42	.600	4.60	1.30	0.31	54	0.60	1.15	2.38	3.60	4.60
71	SPRING	21	7.68	.900	88.80	18.8	4.11	245	1.15	1.55	2.95	4.18	14.9
71	SUMMER	15	3.00	.725	16.00	3.81	0.98	127	0.73	1.05	1.70	3.35	16.0
71	WINTER	17	4.74	1.10	15.50	4.10	0.99	86	1.10	1.50	3.10	6.40	15.5
72	FALL	2	25.3	22.5	28.00	3.89	2.75	15	22.5	22.5	25.3	28.0	28.0
72	SPRING	2	41.5	37.0	46.00	6.36	4.50	15	37.0	37.0	41.5	46.0	46.0
72	SUMMER	3	59.5	43.5	84.00	21.5	12.4	36	43.5	43.5	51.0	84.0	84.0
72	WINTER	2	15.0	13.0	17.00	2.83	2.00	19	13.0	13.0	15.0	17.0	17.0
74	FALL	5	11.1	1.00	17.50	6.13	2.74	55	1.00	11.0	13.0	13.0	17.5
74	SPRING	5	9.89	2.15	26.50	9.96	4.46	101	2.15	3.23	6.10	11.5	26.5
74	SUMMER	5	5.90	1.40	12.50	4.19	1.87	71	1.40	4.10	4.50	7.00	12.5
74	WINTER	3	17.3	15.0	19.00	2.08	1.20	12	15.0	15.0	18.0	19.0	19.0

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Turb_NTU_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
29	FALL	5	13.0	2.43	48.50	19.9	8.89	153	2.43	3.95	4.55	5.53	48.5
29	SPRING	4	3.23	1.70	4.00	1.04	0.52	32	1.70	2.60	3.60	3.85	4.00
29	SUMMER	6	18.5	3.43	70.00	26.2	10.7	141	3.43	4.48	5.65	22.0	70.0
29	WINTER	4	4.50	3.05	6.30	1.39	0.69	31	3.05	3.48	4.33	5.53	6.30
33	FALL	0	.	.	.	.	.	.	.	.	.	.	.
33	SPRING	0	.	.	.	.	.	.	.	.	.	.	.
33	SUMMER	1	0.50	.500	0.50	.	.	.	0.50	0.50	0.50	0.50	0.50
33	WINTER	0	.	.	.	.	.	.	.	.	.	.	.
35	FALL	81	15.6	2.15	63.00	12.4	1.38	80	4.58	7.80	12.0	18.0	40.0
35	SPRING	83	27.6	2.25	175.00	28.2	3.09	102	8.20	12.5	18.5	26.5	75.0
35	SUMMER	82	16.5	3.55	58.50	10.1	1.12	61	4.65	8.80	15.3	22.3	36.1
35	WINTER	72	22.4	3.48	150.00	25.5	3.00	114	6.25	10.2	14.3	24.1	53.5
37	FALL	27	19.3	2.20	91.00	23.0	4.42	119	3.80	5.60	10.8	17.5	76.0
37	SPRING	22	19.5	4.80	52.00	12.3	2.63	63	6.10	10.4	15.6	28.0	44.7
37	SUMMER	23	25.3	2.15	150.00	35.5	7.41	140	4.10	5.75	11.5	26.5	86.7
37	WINTER	21	15.9	4.10	50.25	10.4	2.28	66	6.50	8.15	13.5	19.5	29.5
40	FALL	32	36.2	3.00	113.00	26.3	4.64	72	7.00	17.0	25.5	57.5	73.4
40	SPRING	18	37.2	16.5	80.15	18.5	4.35	50	16.5	21.0	33.5	43.0	80.2
40	SUMMER	53	41.5	4.65	212.00	37.3	5.13	90	5.80	14.0	32.0	52.7	104
40	WINTER	6	31.0	11.0	55.00	19.6	8.01	63	11.0	14.0	25.5	55.0	55.0
45	FALL	25	9.29	2.80	23.75	5.52	1.10	59	3.05	5.95	8.60	10.7	21.0
45	SPRING	26	10.5	.900	28.50	7.07	1.39	67	1.20	5.48	9.25	14.2	23.0
45	SUMMER	35	11.9	1.70	80.50	13.3	2.24	112	2.30	5.30	9.35	14.0	28.3
45	WINTER	22	12.0	4.35	42.40	8.39	1.79	70	4.73	7.70	10.4	13.9	28.2

64	FALL	20	4.75	.250	19.00	5.06	1.13	106	0.25	1.63	2.43	6.85	17.0
64	SPRING	21	6.98	1.10	27.00	6.78	1.48	97	1.30	2.75	4.30	7.60	20.0
64	SUMMER	33	6.42	1.40	16.90	4.07	0.71	63	1.80	3.05	5.10	10.0	13.2
64	WINTER	15	7.25	1.00	32.75	7.77	2.01	107	1.00	2.90	5.18	8.50	32.8
65	FALL	115	10.8	.250	71.68	11.0	1.03	102	1.25	4.10	7.10	14.1	27.0
65	SPRING	152	17.8	.100	128.00	16.8	1.36	94	1.60	7.41	14.0	22.0	43.5
65	SUMMER	173	17.3	.250	116.00	20.6	1.57	119	1.30	6.00	10.2	19.0	72.0

Aggregate Nutrient Ecoregion: IX
Rivers and Streams
Descriptive Statistics by Decade and Season
Parameter Turb_NTU_Median

Eco_ Level_ III	SEASON	N	MEAN	MIN	MAX	STDDEV	STDERR	CV	P5	P25	MEDIAN	P75	P95
65	WINTER	133	18.2	.250	84.00	16.5	1.43	91	1.30	6.40	14.0	25.0	50.3
71	FALL	45	11.8	.250	82.75	13.9	2.07	117	2.00	4.60	7.55	14.5	35.3
71	SPRING	34	20.0	3.00	72.75	17.8	3.05	89	3.95	9.00	12.1	25.5	63.0
71	SUMMER	47	19.0	1.50	199.90	29.9	4.36	157	2.00	4.95	11.5	24.0	40.0
71	WINTER	33	26.1	.250	125.53	28.7	5.00	110	5.35	9.50	18.5	26.1	108
72	FALL	6	28.7	10.0	64.00	19.9	8.13	69	10.0	13.0	25.0	35.0	64.0
72	SPRING	3	20.8	17.0	26.00	4.68	2.70	23	17.0	17.0	19.3	26.0	26.0
72	SUMMER	5	23.7	.000	50.25	19.9	8.92	84	0.00	13.0	18.0	37.0	50.3
72	WINTER	2	22.4	17.0	27.75	7.60	5.38	34	17.0	17.0	22.4	27.8	27.8
74	FALL	10	23.4	7.60	39.25	11.2	3.53	48	7.60	14.3	22.5	35.0	39.3
74	SPRING	16	30.7	4.00	90.00	22.3	5.57	73	4.00	17.0	23.5	38.3	90.0
74	SUMMER	18	41.9	5.00	130.00	36.9	8.69	88	5.00	15.5	30.6	54.0	130
74	WINTER	15	31.4	10.0	93.50	21.2	5.48	67	10.0	17.5	25.0	37.2	93.5

APPENDIX C

Quality Control/Quality Assurance Rules

Support for the Compilation and Analysis of National Nutrient Data

15 Nutrient Ecoregion/Waterbody Type Summary Chapters

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APPENDIX A Process Used to QA/QA the Legacy STORET Nutrient Data Set
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1.0 BACKGROUND

The Nutrient Criteria Program has initiated development of a national Nutrient Criteria Database application that will be used to store and analyze nutrient data. The ultimate use of these data will be to derive ecoregion- and waterbody-specific nutrient criteria ranges. EPA converted STOrage and RETrieval (STORET) legacy data, National Stream Quality Accounting Network (NASQAN) data, National Water-Quality Assessment (NAWQA) data, and other relevant nutrient data from universities and States/Tribes into the database. The data imported into the Nutrient Criteria Database will be used to develop national nutrient criteria ranges.

1.1 Purpose

The purpose of this deliverable is to provide EPA with information regarding the data used to create the statistical reports which will be used to derive ecoregion- and waterbody-specific nutrient criteria ranges for Level III ecoregions. There are fourteen aggregate nutrient ecoregions. Each aggregate nutrient ecoregion is divided into smaller ecoregions referred to as Level III ecoregions. EPA will determine criteria ranges for the waterbody types and Level III ecoregions within the following aggregate nutrient ecoregions:

- Lakes and Reservoirs
  - Aggregate Nutrient ecoregions: 2, 6, 7, 8, 9, 11, 12, 13

- Rivers and Streams
  - Aggregate Nutrient ecoregions: 2, 3, 6, 7, 9, 11, 12, 14

1.2 References

This section lists documents that contain baselines, standards, guidelines, policies, and references that apply to the data analysis. Listed editions were valid at the time of publication. All documents are subject to revision, but these specific editions govern the concepts described in this document.

Nutrient Criteria Technical Guidance Manual: Lakes and Reservoirs (Draft). EPA, Office of Water, EPA 822-D-99-001, April 1999.

Nutrient Criteria Technical Guidance Manual: Rivers and Streams (Draft). EPA, Office of Water, EPA 822-D-99-003, September 1999.

Guidance for Data Quality Assessment: Practical Methods for Data Analysis. EPA, Office of Research and Development, EPA QA/G-9, January 1998.

2.0 QA/QC PROCEDURES

In order to develop nutrient criteria, EPA needed to obtain nutrient data from the states. EPA requested nutrient data from the states and forwarded the data sets to INDUS via e-mail and/or US mail. In addition, EPA tasked INDUS to convert data from three national data sets. EPA provided INDUS with a Legacy STORET extraction to convert into the database. The United States Geologic Survey (USGS) sent INDUS a CD-ROM with NASQAN data to convert. INDUS downloaded NAWQA files from the USGS Web site to convert the data. In total, INDUS converted and imported the following national and state data sets into the Nutrient Criteria Database:

- Legacy STORET
- NAWQA
- NASQAN
- Region 1
- Region 2 - Lake Champlain Monitoring Project
- Region 2 - NYSDEC Finger Lakes Monitoring Program
- Region 2 - NY Citizens Lake Assessment Program
- Region 2 - Lake Classification and Inventory Survey
- Region 2 - NYCDEP (1990-1998)
- Region 2 - NYCDEP (Storm Event data)
- Region 2 - New Jersey Nutrient Data (Tidal Waters)
- Region 5
- Region 3
- Region 3 - Nitrite Data
- Region 3 - Choptank River files
- Region 4 - Tennessee Valley Authority
- Region 7 - Central Plains Center for BioAssessment (CPCB)
- Region 7 - REMAP
- Region 2 - Delaware River Basin Commission (1990-1998)
- Region 3 - PA Lake Data
- Region 3 - University of Delaware
- Region 10
- University of Auburn

As part of the conversion process, INDUS performed a number of Quality Assurance/Quality Control (QA/QC) steps to ensure that the data was properly converted into the Nutrient Criteria Database. Section 2 explains the steps performed by INDUS to convert the data.

2.1 National Data Sets

INDUS converted three national data sets into the Nutrient Criteria Database: Legacy STORET data, NASQAN data, and NAWQA data. A previous EPA contractor performed the extraction of Legacy STORET data and documented the QA/QC procedures used on the data. This documentation is included in Appendix A. INDUS performed minimal QA/QC on the Legacy STORET data set because the previous contractor completed the steps outlined in Appendix A. INDUS and EPA also agreed to convert the NAWQA and NASQAN data sets with minimal QA/QC on the assumption that the source agency, the USGS, QA/QC'd the data.

For each of the three national data sets, INDUS ran queries to determine if 1) samples existed without results and 2) if stations existed without samples. Per Task Order Project Officer (TOPO) direction, these records were deleted from the system. For analysis purposes, EPA determined that there was no need to keep station records with no samples and sample records with no results. INDUS also confirmed that each data set contained no duplicate records.

In addition, INDUS deleted all composite results from the Legacy STORET data. Per TOPO direction, it was decided that composite sample results would not be used in the statistical analysis.

2.2 State Data

Each state data set was delivered in a unique format. Many of the data sets were delivered to INDUS without corresponding documentation. INDUS analyzed each state data set in order to determine which parameters should be converted for analysis. INDUS obtained a master parameter table from EPA and converted the parameters in the state data sets according to those that were present in the EPA parameter table. INDUS converted all of the data elements in the state data sets that mapped directly to the Nutrient Criteria Database; data elements that did not map to the Nutrient Criteria Database were not converted. In some cases, state data elements that did not directly map into the Oracle database were inserted into a comment field within the database. Also, INDUS maintained an internal record of which state data elements were inserted into the comment field.

As part of the data clean-up efforts, INDUS determined whether or not there were any duplicate records in the state data sets and deleted the duplicate records. INDUS checked the waterbody, station, and sample entities for duplicate records. In addition, INDUS deleted station records with no samples and sample records with no results. INDUS also deleted waterbody records that were not associated with a station. In each case, INDUS maintained an internal record of how many records were deleted.

If INDUS encountered referential integrity errors, such as samples that referred to stations that did not exist, or if INDUS was unsure of whether a record was a duplicate, INDUS contacted the agency directly via e-mail or phone to resolve any issues that arose. INDUS saved an electronic

copy of each e-mail correspondence with the states to ensure that a record of the decision was maintained. INDUS also contacted each agency to determine which laboratory methods were used for each parameter.

Finally, INDUS examined the remark codes of each result record in the state data sets. INDUS mapped the remark codes to the STORET remark codes listed in Table 2 of Appendix A. If any of the state result records were associated with remark codes marked as "Delete" in Table 2 of Appendix A, the result records were not converted into the database.

2.3 Laboratory Methods

Many of the state data sets did not contain laboratory method information. In addition, laboratory method information was not available for the three national data sets. In order to determine missing laboratory method information, EPA tasked another contractor to contact the data owners to obtain the laboratory method. In some cases, the data owners responded and the laboratory methods were added to the database.

2.4 Waterbody Name and Class Information

A large percentage of the data did not have waterbody-specific information. The only waterbody information contained in the three national data sets was the waterbody name, which was embedded in the station 'location description' field. Most of the state data sets contained waterbody name information; however, much of the data was duplicated throughout the data sets. Therefore, the waterbody information was cleaned manually. For the three national data sets, the 'location description' field was extracted from the station table and moved to a temporary table. The 'location description' field was sorted alphabetically. Unique waterbodies were grouped together based on name similarity and whether or not the waterbodies fell within the same county, state, and waterbody type. Finally, the 'location description' field was edited to include only waterbody name information, not descriptive information. For example, 110 MILE CREEK AT POMONA DAM OUTFLOW, KS PO-2 was edited to 110 MILE CREEK. Also, if 100 MILE CREEK was listed ten times in New York, but in four different counties, four 100 MILE CREEK waterbody records were created.

Similar steps were taken to eliminate duplicate waterbody records in the state data sets. If a number of records had similar waterbody names and fell within the same state, county, and waterbody type, the records were grouped to create a unique waterbody record.

Most of the waterbody data did not contain depth, surface area, and volume measurements. EPA needed this information to classify waterbody types. EPA attempted to obtain waterbody class

information from the states. EPA sent waterbody files to the regional coordinators and requested that certain class information be completed by each state. The state response was poor; therefore, EPA was not able to perform statistical analysis for the waterbody types by class.

2.5 Ecoregion Data

Aggregate nutrient ecoregions and Level III ecoregions were added to the database using the station latitude and longitude coordinates. If a station was lacking latitude and longitude coordinates or county information, the data were not included in the statistical analysis. Appendix B lists the steps taken to add the two ecoregion types (aggregate and Level III) to the Nutrient Criteria Database. The ecoregion names were pulled from aggregate nutrient ecoregion and Level III ecoregion Geographical Information System (GIS) coverages. In summary, the station latitude and longitude coordinates were used to determine the ecoregion under the following circumstances:

- The latitude and longitude coordinates fell within the county/state listed in the station table.
- The county data was missing.

The county centroid was used to determine the ecoregions under the following circumstances:

- The latitude and longitude coordinates were missing, but the state/county information was available.
- The latitude and longitude coordinates fell outside the county/state listed in the station table. The county information was assumed to be correct; therefore, the county centroid was used.

If the latitude and longitude coordinates fell outside the continental US county coverage file (i.e., the point fell in the ocean or Mexico/Canada), the nearest ecoregion was assigned to the station.

3.0 STATISTICAL ANALYSIS REPORTS

Aggregate nutrient ecoregion tables were created by extracting all observations for a specific aggregate nutrient ecoregion from the nutrient criteria database. Then, the data were reduced to create tables containing only the yearly median values. To create these tables, the median value for each waterbody was calculated using all observations for each waterbody by Level III ecoregion, year, and season. Tables of decade median values were created from the yearly median tables by calculating the median for each waterbody by Level III ecoregion by decade and season.

The Data Source and the Remark Code reports were created using all observations (all reported values). All the other reports were created from either the yearly median tables or the decade median tables. In other words, the descriptive statistics and regressions were run using the median values for each waterbody and not the individual reported values.

Statistical analyses were performed under the assumption that this data set is a random sample. If this assumption cannot be verified, the observations may or may not be valid. Values below the 1st and 99th percentile were removed from the Legacy STORET database prior to the creation of the national database. Also, data were treated according the Legacy STORET remark codes in Appendix A.

The following contains a list of each report and the purpose for creating each report:

- Data Source Created to provide a count of the amount of data and to identify the source(s).
- Remark Codes Created to provide a description of the data.
- Median of Each Waterbody by Year This was an intermediate step performed to obtain a median value for each lake to be used in the yearly descriptive statistics reports and the regression models.
- Median of Each Waterbody by Decade This was an intermediate step performed to obtain a median value for each lake to be used in the decade descriptive statistics.
- Descriptive Statistics Created to provide EPA with the desired statistics for setting criteria levels.
- Regression Models Created to examine the relationships between biological and nutrient variables.

Note: Separate reports were created for each season.

3.1 Data Source Reports

Data source reports were presented in the following formats:

- The number and percentage of data from each data source were summarized in tables for each aggregate nutrient ecoregion by season and waterbody type.

- The number and percentage of data from each data source were summarized in tables for each Level III ecoregion by season and waterbody type.

The 'Frequency' represents the number of data values from a specific data source for each parameter by data source. The 'Row Pct' represents the percentage of data from a specific data source for each parameter.

3.2 Remark Code Reports

Remark code reports were presented in the following formats:

- The number and percentage of data associated with a particular remark code for each parameter were summarized in tables by Level III ecoregion by decade and season.

- The number and percentage of data associated with a particular remark code for each parameter were summarized in tables by Level III ecoregion by year and season.

The 'Frequency' represents the number of data values corresponding to the remark code in the column. The 'Row Pct' represents the percentage of data that was associated with the remark code in that row.

In the database, remark codes that were entered by the states were mapped to Legacy STORET remark codes. Prior to the analysis, the data were treated according to these remark codes. For example, if the remark code was 'K,' then the reported value was divided by two. Appendix A contains a complete list of Legacy STORET remark codes.

Note: For the reports, a remark code of 'Z' indicates that no remark codes were recorded. It does not correspond to Legacy STORET code 'Z.'

3.3 Median of Each Waterbody

To reduce the data and to ensure heavily sampled waterbodies or years were not over represented in the analysis, median value tables (described above) were created. The yearly median tables and decade median tables were delivered to the EPA in electronic format as csv (comma separated value or comma delimited) files.

3.4 Descriptive Statistic Reports

The number of waterbodies, median, mean, minimum, maximum, 5th, 25th , 75th , 95th percentiles, standard deviation, standard error, and coefficient of variation were calculated. The tables (described above) containing the decade median values for each waterbody for each parameter were used to create descriptive statistics reports for:

- Level III ecoregions by decade and season
- Aggregate nutrient ecoregions by decade and season

In addition, the tables containing the yearly median values for each waterbody for each parameter were used to create descriptive statistics reports for:

- Level III ecoregions by year and season

3.5 Regression Models

Simple linear regressions using the least squares method were performed to examine the relationships between biological and nutrient variables in lakes and reservoirs, and rivers and streams. Regressions were performed using the yearly median tables. Chlorophyll(s) in micrograms per liter (ug/L), secchi in meters (m), dissolved oxygen in milligrams per liter (mg/L), turbidity, and pH were the biological variables in these models. When there was little or no data for chlorophyll, then pH or dissolved oxygen was substituted for chlorophyll. Secchi data were used in the lake and reservoir models, and turbidity data were used in the river and stream models. The nutrient variables in these models include: total phosphorus in ug/L, total nitrogen in mg/L, total kjeldahl nitrogen in mg/L, and nitrate and nitrite in mg/L. Regressions were also run for total nitrogen and total phosphorus for ecoregions where both these variables were measured.

Note: At the time of creation of this document only regressions for aggregate nutrient ecoregion 7 for lakes and reservoirs were delivered to the EPA. Regressions for the remaining aggregate nutrient ecoregions will be delivered in August 2000.

4.0 TIME PERIOD

Data collected from January 1990 to December 1999 were used in the statistical analysis reports. To capture seasonal differences, the data were classified as follows:

- Aggregate nutrient ecoregions: 6, 7, and 8

  - Spring: April to May
  - Summer: June to August
  - Fall: September to October
  - Winter: November to March

- Aggregate nutrient ecoregions: 1, 2, 9, 10, 11, 12, and 13

  - Spring: March to May
  - Summer: June to August
  - Fall: September to November
  - Winter: December to February

5.0 DATA SOURCES AND PARAMETERS FOR THE AGGREGATE NUTRIENT ECOREGIONS

This section provides information for the nutrient aggregate ecoregions that were analyzed by waterbody type. Each section lists the data sources for the aggregate nutrient ecoregion including: 1) the data sources, 2) the parameters included in the analysis, and 3) the Level III ecoregions within the aggregate nutrient ecoregions.

Note: For analysis purposes, the following parameters were combined to form Phosphorous, Dissolved Inorganic (DIP):

Phosphorus, Dissolved Inorganic (DIP)
Phosphorus, Dissolved (DP)
Phosphorus, Dissolved Reactive (DRP)
Orthophosphate, dissolved, mg/L as P
Orthophosphate (OPO4_PO4)

5.1 Lakes and Reservoirs

5.1.1 Aggregate Nutrient Ecoregion 2

Data Sources:

Legacy STORET
EPA Region 10

Parameter:

Chlorophyll A, Fluorometric, Corrected	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Phosphorous, Dissolved Inorganic (DIP)	(ug/L)
Dissolved Oxygen (DO)	(mg/L)
Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Nitrogen, Total (TN)	(mg/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Phosphorus, Total (TP)	(ug/L)
Phosphorus, Total Reactive	(ug/L)
SECCHI	(m)
pH	

Level III ecoregions:

1, 2, 4, 5, 9, 11, 15, 16, 17, 19, 21, 23, 41, 77, 78

5.1.2 Aggregate Nutrient Ecoregion 6

Data Sources:

Legacy STORET

Parameters:

Chlorophyll A, Fluorometric, Corrected	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Dissolved Oxygen (DO)	(mg/L)
Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Nitrogen, Total (TN)	(mg/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Phosphorus, Total (TP)	(ug/L)
SECCHI	(m)

Level III ecoregions:

46, 47, 48, 54, 55, 57

5.1.3 Aggregate Nutrient Ecoregion 7

Data Sources:

LCMPD
Legacy STORET
NYCDEP
EPA Region 1

Parameters:

Chlorophyll A, Fluorometric Corrected	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric, Uncorrected	(ug/L)
Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Phosphorous, Dissolved Inorganic (DIP)	(ug/L)
Dissolved Oxygen (DO)	(mg/L)

Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Nitrogen, Total (TN)	(mg/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Phosphorus, Orthophosphate, Total as P	(ug/L)
Phosphorus, Total (TP)	(ug/L)
SECCHI	(m)

Level III ecoregions:

51, 52, 53, 56, 60, 61, 83

5.1.4 Aggregate Nutrient Ecoregion 8

Data Sources:

LCMPD
Legacy STORET
NYCDEP
NYCDEC
EPA Region 1
EPA Region 3

Parameters:

Chlorophyll A, Fluorometric, Corrected	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric, Uncorrected	(ug/L)
Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Chlorophyll B	(ug/L)
Chlorophyll C	(ug/L)
Phosphorous, Dissolved Inorganic (DIP)	(ug/L)
Dissolved Oxygen (DO)	(mg/L)
Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Nitrogen, Total (TN)	(mg/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Phosphorus, Total (TP)	(ug/L)
SECCHI	(m)

Level III ecoregions:

49, 50, 58, 62, 82

5.1.5 Aggregate Nutrient Ecoregion 9

Data Sources:

Auburn University
Legacy STORET
EPA Region 4

Parameters:

Chlorophyll A, Fluorometric, Corrected	(ug/L)
Chlorophyll A, Pheophytin	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric, Uncorrected	(ug/L)
Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Phosphorous, Dissolved Inorganic (DIP)	(ug/L)
Dissolved Oxygen (DO)	(mg/L)
Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Nitrogen, Total (TN)	(mg/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Phosphorus, Total (TP)	(ug/L)
SECCHI	(m)

Level III ecoregions:

29, 33, 35, 37, 40, 45, 64, 65, 71, 72, 74

5.1.6 Aggregate Nutrient Ecoregion 11

Data Sources:

Auburn University
Legacy STORET
NYSDEC
EPA Region 3
EPA Region 4

Parameters:

Chlorophyll A, Fluorometric, Corrected	(ug/L)
Chlorophyll A, Pheophytin	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric, Uncorrected	(ug/L)

Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Phosphorous, Dissolved Inorganic (DIP)	(ug/L)
Dissolved Oxygen (DO)	(mg/L)
Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Nitrogen, Total (TN)	(mg/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Phosphorus, Total (TP)	(ug/L)
SECCHI	(m)

Level III ecoregions:

36, 38, 39, 66, 67, 68, 69, 70

5.1.7 Aggregate Nutrient Ecoregion 12

Data Sources:

Legacy STORET

Parameters:

Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Dissolved Oxygen (DO)	(mg/L)
Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Nitrogen, Total (TN)	(mg/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Phosphorus, Total (TP)	(ug/L)
SECCHI	(m)

Level III ecoregions:

75

5.1.8 Aggregate Nutrient Ecoregion 13

Data Sources:

Legacy STORET

Parameters:

Chlorophyll A, Fluorometric, Corrected	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Dissolved Oxygen (DO)	(mg/L)
Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Nitrogen, Total (TN)	(mg/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Phosphorus, Total (TP)	(ug/L)
SECCHI	(m)

Level III ecoregions:

76

5.2 Rivers and Streams

5.2.1 Aggregate Nutrient Ecoregion 2

Data Sources:

Legacy STORET
NASQAN
NAWQA
EPA Region 10

Parameters:

Chlorophyll A, Fluorometric, Corrected	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Phytoplankton, chromotographic- fluorometric	(ug/L)
Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Chlorophyll B, Phytoplankton, chromotographic- fluorometric	(ug/L)
Phosphorous, Dissolved Inorganic (DIP)	(ug/L)
Dissolved Oxygen (DO)	(mg/L)
Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Phosphorus, Orthophosphate, Total as P	(ug/L)
Phosphorus, Total (TP) Reactive	(ug/L)
Nitrogen, Total (TN)	(mg/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Phosphorus, Total (TP)	(ug/L)
Turbidity	(FTU)

Turbidity	(JCU)
Turbidity	(NTU)

Level III ecoregions:

1, 2, 4, 5, 8, 9, 11, 15, 16, 17, 19, 21, 23, 41, 77, 78

5.2.2 Aggregate Nutrient Ecoregion 3

Data Sources:

Legacy STORET
NASQAN
NAWQA
EPA Region 10

Parameters:

Chlorophyll A, Fluorometric, Corrected	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Phytoplankton, chromotographic- fluorometric	(ug/L)
Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Chlorophyll B, Phytoplankton, chromotographic- fluorometric	(ug/L)
Phosphorous, Dissolved Inorganic (DIP)	(ug/L)
Dissolved Oxygen (DO)	(mg/L)
Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Nitrogen, Total (TN)	(mg/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Phosphorus, Total (TP)	(ug/L)
Turbidity	(FTU)
Turbidity	(JCU)
Turbidity	(NTU)

Level III ecoregions:

6, 10, 12, 13, 14, 18, 20, 22, 24, 79, 80, 81

5.2.3 Aggregate Nutrient Ecoregion 6

Data Sources:

Legacy STORET
NASQAN
NAWQA
EPA Region 5
EPA Region 7

Parameters:

Chlorophyll A, Fluorometric, Corrected	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Phytoplankton, chromotographic- fluorometric	(ug/L)
Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Chlorophyll B, Phytoplankton, chromotographic- fluorometric	(ug/L)
Phosphorous, Dissolved Inorganic (DIP)	(ug/L)
Dissolved Oxygen (DO)	(mg/L)
Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Nitrogen, Total (TN)	(mg/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Organic, Phosphorus	(ug/L)
Phosphorus, Total (TP)	(ug/L)
Phosphorus, Orthophosphate, Total as P	(ug/L)
Turbidity	(FTU)
Turbidity	(JCU)
Turbidity	(NTU)

Level III ecoregions:

46, 47, 48, 54, 55, 57

5.2.4 Aggregate Nutrient Ecoregion 7

Data Sources:

LCMPD
Legacy STORET
NASQAN
NAWQA
NYCDEP

Parameters:

Chlorophyll A, Fluorometric, Corrected	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric, Uncorrected	(ug/L)
Chlorophyll A, Phytoplankton, chromotographic- fluorometric	(ug/L)
Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Chlorophyll B, Phytoplankton, chromotographic- fluorometric	(ug/L)
Phosphorous, Dissolved Inorganic (DIP)	(ug/L)
Dissolved Oxygen (DO)	(mg/L)
Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Nitrogen, Total (TN)	(mg/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Organic, Phosphorus	(ug/L)
Phosphorus, Orthophosphate, Total as P	(ug/L)
Phosphorus, Total (TP)	(ug/L)
Turbidity	(FTU)
Turbidity	(JCU)
Turbidity	(NTU)

Level III ecoregions:

51, 52, 53, 56, 60, 61, 83

5.2.5 Aggregate Nutrient Ecoregion 9

Data Sources:

Auburn University
Legacy STORET
NASQAN
NAWQA
EPA Region 3
EPA Region 5
EPA Region 7

Parameters:

Chlorophyll A, Fluorometric, Corrected	(ug/L)
Chlorophyll A, Phytoplankton, chromotographic- fluorometric	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric, Uncorrected	(ug/L)

Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Chlorophyll B, Phytoplankton, chromotographic- fluorometric	(ug/L)
Chlorophyll B, Phytoplankton, Spectrophotometric	(ug/L)
Phosphorous, Dissolved Inorganic (DIP)	(ug/L)
Dissolved Oxygen (DO)	(mg/L)
Organic, Phosphorus	(ug/L)
Phosphorus, Orthophosphate, Total as P	(ug/L)
Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Nitrogen, Total (TN)	(mg/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Phosphorus, Total (TP)	(ug/L)
Turbidity	(FTU)
Turbidity	(JCU)
Turbidity	(NTU)

Level III ecoregions:

29, 33, 35, 37, 40, 45, 64, 65, 71, 72, 74

5.2.6 Aggregate Nutrient Ecoregion 11

Data Sources:

Auburn University
Legacy STORET
NASQAN
NAWQA
EPA Region 3
EPA Region 5
EPA Region 7

Parameters:

Chlorophyll A, Fluorometric, Corrected	(ug/L)
Chlorophyll A, Phytoplankton, chromotographic- fluorometric	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric, Uncorrected	(ug/L)
Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Chlorophyll B, Phytoplankton, chromotographic- fluorometric	(ug/L)
Phosphorous, Dissolved Inorganic (DIP)	(ug/L)
Dissolved Oxygen (DO)	(mg/L)
Organic, Phosphorus	(ug/L)

Phosphorus, Orthophosphate, Total as P	(ug/L)
Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Nitrogen, Total (TN)	(mg/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Phosphorus, Total (TP)	(ug/L)
Turbidity	(FTU)
Turbidity	(JCU)
Turbidity	(NTU)

Level III ecoregions:

36, 38, 39, 66, 67, 68, 69, 70

5.2.7 Aggregate Nutrient Ecoregion 12

Data Sources:

Legacy STORET
NASQAN
NAWQA

Parameters:

Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric, Uncorrected	(ug/L)
Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Chlorophyll B, Phytoplankton, Spectrophotometric	(ug/L)
Phosphorous, Dissolved Inorganic (DIP)	(ug/L)
Dissolved Oxygen (DO)	(mg/L)
Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Nitrogen, Total (TN)	(mg/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Phosphorus, Orthophosphate, Total as P	(ug/L)
Phosphorus, Total (TP)	(ug/L)
Turbidity	(FTU)
Turbidity	(NTU)

Level III ecoregions:

75

5.2.8 Aggregate Nutrient Ecoregion 14

Data Sources:

Legacy STORET
NASQAN
NAWQA
NYCDEP
EPA Region 1
EPA Region 3

Parameters:

Chlorophyll A, Fluorometric, Corrected	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric Acid	(ug/L)
Chlorophyll A, Phytoplankton, Spectrophotometric, Uncorrected	(ug/L)
Chlorophyll A, Trichromatic, Uncorrected	(ug/L)
Phosphorous, Dissolved Inorganic (DIP)	(ug/L)
Dissolved Oxygen (DO)	(mg/L)
Nitrite and Nitrate, (NO2+NO3)	(mg/L)
Phosphorus, Orthophosphate, Total as P	(ug/L)
Nitrogen, Total Kjeldahl (TKN)	(mg/L)
Nitrogen, Total (TN)	(mg/L)
Phosphorus, Total (TP)	(ug/L)
Turbidity	(FTU)
Turbidity	(JCU)
Turbidity	(NTU)

Level III ecoregions:

59, 63, 84

APPENDIX A

Process Used to QA/QA the Legacy STORET Nutrient Data Set

1. STORET water quality parameters and Station and Sample data items were retrieved from USEPA's mainframe computer. Table 1 lists all retrieved parameters and data items.

TABLE 1: PARAMETERS AND DATA ITEMS RETRIEVED FROM STORET

Parameters Retrieved (STORET Parameter Code)	Station Data Items Included (STORET Item Name)	Sample Data Items Included (STORET Item Name)
TN - mg/l (600) TKN - mg/l (625) Total Ammonia (NH3+NH4) - mg/l (610) Total NO2+NO3 - mg/l (630) Total Nitrite - mg/l (615) Total Nitrate - mg/l (620) Organic N - mg/L (605) TP - mg/l (665) Chlor *a* - ug/L (spectrophotometric method, 32211) Chlor *a* - ug/L (fluorometric method corrected, 32209) Chlor *a* - ug/L (trichromatic method corrected, 32210) Secchi Transp. - inches (77) Secchi Transp. - meters (78) +Turbidity JCUs (70) +Turbidity FTUs (76) +Turbidity NTUs field (82078) +Turbidity NTUs lab (82079) +DO - mg/L (300) +Water Temperature (degrees C, 10/degrees F, 11)	Station Type (TYPE) Agency Code (AGENCY) Station No. (STATION) Latitude - std. decimal degrees (LATSTD) Longitude - std. decimal degrees (LONGSTD) Station Location (LOCNAME) County Name (CONAME) State Name (STNAME) Ecoregion Name - Level III (ECONAME) Ecoregion Code -Level III (ECOREG) Station Elevation (ELEV) Hydrologic Unit Code (CATUNIT) RF1 Segment and Mile (RCHMIL) RF1ON/OFF tag (ONOFF)	Sample Date (DATE) Sample Time (TIME) Sample Depth (DEPTH) Composite Sample Code (SAMPMETH)

+ If data record available at a station included data only for this or other such marked parameters, data record was deleted from data set.

The following set of retrieval rules were applied to the retrieval process:

- Data were retrieved for waterbodies specified only as 'lake', 'stream', 'reservoir', or 'estuary' under "Station Type" parameter. Any stations specified as 'well,' 'spring,' or 'outfall' were eliminated from the retrieved data set.

- Data were retrieved for station types described as 'ambient' (e.g., no pipe or facility discharge data) under the "Station Type" parameter.

- Data were retrieved that were designated as 'water' samples only. This includes 'bottom' and 'vertically integrated' water samples.

- Data were retrieved that were designated as either 'grab' samples and 'composite' samples (mean result only).
- No limits were specified for sample depths.
- Data were retrieved for all fifty states, Puerto Rico, and the District of Columbia.
- The time period specified for data retrieval was January 1990 to September 1998.
- No data marked as "Retired Data" (i.e., data from a generally unknown source) were retrieved.
- Data marked as "National Urban Runoff data" (i.e., data associated with sampling conducted after storm events to assess nonpoint source pollutants) were included in the retrieval. Such data are part of STORET's 'Archived' data.
- Intensive survey data (i.e., data collected as part of specific studies) were retrieved.

2. Any values falling below the 1st percentile and any values falling above the 99th percentile were transformed into 'missing' values (i.e., values were effectively removed from the data set, but were not permanently eliminated).

3. Based on the STORET 'Remark Code' associated with each retrieved data point, the following rules were applied (Table 2):

TABLE 2: STORET REMARK CODE RULES

STORET Remark Code	Keep or Delete Data Point
blank - Data not remarked.	Keep
A - Value reported is the mean of two or more determinations.	Keep
B - Results based upon colony counts outside the acceptable ranges.	Delete
C - Calculated. Value stored was not measured directly, but was calculated from other data available.	Keep
D - Field measurement.	Keep

E - Extra sample taken in compositing process.	Delete
F - In the case of species, F indicates female sex.	Delete
G - Value reported is the maximum of two or more determinations.	Delete
H - Value based on field kit determination; results may not be accurate.	Delete
I - The value reported is less than the practical quantification limit and greater than or equal to the method detection limit.	Keep, but used one-half the reported value as the new value.
J - Estimated. Value shown is not a result of analytical measurement.	Delete
K - Off-scale low. Actual value not known, but known to be less than value shown.	Keep, but used one-half the reported value as the new value.
L - Off-scale high. Actual value not known, but known to be greater than value shown.	Keep
M - Presence of material verified, but not quantified. Indicates a positive detection, at a level too low to permit accurate quantification.	Keep, but used one half the reported value as the new value.
N - Presumptive evidence of presence of material.	Delete
O - Sample for, but analysis lost. Accompanying value is not meaningful for analysis.	Delete
P - Too numerous to count.	Delete
Q - Sample held beyond normal holding time.	Delete
R - Significant rain in the past 48 hours.	Delete
S - Laboratory test.	Keep
T - Value reported is less than the criteria of detection.	Keep, but replaced reported value with 0.

U - Material was analyzed for, but not detected. Value stored is the limit of detection for the process in use.	Keep, but replaced reported value with 0.
V - Indicates the analyte was detected in both the sample and associated method blank.	Delete
W - Value observed is less than the lowest value reportable under remark "T."	Keep, but replaced reported value with 0.
X - Value is quasi vertically-integrated sample.	No data point with this remark code in data set.
Y - Laboratory analysis from unpreserved sample. Data may not be accurate.	Delete
Z - Too many colonies were present to count.	Delete
If a parameter (excluding water temperature) value was less than or equal to zero and no remark code was present, the value was transformed into a missing value. Rationale - Parameter concentrations should never be zero without a proper explanation. A method detection limit should at least be listed.	

4. Station records were eliminated from the data set if any of the following descriptors were present within the "Station Type" parameter:

- **MONITR -** Source monitoring site, which monitors a known problem or to detect a specific problem.
- **HAZARD -** Site of hazardous or toxic wastes or substances.
- **ANPOOL -** Anchialine pool, underground pools with subsurface connections to watertable and ocean.
- **DOWN -** Downstream (i.e., within a potentially polluted area) from a facility which has a potential to pollute.
- **IMPDMT -** Impoundment. Includes waste pits, treatment lagoons, and settling and evaporation ponds.
- **STMSWR -** Storm water sewer.
- **LNDFL -** Landfill.
- **CMBMI -** Combined municipal and industrial facilities.
- **CMBSRC -** Combined source (intake and outfall).

Rationale - these descriptors potentially indicate a station location that at which an ambient water sample would not be obtained (i.e., such sampling locations are potentially

biased) or the sample location is not located within one of the designated water body types (i.e, ANPOOL).

5. Station records were eliminated from data set if the station location did not fall within any established cataloging unit boundaries based on their latitude and longitude.

6. Using nutrient ecoregion GIS coverage provided by USEPA, all station locations with latitude and longitude coordinates were tagged with a nutrient ecoregion identifier (nutrient region identifiers are values 1 - 14) and the associated nutrient ecoregion name. Because no nutrient ecoregions exist for Alaska, Hawaii, and Puerto Rico, stations located in these states were tagged with "dummy" nutrient ecoregion numbers (20 = Alaska, 21 = Hawaii, 22 = Puerto Rico).

7. Using information provided by TVA, 59 station locations that were marked as 'stream' locations under the "Station Type" parameter were changed to 'reservoir' locations.

8. The nutrient data retrieved from STORET were assessed for the presence of duplicate data records. The duplicate data identification process consisted of three steps: 1) identification of records that matched exactly in terms of each variable retrieved; 2) identification of records that matched exactly in terms of each variable retrieved except for their station identification numbers; and 3) identification of records that matched exactly in terms of each variable retrieved except for their collecting agency codes. The data duplication assessment procedures were conducted using SAS programs.
Prior to initiating the data duplication assessment process, the STORET nutrient data set contained:

41,210 station records
924,420 sample records

- <u>Identification of exactly matching records</u>
All data records were sorted to identify those records that matched exactly. For two records to match exactly, all variables retrieved had to be the same. For example, they had to have the same water quality parameters, parameter results and associated remark codes, and have the same station data item and sample data item information. Exactly matching records were considered to be exact duplicates, and one duplicate record of each identified matching set were eliminated from the nutrient data set. A total of 924 sample records identified as duplicates by this process were eliminated from the data set.

- <u>Identification of matching records with the exception of station identification number</u>
All data records were sorted to identify those records that matched exactly except for their station identification number (i.e., they had the same water quality

parameters, parameter results and associated remark codes, and the same station and sample data item information with the exception of station identification number). Although the station identification numbers were different, the latitude and longitude for the stations were the same indicating a duplication of station data due to the existence of two station identification numbers for the same station. For each set of matching records, one of the station identification numbers was randomly selected and its associated data were eliminated from the data set. A total of 686 sample records were eliminated from the data set through this process.

- Identification of matching records with the exception of collecting agency codes
  All data records were sorted to identify those records that matched exactly except for their collecting agency codes (i.e., they had the same water quality parameters, parameter results and associated remark codes, and the same station and sample data item information with the exception of agency code). The presence of two matching data records each with a different agency code attached to it suggested that one agency had utilized data collected by the other agency and had entered the data into STORET without realizing that it already had been placed in STORET by the other agency. No matching records with greater than two different agency codes were identified. For determining which record to delete from the data set, the following rules were developed:

  - If one of the matching records had a USGS agency code, the USGS record was retained and the other record was deleted.
  - Higher level agency monitoring program data were retained. For example, federal program data (indicated by a "1" at the beginning of the STORET agency code) were retained against state (indicated by a "2") and local (indicated by values higher than 2) program data.
  - If two matching records had the same level agency code, the record from the agency with the greater number of overall observations (potentially indicating the data set as the source data set) was retained.

  A total of 2,915 sample records were eliminated through this process.

As a result of the duplicate data identification process, a total of 4,525 sample records and 36 individual station records were removed from the STORET nutrient data set. The resulting nutrient data set contains the following:

41,174 station records
919,895 sample records

APPENDIX B

Process for Adding Aggregate Nutrient Ecoregions and Level III Ecoregions

Steps for assigning Level III ecoregions and aggregate nutrient ecoregion codes and names to the Nutrient Criteria Database (performed using ESRI's ARCView v 3.2 and its GeoProcessing Wizard). This process is performed twice; once for the Level III ecoregions and once for the aggregate nutrient ecoregions:

- Add the station .dbf data table, with latitude and longitude data, to project by 'Add Event Theme'
- Convert to the shapefile format
- Create 'stcojoin' field, populate the 'stcojoin' field with the following formula: 'County.LCase+State.LCase'
- Add field 'stco_flag' to the station shapefile
- Spatially join the station data with the county shapefile (cntys_jned.shp)
- Select 'stcojoin' (station shapefile) field = 'stco_join2' (county shapefile) field
- Calculate stco_flag = 0 for selected features
- Step through all blank stco_flag records, assign the appropriate stco_flags, see list on the following page
- Select all stco_flags = 4 or 7, switch selection
- Calculate ctyfips (station) to cntyfips (county)
- Stop editing and save edits, remove all joins
- Add in 2 new fields 'x-coord1' and 'y-coord1' into station table
- Select all stco_flags = 1, 2, and 6
- Link county coverage with station coverage
- Populate 'x-coord1' and 'y-coord1' with 'x-coord' and 'y-coord' from county coverage
- Select all stco_flags = 1, 2, and 6, export to new .dbf file
- Add new .dbf file as event theme
- Convert to shapefile format
- Add the following fields to both tables (original station and station126 shapefiles): 'eco_omer', 'name_omer', 'dis_aggr', 'code_aggr', 'name_aggr'
- Spatially join station126 and eco-omer coverage
- Populate the 'eco_omer' field with the 'eco' value
- Repeat the previous step using the nearest method (line coverage) to determine ecoregion assignment for the line coverage, if some records are blank
- Spatially join the ecoregion line coverage to station coverage, link the LPoly# (from the spatially joined table) to Poly# (of the ecoregion polygon coverage)
- Populate the Eco fields with the appropriate information.
- Follow the same steps to the Rpoly#
- Remove all table joins
- Link the useco-om table with station126 table and populate 'name-omer' field
- Spatially join station aggr coverage and populate the rest of the fields. Follow the same procedures as outlined above
- Remove all joins
- Make sure the new Eco field added into the station126 shapefile are different than

the ones in the original station shapefile

- Join station126 and station coverage by station-id
- Populate all the Eco fields in the original station coverage
- Remove all joins
- Save table
- Make sure that all ctyfips records are populated; the county shapefile may have to be joined to populate the records, if the stco_flag = 4
- Create 2 new fields, 'NewCounty' and 'NewState'
- Populate these new fields with a spatial join to the county coverage
- Select by feature (ecoregion shapefile) all of the records in the station shapefile
- Switch selection (to get records outside of the ecoregion shapefile)
- If any of the selected records have stco_flag = 0 (they are outside the ecoregion shapefile boundary), calculate them to stco_flag = 3

stco_flags (state/county flags in order of importance)

0 The state and county values from the data set matched the state and county values from the spatial join.
(Ecoregions were assigned based on the latitude/longitude coordinates.)

1 The state and county values from the data set did not match the state and county values from the spatial join, but the point was inside the county coverage boundary.
(Ecoregions were assigned based on the county centroid.)

2 The state and county values from the data set did not match the state and county values from the spatial join because the point was outside the county coverage boundary; therefore, there was nothing to compare to the point (i.e., the point falls in the ocean/Canada/Mexico). This occurred for some coastal samples.
(Ecoregions were assigned based on the county centroid.)

3 The state and county values from the data set matched the state and county from the spatial join, but the point was outside the ecoregion boundary.
(Ecoregions were assigned to the closest ecoregion to the point.)
(No ecoregions were assigned to AK, HI, PR, BC, and GU.)

4 Latitude/longitude coordinates were provided, but there was no county information.
(Ecoregions were assigned based on the latitude/longitude coordinates.)

5 The state and county values from the data set did not match the state and county values from the spatial join due to spelling or naming convention errors.
The matches were performed manually.
(Ecoregions were assigned based on the latitude/longitude coordinates.)

6 No latitude/longitude coordinates were provided, only state and county information was available.
(Ecoregions were assigned based on the county centroid.)

7 No latitude/longitude coordinates were provided, only state information was available; therefore, no matches were possible.
(Ecoregions were not assigned. Data is not included in the analysis.)

APPENDIX C

Glossary

Coefficient of Variation- Equal to the standard deviation divided by the mean multiplied by 100.

Maximum- The highest value.

Mean- The arithmetic average.

Median- The 50th percentile or middle value. Half of the values are above the median, and half of the values are below the median.

Minimum- The lowest value.

Standard Deviation- Equal to the square root of the variance with the variance defined as the sum of the squared deviations divided by the sample size minus one.

Standard Error- Standard error of the mean is equal to the standard deviation divided by the square root of the sample size.

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Recommended biological and water quality limits for trout fishery and trout spawning waters in the Wellington Region

May 2013

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2	July 2012	Final draft	Dr John Hayes
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Cover photographs: Top: Ruamahanga River at Mt Bruce (Photograph by Summer Greenfield); Bottom Left: Mangatarere Stream downstream of Dalefield Road (photograph by Olivier Ausseil); Bottom Right: Hutt River at Te Marua Photograph by Summer Greenfield)

EXECUTIVE SUMMARY

Greater Wellington Regional Council (GWRC) is in the process of developing technical recommendations to support its second generation Regional Plan. This report is one of a series of technical reports on the Wellington Region's streams and rivers, destined to inform and support the policy development process, in particular the development of biological and water quality limits in relation to different management purposes.

Trout fishery is one of the management purposes identified in GWRC's proposed Regional Policy Statement (RPS). A number of other freshwater management purposes, such as aquatic ecosystem health, contact recreation, amenity and stock drinking water have also been identified in the Wellington Region. Separate technical reports make recommendations for biological and water quality limits in relation to these management purposes.

"Trout fishery" as a management purpose includes a number of aspects, some directly relating to the requirements of healthy trout populations (e.g. habitat, food, reproduction) and some relating to the "human" aspects of a trout fishery, such as aesthetic, amenity and natural character values, "fishability", quality or quantity of fish, etc. Where possible, this report recommends biological and water quality limits in relation to these different aspects. Limits are recommended in relation to key biological and water quality determinands that were considered to have direct relevance to the state of trout fisheries and trout spawning.

Under the current provisions of the proposed RPS, the trout fishery management purpose applies in some selected waterbodies and comes in addition to the aquatic ecosystem management purpose. As a result, the limits recommended in this report are in addition to the biological and water quality limits recommended for water to be managed for aquatic ecosystem health (Greenfield, 2013a and 2013b; Ausseil, 2011a).

The biological and water quality limits recommended in this report for waters to be managed for trout fishery in the Wellington Region are summarised in Table A.

In order to present a comprehensive and consistent set of recommended biological and water quality limits for each water body, catchment or any other freshwater "management unit" that may be defined, for inclusion in the regional plan, the following steps are recommended:

- identify and compile the management purposes that apply to each "management unit";
- compile all the biological and water quality limits that apply to each management purpose in each "management unit";
- for each biological and water quality determinand, identify a limit that will enable the maintenance of all management purposes.

Further work is also recommended in relation to the development of in-stream sedimentation limits and the application of the recommended limits in the Regional Plan and subsequent resource management processes.

Table A: Summary of recommended biological and water quality limits for waters managed for trout fishery and trout spawning purposes.

Water quality determinand	Trout Fishery Class	Recommended limit	Limit application
MCI (minimum score)	Locally significant	100	Year round, all river flows
	Regionally significant	120	
	Trout spawning	120	
QMCI change (maximum % change)	All	20%	Year round, all river flows
Periphyton biomass (mg Chlorophyll *a*/m^2)	All	120 mg/m^2	Year round, River flows< 3 × median
Periphyton cover (%stream bed, filam. algae >2cm long)	All	30%	Year round, River flows< 3 × median
Temperature (°C, Daily maximum)	Locally significant	24°C	Year round, all river flows
	Regionally significant	19°C	
	Trout spawning	11°C	May - October
Temperature change (°C, maximum change)	Locally significant	±3°C	Year round, all river flows
	Regionally significant	±2°C	
	Trout spawning	±3°C	May - October
pH (pH units, Range)	Locally significant	6.0 to 9.0	Year round, all river flows
	Regionally significant	6.3 to 8.4	
	Trout spawning	6.3 to 8.4	May - October
pH Change (pH units, maximum change)	Locally significant	±0.5	Year round, all river flows
	Regionally significant	±0.5	
	Trout spawning	±0.5	May - October
DO (% saturation , daily minimum)	Locally significant	70%	Year round, all river flows
	Regionally significant	80%	
	Trout spawning	80%	May - October
$ScBOD_5$ (mg/L, maximum daily average)	All	2 mg/L	Year round, River flows< median
POM (mg/L, maximum average)	All	5 mg/L	Year round, River flows< median
Visual clarity (m, minimum)	Locally significant	2.0 m	Year round, River flows< median
	Waikanae River	2.0 m	
	Wainuiomata River	2.0 m	
	Ruamahanga River	3.0 m	
	Waiohine River	2.5 m	
	Hutt River	2.1 m	
Visual clarity change (% change, maximum)	Locally significant	33%	Year round, all river flows
	Regionally significant	20%	
Total Ammonia-N (**Chronic**) (mg/L, maximum average concentration at pH=8.0, Temp=20°C)	All	0.916 mg/L	Year round, all river flows
Other toxicants (protection level)	Locally significant	95%	Year round, all river flows
	Regionally significant	99%	
	Trout spawning	99%	

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1. Introduction

1.1. Background

Greater Wellington Regional Council (GWRC) is in the process of developing technical recommendations to support its second generation Regional Plan. This report is one of a series of technical reports on the Greater Wellington Region's streams and rivers, destined to inform and support the policy development process, in particular the development of biological and water quality limits in relation to different management purposes.

The term "limit" is used here as a generic term to describe a numeric or narrative threshold that defines a particular state for a river or stream. The way in which these limits will be used in the Regional Plan is a policy decision and is outside the scope of this report. In particular, it is important to note that since this report was initiated, the form of GWRC's regional plan process has changed from a 'traditional' single stage plan process to a two-stage 'collaborative' process. It is expected the two-stage process will involve firstly a regional plan which will include river and stream objectives appropriate at a regional scale and secondly collaborative development of catchment or 'whaitua' based river and stream objectives and resource use limits. This means that some of the in-stream 'limits' identified in this report will be used to inform the first stage, i.e. the definition of regional scale river and stream objectives, while some will be considered during the collaborative 'whaitua' second stage. Identification of at what stage the limits proposed here will be considered is outside the scope of this report.

Trout fishery is one of the management purposes identified in GWRC's proposed Regional Policy Statement (RPS). "Trout fishery" as a management purpose includes a number of aspects, some directly relating to the requirements of healthy trout populations (e.g. habitat, food, reproduction) and some relating to the "human" aspects of a trout fishery, such as aesthetic, amenity and natural character values, "fishability", quality or quantity of fish, etc.

This report should be read in conjunction with the other reports in the series, which recommend biological and water quality limits for waters managed for aquatic ecosystem (Greenfield, 2013a and 2013b; Ausseil, 2013b) and contact recreation, amenity and stock drinking water values (Ausseil, 2013a). One should also refer to the report that recommends in-stream nutrient limits (Ausseil, 2013c) to give effect to the different periphyton biomass and cover limits defined in relation to the management purposes mentioned above.

1.2. Aim and scope of this report

As stated above, the primary aim of this report is to recommend biological and water quality limits for waters to be managed for trout fishery or trout spawning purposes in the Wellington Region. Limits are recommended in relation to key biological and water quality determinands that were considered to have direct relevance to the state of trout fisheries and trout spawning, as summarised in Table 1. Key determinands, or groups of determinands, include physico-chemical stressors, such as water temperature, pH, clarity and dissolved oxygen, sediments, toxicants, nutrients, and biological indicators relating to macroinvertebrate and periphyton communities.

The recommendations relating to water quality limits for deposited sediments and toxicants (other than ammonia) are kept general in this report. Detailed examination of toxicant guidelines is undertaken as part of a separate project (Pawson and Milne, 2011). Since this report was initiated in 2010 and primarily written in 2010/2011, guidelines published after that time have not been considered in this report. This concerns in particular the sediment assessment protocols (Clapcott *et al*. 2011), the review of the instream plant and nutrient guidelines (Matheson *et al.*, 2012) and some additional work undertaken by NIWA on nitrate toxicity (e.g. Hickey, 2013). Similarly this report does not reference or consider recent changes in

Regional Plan provisions (such as summarised in Table 3) and/or recent technical work on water quality limits (e.g. Uytendaal and Ausseil, 2013).

For the purpose of this report, it was necessary to identify and classify the streams and rivers that support significant trout fisheries and trout spawning in the Wellington Region. This was primarily based on information provided by the Wellington Fish and Game Council, but it should be noted that additional checks and consultation may be required to refine the identification of trout fishery and trout spawning values in the region.

Finally, the maintenance or protection of significant trout fisheries certainly does not depend entirely on maintaining biological or water quality determinands between certain limits; other aspects, such as the quality of riparian and in-stream habitat, and the management of trout population are essential but fall outside the scope of this report.

1.3. Policy context

1.3.1. RMA

The purpose of the Resource Management Act (RMA) (1991) is to promote the sustainable management of the natural and physical resources. This particularly includes "safeguarding the life-supporting capacity of […] water […] and ecosystems" and "avoiding, remedying or mitigating any adverse effect of activities on the environment".

Other Sections of Part 2 of the RMA identify matters that directly relate to different aspects of the trout fishery. For example, Section 6 identifies matters of national importance, including the preservation of the natural character of rivers and lakes, the protection of outstanding features and landscapes and the maintenance and enhancement of public access, which are relevant to the quality of the fishing experience. Part 7 identifies other matters that are also relevant to trout fishery values, including the maintenance and enhancement of amenity values, intrinsic values of ecosystems and the protection of the habitat of trout and salmon.

Sections 70(1) and 107(1) set five narrative standards with respect to permitted and consented discharges to water or to land. These standards relate to different potential impacts of a discharge, ranging from visual impact to adverse effects on aquatic life.

Section 69 enables the following approaches to rules relating to water quality:

- Section 69(1) refers to Schedule 3, which defines 11 water classes, corresponding to management purposes. Schedule 3 defines a suite of numerical or narrative water quality standards for each class. Section 69(1) also gives mandate to the Regional Councils to use and apply these classes and narrative water quality standards in Regional Plans. Where the Council is of the opinion that these standards are not adequate or appropriate, it may define more stringent or specific water quality standards;
- Section 69(2) allows the Regional Council to define new classes where it is not satisfied that the classes/standards defined in Schedule 3 provide for certain management purposes.

In addition, Section 69(3) prohibits the setting of standards in a plan which result or may result in a reduction of the quality of the water in any waters at the time of the public notification, unless it is consistent with the purpose of the Act to do so.

Table 1: Summary of water quality determinands relevant to the Trout Fishery (TF) and Trout Spawning (TS) management purposes.

Main issue	Water quality determinand	Management purpose	Notes
Physico-chemical stressors	pH	TS, TF	High or low pH can have detrimental effects on trout growth; extreme pH can cause direct toxic effects
	Temperature	TF, TS	Elevated water temperature can cause direct effects on trout behaviour (e.g. feeding), growth, spawning, egg development and survival; Elevated temperature can also alter macroinvertebrate communities, affecting one of the trout's food sources.
	Dissolved oxygen (DO)	TF, TS	Water column DO for adult trout, intra-gravel DO for trout spawning
	Water clarity	TF	Essential for sight-feeding trout
Toxicants	Ammonia	TF, TS	Acute and chronic toxic effects on trout
	Nitrate	TF, TS	Acute and chronic toxic effects on trout
	Other toxicants	TF, TS	Relevant to both TF and TS Only general recommendations in this report – refer to Pawson and Milne (2011)
Sediment	Turbidity	TF	Often used as a surrogate for water clarity and SS
	Total Suspended Solids	TF	Indicator of clarity and sedimentation
	Deposited fine sediments	TF, TS	Particularly relevant to TS, but also to TF through effects on macroinvertebrates
Organic enrichment and Eutrophication	Algal biomass	TF, TS	Effects on macroinvertebrates, DO and pH
	Algal cover	TF, TS	Effects on aesthetic and trout fishing experience (e.g. long filamentous algae foul fishing lines)
	Heterotrophic growths	TF, TS	Effects on dissolved oxygen, macroinvertebrate communities and aesthetic values
	Organic matter (BOD, COD, TOC, DOC, etc…)	TF, TS	Effects on dissolved oxygen and heterotrophic growths
	Dissolved nutrients (DIN, DRP)	TF, TS	Promote algal growth Covered in a separate report (Ausseil, 2011c)
Macroinvertebrate communities	MCI/QMCI	TF TS	Relevant as source of food for all life stages of trout, and as general water quality/ecosystem health indicator.
	MCI/QMCI change	TF TS	

The narrative standards in Schedule 3 to the RMA provide essential guidance for the definition of water quality limits in the context of this report. Of particular relevance to this report, are the "Fishery" and "Fish Spawning" management purposes and standards. They read as follows:

"2.
Class F Water (being water managed for fishery purposes)
(1) The natural temperature of the water
(a) Shall not be changed by more than 3° Celsius. and
(b)Shall not exceed 25° Celsius
(2) The concentration of dissolved oxygen shall exceed 80% of saturation concentration
(3) Fish shall not be rendered unsuitable for human consumption by the presence of contaminants.
3.
Class FS Water (being water managed for fish spawning purposes)
(1) The natural temperature of the water shall not be changed by more than 3° Celsius. The temperature of the water shall not adversely affect the spawning of the specified fish species during the spawning season.
(2) The concentration of dissolved oxygen shall exceed 80% of saturation concentration.
(3) There shall be no undesirable biological growths as a result of any discharge of a contaminant into the water."

1.3.1. National Policy Statement – Freshwater Management 2011

On 12th May 2011, a National Policy Statement (NPS) for freshwater management was gazetted. The NPS's preamble identifies recreational activities as one of the national values of freshwater. It also identifies values that:

"relate to recognising and respecting fresh water's intrinsic values for: safeguarding the life-supporting capacity of water and associated ecosystems; and sustaining its potential to meet the reasonably foreseeable needs of future generations. Examples of these values include:

- *the interdependency of the elements of the freshwater cycle*
- *the natural form, character, functioning and natural processes of water bodies and margins, including natural flows, velocities, levels, variability and connections*
- *the natural conditions of fresh water, free from biological or chemical alterations resulting from human activity, so that it is fit for all aspects of its intrinsic values*
- *healthy ecosystem processes functioning naturally*
- *healthy ecosystems supporting the diversity of indigenous species in sustainable populations […]"*

It is interesting to note that none of the values identified in the NPS explicitly relates to exotic sport fisheries such as trout fisheries, although trout fishery is a recreational activity and trout populations depend heavily on the healthy functioning of aquatic ecosystems.

The NPS contains five main parts relating to: A. Water quality, B. Water Quantity, C. Integrated Management, D. Tangata whenua role and interests and E. Progressive implementation programme. In Part A. (Water quality), Objectives A1 and A2 set the overall objectives, whilst Policy A1 directs every regional council to establish freshwater objectives and set freshwater quality limits for all bodies of fresh water in their region. Policy A2 directs the regional councils to set targets where water bodies do not meet the freshwater objectives.

The text of Objectives A1 and A2 and Policies A1 and A2 is reproduced below for use of reference.

"Objective A1
To safeguard the life-supporting capacity, ecosystem processes and indigenous species including their associated ecosystems of fresh water, in sustainably managing the use and development of land, and of discharges of contaminants.

Objective A2
The overall quality of fresh water within a region is maintained or improved while:
a) protecting the quality of outstanding freshwater bodies
b) protecting the significant values of wetlands and
c) improving the quality of fresh water in water bodies that have been degraded by human activities to the point of being over-allocated.

Policy A1
By every regional council making or changing regional plans to the extent needed to ensure the plans:
a) establish freshwater objectives and set freshwater quality limits for all bodies of fresh water in their regions to give effect to the objectives in this national policy statement, having regard to at least the following:
i) the reasonably foreseeable impacts of climate change
ii) the connection between water bodies
b) establish methods (including rules) to avoid over-allocation.

Policy A2
Where water bodies do not meet the freshwater objectives made pursuant to Policy A1, every regional council is to specify targets and implement methods (either or both regulatory and non-regulatory) to assist the improvement of water quality in the water bodies, to meet those targets, and within a defined timeframe."

1.3.2. Existing Regional Policy

GWRC has an operative Regional Freshwater Plan (1999) with specific policies that manage the water quality of all surface water bodies for the following identified purposes:

- aquatic ecosystems (all water bodies)
- contact recreation (identified water bodies)
- natural state (identified water bodies)
- trout fishery and fish spawning (identified water bodies)
- water supply (identified water bodies).

Both narrative and prescriptive receiving water quality guidelines associated with each water quality purpose are identified in appendices that are linked to each relevant policy (although the guidelines are very limited, reflecting the date of the plan). Some water bodies that are known to be degraded are identified separately as needing enhancement, so that water quality guidelines for aquatic ecosystems, contact recreation or fishery and fish spawning purposes are met.

1.3.3. Greater Wellington's proposed Regional Policy Statement

GWRC's proposed Regional Policy Statement sets the proposed directions for the management of natural resources in the region, including freshwater quality (GWRC, 2010)[1]. Of particular relevance to this work is:

Policy 11

"Regional Plans will establish limits for water quality, flows and water levels that safeguard aquatic habitats and ecosystems in water bodies.
The narrative standard for aquatic ecosystems in the Third Schedule to the Resource Management Act will be used as the basis for safeguarding what is needed for aquatic ecosystem protection in terms of water quality."

Policy 11 also indicates that some water bodies may also be managed for other purposes, such as trout fishery, contact recreation, water supply, groundwater protection or cultural purposes. Where more than one management purposes is assigned to a waterbody, water quality "*shall not be less than the limits established for aquatic ecosystem health*".

Appendix 1 of the RPS lists the rivers and lakes with significant amenity and recreational values, including fishing. This list is reproduced in Table 2 below.

Table 2: Rivers and lakes with significant amenity and recreational values, as identified in Appendix 1, Table 15 of GWRC's proposed RPS (GWRC 2010).

River or lake	Recreational uses
Lake Waitawa (Forest Lakes)	kayaking, windsurfing, sailing
Otaki River	fishing, swimming, kayaking, canoeing, tubing, rafting, picnicking, camping
Waikanae River	fishing, swimming, camping
Kaiwharawhara Stream	picnicking, walking, running
Korokoro Stream	walking, running, mountain biking
Hutt River	fishing, swimming, kayaking, canoeing, tubing, rafting, power boating, radio controlled boats, jet skis, picnicking, walking, running, mountain biking
Pakuratahi River	fishing, swimming, picnicking
Akatarawa River	fishing, swimming, kayaking, bird watching, picnicking, walking, running, mountain biking, trail biking, horse riding, 4-wheel driving
Upper Gollan's Stream (including Butterfly Creek	picnicking, tramping walking, running, bird watching
Wainuiomata River	fishing, swimming, canoeing, kayaking, walking, horse riding
Orongorongo River	fishing, tramping
Kohangapiripiri and Kohangatera Lakes	bird watching, picnicking, walking, mountain biking
Ruamahanga River	fishing, swimming, kayaking, canoeing, tubing, rafting, power boating, jet skiing, picnicking, walking, duck shooting
Tauherenikau River	fishing, swimming, walking, picnicking, rafting
Waingawa River	fishing, swimming, kayaking, tubing, rafting, walking
Waiohine River	fishing, swimming, kayaking, canoeing, tubing, rafting, camping
Kopuaranga River	fishing
Waipoua River	fishing, swimming, running, trail biking
Henley Lake, Masterton	kayaking, dragon boating, radio controlled boats, picnicking, running, biking

[1] GWRC's Regional Policy Statement became operative in April 2013.

River or lake	Recreational uses
Lake Wairarapa	fishing, kayaking, canoeing, boating, duck shooting, bird watching, walking, photography

1.3.4. Other regional policy statements and regional plans

Most Regional Councils in New Zealand have produced regional policy statements and regional plans. Although most regional policy statements and regional plans identify management objectives and/or values associated with waterbodies, only a relatively small number of regions have operative or proposed numerical water quality standards or limits (Table 3). One of the first regional plans to contain numerical water quality standards was the Manawatu Catchment Water Quality Regional Plan, which became operative in 1998. The Waikato Regional Plan (2007) also contains a small number of numerical water quality standards, primarily relating to the protection of recreational values (contact recreation and trout fishery).

More recently, Canterbury's Proposed Natural Resources Regional Plan (April 2011 version) contains numerical water quality and ecological objectives relating to the protection of a number of management purposes, including significant habitat for salmonids (trout and salmon).

The Regional Water Plan for Southland (2010) contains water quality standards to ensure that the water bodies are suitable for a number of values, including trout and natural character (which includes aesthetics). One of the objectives (Objective 4) of the Plan is to achieve measurable improvement in surface water quality in four of its stream/river classes. Objective 4 sets a minimum of 10 % improvement over 10 years in levels of four key water quality determinands: microbiological contaminants, nitrate, phosphorus and clarity.

The Manawatu-Wanganui combined Regional Policy Statement and Regional Plan, the Proposed One Plan, was notified in 2008. Submissions on the notified plan were heard and the panel decision released in August 2010. The One Plan (2010) includes a framework of 19 river values (ecological, recreational and cultural, consumptive use and social and economic values) and water quality targets, superimposed over a spatial framework constituted of 44 water management zones and 117 water management sub-zones. The values framework identifies 3 classes of trout fisheries (Outstanding/ Regionally Significant/ Other trout fishery) and trout spawning waters. The One Plan is currently under appeal to the Environment Court.

1.4. Biological and water quality limits

Biological and water quality numerical thresholds can be expressed in a number of ways in regional plans: as objectives, limits, standards, targets or guidelines. The actual term used for each threshold, and its applicability in different circumstances will be defined by the regional planning framework (RPS and Regional Plan). This report is a technical report, and it is outside its scope to make detailed recommendations regarding the policy framework.

This report generally uses the term "limits" in relation to biological and water quality thresholds, although the use of these limits as actual standards directly applicable to consented activities is suggested where particularly relevant.

1.5. Management purposes

Policy 11 in the proposed RPS indicates that water bodies shall be managed as a minimum for the purpose of maintaining or enhancing aquatic ecosystem health. Policy 11 indicates that some water bodies may also be managed for other purposes, such as trout fishery, contact recreation, water supply, groundwater protection or cultural purposes. Where more than one management purposes is assigned to a waterbody, water quality "*shall not be less than the limits established for aquatic ecosystem health*".

This report makes recommendations for biological and water quality limits in relation to the maintenance and/or protection of trout fishery and trout spawning values in the Wellington region. Under the current provisions of the proposed RPS, management purposes associated with recreational activities such as fishing apply to identified water bodies and come in addition to the aquatic ecosystem management purpose. As a result, any biological or water quality limit defined in relation to the protection of trout fishery and trout spawning values will only become applicable if it brings an additional level of protection to the waterbody. Where the limits defined in this report in relation to, say, trout fishery, are less stringent than those defined for the protection of aquatic ecosystems, they will be superseded by the aquatic ecosystems limits. More generally, the biological and water quality limits recommended in this series of technical reports in relation to different management objectives or values will have to be collated in order to present a coherent set of limits for each water body in the Region. This exercise is beyond the scope of this report.

Table 3: Summary of numerical water quality standards, guidelines or targets for trout fishery and trout spawning waters in selected operative or proposed regional plans.

Region	Plan	Values/	Determinand	Limit	Comment
Manawatu-Wanganui	Manawatu Catchment Water Quality Regional Plan	"General" standards	Water clarity (change)	30%	MCWQ Rule 1 "general" standards are a numerical translation of Section 70(1) and 107(1) of the Act Standards apply at all times
			Water colour (change)	10 points (Munsell scale)	
			Euphotic depth	20% reduction	
			Total Ammonia-N	0.8 mg/L at T ≥ 15°C 1.1 mg/L at T < 15°C	
			$ScBOD_5$	2 g/m^3	
		Contact Recreation standards	Sewage Fungus	No visible growth	MCWQ Rule 2 Standards are primarily for the purpose of contact recreation, which includes aesthetics (i.e. relevant to the trout fishery management purpose). These standards apply in addition to Rule 1 standards, at or below half median flows
			(POM)	5 g/m^3	
			Periphyton cover	40% (mats + filam. >2cm)	
			Periphyton biomass	100 mg/m^2 (*Chlorophyll a*)	
			Water clarity	1.6m	
		Fishery Standards	Temperature *(max daily)*	25°C	MCWQ Rule 3 Standards apply in addition to Rule 1 and Rule 2 standards, at or below half median flows. Standards apply to identified trout fishery rivers
			Temperature *(change)*	±3°C	
			DO *(min. daily)*	80%	
			Human consumption	RMA Class F(3) standard	
		Fish Spawning Standards	Temperature *(change)*	±3°C	MCWQ Rule 4 Standards apply in addition to Rule 1 and Rule 2 standards, at or below half median flows. Standards apply to identified trout spawning streams
			DO *(min. daily)*	80%	
			Biological growths	No undesirable biological growths	
			Sedimentation	No significant deposition of sediment or particulate organic matter	

Region	Plan	Values/	Determinand	Limit	Comment
	One Plan (2010)	Trout fishery (3 classes)	pH	[7 - 8.2] to [7 -8.5]	Apply at all times. Water management zone-specific target
			Temperature *(max daily)*	19°C to 24°C	
			DO *(min. daily)*	70% to 80%	
			$ScBOD_5$ (*monthly average*)	1.5 to 2 mg/L	Applies at flows below 20th flow exceedance percentile. Water management zone-specific target
			POM (average)	5 mg/L	Applies at flows below median flow. Identical target for all water management zones
			QMCI	20% change	Applies at all times. Identical target for all water management zones
			MCI	100 to 120	Applies at all times. Water management zone-specific target
			Periphyton biomass	120 mg/m^2 (*Chlorophyll a*)	
			DRP	0.006 to 0.015 mg/L	Applies at flows below 20th flow exceedance percentile. Water management zone-specific target
			DIN	0.070 to 0.444 mg/L	
			Total Ammonia-N	0.320 to 0.400 mg/L	Average concentration, applies at all times
				1.7 to 2.1 mg/L	Maximum concentration, applies at all times.
			Toxicants	95 to 99 %	2000 ANZECC Guidelines protection level
			Water clarity	2 to 3.4m	Applies at flows below median. Water management zone-specific target
			Water clarity change	20 to 30%	Applies at all times. Water management zone-specific target
		Trout Spawning	Temperature *(max daily)*	11°C	Applies 1 May to 30 September to specified sties/reaches with identified trout spawning value
			Temperature *(change)*	±2°C	
			DO *(min. daily)*	80%	
			Sedimentation	No measurable increase of deposited sediment or particulate organic matter	
			Toxicants	99 %	
Southland	Regional Water Plan for Southland	Native fish Aquatic habitat Trout	pH	[6.5 – 9.0] to [7.2 - 8.0]	Applies at all times. Class-specific standard
			Temperature *(max daily)*	21°C to 23°C	Applies at all times. Class-specific standard
			Temperature (change)	1 to 3°C	Allowable temperature changes depends on background temperature

Region	Plan	Values/	Determinand	Limit	Comment
			DO *(min. daily)*	80% to 99% 5 to 6 mg/L	Applies at all times. Class-specific standard
			Total Ammonia-N	0.32 to 0.9 mg/L	pH–dependant standard Applies at all times. Class-specific standard
			Periphyton biomass	50 to 120 mg/m^2 (*Chlo. a*) 35 g/m^2 (AFDW)	Applies at all times. Class-specific standard
			Periphyton cover	30% (filamentous. >2cm)	Applies at all times. Class-specific standard
			Sewage Fungus	No visible growth	This standard applies to within the zone of reasonable mixing
			Water clarity	1.6 to 3 m	Applies at flows below median flow. Class-specific standard
			sQMCI	4.5 to 5.5	Applies at all times. Class-specific standard
			MCI	90 to 100	
Canterbury	Natural Resources Regional Plan (NRRP – October 2010)		pH	6.5 to 8.5	Standard, applicable to consented activities
			Temperature *(max daily)*	20°C	Objective
			Temperature (change)	2°C	Standard, applicable to consented activities
			DO *(min. daily)*	70% to 90%	Numerical objective depends on waterbody class
			Toxicants	90 to 99 %	2000 ANZECC Guidelines protection level, Class-spcific standard applicable to consented activities
			Periphyton biomass	50 to 200 mg/m^2 (Chlorophyll *a*)	Numerical objective depends on waterbody class
			Periphyton cover	10 to 30% (filamentous >2cm)	Numerical objective depends on waterbody class
			Macrophyte cover	20 to 30% (emergent) 30 to 60% (total)	Numerical objective depends on waterbody class
			Deposited sediment	10 to 40% cover	Numerical objective depends on waterbody class
			QMCI	3.5 to 6	Numerical objective depends on waterbody class
			Water clarity change	20 to 35%	Class-specific standard, applicable to consented activities
			Water colour change	5 to 10 pts (Munsell Scale)	

Region	Plan	Values/	Determinand	Limit	Comment
Waikato	Waikato Regional Plan (2007)	CR (CR Water Class)	Sewage Fungus	No visible growth	Set as a standard for Contact Recreation Class
			Periphyton cover	25%	Set as a Policy (Policy 6) for Contact Recreation Class
				40%	Set as a standard for Contact Recreation Class
			Periphyton biomass	100 mg/m^2 (Chlorophyll a)	
			Water clarity	1.6m	Set as a standard for Contact Recreation Class
			Other contaminants	Narrative standard	"The water shall not be rendered unsuitable for contact recreation activities by the presence of contaminants"
		Significant Trout Fisheries and Trout Habitat	Temperature *(max daily)*	20°C	Permitted activity rule standards for significant trout fishery and trout habitat waters
			Temperature (change)	3°C	
			Ammoniacal – Nitrogen	0.88 g/m^3	
			DO *(min. daily)*	80%	No change allowed if DO is already below 80%
			Human consumption	RMA Class F(3) standard	
			TSS increase	10%	Permitted activity rule standards
			TSS in discharge	100 mg/L	Permitted activity rule standards for significant trout fishery and trout habitat waters
			TSS in receiving environment	25 mg/L	
		Trout Spawning	Temperature *(max daily)*	12°C	Applies May to September

2. Data and methods

2.1. Monitoring data

The development of water quality limits recommended in this report was supported by monitoring data and data summaries provided by GWRC. These monitoring data were collected as part of GWRC's River State of the Environment (RSoE) monitoring programme during the period July 2004 to June 2009. GRWC's RSoE monitoring programme for this period included 56 river/stream sites across the Wellington region[2]. A number of these sites are located on regionally and locally significant trout fisheries (Table 4) and/or on or near trout spawning grounds (Appendix A).

GWRC also continuously monitors river flow at 42 sites across the region. However, only 19 of these sites are directly associated with a SoE water quality monitoring site. GWRC has therefore undertaken work to provide flow estimates at many water quality sites. To provide an informative dataset for this work, GWRC have developed flow estimates for an extra 33 sites. The following data were made available for this study:

- Mean daily flow on each sampling day, available at 12 sites;
- A flow category estimate on each sampling day, given as one of four flow categories: below half median flow, half median flow to median flow, median flow to three times median flow and above three time median flow. Flow category data were available for 45 sites (including the 12 sites where mean daily flow data were available).

2.2. Trout fisheries in the Wellington Region

The Wellington region contains a number of significant trout fisheries. In particular, the Hutt and Ruamahanga Rivers attract a large number of anglers each year. A number of their tributaries, such as the Waiohine River also have a reputation for being excellent backcountry trophy fisheries. These are primarily brown trout (*Salmo trutta*) fisheries, although rainbow trout (*Oncorhynchus mykiss*) are also present. Chinook Salmon (*Oncorhynchus tshawytscha)* have also been anecdotally reported in the Wellington Harbour, Lower Hutt River and Lake Onoke, but there are currently no significant salmon fisheries in the Wellington region.

In consultation with the Wellington Fish and Game Council, the known significant trout fisheries in the Wellington region have been identified and categorised as:

- "Regionally Significant": this includes the main stems of the Hutt and Ruamahanga Rivers, as well as the Waiohine, Waikanae and Wainuiomata Rivers. These rivers are the most utilised in the region: based on National Angler Survey (NAS) data, they account together for more than 85% of the trout fishing activity in the region's rivers and streams (Table 4);
- "Locally Significant", which includes all other trout fisheries in the Region, i.e. fisheries with lower angler use than the regionally significant fisheries, and fisheries that are anecdotally known to be regularly used but for which no NAS survey data were available.

This approach is similar to that of the One Plan, although an additional class for nationally and internationally significant trout fisheries is defined in the One Plan (Ausseil and Clark, 2007a). No nationally or internationally significant trout fisheries were identified in the Wellington region.

Spawning grounds are essential in sustaining trout fisheries, and known spawning streams were also identified, as summarised in Appendix A.

[2] Monitoring at RS01 (Mangapouri Stream at Rahui Rd) was discontinued in October 2009. As a result, the SoE water quality monitoring network currently comprises 55 sites.

Table 4: Significant trout fisheries in the Wellington Region, angler usage estimates (angler days) from the NAS, and GWRC's RSoE water quality monitoring sites. RSoE sites in grey-shaded cells are reference/ low impact sites. Angler days data from the National Angler Survey. (-): no data.

Class	River/Stream	Angler days per year			RSoE Sites	
		1994/95	2001/02	2007/08	No	Name
Regionally significant	Waikanae River	750	420	1,420	RS09	Waikanae @ Mangaone Walkway
					RS10	Waikanae @ Greenaway Rd
	Wainuiomata River	2,390	750	1,560	RS28	Wainuiomata @ Manuka Track
					RS29	Wainuiomata u/s White Bridge
	Ruamahanga River	7,390	6,910	6,540	RS31	Ruamahanga @ McLays
					RS32	Ruamahanga @ Te Ore Ore
					RS33	Ruamahanga @ Gladstone
					RS34	Ruamahanga @ Pukio
	Waiohine River	1,330	960	860	RS47	Waiohine @ Gorge
					RS48	Waiohine at Bicknells
	Hutt River	19,960	6,160	3,790	RS20	Hutt River @ Te Marua
					RS21	Hutt River @ Manor Park
					RS22	Hutt River @ Boulcott
Locally Significant	Otaki River	690	350	700	RS05	Otaki @ Pukehinau
					RS06	Otaki @ Mouth
	Taueru River	50	140	300	RS36	Taueru @ Castlehill
					RS37	Taueru @ Gladstone
	Huangarua River	-	60	60	RS51	Huangarua @ Ponatahi Bridge
	Kopuaranga River	520	520	310	RS38	Kopuaranga @ Stewarts
	Tauherenikau River	360	220	160	RS55	Tauherenikau @ Websters
	Waingawa River	430	140	140	RS41	Waingawa @ South Rd
	Mangatarere Stream	260	160	-	RS50	Mangatarere at SH2
	Beef Creek	-	-	-	RS49	Beef Creek @ headwaters
	Waipoua River	140	260	80	RS40	Waipoua @ Colombo Rd
	Akatarawa River	70	320	220	RS25	Akatarawa @ Hutt Confluence
	Whakatikei River	70	80	20	RS26	Whakatikei @ Riverstone
	Pakuratahi River	50	50	-	RS23	Pakuratahi Below Farm Creek
	Mangaroa River	120	10	-	RS24	Mangaroa @ Te Marua
	Orongorongo River	-	40	-	RS30	Orongorongo River
	Mangaone Stream	-	-	-	RS07	Mangaone @ Sims Rd
	Waitohu Stream	-	-	70	RS03	Waitohu @ Forest Park
	Makara Stream	100	70	-	RS17	Makara @ Kennels
	Karori Stream	120	-	-	RS18	Karori @ Makara Peak
	Kaiwharawhara S.	20	-	-	RS19	Kaiwharawhara @ Ngaio Gorge
	Korokoro Stream	20	-	-	-	-
	Pahaoa River	-	10	-	-	-
	Wainuioru River	-	-	-	-	-

2.3. General approach and level of protection

As explained in Section 2.2 of this report, two classes of significant trout fisheries were identified in the Wellington region: regionally and locally significant trout fisheries. The overall philosophy of the approach taken in this report is to use this classification to define water quality and biological limits corresponding to two levels of protection:

- in "locally significant" trout fisheries, the limits aim at maintaining biological and water quality within the "tolerable to good" range of conditions, to enable the long-term survival of trout populations;
- in "regionally significant" trout fisheries, the recommended objectives and standards are based on a higher level of protection, aiming at corresponding to "good to excellent" conditions for trout and other components of the aquatic ecosystem that are key to maintaining good trout populations.

Only one class of trout spawning (TS) waters have been identified following consultation with the Wellington Fish and Game Council. This results in only one recommended level of protection common to all TS waters in the region. In this report, the corresponding biological and water quality limits are purposely placed at a relatively conservative level, to reflect the role of these waters in sustaining significant trout fisheries. However, the blanket application of the recommended limits to all TS waters may result in un-necessarily stringent restrictions, and there may be grounds for relaxation of the recommended limits on a case-by-case basis, for example, in spawning grounds that are seldom used by trout, or that are demonstrated to be only minor contributors to the recruitment in a particular trout fishery.

Trout spawning, egg development and hatching and alevin (larvae) development are highly seasonal. The spawning and incubation period may vary from year to year and is different for brown and rainbow trout, brown trout being autumn spawners and rainbows primarily spring spawners, although rainbows sometimes exhibit extended spawning from May to November or even later (e.g. Taupo). Nevertheless, in most catchments where brown and rainbow trout co-occur, May to October covers the combined spawning period but is generally inside the May to October period (Hay *et al.*, 2006). In those catchments where brown just brown trout occurs, May to October includes the spawning season and embryo incubation period; with most fry emerging over September – October. Where spring spawning rainbow trout are present, the incubation period usually extends to early December. Trout fisheries in the Hutt and Ruamahanga catchments are dominated by brown trout, although rainbow trout are present. It is recommended that the biological and water quality limits defined in this report for trout spawning waters generally be applied during the May to October (inclusive) period, primarily to protect brown trout spawning and incubation periods, but consideration could be given to extending this period to November in some waters if/where protection of the incubation of late spawning rainbow trout was considered particularly important for the maintenance of the fishery.

Certain water quality limits may only need to apply in some places, at some times of the year, and/or under some river flow conditions. Where required, this report includes recommendations relating to the location and timing of their applicability.

2.4. Determinands

A number of measurable biological, water quality and habitat determinands are relevant to the protection of the values associated with trout fisheries and spawning. They are either directly relevant to the trout's physiological requirements at different life stages (e.g. temperature, toxicants), their physical habitat (e.g. water clarity, deposited sediments), or macroinvertebrates (a major food source for trout).

As indicated above, trout spawning and egg and juvenile development are highly seasonal. Trout spawning streams also tend to occur in smaller tributaries of the larger waterbodies that constitute the actual fishery. Finally, trout eggs and embryos have specific water quality and habitat requirements. For

these reasons, separate sets of limits are defined in this report for trout spawning waters and the two classes of trout fisheries. Where both trout fishery and trout spawning values have been identified for a given stream or river, it is recommended that the trout spawning limits apply in addition to the trout fishery limits.

A number of determinands also directly relate to the "human" aspects of the trout fishery, i.e. things that will influence the quality of the fishing experience, such as water clarity or periphyton cover. Table 1 summarises the determinands selected, the management purpose(s) they apply to and the reasons for their selection. It is noted that, to a large extent, this list of determinands is consistent with those recommended by Hayward *et al*. (2009) and Ausseil and Clark (2007b) for the protection of trout fishery values in the Canterbury and Manawatu-Wanganui regions respectively.

3. Recommended water quality and biological limits

3.1. Macroinvertebrate communities

3.1.1. MCI limits

The composition of aquatic macroinvertebrate communities is commonly used in New Zealand as a biomonitoring tool to assess the likely level of ecosystem degradation or enrichment (Stark, 1985). Because of their continuous presence in the stream or river, and their sensitivity to a number of water quality and habitat "issues" (such as organic enrichment, eutrophication, sedimentation, toxicants), the state of macroinvertebrate communities constitutes an integrated indicator of ecosystem "health".

A number of indices, such as the Macroinvertebrate Community Index (MCI), the Quantitative Macroinvertebrate Community Index (QMCI), and the proportion of ephemeroptera (mayflies), plecoptera (stoneflies) and trichoptera (caddisflies) numbers or taxa (%EPT, %EPT taxa) are commonly used to assess and summarise the state of macroinvertebrate communities in a number of resource management situations in New Zealand.

The macroinvertebrate species that score highly in the calculation of the different macroinvertebrate community indices are also generally good quality prey for drift feeding trout. The different indices therefore have the potential to provide an indication of the relative availability of trout food (Hay *et al*., 2006).

The use of the MCI in the definition of numerical objectives or standards for the protection of trout fishery values was recommended by Hay *et al.* (2006), and more recently by Quinn (2009) for the protection of ecological and recreational values (including trout fishery and trout spawning) in the Manawatu-Wanganui Region.

Greenfield (2013a) identified objectives relating to macroinvertebrate communities for the protection of aquatic ecosystems. These numerical objectives are also based on the Macroinvertebrate Community Index (MCI) as the indicator of macroinvertebrate community health. The MCI is the most commonly used indicator of macroinvertebrate community health in large-scale monitoring and reporting in New Zealand, such as State of the Environment monitoring and reporting undertaken by Regional Councils and TLAs[3]. It is therefore recommended that the MCI be used for the definition of numerical limits for the protection of the trout fishery values in the Wellington region.

For regionally significant trout fisheries, a minimum MCI score of 120, indicative of clean water (Stark, 1985) is recommended. For other, locally significant trout fisheries, a MCI score of 100 (indicative of

[3] Territorial and Local Authorities

possible mild pollution) is recommended (Table 5). These recommendations are consistent with those of Hay *et al*. (2006).

The MCI is sensitive to fine sediment and organic enrichment – both of which combine to reduce DO concentration in the substrate where trout eggs are deposited. A high MCI score (120) is also recommended as a limit for trout spawning streams, applicable during the main spawning and egg and alevin (larvae) development season (May – October).

It should be noted however, that these limits are general recommendations, which may not be realistically achieved in some stream or river types. It is recommended that these limits be checked against those recommended in relation to the aquatic ecosystem management purpose (Greenfield, 2013a) when the limits recommended for the different management purposes are compiled for each water quality management unit (e.g. stream, river, sub-catchment or catchment).

3.1.2. QMCI change limits

Specific activities, such as point-source discharges or works in the beds of rivers and streams, can have a direct detrimental impact on macroinvertebrate communities. In this context, it is recommended to also define numerical limits relating to changes in macroinvertebrate community health. These limits would be well suited for use as standards, directly applicable to specific activities.

Whilst MCI is well suited to SoE reporting and the setting of management objectives or targets, QMCI is considered better adapted to direct comparisons between different sets of data collected to assess the effects of a specific activity, such as upstream/downstream comparisons. Because it is a quantitative rather than a qualitative (like the MCI) index, the QMCI is considered less likely to be influenced by upstream macroinvertebrate communities [4], and more able to detect changes in community composition (Quinn, 2009). Stark and Maxted (2007) also maintained that QMCI (and SQMCI) were more suited to compliance monitoring than SoE monitoring.

For both regionally and locally significant trout fisheries, as well as for trout spawning streams, a maximum QMCI change of 20% as a result of a specific activity or a group of activities, is recommended as a standard. This threshold is consistent with what was recommended for the protection of the aquatic ecosystem values (Greenfield, 2013a), and essentially corresponds to a degree of change that is generally ecologically significant, can be statistically detected with an acceptable level of sampling effort and can be tested using relatively simple statistical methods (Stark, 2010).

3.1.3. Application of macroinvertebrate limits

The overall health of macroinvertebrate communities within a stream or river system is governed by a number of catchment-wide processes and activities. The MCI score is an indicator of overall macroinvertebrate community health, and is well suited for use in general SoE reporting.

The recommended QMCI change limits relate to the degree of change in space or time, in the overall health of macroinvertebrate communities. As such, they appear particularly well suited to situations where the effects of a specific activity are being assessed, e.g. upstream/downstream of a discharge.

Although good practice requires that macroinvertebrate communities be sampled following stable flow conditions, macroinvertebrates and trout live in the streams and rivers year-round, and at all flow conditions. This includes trout spawning waters, which generally are important rearing grounds for juvenile trout beyond the main spawning season. Thus, it is recommended that all macroinvertebrate limits should apply at all times.

[4] In the context of upstream/downstream comparisons, downstream MCI is more easily influenced by a small numbers of macroinvertebrate species that may drift from the upstream site.

Table 5: Recommended MCI and QMCI change limits for locally and regionally significant trout fisheries and trout spawning streams in the Wellington Region. The "measured range" corresponds to the range of median MCI recorded at RSoE sites (2004-2009 period).

Management Objective	MCI (minimum score)	Measured range		QMCI change (%change)
		Reference	Impacted	
Locally significant trout fishery	100	115-150	78-147	20%
Regionally significant trout fishery	120	133-154	79-150	20%
Trout spawning (year-round)	120	123-153	78-147	20%

3.2. Periphyton

Excessive periphyton growth can have detrimental effects on benthic habitat quality and macroinvertebrates, which can in turn influence trout growth and abundance (Jowett, 1992; Hayes *et al*. 2000). It can also cause wide daily changes in pH (Section 3.4) and dissolved oxygen concentration, which can also have detrimental effects on trout.

Excessive algal growth can also have a direct effect on the quality of the fishing experience for the angler. Long filamentous algae can become a nuisance by fouling fishing lures and lines. Excessive long filamentous algae and thick mats are unsightly and can also adversely affect the quality of the angling experience.

Biological limits associated with other types of biological growths, including heterotrophic growths (sewage fungus) and macrophytes are considered in a separate report for waters to be managed for aquatic ecosystem health (Greenfield, 2013b). It is considered that the limits recommended in relation to aquatic ecosystem health are also suitable for Trout Fishery and Trout Spawning waters, thus no additional limits are recommended.

The New Zealand Periphyton Guidelines (Biggs, 2000) define maximum periphyton biomass and cover in relation to trout habitat and angling. These guidelines have become widely accepted in New Zealand, and they are recommended for inclusion in GWRC's Regional Plan (Table 6).

The New Zealand Periphyton Guidelines recommend a maximum Ash-Free Dry Weight (AFDW) of 35 g/m^2 for the protection of trout habitat and angling, which corresponds approximately to 120 mg chlorophyll *a* $/m^2$ for communities dominated by filamentous algae, and 200 mg chlorophyll *a* $/m^2$ for communities dominated by mat-forming cyanobacteria and /or diatoms. Application of the guidelines using the two chorophyll *a* thresholds may give rise to problems in common situations where periphyton communities are mixed filamentous/cyanobacteria/diatoms assemblages. For simplicity, a unique biomass limit (120 mg chlorophyll *a* $/m^3$) could be used, for the protection of both classes of trout fisheries and trout spawning waters, but this may be un-necessarily conservative where/when the periphyton communities are heavily dominated by cyanobacteria (e.g. Phormidium-dominated periphyton at times in the Hutt River). For this reason, the AFDW guideline, is recommended for the protection of trout habitat and angling.

It is noted however that the periphyton biomass limits recommended by Greenfield (2013b) for the protection of aquatic ecosystem values are based on chlorophyll *a* concentrations that are not dependent on the composition of the periphyton community. It is probable that these limits will supersede the periphyton biomass thresholds recommended in this report in many locations.

3.2.1. Application of periphyton limits

Periphyton biomass influences macroinvertebrate communities, which are in turn an essential food source for trout year-round. It is therefore recommended that the periphyton biomass limits apply year-round, at all river flows.

The periphyton cover limits relate more directly to the usage humans make of the fishery (aesthetic and "fishability" aspects). For this reason, it is recommended that the periphyton cover limits apply to the open fishing season, which can vary depending on the stream or river (some fisheries are open year-round, whilst some are subject to seasonal closures).

Table 6: Recommended periphyton biomass and cover limits for locally and regionally significant trout fisheries and trout spawning streams in the Wellington Region. The "measured range" corresponds to the range of median periphyton biomass recorded at RSoE sites (2004-2009 period).

Management Objective	Periphyton biomass			Measured range (mg Chlo *a*/m²)		Periphyton cover (%stream bed, Filamentous algae >2cm long, during the fishing season)
	AFDW (g/m²)	Filam. Algae (mg Chlo *a*/m²)	Mats (mg Chlo *a*/m²)	Reference	Impacted	
Locally significant trout fishery	35	120	200	1-6	3-293	30%
Regionally significant trout fishery	35	120	200	0-9	1-57	30%
Trout spawning (May – October)	35	120	200	1-9	3-293	30%

3.3. Water temperature

3.3.1. Generalities

The functioning of aquatic ecosystems, their biological, chemical and physical processes, are closely regulated by water temperature. An organism's food consumption, metabolism, growth, reproduction, mobility, migration patterns and survival may all be influenced by changes in ambient water temperature (ANZECC, 2000; Hokanson et al., 1977, Elliott, 1994). Temperature changes may occur as part of natural diurnal and seasonal cycles, or as a consequence of human activities. Water temperature in a stream or river typically fluctuates diurnally around a seasonal daily mean, with a faster rise to the mid-afternoon daily maximum temperature than fall to the daily minimum near dawn (Davies-Colley and Wilcock, 2004).

Excess heat or cold are considered to be forms of thermal pollution. Anthropogenic point sources of thermal pollution can include discharges of relatively warm (e.g. industrial cooling water) or cold (bottom water from dams) water. Loss of riparian vegetation, water abstraction and global warming may also lead to temperature increases in streams, representing the non-point source component of thermal pollution.

3.3.2. Effects of temperature on trout

Similarly to a number of other general water quality determinands (such as pH and dissolved oxygen), the temperature requirements of trout are well documented in the scientific literature. The intention in this report is not to provide a comprehensive literature review on the effects of temperature on trout; rather it is to focus on the definition of water quality limits corresponding to the levels of protection sought for the different classes of trout fisheries and trout spawning waters.

Rainbow and brown trout are cold water species, with limited tolerance to high water temperature. The growth optima are 14 °C to 17 °C for brown trout and 16 °C to 18°C for rainbow trout, depending on whether the diet comprises invertebrates or fish (the higher optima are for fish diet) (Hay *et al.*, 2006). These optima are for trout that are not food limited (i.e., that are able to feed to satiation). When food is limited the optimal temperature for growth declines (Elliott 1994). Behavioural disturbances, such as cessation of feeding, can be expected at temperatures above 19 °C for both brown and rainbow trout (Elliott, 1994; Hay *et al.*, 2006).

The incipient lethal temperature (i.e. that can be tolerated for a prolonged period) for brown trout increases with acclimation to a plateau at 24.7°C, and 26.2°C for rainbow trout. The ultimate lethal temperatures (i.e. that cannot be tolerated even for a short period of time) are 29.7 °C and 30 °C for brown and rainbow trout respectively (Elliott, 1994 and 1995). Trout are less tolerant to high temperatures when they are not slowly acclimatised to the maximum temperature, i.e. they are sensitive to sudden changes in temperatures.

Trout eggs are also sensitive to temperature changes and high temperatures. Reported temperature optimum for trout spawning, egg development and hatching range between 1 and 12°C, with maximum temperatures of 15-16°C. Large-scale modelling also indicates that high winter temperatures may limit brown trout recruitment in New Zealand rivers (Scott & Poynter 1991; Jowett 1992; both cited in Hay *et al.* 2006).

3.3.3. Recommended limits

The available scientific literature indicates that trout and trout eggs are sensitive to both temperature changes and high temperatures. It is thus recommended to establish numerical thresholds for daily maximum water temperature and a maximum relative change in water temperature.

Water temperature in streams or rivers generally largely depends on catchment-wide processes, and it is recommended that daily maximum temperature limits be used in the Regional Plan as overall limits, particularly suited for SoE reporting purposes. Sudden, localised temperature changes that may result from a specific activity may be best controlled by way of directly enforceable temperature change standards.

The RMA Third Schedule standards for waters managed for fisheries set a maximum temperature of 25°C. However, a number of scientific studies suggest that, although a temperature of 25°C can probably be tolerated by rainbow trout, and, to a lesser extent, by brown trout, sub-lethal effects are likely at lower temperatures, between 19°C and 25°C.

As defined in Section 2.3, the overall philosophy for the definition of water quality limits for regionally significant trout fisheries is that they should remain close to the trout's optimum requirements. A temperature limit of 25°C is unlikely to provide the relatively high level of protection sought, and a maximum temperature of 19°C is recommended for regionally significant trout fisheries. This recommendation is consistent with those of Hay *et al.* (2006).

A lesser level of protection is sought for locally significant trout fisheries. Water temperature below 24°C would ensure that lethal effects of high water temperatures are avoided. This is also the limit recommended by Hay *et al.* (2006) for the protection of locally significant trout fisheries in the Manawatu-Wanganui Region. A similar daily maximum water temperature limit of 24°C is recommended for locally significant trout fisheries.

A maximum water temperature of 11°C between May and October is recommended for trout spawning waters. This temperature limit is close to the optimum temperature for trout eggs and should avoid any significant adverse effects of the water temperature on trout spawning success.

The RMA Third Schedule sets a maximum water temperature change of 3°C for both fishery and fish spawning waters. It is recommended that this threshold be used as a standard in the Regional Plan in

relation to specific activities, such as discharges to water or water abstraction, and that this standard apply within the bounds of the maximum temperature limit.

Table 7: Recommended water temperature and water temperature change limits for locally and regionally significant trout fisheries and trout spawning streams in the Wellington Region. The "measured range" corresponds to the range of 95th percentile of water temperature data recorded monthly at RSoE sites (2004-2009 period).

Management Objective	Recommended maximum temperature (daily maximum)	Recommended Temperature change standard (°C)	Measured range (95th percentile)	
			Reference	Impacted
Locally significant trout fishery	24°C	±3°C	15.6 – 17.7	15.4 – 23.6
Regionally significant trout fishery	19°C	±3°C	14.4 – 16.2	19.0 – 22.15
Trout spawning (May – October)	11°C	±3°C	10.0 – 11.3	11.2 – 15.7

3.3.4. Application of water temperature limits

The maximum daily water temperature and water temperature change limits recommended above for trout fisheries should apply at all times/all river flows. The limits relating to trout spawning waters should apply between May and October, at all river flows.

For general State of the Environment reporting, it is recommended that compliance with the daily maximum water temperature limits be assessed against the 95th percentile of data collected year-round for monthly RSoE data.

Both daily maximum water temperature and water temperature change limits are recommended. The way these two limits are intended to work is for the temperature change limit to apply within the bounds of the daily maximum temperature limit. In other words, if the background water temperature is, say, 18°C and the water temperature limits are 19°C (maximum daily) and ±3°C (change), then the temperature should be allowed to increase to 19°C, not 21°C, unless site/case-specific investigations show that the effects of doing so are acceptable.

3.4. Water pH

3.4.1. Generalities

pH is a measure of water acidity or alkalinity, on a scale of 0 (extremely acidic) to 14 (extremely alkaline). Pure distilled water is neutral at pH 7. Most natural freshwaters have a pH in the range 6.5-8.5, whilst the pH of marine waters is close to 8.2 (ANZECC, 2000).

pH is a central determinand in natural waters and interacts with other major physico-chemical or biological determinands of freshwater ecosystems. For example:

- Low or high pH can cause direct adverse effects on aquatic life;
- Changes in pH affect the bioavailability (hence the toxicity) of numerous toxicants. For example, a lowering in pH increases the bioavailability of certain metals, such as aluminium and cadmium, and inorganic toxicants such as hydrogen sulphide. Conversely, ammonia toxicity increases with pH (and temperature);
- pH can follow more or less pronounced diurnal changes controlled by in-stream primary production (photosynthesis), with minima generally observed at dawn and maxima late afternoon. During the day, the algal production uses CO_2 faster than it can be replaced from the atmosphere, causing the

dominant CO_2/HCO_3^- equilibrium[5] to be displaced so that the pH is increased. As a result, pH maxima in streams are generally observed during low river flow conditions in the late afternoon.

3.4.2. Bibliography

Raleigh *et al.* (1986) suggest that the tolerable pH range for brown trout is between 5 and 9.5, with an optimal range of 6.7 to 7.8. Kwak and Waters (1997) found a positive correlation between salmonids production and alkalinity in North American streams, i.e. salmonid production is lower in acidic waters. Hay *et al.* (2006) suggest that maintaining pH within a circum-neutral range should avoid any adverse effects on trout, although guidelines may need to account for the natural range of pH in each system.

3.4.3. Recommended water quality limits

Similarly to what is recommended for water temperature, it is recommended to establish numerical thresholds for absolute pH values as overall limits, whilst maximum pH relative change limits as a result of an activity could be used as a standard.

As defined in Section 2.3 of this report, the overall philosophy for the definition of water quality limits for regionally significant trout fisheries is that they should remain close to the trout's optimum. In terms of pH, the trout's optimal range is 6.7 to 7.8. As per Hay *et al.*'s (2006) recommendations, it should also remain circum-neutral (around 7), whilst accounting for the natural range of pH in each system. Reference sites are available in both regionally (4 sites) and locally significant trout fisheries (5 sites). The range of pH observed at these sites is representative of natural conditions for these rivers.

The 5th percentile of the data distribution (i.e. the lower end of the range for each site) at the regionally significant trout fishery reference sites is 6.4 to 6.8, and the 95th percentiles range from 7.8 to 7.9. A pH range of 6.4 to 7.9 could therefore be recommended. However, the SoE data is unlikely to capture the whole range of natural variations, and it seems reasonable to consider extending both ends of this range to account for this natural variability. The lowest 5th percentile across all RSoE sites is a pH 6.3, indicating that a lower bound of 6.3 would probably adequately capture the natural lower end of the range. The upper end of the range could be extended by 0.5 pH units (the recommended pH change limit) to 8.4 (Table 8). This range would ensure that the pH remains circum-neutral, and close to optimal pH range for trout, whilst still accounting for natural variability across the different systems covered by the regionally significant trout fishery management purpose. The same range is recommended for trout spawning waters.

Water quality limits for locally significant trout fisheries should ensure that water quality is not outside the range of tolerable values (refer to Section 2.3). Based on data collected at reference sites, the natural pH in these rivers appear to range from 6.6 to 7.9. A range of 6.0 to 9.0 would ensure that the pH remains well within the tolerable pH range defined by Raleigh *et al.* (1986), whilst still allowing a wider variation than in regionally significant trout fisheries (Table 8).

In addition to the above management targets, a standard allowing a maximum pH change of 0.5 units after reasonable mixing as a result of a given activity is recommended. This recommended standard is consistent with the recommendations of the ANZECC (1992) guidelines.

[5] $HCO_3^- + H^+ \leftrightarrow CO_2 + H_2O$

Table 8: Recommended pH range and pH change limits for locally and regionally significant trout fisheries and trout spawning streams in the Wellington Region. The "measured range" corresponds to the range of 5th -95th percentile of water temperature data recorded monthly at RSoE sites (2004-2009 period).

Management Objective	Recommended pH range limits	Recommended pH change limits	Measured range (5th and 95th percentiles)	
			Reference	Impacted
Locally significant trout fishery	6.0 to 9.0	±0.5	6.6 – 7.3 to 7.8 – 8.0	6.3 – 7.7 to 7.4 – 8.8
Regionally significant trout fishery	6.3 to 8.4	±0.5	6.4 - 6.8 to 7.8 – 7.9	6.3 – 7.1 to 7.7 – 9.1
Trout spawning (May –October)	6.3 to 8.4	±0.5	6.6 – 7.0 to 7.8 – 8.3	6.2 – 7.7 to 7.5 - 8.9

3.4.1. Application of water pH limits

The pH range and pH change limits recommended above for trout fisheries should apply at all times/all river flows. The limits relating to trout spawning waters should apply between May and October, at all river flows.

For general State of the Environment reporting, it is recommended that compliance with the pH range limits be assessed against the 5th-95th percentile of data collected year-round.

Similarly to water temperature, the pH change limits are intended to apply within the bounds of the pH range limits.

3.5. Dissolved Oxygen

3.5.1. Generalities

Dissolved oxygen (DO) is essential for aerobic forms of river life, including most plants and animals. As explained by Davies-Colley and Wilcock (2004), the dissolved oxygen concentration at any point in time will be a resulting balance between a number of processes:

- Oxygen-consuming respiration by aquatic life (bacteria, plants and animals);
- Oxygen-producing photosynthesis by aquatic plants and cyanobacteria; and
- Exchanges between the water and the atmosphere that tend to re-establish equilibrium at "saturation" level (in turn largely dependent on the water temperature). This process (reaeration) is mostly controlled by the degree of turbulent mixing occurring. Thus, a swift-flowing river is well re-aerated, whereas a sluggish stream has poor uptake of atmospheric oxygen.

The DO concentration in the water is subject to diurnal variations governed by the three processes above, leading to maximum levels (which can be significantly higher than the equilibrium 100% saturation) in mid-afternoon when photosynthesis is at maximum intensity, and minimum levels at dawn (after a whole night of oxygen consuming respiration, and no photosynthesis). Low levels of DO can be a major stressor to aquatic life, including fish, invertebrates and micro-organisms, which depend upon oxygen for their efficient functioning.

3.5.2. Bibliography

The DO requirements of trout and other salmonids are well documented in the scientific literature. Rainbow trout are more sensitive to low dissolved oxygen concentrations than most New Zealand native fish species (Dean and Richardson, 1999). The incipient lethal DO concentration for both brown and rainbow trout is approximately 3 mg/L (Raleigh *et al.*, 1984 and 1986). Dean and Richardson (1999) also observed some mortality and consistent surfacing behaviour at DO concentrations of 3 mg/L and some

surfacing behaviour at 5 mg/L DO concentration. Free swimming trout can tolerate DO concentrations of 5 to 5.5 mg/L, but the saturation should be at least 80%. (Hay *et al.*, 2006).

The effects of DO and water temperature on trout are interlinked: the oxygen requirements of salmonids increase with water temperature due to increased metabolism (Elliott, 1994). The oxygen saturation also depends on temperature. The link between temperature and DO is essential in the context of this work, where temperature objectives are also defined.

The ANZECC (1992) guidelines recommend a minimum DO concentration of 6 mg/L and 80% saturation. Hay *et al.* (2006) suggest that these limits should be seen as short-term exposure levels (i.e. days), as data suggests that long-term exposure to DO levels of 6 mg/L can chronically impair the growth of salmonids (BCME, 1997). The British Columbia Ministry of the Environment (BCME) guidelines set a minimum long-term concentration (30 day average) of 8 mg/L for best protection of salmonids waters.

The USEPA (1986) DO criteria associates minimum DO concentrations with a degree of impairment of the trout fishery, both for waters containing and not containing early life stages (Table 9).

With regards to trout spawning waters, the USEPA (1986) criteria identify a minimum DO concentration of 9 mg/L in the water column to maintain near optimum conditions ("slight impairment"). The BCME (1997) defines a similar guideline, which is also recommended by Hay *et al.* (2006) for the protection of trout spawning waters in the Manawatu-Wanganui Region.

Table 9: Dissolved oxygen (DO) concentrations (mg/L) recommended by the USEPA to confer five levels of protection for waters containing adult and juvenile (early life stages) salmonids (adapted from Dean and Richardson 1999), and DO saturation corresponding to the "adults" water column DO concentrations at different temperatures.

Degree of impairment acceptable	Early life stages		Adults	Saturation at			
	Water column DO (mg/L	Intra-gravel DO (mg/L)	Water column DO (mg/L)	10 °C	16 °C	19 °C	24 °C
None	11	8	8	71	81	86	95
Slight	9	6	6	53	61	65	71
Moderate	8	5	5	44	51	54	59
Severe	7	4	4	35	41	43	48
Acute	6	3	3	27	30	32	36

3.5.3. Recommended DO limits

The Third Schedule of the RMA defines that "the concentration of dissolved oxygen shall exceed 80% saturation concentration dissolved oxygen concentration saturation" in class F (fisheries) waters. This section of the report therefore examines the suitability of using the 80% threshold as a limit for trout fishery and trout spawning waters in the Wellington Region.

The recommended water temperature objective for regionally significant trout fisheries is 19°C (Section 3.3 of this report). At this temperature, a DO saturation of 80% corresponds to a concentration of 7.5 mg/L, which is just under the USEPA limit for "no impairment" (8mg/L) for adult trout and well above the "slight impairment" limit (6 mg/L), which corresponds well to the level of protection sought for regionally significant trout fisheries, as defined in Section 2.3 of this report.

With regards to locally significant trout fisheries, the literature establishes that a DO concentration of 6 mg/L is generally acceptable to trout, although long-term exposures to concentrations near 6 mg/L may lead to sub-lethal effects, such as decreased growth rates. The ANZECC (1992) Guidelines recommended

a minimum DO concentration of 6 mg/L. At a water temperature of 24°C (the recommended standard for locally significant trout fisheries in this report), 6 mg/L corresponds to a saturation of 71% (Table 9). A minimum saturation of 70% would ensure that DO concentrations remain above 6 mg/L as long as the temperature does not exceed 24°C. At temperatures in excess of 24°C, 70% saturation will correspond to DO concentrations below 6 mg/L, which may compound the direct effects of high water temperatures.

The maximum recommended temperature for trout spawning waters is 11°C during the May to October period. At 11°C, a concentration of 9 mg/L corresponds approximately to 80% saturation. A minimum saturation of 80% would ensure that DO concentrations remain above the 9 mg/L limit (recommended by Hay *et al*., 2006) at water temperatures below 11°C. This limit is consistent with the RMA Third Schedule standard for fisheries waters, and is consequently recommended for GWRC's Regional Plan (Table 10).

Table 10: Recommended Dissolved Oxygen (DO) saturation limits for locally and regionally significant trout fisheries and trout spawning streams in the Wellington Region. The "measured DO" columns reports the range of 5th percentile of monthly DO saturation data measured at RSoE sites (2004-2009 period).

Management Objective	Recommended minimum DO limit (% saturation)	Measured DO (5th percentiles at individual sites)	
		Reference	Impacted
Locally significant trout fishery	70%	85 - 93%	55 - 92%
Regionally significant trout fishery	80%	86 – 94%	81 - 94%
Trout spawning (May –October)	80%	82 – 96%	80 - 94%

3.5.4. Application of DO limits

Dissolved oxygen being indispensable to most superior forms of aquatic life, it is recommended that the DO saturation objectives apply at all times, at all river flows.

The limits recommended above are daily minima, and compliance against them should be assessed accordingly.

A significant point to note is that day-time instantaneous ("spot") measurements, generally taken as part of GWRC's routine SoE monitoring programme, only provide a snapshot of the DO concentration in the river at the time of sampling, but provide little information on the daily minimum concentrations. As such, they are of limited value in terms of SoE reporting or to assess compliance with the DO objectives. Although low daytime DO measurements do indicate a possible significant issue, reasonably high concentrations do not mean that the DO concentration remains acceptable at night.

Ideally, continuous monitoring records should be obtained at least during summer, although spot measurements taken at or near dawn can provide a useful measure of daily minimum DO concentration/saturation.

The existing SoE "spot measurement" DO data can still be useful in identifying existing issues associated with DO, although it will not enable a thorough assessment at all sites. Basically daytime "spot" measurements that regularly fall below the saturation guideline strongly indicate the existence of a DO issue. The opposite is not true however: high daytime DO readings are inconclusive. As a result the lower end of the range of values measured at each site (5th percentile is recommended in this case) should be compared with the recommended limits and used as a trigger for further investigations.

3.6. Organic matter

Water quality limits relating to soluble carbonaceous five-day biochemical oxygen demand ($ScBOD_5$) and particulate organic matter (POM) have been recommended in relation to the Aquatic Ecosystem management purpose (Ausseil, 2011b). The same numerical limits are recommended for both regionally and locally significant trout fisheries in the Wellington Region:

- a maximum monthly average $ScBOD_5$ concentration of 2 mg/L, applicable under base flow conditions (below median flow); and
- a maximum monthly average POM concentration of 5 mg/L, also applicable under base flow conditions (below median flow).

The above water quality limits are recommended for inclusion in the Regional Plan, but only in relation to point source discharges.

$ScBOD_5$, and to a lesser extent POM, analysis are relatively expensive and it is not recommended that routine monitoring of $ScBOD_5$ or POM be undertaken across the region (e.g. as part of the RSoE monitoring programme) in response to including this limit in the Regional Plan. Monitoring of these determinands should only be undertaken on a case-by-case basis, generally in relation to an existing or suspected point-source discharge.

3.7. Water clarity

3.7.1. Background

Water clarity refers to light transmission through water, and has two important aspects: visual clarity (sighting range for humans and aquatic animals) and light penetration for growth of aquatic plants (Davies-Colley and Smith, 2001; Davies-Colley *et al.*, 2003). Changes (generally reduction) of water clarity can affect a number of values associated with streams and rivers, including recreational, amenity, and, of relevance to this report, aquatic life (including trout) values.

Trout are visual predators and feeding on drifting prey (drift feeding) is the predominant foraging behaviour in most rivers, especially those of moderate to steep gradient (Hay *et al.*, 2006), typical of river trout fisheries in the Wellington region. Decreases in visual clarity, or equivalent increases in water turbidity, have been shown to reduce foraging efficiency (i.e. more energy is spent consuming the same amount of prey, or less prey are consumed). Sweka and Hartman (2001) showed that increased turbidity had no significant effect on brook trout mean daily prey consumption but resulted in a significant reduction in growth rates. This was because trout abandoned drift feeding in favour of active searching - which is energetically more expensive - as turbidity increased. Bioenergetic models, described in Hay *et al.* (2006) have been developed to link foraging efficiency with water turbidity or clarity. Water clarity/turbidity and suspended solids can also have an influence on plant/algae and macroinvertebrate communities (Ryan, 1991; Quinn and Hickey, 1990), which in turn may affect trout populations.

Water clarity is also a significant attribute of a number of river trout fisheries. Poor water clarity reduces angling opportunities and the range of usable angling methods, such as fly fishing and casting to sighted sight fishing opportunities. Poor water clarity may also reduce the aesthetic values of the stream or river and may be inconsistent with angler's expectations; it is generally perceived as having a negative impact on the quality of the angling experience.

Three water clarity determinands are commonly monitored in relation to particles present in the water column: visual water clarity, turbidity and total suspended solids (TSS).

- Visual clarity is generally measured using the "black disc" method, which determines the underwater horizontal sighting range of a black disc.

- TSS is a direct measurement of the concentration of sediment suspended in the water column. As such, it is the best determinand to estimate sediment loads transported by a waterway.
- Turbidity is an index of light scattering by suspended particles that is widely used in scientific monitoring and research. Turbidity can be measured in a water sample, which means physical conditions at the site (poor light conditions, small streams) do not prevent measurement. Importantly, turbidity probes allow continuous turbidity monitoring.

Provided sufficient data are collected, robust site-specific correlations can be drawn between the three determinands. As a result, continuous turbidity probes are particularly useful monitoring tools, as they enable the indirect (i.e. via statistical correlations) continuous monitoring of TSS, in turn enabling the estimation of sediment loads transported by a waterway. Continuous turbidity monitoring also enables the indirect continuous monitoring of visual clarity.

In a review of the available scientific literature, Davies-Colley and Smith (2001) assessed the suitability of the three indicators for use in water quality applications, including environmental standards. The use of TSS is not recommended in the context of water quality values protection, as much of the impact while sediment remains suspended is related to its light attenuation, which reduces visual range in water and light availability for photosynthesis. Thus measurement of the optical attributes of suspended matter in many instances is more relevant than measurement of its mass concentration. Turbidity is a widely used, simple, cheap instrumental surrogate for suspended sediments that also relates more directly than mass concentration to optical effects of suspended matter. However, turbidity is only a relative measure of scattering that has no intrinsic environmental relevance until calibrated to a "proper" scientific quantity. The authors conclude that visual clarity or beam attenuation should supplant Nephlometric turbidity in many water quality applications, including environmental standards.

Visual clarity limits have also been defined (in preference to turbidity or TSS limits) in most recent regional plans that contain river water quality limits, including the Regional Water Plan for Southland, the Canterbury NRRP, and Manawatu-Wanganui's One Plan.

Based on the above considerations and because of its direct relevance to the sight feeding of trout and the aesthetic and angling experience values, it is recommended that visual clarity, measured as the horizontal sighting range of a black disc, be used for the definition of limits in relation to the trout fishery management purpose.

Trout spawning, and in particular the development and hatching of eggs is sensitive to the amount of sediment deposited in and on the gravels that constitute trout spawning grounds, rather than to sediments suspended in the water column. As a result, it is recommended to define narrative, and then numerical limits relating to deposited sediments for the protection of trout spawning (refer to Section 3.8 of this report), rather than water clarity limits.

3.7.2. Recommended visual clarity limits

RMA Sections 70 and 107 set that discharges of contaminants into water shall not give rise to "any conspicuous change in the colour or visual clarity" in the receiving waters. The Ministry for the Environment Water Quality Guidelines No. 2 (MfE, 1994) provide guidance as to what degree of water clarity change constitutes a "conspicuous change": 20% change in waters where visual clarity is an important characteristic of the waterbody, and 33% to 50% in other waters.

As indicated above, water clarity not only influences the biological (i.e. trout themselves and macroinvertebrates) but also the human aspects of trout fisheries. It is thus postulated that water clarity is likely to be an important characteristic for trout fishery waters, particularly regionally significant trout fishery waters. The following limits setting maximum change in visual clarity as a result of a given activity are recommended (Table 11):

- 20% water clarity change in regionally significant trout fisheries; and

- 33% water clarity change for locally significant trout fisheries.

It is expected that water clarity change limits will adequately cover potential issues associated with changes in water colour and euphotic depth, except in exceptional cases (Davies-Colley, 2009).

Hay *et al.* (2006) predict that a 5 to 10% reduction in the foraging area of drift feeding trout (compared with clear water conditions, of 5m black disc clarity) would correspond to approximately 4.75 and 3.75m black disc clarity. The authors recommend water clarity thresholds of 5m for outstanding and regionally significant trout fisheries to maintain optimum drift feeding conditions, and 3.5m for the locally significant trout fisheries, to maintain reaction distances of drift-feeding trout at reasonable levels. These guidelines should apply only under base flow conditions (i.e. below median flow). The authors acknowledge however, that these thresholds may need to be adapted to local conditions and values. For example, the thresholds may need to be decreased in situations where they may not be attainable due to catchment characteristics, such as underlying geology, and may need to be set at more conservative levels in other situations.

As a result, the reference, or natural, conditions pertaining to each trout fishery needs to be considered in the definition of water clarity objectives. Reference conditions are available for all regionally significant trout fisheries and for some of the locally significant trout fisheries (Table 11). The setting of water clarity objectives that exceed reference conditions would be unattainable, and would carry the risk of setting unreasonable expectations, and is not recommended. The acceptable change in water clarity can also be used to define an acceptable degree of departure (or change) from reference conditions. A 33% departure from reference conditions would result in water clarity objective of 1.7m for the Otaki River and Waitohu Stream. A 20% departure from reference conditions would result in the following water clarity limits in regionally significant trout fisheries:

- 1.8 m for the Waikanae and Wainuiomata Rivers;
- 3.3m for the Ruamahanga River;
- 2.5m for the Waiohine River;
- 2.1m for the Hutt River.

Angling opportunities are considerably reduced when the visual clarity is less than 2m (Peter Taylor, pers. comm. in Ausseil and Clark 2007b); a visual clarity of 2m should also maintain the foraging area of trout when they are feeding on average-sized preys (12 mm or less) (Hay *et al.*, 2006). A visual clarity limit of 2m is recommended as the bottom line for all trout fisheries (locally and regionally significant in the region). This threshold is met at all currently monitored reference/low pressure sites.

A water clarity of 3m maintains good sight feeding range for drift-feeding trout (Hay *et al*., 2006), and also maintains good angling opportunities, including sight-fishing (Ausseil and Clarke, 2007b). A visual clarity limit of 3.0 m could therefore be recommended for regionally significant trout fisheries and is recommended where reference conditions are significantly better (e.g. the Ruamahanga River). However, a water clarity objective of 3m would be very close to the reference conditions measured in the upper Waiohine River (3.1m), and would only allow a minimal departure from reference conditions. Consequently, a limit of 2.5m (corresponding to a 20% decrease from reference conditions) is recommended for the Waiohine River. A water clarity limit of 3m would not be met at reference/low pressure sites on the Waikanae, Wainuiomata and Hutt Rivers (assessed against the 20th percentile of the base flow visual clarity data distribution), so would be unrealistic for these systems. It is recommended to revert to the 2m bottom-line limit for trout fisheries as described above for the Waikanae and Wainuiomata Rivers, and an objective of 2.1m for the Hutt River, corresponding to a 20% departure from its reference conditions.

Table 11: Recommended water clarity limits for individual rivers to be managed for trout fishery purposes. The "measured visual clarity" corresponds to the range of 20th percentile of monthly black disc sighting range data recorded monthly at RSoE sites (2004-2009 period).

Management Objective	River/Stream	Recommended water clarity limit (m)	Measured visual clarity (m) (20th percentiles at flows below median at individual RSoE sites)	
			Reference	Impacted
Locally significant trout fishery	All	2.0m	2.5m (RS05, RS03)	1.3m (RS04) - 2.2m (RS06)
Regionally significant trout fishery	Waikanae River	2.0 m	2.3m (RS09)	1.5m (RS10)
	Wainuiomata River	2.0m	2.2m (RS28)	1.2m (RS28)
	Ruamahanga River	3.0m	4.1m (RS31)	0.8 - 1.5m (RS32-34)
	Waiohine	2.5m	3.1m (RS47)	0.4m (RS48)
	Hutt River	2.1m	2.7m (RS20)	1.8 – 1.9m (RS21-22)

3.7.3. Notes on monitoring methods and compliance assessment

The most common method of measuring visual clarity in rivers in New Zealand is by measuring the horizontal sighting range of a black disc (Davies-Colley, 1988). It is a simple field method that can be used to directly estimate the beam attenuation coefficient, the primary factor controlling underwater visual ranges for both humans and aquatic animals (Davies-Colley, 1988; Davies Colley *et al.*, 2003). The direct black disc measurement can be limited by high turbidity and/or physical conditions at the sites (e.g. very small, shallow streams). In these cases, visual clarity can be measured ex-situ in a steel trough. These measurements have been shown to be closely correlated with both in-situ measurements and the beam attenuation coefficient (Davies-Colley and Smith, 1992).

Another out-of-stream method uses a 1m long clear plastic tube, with a small black disc sliding inside the tube. This method was originally developed as part of the Stream Health Monitoring and Assessment Kit (SHMAK) (Biggs *et al.*, 2002). The clarity tube measurements have been shown to be correlated with in-situ clarity measurements, particularly in relatively low water clarity environments (Kilroy and Biggs, 2002).

Nephlometric turbidity provides a relative measure of light scattering and has no direct environmental relevance (Davies-Colley, 1991). Turbidity and water clarity and turbidity and total suspended solids are generally well correlated, although specific relationships vary between rivers. Turbidity probes can be directly installed on site and provide a continuous turbidity record. Turbidity monitoring, in particular continuous monitoring, can be a very useful way of providing a continuous (including at night) assessment of compliance with water clarity limits, provided that specific turbidity/water clarity relationships are established at each site.

All three methods above are acceptable as surrogates for direct visual clarity measurements, within their respective field of application, and it is recommended that any Regional Plan limit or standard allow for the use of these methods where dictated by conditions.

The recommended limits should apply year-round under base flow conditions, i.e. below median flow. Compliance should be assessed such that a site will be deemed to comply with the recommended objective if 80% or more of the measurements undertaken at this site when the flow is below median flow are better than the recommended objective. In practice, this means comparing the limit with the 20th percentile of the data collected at the site when the flow is at or below median flow.

The RMA S107 and S70 standards relating to conspicuous change in water colour or clarity do not specify any acceptable frequency or duration of breach of these standards. The recommended water

clarity change standards may thus be applied to single water clarity measurements. It is noted however, that specific situations may require a modification or relaxation of this standard. For example, in situations where a conspicuous change in water clarity is inevitable as the result of an activity (e.g. infrastructure works in the bed of a river), then a duration or frequency at which the standard may be breached may need to be defined (e.g. 8 hours in a row, or 2 hours after the cessation of the works).

3.8. Deposited sediments

The deposition of fine sediment on and in the bed of streams and rivers can affect a number of values, including ecological, and aesthetic/recreational values (Matthaei *et al.*, 2006; Ryan, 1991; Death *et al.*, 2003). Although there seems to be a general acceptance of the significance of the issue in New Zealand, there are no nationally accepted protocols for the measurement of deposited sediments, or guidelines to interpret the results in relation to ecological values. In this situation it is difficult to provide robust recommendations in relation to acceptable levels of deposited sediments.

Nuisance /excessive fine sediment deposition can occur as a result of accelerated erosion within a given catchment, generally influenced by catchment-wide processes such as geological characteristics and landcover/landuse. In this context it is recommended that numerical objectives or targets be defined for each catchment/river type, setting the general level of fine sediment deposition at which each waterbody should be managed.

Sediment deposition can also occur as a result of specific activities, such as earthworks, river works or stock crossing. In this context, it seems appropriate that specific standards be defined in relation to these activities, possibly defining a maximum level of change in fine sediment deposition that may result from a given activity.

A Regional Council-led Envirolink Tools project is currently underway to develop national protocols for the measurement of fine sediment deposition, and national guidelines for the protection of a number of common river values, including macroinvertebrate communities and trout spawning. As this project is expected to be completed in late 2011, it is recommended to await its outcome prior to making detailed recommendations for GWRC's Regional Plan. In any case, it is recommended that RMA Third Schedule standards for class AE be used to guide the definition of numerical thresholds:

"(2) The following shall not be allowed if they have an adverse effect on aquatic life:
(b) any increase in the deposition of matter on the bed of the water body or coastal water:"

3.9. Ammonia

3.9.1. Background

Ammonia is a common pollutant in raw or treated domestic, agricultural and industrial wastewater, and can be toxic to many aquatic species. Ammonia is a toxicant, but also a directly bioavailable nutrient[6]. This report only considers the potential effects of ammonia as a toxicant; aspects relating to ammonia as a nutrient are covered in a separate report (Ausseil, 2011c).

When in solution in the water, ammonia occurs under two main chemical forms: the ammonium cation (NH_4^+) and unionised ammonia (NH_3). The respective proportion of these two forms is determined by a chemical equilibrium governed by pH and temperature. The higher the pH and temperature, the higher the proportion of unionised ammonia. Unionised ammonia being much more toxic to aquatic life, the toxicity of total ammonia (being the sum of unionised and ionised forms) increases with pH and/ or temperature.

[6] Total ammoniacal-nitrogen is one of the components of Dissolved Inorganic Nitrogen (DIN), which also contains nitrate- and nitrite- nitrogen.

In setting ammonia limits, the pH and temperature dependency of ammonia toxicity must be carefully considered.

3.9.2. Bibliography

Ammonia being a very common water pollutant, its toxicity to aquatic biota, including trout and other fish and macroinvertebrates is well studied. The report recommending water quality limits for waters to be managed for aquatic ecosystems (Ausseil, 2011b) provides a bibliographic summary of ammonia toxicity on New Zealand native fish and macroinvertebrates. The ANZECC (2000) guidelines and USEPA (1999) ammonia criteria documents provide excellent summaries of the effects of ammonia on different components of aquatic ecosystems, including trout, and the reader is encouraged to refer to these documents for additional information.

3.9.1. Recommended ammonia limits

The ANZECC (2000) guidelines were based on the toxicity studies available at the time, and recommend a default trigger value based on a concentration of 0.035 mg/L (35 ppb) as unionised ammonia-N for the 95% protection level. It is considered that the ANZECC (2000) 95% protection level trigger value will adequately protect trout and other components of the ecosystem that are key to maintaining healthy trout populations, including macroinvertebrate communities. It is recommended to use this trigger value as the basis for the chronic total ammonia-N concentration limits for GWRC's Regional Plan. The limit recommended) is based on an unionised ammonia-N concentration of 0.035 mg/L, corresponding to approximately 0.916 mg/l as total ammonia-N at pH=8 and water temperature =20°C..

Because of the pH and temperature dependency of ammonia toxicity, the pH and temperature measured at the time and place of sampling should be used to calculate the percentage of unionised ammonia in the sample, and the result compared with the recommended limits. The ANZECC (2000) guidelines provide the necessary equations. Table 12, **Error! Reference source not found.** and **Error! Reference source not found.** provide examples of Total ammonia-N limits at different water pH and temperatures

Ausseil (2011b) recommends the inclusion of an acute total ammonia-N concentration limit for waters to be managed for Aquatic Ecosystem health (i.e. all streams and rivers), to apply in cases where the exposure to ammonia is of known short duration. These limits would provide adequate protection to trout and macroinvertebrate communities, and no additional limits are recommended specifically for trout fishery and trout spawning waters.

Table 12: Recommended <u>chronic</u> total ammonia-N concentration (mgN/L) limit for trout fisheries, at different water pH and temperature.

		Temperature			
		15°C	**20°C**	**25°C**	**30°C**
pH	**6.5**	40	28	19	14
	7	13	8.8	6.2	4.4
	7.5	4.1	2.8	2.0	1.4
	8	1.314	0.916	0.649	0.469
	8.5	0.440	0.314	0.229	0.172
	9	0.163	0.123	0.096	0.078

3.9.2. Application of ammonia limits

All ammonia concentration limits should apply at all river flows. The chronic limit should be applied to situations with constant or variable and/or repetitive exposures (e.g. for a given duration every day) to ammonia occurring for extended periods (e.g. more than four days in a row). This number should be compared with the average concentration of total ammonia nitrogen, calculated over a period exceeding four days.

It is recommended that the chronic exposure limit be considered the default limit, but it is also recommended that the plan provide for the use of an acute limit, for situations where the exposure to ammonia is of known short duration. This limit should not be exceeded for more than one hour (in effect it means that it is applicable to individual samples, as it is very rare to have more than one sample taken in less than one hour).

3.10. Other toxicants

A very large number of other toxicants, including metals and organic micro-contaminants (such as pesticides, hydrocarbons, etc.), may be released in the aquatic environment, and cause toxic effects. Listing them and defining concentration limits for each of them is beyond the scope of this report. The general recommendation in this report is to use the trigger values provided in Table 3.4.1 of the ANZECC (2000) guidelines, with the level of protection recommended below. Detailed examination of toxicant guidelines is undertaken as part of a separate project (Pawson and Milne, 2011).

The ANZECC (2000) water quality guidelines define different protection levels, depending on the type of receiving environment. The approach is based on calculations of a probability distribution of aquatic toxicity end-points, and attempts to protect a pre-determined percentage of species. A percentage of species protected of 95% is generally used, but the approach enables quantitative alteration of protection levels.

The 95% protection level is the most commonly applied level of protection, and should be applied to "slightly to moderately disturbed" ecosystems. The ANZECC (2000) guidelines recommend the use of a higher (99%) protection level as the default value for ecosystem with high conservation values. Finally, the ANZECC (2000) guidelines recognise that it can be appropriate, depending on the state of the ecosystem, the management goals and in consultation with the community, to apply less stringent protection levels (90% or 80%), as intermediate targets for water quality improvement.

Hay *et al.* (2006) note that trout are generally located towards the more sensitive end of the continuum of sensitivity to toxic substances in the environment, and accordingly, recommend that the 99% protection level should provide for the protection of the trout fishery values. This is particularly the case for juvenile trout, and it is recommended that the 99% protection level should generally be applied to trout spawning waters. It is also recommended that this level of protection generally be applied to regionally significant trout fisheries, in line with the relatively high level of protection sought for these waters (Section 2.3 of this report). It is recommended that the 95% protection level be applied to locally significant trout fisheries, corresponding to a slightly lower level of protection than recommended for regionally significant trout fisheries.

It is noted however, that these are general recommendations, and that it may be useful to be able to review them on a case-by-case basis, for example to provide for a lower protection for a given water body and/or in relation to a given toxicant level if information available allows one to determine that this would not result in a significant degradation of the trout fishery or trout spawning values. It is recommended that the provisions of the Regional Plan allow for such flexibility.

3.10.1. Application of toxicant limits

It is essential to note that the numerical limits provided in Table 3.4.1 of the ANZECC (2000) guidelines are "trigger values", and are not intended to be used as absolute water quality limits or standards. They "represent the best current estimates of the concentrations of chemicals that should have no significant adverse effects on the aquatic ecosystems" (ANZECC 2000, Section 3.4.3). The ANZECC (2000) guidelines provide a risk-based decision scheme for applying the guideline trigger values. The process is summarised in Figure 3.4.1, p 3.4-14 of the guidelines document. Basically the recommended process involves comparing the expected contaminant concentration with the default trigger guideline value. If the expected contaminant concentration is below the guideline, this indicates a low risk of significant adverse effects on the aquatic ecosystems. If the contaminant concentration exceeds the guideline, this indicates a potential risk, and the guideline trigger values should be reviewed in the light of site-specific factors and/or a site-specific guideline should be calculated. If the site-specific guideline is still exceeded, the ANZECC framework recommends that either further investigation of the risk of effects (e.g. direct toxicity assessments) or remediation action be undertaken.

These considerations have direct implications when considering the translation of these trigger values into the policy framework, and into resource consent conditions.

The ANZECC (2000) guidelines could be used as thresholds helping the determination of an activity's status, with non-compliance with the trigger value leading to a change in activity status (e.g. from discretionary to non-complying as in the Canterbury Regional Plan), and the risk of environmental effects should be refined through the application process. Caution should also be exerted when considering translating ANZECC trigger values directly into resource consent conditions, to ensure that limits imposed through the consent conditions are consistent with the intent of the ANZECC (2000) guidelines. In particular:

- trigger value concentrations should be applied to the bioavailable (not total) fraction of toxicants; and
- most of ANZECC (2000) Table 3.4.1 trigger values are chronic exposure values, and should, as a first approach, be compared with the median value of monitoring results. Requiring an absolute compliance with a chronic toxicity threshold is likely to be inconsistent with the intent underpinning the number.

The other important point to note is that the ANZECC (2000) guidelines are currently under review. The review, and the release of the updated guidelines are likely to be a relatively lengthy and staged (i.e. individual guidelines may be released as work is completed) process. It is recommended that sufficient flexibility be built into GWRC's Regional Plan to allow for the use of revised guidelines as they become available.

4. Conclusions and recommendations

4.1. Summary

The biological and water quality limits recommended in this report for waters to be managed for trout fishery and trout spawning purposes in the Wellington Region are summarised in Table 13.

A number of other freshwater management purposes, such as aquatic ecosystem, contact recreation, amenity and stock drinking water have also been identified in the Wellington Region. Separate technical reports make recommendations for biological and water quality limits in relation to these management purposes.

In order to present a comprehensive and consistent set of recommended biological and water quality limits for each water body, catchment or any other freshwater "management unit" that may be defined, for inclusion in the regional plan, the following steps are recommended:

- identify and compile the management purposes that apply to each "management unit";
- compile all the biological and water quality limits that apply to each management purpose in each "management unit";
- for each biological and water quality determinand, identify a limit that will enable the maintenance of all management purposes.

4.2. Further work

It is also recommended that existing stream and river monitoring data be compared with the limits recommended in the different reports in this series, to assess the current state of the region's streams and rivers in relation to the different management purposes.

This report presents some preliminary work undertaken to identify and classify the streams and rivers that support significant trout fisheries and trout spawning in the Wellington Region. This work was primarily based on data and information provided by the Wellington Fish and Game Council, but it is recommended that additional checks and consultation be undertaken to refine the identification of trout fishery and trout spawning values in the region.

As indicated previously in this report, further work is required in relation to the definition of in-stream limits for deposited sediments, for both trout fishery and trout spawning waters. This work should follow the release of national guidelines for in-stream sedimentation, anticipated in late 2011.

This report recommends in-stream biological and water quality limits for waters to be managed for trout fishery and trout spawning purposes. Other aspects, such as those relating to the management of the riparian and in-stream physical habitat (e.g. riparian vegetation, works in the beds of rivers, etc.), or the management of trout population are also essential for the management of the trout fishery values. It is recommended that the management of these aspects be considered for their potential incorporation in the Regional Plan.

4.3. Application of limits

This report makes technical recommendations relating to biological and water quality limits. Their inclusion in a Regional Plan will inevitably lead to a number of issues and questions. The policy response to these questions (which is beyond the scope of this report) will essentially determine the status and applicability of the biological and water quality limits within the regulatory and non-regulatory components of the Regional Plan. Issues commonly arising in a number of resource management processes include:

- Where water quality exceeds (i.e. is better than) a given water quality limit (or objective, target or standard), should an activity or a group of activities be allowed to degrade water quality down (or up) to the limit?
- Where water quality is worse than the water quality limits, should an activity or a group of activities be allowed to degrade water quality further?
- Where water quality is much worse than the water quality limits, should the water quality limits apply, or should management "targets" be set, some way between the current state and the water quality limit?
- How should limits be translated into consent conditions? The consenting process is designed to assess each application on its own merits, and account for site- or activity-specific conditions. However, to ensure consistency in the way similar activities are treated, it is recommended that a

practice guide document be produced, to provide guidance on how numerical limits should be included in consent conditions.

Table 13: Summary of recommended biological and water quality limits for waters managed for trout fishery and trout spawning purposes.

Water quality determinand	Trout Fishery Class	Recommended limit	Limit application
MCI (minimum score)	Locally significant	100	Year round, all river flows
	Regionally significant	120	
	Trout spawning	120	
QMCI change (maximum % change)	All	20%	Year round, all river flows
Periphyton biomass (mg Chlorophyll *a* / m^2)	All	120 mg/m^2	Year round, River flows< 3 × median
Periphyton cover (%stream bed, filam. algae >2cm long)	All	30%	Trout fishing season, River flows< 3 × median
Temperature (°C, Daily maximum)	Locally significant	24°C	Year round, all river flows
	Regionally significant	19°C	
	Trout spawning	11°C	May - October
Temperature change (°C, maximum change)	Locally significant	±3°C	Year round, all river flows
	Regionally significant	±2°C	
	Trout spawning	±3°C	May - October
pH (pH units, Range)	Locally significant	6.0 to 9.0	Year round, all river flows
	Regionally significant	6.3 to 8.4	
	Trout spawning	6.3 to 8.4	May - October
pH Change (pH units, maximum change)	Locally significant	±0.5	Year round, all river flows
	Regionally significant	±0.5	
	Trout spawning	±0.5	May - October
DO (% saturation , daily minimum)	Locally significant	70%	Year round, all river flows
	Regionally significant	80%	
	Trout spawning	80%	May - October
$ScBOD_5$ (mg/L, maximum daily average)	All	2 mg/L	Year round, River flows< median
POM (mg/L, maximum average)	All	5 mg/L	Year round, River flows< median
Visual clarity (m, minimum)	Locally significant	2.0 m	Year round, River flows< median
	Waikanae River	2.0 m	
	Wainuiomata River	2.0 m	
	Ruamahanga River	3.0 m	
	Waiohine	2.5 m	
	Hutt River	2.1 m	
Visual clarity change (% change, maximum)	Locally significant	33%	Year round, all river flows
	Regionally significant	20%	
Total Ammonia-N (**Chronic**) (mg/L, maximum average concentration at pH=8.0, Temp=20°C)	All	0.916 mg/L	Year round, all river flows
Other toxicants (protection level)	Locally significant	95%	Year round, all river flows
	Regionally significant	99%	
	Trout spawning	99%	

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APPENDICES

Appendix A:

Identified trout spawning waters in the Wellington Region, and GWRC's RSoE water quality monitoring sites. RSoE sites in grey –shaded cells are reference/ low impact sites. Data from Wellington Fish and Game Council.

Catchment	River/Stream	Tributary/Stream	RSoE Sites	
			No	Name
Hutt	Akatarawa River	Akatarawa River	RS25	Akatarawa @ Hutt Confluence
		Akatarawa West		
		Deadwood Stream		
		Frances Stream		
	Whakatikei River	Whakatikei River	RS26	Whakatikei @ Riverstone
		Wainui Stream		
		Flightys Creek		
	Pakuratahi River	Pakuratahi River	RS23	Pakuratahi Below Farm Creek
		Farm Creek		
		Rimutaka Stream		
	Mangaroa River	Mangaroa River	RS24	Mangaroa @ Te Marua
		Collins Stream		
		Cooleys Stream		
		Collets Stream		
		Narrow Neck Stream		
		Plateau Stream		
	Hutt River	Moonshine Stream		
		Birchville Stream		
		Hutt River	RS20	Hutt River @ Te Marua
			RS21	Hutt River @ Manor Park
			RS22	Hutt River @ Boulcott
Ruamahanga	Ruamahanga River	Ruamahanga River (upper reaches)	RS31	Ruamahanga @ McLays
			RS32	Ruamahanga @ Te Ore Ore
	Huangarua River	Huangarua River	RS51	Huangarua @ Ponatahi Bridge
		Whangaehu Stream		
		Ruakokoputuna Stream		
	Kopuaranga River	Kopuaranga River		
	Taueru River	Taueru River	RS36	Taueru @ Castlehill
			RS37	Taueru @ Gladstone
		Tupurupuru Stream		
	Waingawa River	Waingawa River	RS41	Waingawa @ South Rd
		Atiwhakatu Stream		
		Blakes Stream		
	Waiohine River	Waiohine River	RS47	Waiohine @ Gorge
			RS48	Waiohine at Bicknells
		Mangatarere Stream	RS50	Mangatarere at SH2
		Kaipaitangata Stream		
		Beef Creek	RS49	Beef Creek @ headwaters
		Enaki Stream		
		Papawai Stream		

Catchment	River/Stream	Tributary/Stream	RSoE Sites	
			No	Name
Others	Tauherenikau River	Tauherenikau River (upper reaches)	RS55	Tauherenikau @ Websters
	Wainuiomata River	Wainuiomata River	RS28	Wainuiomata @ Manuka Track
			RS29	Wainuiomata u/s White Bridge
		Catchpool Stream		
	Kaiwharawhara Stream	Kaiwharawhara Stream	RS19	Kaiwharawhara @ Ngaio Gorge
	Otaki River	Otaki River	RS05	Otaki @ Pukehinau
			RS06	Otaki @ Mouth
		Waiotauru Stream		
		Pukeatua Stream		
		Rahui Stream		
		Pukehinau Stream		
		Waitatapia Stream		
	Waikanae River	Waikanae River	RS09	Waikanae @ Mangaone Walkway
			RS10	Waikanae @ Greenaway Rd
	Waitohu Stream	Waitohu Stream	RS03	Waitohu @ Forest Park

Appendix B:

Peer review comments from Dr John Hayes (Cawthron Institute) were received in the form of a "track-changes" version of the draft report. The table below summarises the comments from Dr Hayes and the author's response.

Comments from Dr John Hayes			Response from author	
No.	**Reference**	**Comment**	**Comment**	**Action**
1	Executive Summary	Specify what category of periphyton. 120 mg/m^2 applies to filamentous algae. No limit is recommended for diatoms and cyanobacteria (Hay et al. 2006) recommended 200 mg/m^2 for these based on Biggs 2000.	Refer to response to comment 9 below	Changed to 35 g/m^2 AFDW
2	Section 2.2 and Table 4	Replace angler survey by "National Angler Survey (NAS)" and consequential wording suggestions	Agree	Changes made to text as per suggestions
3	Section 2.3	Suggested additions to text to better characterise timing of brown vs. rainbow trout spawning	Agree	Changes made to text as per suggestions
4	Section 2.3	Give consideration to extending the period of application of the recommended water quality limits for trout spawning waters limit to include November for rivers in which rainbow trout occur	Agree in principle, however the key trout fisheries in the Wellington Region, i.e. the Ruamahanga and Hutt Rivers and their tributaries are primarily recognised as brown trout fisheries.	No change to the recommended limits Added a comment suggesting that consideration should be given to extending the period of application of the recommended water temperature limit if/where protection of the incubation of late spawning rainbow trout was considered particularly important for the maintenance of the fishery
5	Section 2.4, second para.	The eggs/embryos – not the fry – have lower temperature requirements than free swimming trout.	Agree	Replace "fry" by "embryo"
6	Section 3.1.1 (MCI Limits)	In relation to the recommended MCI limit for trout spawning waters: Some more justification for this would be helpful rather than just the MCI being indicative of clean water. More importantly the MCI is sensitive to fine sediment and organic enrichment – both of which combine to reduce DO concentration in the substrate where trout eggs are deposited.	Agree	Comment added as suggested
7	Section 3.2 First para	Add references to Jowett, 1992 and Hayes et al. 2000	Agree	Added references to Jowett, 1992 and Hayes et al. 2000
8	Section 3.2, third para	Referring to the NZ periphyton Guidelines (Biggs, 2000): "But they are based on little more than expert opinion for trout fisheries. They actually need to be underpinned by a more thorough analysis. Barry Biggs developed the trout periphyton guidelines by rating a	Agree – however to the author's knowledge no other guidelines have been developed that might supersede the Biggs (2000) guidelines. The Biggs (2000) guidelines are widely used in RMA	No changes made

Comments from Dr John Hayes			Response from author	
No.	**Reference**	**Comment**	**Comment**	**Action**
		few rivers covering a gradients in peripyton biomass and chlorophyl a concentrations by the quality of their trout fisheries (based on expert opinion) and to set the trout fishery standards"	processes, including regional plans and consents/compliance processes	
9	Sectiom 3.2, fourth para.	The implication is that 120 mg Chl a /m^2 limit applies to all algal communities whereas in Biggs's periphyton guidelines it applied to filamentous algae. This limit could be accused as being environmentally conservative when applied to diatom/cyanobacteria given Biggs guideline for these was 200 mg/m^2 Chl a. The 120 mg limit may come under the spot light in submissions where dairy enrichment is affecting N&P and periphyton. There needs to be a convincing reason for it. The Environment Court decision on the One Plan in respect of this matter will be a useful guide. The periphyton limits summary in Hay et al. (2006) includes both types of algal community (i.e., or Lowland streams, Diatoms and cyanobacteria: 200 mg/m^2 Chlorophyll a 35 g/m^2 AFDW Filamentous algae: 120 mg/m^2 Chlorophyll a 35 g/m^2 AFDW) Hay et al. also recommend a more stringent limit for upland streams 50 mg/m^2 Chlorophyll a maximum 15 mg/m^2 Chlorophyll a mean monthly These are all based on Bigg's periphyton guidelines. Recent research on cyanobacteria at Victoria University & Cawthron might also be useful for informing cyanobacteria limits (contact Susie Woods or Mark Heath) http://researcharchive.vuw.ac.nz/bitstream/handle/10063/1102/thesis.pdf?sequence=1	Agree with regards to the potential for the recommended limit potentially being overly conservative when/where periphyton communities are heavily dominated by mat-forming species, as is commonly the case in the Hutt River. However, the difficulties of applying the Biggs (200) recommended chlorophyll a limits in situations where the periphyton communities are mixed filamentous/mats assemblages are also noted.	Recommend using the Biggs (2000) 35 g/m^2 AFDW threshold, noting however the possible inconsistencies with the chlorophyll a limits recommended by Greenfield (2013b) for the protection of aquatic ecosystem values.
10	Section 3.3.1, first para	Include references to Hokanson et al 1977 and Elliott, 1994 Editorial changes in text	Agree	References added Changes made to text as per suggestions
11	Section 3.3.2, second paragraph	Suggested additions to text relating to the relationship between growth temperature optima depending on fish diet and food limitation.	Agree	Changes made to text as per suggestions
12	Section 3.3.2, last paragraph	Add references to Scott and Poynter 1991 and Jowett 1992 as cited in Hay et al. 2006	Agree	Changes made to text as per suggestions
13	Section 3.3.3	The Hokanson et al (1977) paper cited in Hay et al. (2006) provides the best rationale for justifying a maximum temperature	Agree	No changes made to report, but point noted.

Comments from Dr John Hayes			Response from author	
No.	**Reference**	**Comment**	**Comment**	**Action**
		limit. It defines for both a constant and daily fluctuating temperature regime the temperature at which the production of a rainbow trout population will be zero. This takes account of individual growth rate and probability of survival. The latter point is important and always overlooked in rationales for setting maximum temperatures. Survival of fish declines with increasing temperature above the temperature preferenda (usually similar to the optimal temperature for growth). When the population production (mean individual growth x number of fish in the population) is used as a measure of population fitness the growth and survival can be combined and plotted against temperature to find the point at which population biomass rate of increase = zero. Hokanson et al. estimated that the maximum temperature at which a rainbow trout population can be expected to maintain its weight (biomass) was a constant temperature of 23°C and a fluctuating [daily] mean temperature of 21 °C ($i.e.$ the temperature at which population production is zero). Hay et al. (2006a) suggested that given the differences in temperature preference between the species, an equivalent zero production temperature for brown trout is likely to be 19 °C.		
14	Section 3.3.3, Table 7	These limits apply to brown trout, but given that there are no rainbow trout only rivers in the Wellington region they are appropriate. In rainbow trout only rivers in NZ the limits could be slightly higher.	Agree	No changes made to report, but point noted.
15	Section 3.3.4	Consider including November for rivers that also support rainbow trout (unless F&G have information on rainbow trout spawning and fry emergence periods that would argue otherwise.	Refer to response to comment 4 above	No changes made to report.
16	Section 3.7.1	I think it is worth following this sentence with the explanation in Hay et al. (2006) – see below This is because trout abandoned drift feeding in favour of active searching - which is energetically more expensive - as turbidity increased	Agree	Changes made to text as per suggestions
17	Section 3.7.1, 3rd paragraph, 1st sentence	Meaning unclear. Do you mean that water clarity is an important attribute influencing angling/fishabilty?	Yes, this is what is meant. The two sentences that follow this statement are, in the author's view, sufficient and appropriate to clarify its meaning: "Poor water	No changes made to report.

Comments from Dr John Hayes			Response from author	
No.	**Reference**	**Comment**	**Comment**	**Action**
			clarity reduces angling opportunities and the range of usable angling methods, such as fly fishing and casting to sighted fish. Poor water clarity may also reduce the aesthetic values of the stream or river and may be inconsistent with angler's expectations; it is generally perceived as having a negative impact on the quality of the angling experience"	
18	Section 4.7.2	I made some calculations using the reaction distance and foraging area equations and the black disc vs NTU relationship in Fig.3 of Davies-Colley & Close 1990. A 20% reduction in black disc equates to about 50% increase in NTU and this results in about 13 – 20% reduction in the foraging area of a 50 cm drift feeding trout eating 12 mm prey over the black disc range 1 – 4m. Percent reduction in foraging area is higher at lower black disc (i.e., at higher NTU). I did the same calculations for 33% reduction in black disc. This equates to 82.5% increase in NTU resulting in 20-29% reduction in foraging area over the black disc range 1 – 4m. These reductions in foraging area will be proportional to energy intake and growth. So if you framed the question in these terms would you feel comfortable with imposing a water clarity change limit that could reduce the profitability of drift feeding by trout by 13-20% or 20-29% - and potentially the growth of trout by the same amounts?	This is useful information that was not available to the author's at the time of writing the report. It is noted the author understands that actual reductions in growth rates will to a large extent depend on the duration of the reduction in the foraging area and the ability for the trout to fulfil its energetic requirements, e.g. by switching to alternative food sources or feeding behaviour. With regards to the duration of the reduction in foraging area/growth, it is noted that the limit is recommended in the context of point-source discharges, immediately downstream of the zone of reasonable mixing. As specified in Section 3.7.3, it is the author's recommendation that these limits be applied to single water clarity measurements. As a result, the long-term median reductions in water clarity (and associated reductions in foraging area) will have to be significantly lesser than the limit in order to comply at all times.	No changes made to report, but point noted.
19	Table 11	The method used to estimate reference clarity conditions should be given. The reference clarities presented in Table 11 look low to me. Are these reference estimates free from pastoral and exotic forestry land use? Clear-water rivers are usually able to be drift-dived for counting trout. The minimum clarity for drift diving is 4 m. So only one of the rivers listed in Table 11, the Ruamahanga, would qualify for that status. If reference condition is not actually reference, but rather something lower owing to past land use change, then further degradation by 20% could be argued to be not protective.	The numbers provided in Table 11 are 20th percentiles of the water clarity distribution at flows below the median flow. This is because it is recommended that the minimum water clarity limits should be met at least 80% of the time at flows below median flow. This is probably the reason why the numbers look low – median values are significantly higher for the same sites. Reference sites utilised in the report to define reference conditions are "true" reference sites, based on >95% native cover in their catchment.	No changes made to report.

Comments from Dr John Hayes			Response from author	
No.	**Reference**	**Comment**	**Comment**	**Action**
20	Section 3.9.1 First paragraph	Note that concentrations of unionised ammonia-N as low as 0.013 mg/L have been associated with reduced growth in juvenile rainbow trout at temperatures > 16oC (Linton et al. 1997). And there are other studies cited in the same paper reporting sub-lethal effects of ammonia on growth rate. Linton TK, Reid SD, Wood CM. 1997. The metabolic costs and physiological consequences to juvenile rainbow trout of a simulated summer warming scenario in the presence and absence of sublethal ammonia. Transactions of the American Fisheries Society 126: 259-272.	The ANZECC Guidelines trigger values derivation method make use of suitable data available at the time, including the Linton et al. 1997 and studies referenced in that article. It is the author's understanding that for each species the geometric mean of the chronic values is used in the trigger value derivation, which may explain why some studies may have effects at lower concentrations, and that data relating to several salmonid species entered the calculations of the "high reliability" trigger value. The ANZECC guidelines note that the 95% protection species level is considered sufficiently protective of most slightly-moderately disturbed system, although the figure may not be sufficiently protective of the freshwater clam. It was beyond the scope of this report to provide a review of the ANZECC Guidelines trigger values. It is also noted that higher species protection level (99%) has been recommended for some aquatic ecosystem classes, which cover most upland sections of the region's regionally significant trout fisheries.	No changes made to report.
21	Section 3.9.1, second paragraph	The acute limits from Ausseil (2011b) should be repeated in the present report. Or is that what the Acute limit column in Table 12 is?	Agree	Table 12 removed
22	Section 3.9.1, second paragraph and table 12	Are these the actual limits proposed for the sites/rivers or what has been measured. It looks like the latter given that 0.9 mg/l total ammonia N has been recommended as the chronic ammonia limit in Table 13. But neither the 0.9 mg/l limit to the Chronic and Acute Total ammonia limits listed in Table 12 are put in context of the 0.035 mg/l unionised ammonia ANZEC 2000 guideline above which you appear to support. I am confused?	Agree re. the potential for confusion. The limit recommended is 35 ppb of unionised ammonia. There are several ways to express this limit.	For consistency with other reports, the limit is expressed as a total ammonia-N concentration at a given water temperature (20˚C) and pH (8), and a table has been included to provide examples of the corresponding total ammonia-N concentrations at different pH/temperature combinations.

Filamentous Algae Monitoring Pilot Program:

West Virginia Rivers of the Potomac River Basin

Report to the West Virginia Department of Environmental Protection,

Division of Water and Waste Management

by

A. Griggs, J. Cummins, and C. Buchanan

Interstate Commission on the Potomac River Basin

DRAFT

December 12, 2012

ICPRB Report 12-07

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Filamentous Algae Monitoring Pilot Program: West Virginia Rivers of the Potomac River Basin

Background

Since 2007, the West Virginia Department of Environmental Protection has been observing and evaluating the breadth and causes of filamentous green algae blooms within the state. In the Eastern Panhandle, blooms of filamentous green algae (FGA) have manifested in certain rivers of the Potomac Basin. WVDEP asked the Interstate Commission on the Potomac River Basin (ICPRB) to assist WVDEP in documenting FGA blooms in the West Virginia portions of the South Branch Potomac, Cacapon, and Shenandoah rivers. Following the protocols established for monitoring filamentous algae in other parts of the state, ICPRB collected a suite of water quality parameters and performed monthly algal abundance measures at 13 sites in the three rivers between July and October 2012.

Collection Methods

The WVDEP filamentous algae monitoring protocols consist of bi-monthly water chemistry samples, monthly quantitative algae coverage estimates, and a qualitative habitat survey. A total of seven visits were made to each sampling site between July and October 2012 (Table 1). Six sampling sites are located on the Cacapon River between Largent and Wardensville, one on the North River at Forks of Cacapon, two on the lower Shenandoah River near the West Virginia state line, and four on the South Branch Potomac, above and below the town of Moorefield, WV (Table 2). Water chemistry parameters included nitrate, nitrite, total phosphorous, dissolved phosphorous, total alkalinity, magnesium, calcium, and total suspended solids. In-situ water quality parameters included water temperature, pH, specific conductivity, dissolved oxygen, and turbidity. The ICPRB field crew consisted of two biologists during routine water chemistry sample rounds, and three on algal measurement rounds. ICPRB staff Adam Griggs was project leader; ICPRB staff Jim Cummins and summer intern Jason Kinder assisted in the project. Sites were generally sampled one river at a time, traveling sequentially either upstream or downstream, depending upon the route. However, sample handling time requirements and available overnight accommodations influenced the final sampling route. Generally, overnight accommodations consisted of camping at grounds along the sample route.

Sampling Round	Sampling Dates
JUL-1	Jul 18-20
AUG-1	Aug 4-5
AUG-2	Aug 16-17
AUG-3	Aug 29-30
SEP-1	Sep 12-13
SEP-2	Sep 25-26
OCT-1	Oct 10-11

Table 1. Sample rounds and dates.

Site Name	Site ID	Site Location Description
CA-LRGNT	CA-1	Cacapon River at Rt. 9 in the town of Largent
CA-FRKS	CA-2	Cacapon River at Rt. 127 / Forks of Cacapon
CA-D-CPBRG	CA-3	Cacapon River downstream of Capon Bridge off Cold Stream Road
CA-CAPBRG	CA-4	Cacapon River at Rt. 50 in Capon Bridge
CA-LWR-WARDS	CA-5	Cacapon River at Rt. 259 below Wardensville
CA-UPR-WARDS	CA-6	Cacapon River at Kotz Farm Ford above Wardensville
NO-FRKS	NO-1	North River at Gaston Rd. / Forks of Cacapon
SBR-LWR-TRGH	SB-1	South Branch at Harmison's Landing
SBR-UPR-TRGH	SB-2	South Branch at South Branch Wildlife Management Area
SBR-LWR-MRFLD	SB-3	South Branch at Rt. 220/28 crossing below Moorefield
SBR-UPR-MRFLD	SB-4	South Branch at Fisher Rd above Moorefield.
SHEN-LWR	SH-1	Shenandoah at Rt. 340 near Harper's Ferry
SHEN-UPR	SH-2	Shenandoah at Ann Lewis Road near state line

Table 2. Sampling site names, abbreviated IDs, and locations.

Rapid physical habitat assessment

The WVDEP Filamentous Algae Monitoring Form was completed in the field by the project leader. Global Positioning System (GPS) coordinates were taken using a Garmin Etrex20 on the first and most subsequent field visits. If for any reason the sampling location was moved, the GPS coordinates recorded reflect that change. The photograph recordation form was not used as pictures were being uploaded to a Dropbox© account shared with WVDEP staff. The pictures were arranged in folders according to site and sampling date. A site map was drawn on the first visit indicating the water quality sampling location and algae transect. Precipitation history was not always reported in the field; relevant monthly USGS gage hydrographs are included in Appendix B. Qualitative abundance observations of periphyton, moss, and aquatic vascular plants were made on each site visit. Aquatic vascular plant abundance was interpreted as being submerged aquatic vegetation and ubiquitously occurring water willow was not considered for this parameter.

Filamentous algae abundance measurements

Percent algae coverage measurements were performed according to Standard Operating Procedures (SOP) provided by WVDEP. Due to the equipment available, length was recorded in meters and depths in inches. All values were appropriately converted to metric scale on the percent algae calculation spreadsheet file, which was modified from that provided by WVDEP to receive the measurements as recorded. The modified percent algae coverage calculation spreadsheets and associated data are provided separately as a Microsoft Excel© file.

In-situ water quality

Water-chemistry sample bottles were provided pre-fixed by the contracted analysis laboratory. At each sampling location, the large un-fixed TSS/Alkalinity container was used to collect water samples and pour them into the pre-fixed containers. The collection container was rinsed 3 times mid-stream (if possible) and samples were collected facing upstream. Filtering for the dissolved phosphorous sample

was performed using a Nalgene© filter funnel cup, Nalgene© vacuum flask, 47 mm 0.45 µm cellulose-nitrate filter papers and a hand-operated vacuum pump. The vacuum flask and filter apparatus were also rinsed 3 times mid-stream prior to filtering. Otherwise, samples were collected according to WVDEP Standard Operating Procedures.

Sample handling

Water chemistry samples were labeled with a permanent marker and immediately stored on ice. All samples were delivered to Reliance Laboratories in Martinsburg, WV within 48 hours and typically well before the 48-hr hold time for the un-fixed Nitrate/Nitrite samples. Chain-of-custody forms were filled out at the laboratory and a copy was retained for file. On the last sampling round, a complete set of duplicates were collected and the duplicate set was delivered to West Virginia Department of Agriculture office in Moorefield, WV to be picked up by a second water quality laboratory.

Completeness

All 13 stations identified by WVDEP personnel were sampled throughout the study period. In addition, regular photographs and a single water chemistry sample were taken at another site on the Cacapon River, between Capon Bridge, WV and Wardensville, WV. With the exception of the first sampling visit, which required extra time for site establishment, all sites were monitored within a consecutive 2-day period. Water chemistry samples were collected at every visit. On the final sampling round, dissolved phosphorous samples were not collected due to contamination of the filters in the field. In-situ water quality was collected at every site using a Hydrolab DS-5 or a Hydrolab MS-5. The Dissolved Oxygen Sensor on the ICPRB DS-5 was malfunctioning and a loaner multi-parameter sonde (MS-5) was requested from Hach-Hydromet to complete the field season. Algae transects were performed monthly at every site except the two Shenandoah River sites. At the lower Shenandoah Rt. 340 site, river width and hazardous wading conditions prohibited measurements to be performed. At the upper Shenandoah site, river width was problematic but surveys were generally not performed because no filamentous green algae were observed. Occasionally, water clarity or visual surface disturbance due to precipitation prevented performing the visual assessment at certain sites.

Data Analysis

Analysis methods

Data were entered into MS Excel for exploratory analyses. A copy of this dataset is provided electronically to WVDEP along with this report. All analyses were performed using R and analysis scripts are also provided. Water quality parameters were evaluated across station, river system, and sample date using box plots. Basic numerical summaries were used to describe water quality attributes across river basins and a Kruskal-Wallis test was used to look for significant differences between them. Attempts to evaluate stressor-response relationships between the qualitative primary production values and the quantitative algae coverage estimates were explored, but were greatly limited by low sample sizes of the response variables. Associations with a qualitative Sum of Primary Producers variable were also explored by summing the FGA, periphyton, mosses, and aquatic vegetation scores. Quality Assurance concerns were raised about the phosphorus measurements made by Reliance Laboratories

(see below). As a result, results recorded as non-detect (ND) were analyzed as null values in order to control for false negatives. As a result, total phosphorous data used in analyses includes sample duplicate results reported by a second lab during the last sample round. See the project suggestions section below for further details.

Water chemistry

Water chemistry parameters differed across sampling stations, river basins, and season. Water chemistry conditions were generally similar within river basin, but differed among certain parameters between river basins. For example, the two Shenandoah sites had overall higher concentrations of total alkalinity, specific conductivity, magnesium, and nitrate compared to the Cacapon and South Branch sites. Cacapon river sites had generally lower concentrations of every measured parameter and lower conductivities compared to the other river basins. The South Branch sites, specifically those below the Moorefield WWTP seemed to have higher calcium and phosphorous concentrations compared to the other river basins (See Figure 1 and Appendix A).

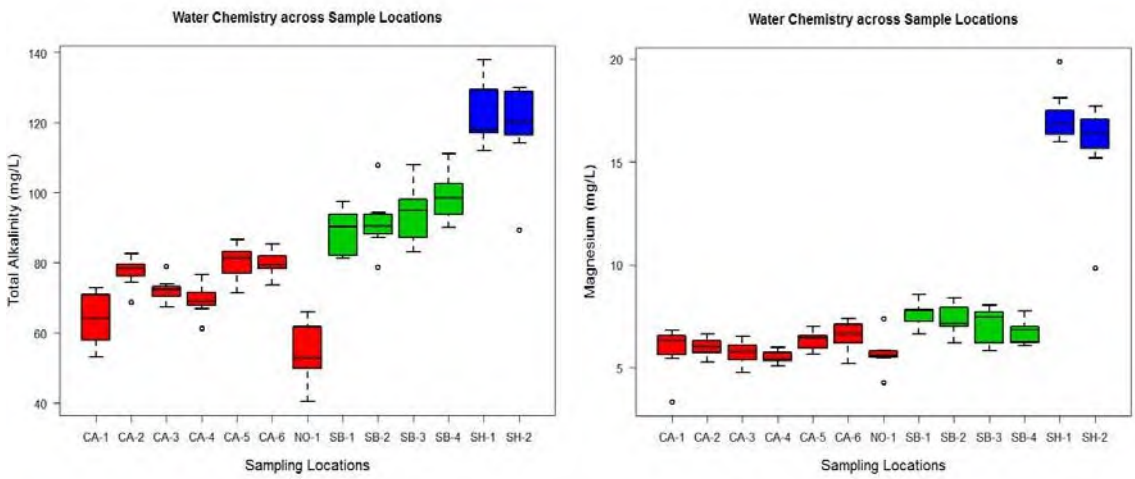


Figure 1. Total Alkalinity and Magnesium concentrations (mg/L) among the 13 sampling locations.

The Kruskal-Wallis analysis of variance found that samples collected from the four river basins differed in most water chemistry parameters (Table 3). Water chemistry concentrations also varied over time and displayed somewhat predictable patterns, such as decreasing water temperature over the season. Concentrations of dissolved pollutants such as nitrate generally

Parameter	Cacapon	North	Shenan-doah	South Branch	Chi-square	P value
ALK	73.8	52.83	119.5	93.1	72.8046	1.07E-15
CA	29.3	18.5	38.4	43.8	65.0509	4.89E-14
DO	12.31	11.385	10.905	11.84	7.6964	0.05272
DP	0.02	0.02	0.025	0.055	4.208	0.2399
MG	5.98	5.58	16.65	7.065	56.6967	2.98E-12
NO2	0.14	0.33	0.34	0.267	4.8181	0.1856
NO3	0.43	0.42	0.88	0.73	31.7936	5.79E-07
PH	8.61	8.01	8.75	8.93	24.8685	1.65E-05
SPCOND	187.9	154.25	325.65	256.5	71.2445	2.31E-15
TP	0.02	0.0295	0.02	0.165	11.1268	0.01106
TSS	4	6	7	3.5	8.1195	0.04361
WTEMP	23	22.5	25.7	24.6	4.7668	0.1897

Table 3. Kruskal-Wallis analysis of variance for the sampled water chemistry among the river basins.

increased over time, possibly due to reduced dilution of pollutants as flows trended downward over the summer season (See Figure 2 and Appendix A, B).

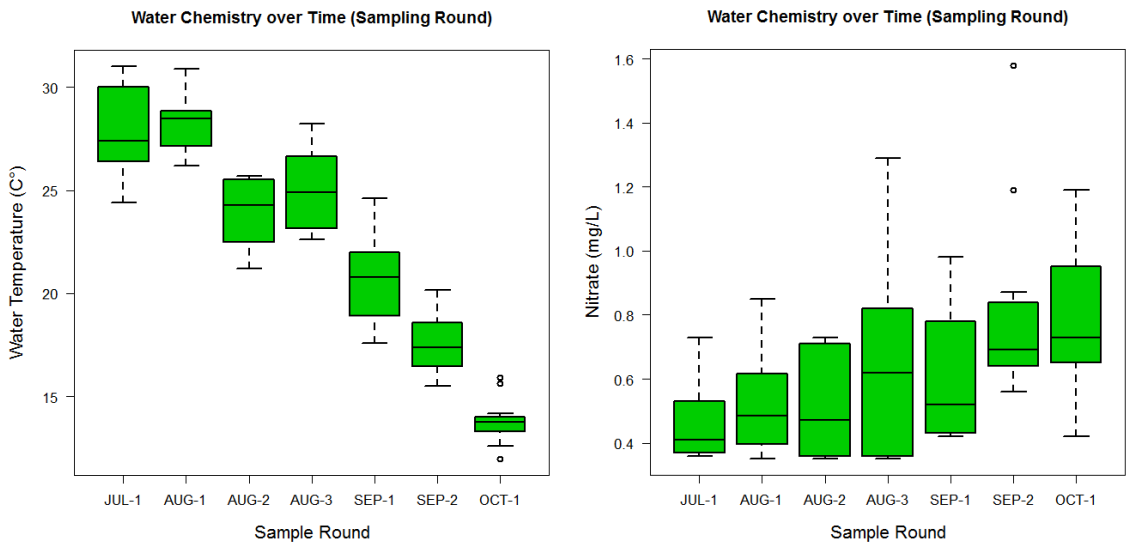


Figure 2. Water Temperature (C°) and Nitrate (mg/L) over time.

Algal measurements and observations

Filamentous algae were generally most abundant toward the beginning of the sampling season, but fluctuated in abundance from site to site over the remaining period (Figure 3, See Appendix C for representative pictures of each site). The most algae observed in a transect was 37.63% at the Upper Moorefield site on the South Branch on July 18, 2012. Subsequent visits found almost no algae in the channel at this site however an upstream tributary with cattle in the stream was often manifesting floating algal mats. The Lower Moorefield site had the most consistent algae coverage throughout the season, ranging from 12% to 23%. This site also had extreme amounts of submerged aquatic vegetation (SAV). Water milfoil, water stargrass, *Elodea*, and *Hydrilla* were all common with stargrass and milfoil dominating the site throughout the sampling season. SAV beds were extensive and persistent throughout the sampling season (See pictures in Appendix C). Filamentous algae were mostly constricted to an embayment on the river-left bank where it often formed floating mats. Submerged aquatic vegetation was observed at all sites except for the Upper Shenandoah site. This site had light periphyton

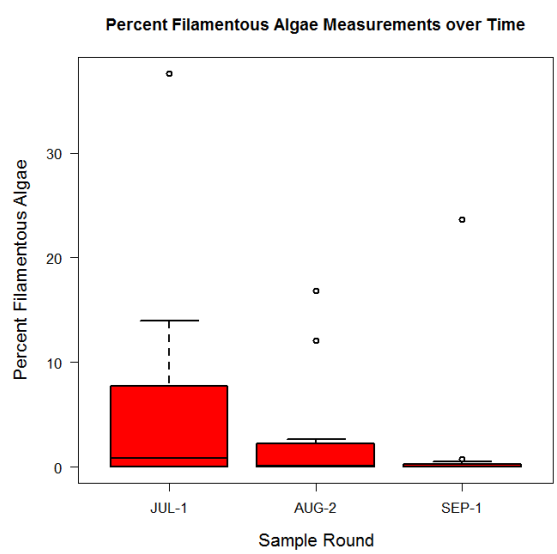


Figure 3. Percent algae coverage estimates over time.

coverage and other typical primary production communities were also conspicuously rare or absent. The North River site was dominated by a submerged vascular plant of unknown identity. No algae or other SAV species were observed. Towards the end of the sampling season, a brown slime or algae began to manifest on cobble and boulder at many sites. This biofilm was neither filamentous green algae, vascular plant, or moss, and was therefore not captured in the qualitative information on the field monitoring sheet.

Suggested alterations or additions to the filamentous algae monitoring program

Monitoring protocols

Overall, the filamentous algae monitoring form used in this monitoring program is comprehensive, straightforward, and suits the program well. During completion of the 2012 monitoring season, ideas for possible improvement or clarification were noted. At the bottom of page 2 of the sampling form, the abundance of various primary producer groups are recorded by assigning scores of 0 – 4 for periphyton, filamentous algae (green), aquatic vascular plants, and aquatic mosses. We suggest that the "aquatic vascular plants" entry be clarified as to whether emergent vegetation should be considered along with submerged vegetation types. We also suggest that WVDEP consider submerged vegetation only, as submerged vegetation competes with algae for benthic substrate more often than do littoral emergent plants. Additionally, the ubiquitous emergent water willow (*Justicia americana)* often dominates littoral zones and can inflate the aquatic vegetation score above what should represent the in-stream condition. There were several occasions when non-filamentous green algae were observed. Blue-green algae (cyanobacteria) and possible brown-algae were encountered with fair regularity. We acknowledge that the protocol is designed to capture filamentous green algae, however, observations of algae communities in general may prove important over time in discerning the underlying drivers of filamentous algae blooms. Further, we suggest an attempt to identify algae types whenever possible, either through field-identification or the collection and preservation of samples. Certain genera of algae are readily identifiable in the field, such as *Hydrodicton sp*. and training or the addition of field-keys could greatly assist in identification (Figure 4).

Figure 4. *Hydrodicton sp*. collected at the Upper Moorefield site on the South Branch Potomac.

The water chemistry parameters collected for the filamentous algae monitoring program are intended to capture ionic composition and nutrient concentrations that may limit or encourage algal growth and largely do so. We suggest that the necessary nitrate species components be sampled, in addition to the nitrate-nitrite which is currently analyzed, to calculate a total nitrogen measure. Total Kjeldahl Nitrogen

(TKN) would measure organic forms of nitrate, ammonia, and ammonium which may be constituents present in the effluent of WWTPs and other sources. Sampling and calculating a TN measure would be a improved measure of overall trophic condition of the sampled waterbodies. In addition, we suggest considering the addition of dissolved organic carbon, which can be a limiting agent of algal production, and heavy metals such as copper or zinc, which can inhibit algal growth even in eutrophic conditions.

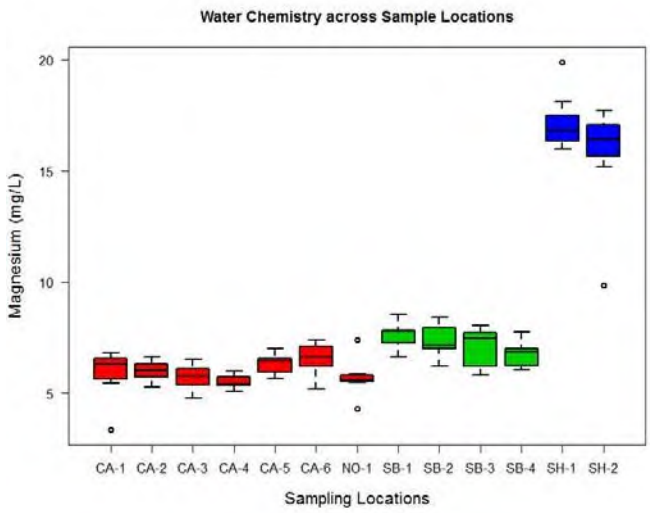
Sampling logistics

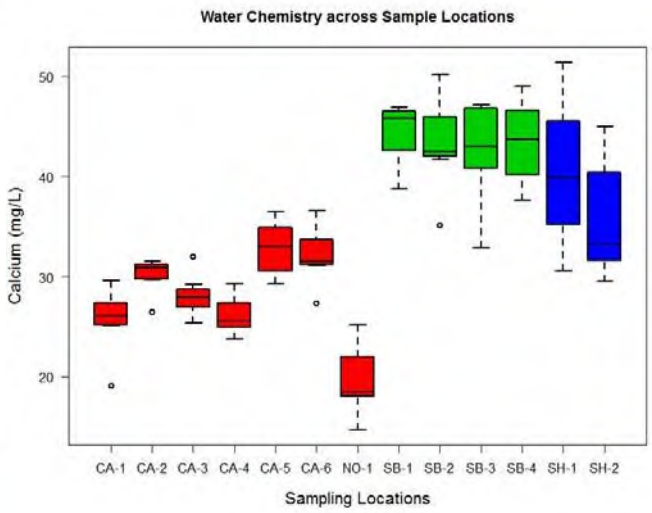
The sampling locations chosen to evaluate algal conditions on the Shenandoah, Cacapon, and South Branch Rivers are both convenient and spatially comprehensive. However, persistent algal blooms on the Cacapon River between Wardensville and Capon Bridge were observed but not captured by the locations currently sampled (Figure 5, Appendix C). We suggest adding a site in this reach to document these persistent blooms. In addition, due to the high nutrients levels observed below the Moorefield, W.Va. WWTP, and only proportional nutrient-assimilation observed between there and Romney, W. Va., we suggest adding sites downstream of Romney to observe any potential algal manifestations in that section of river.

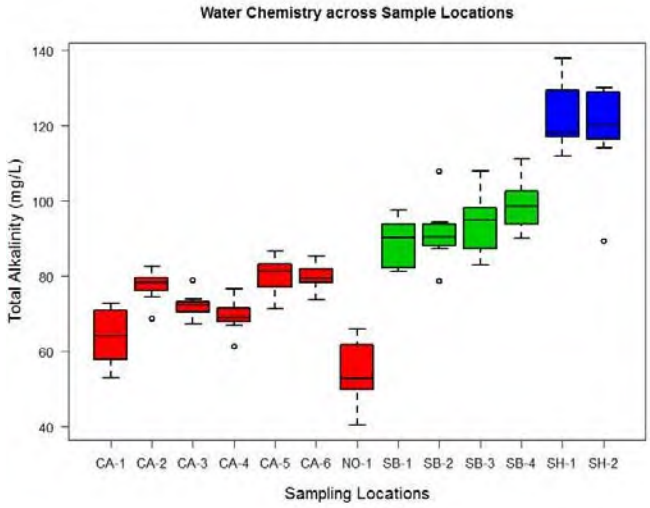
Figure 5. Filamentous algae on the Cacapon River between Wardensville and Capon Bridge, W.Va.

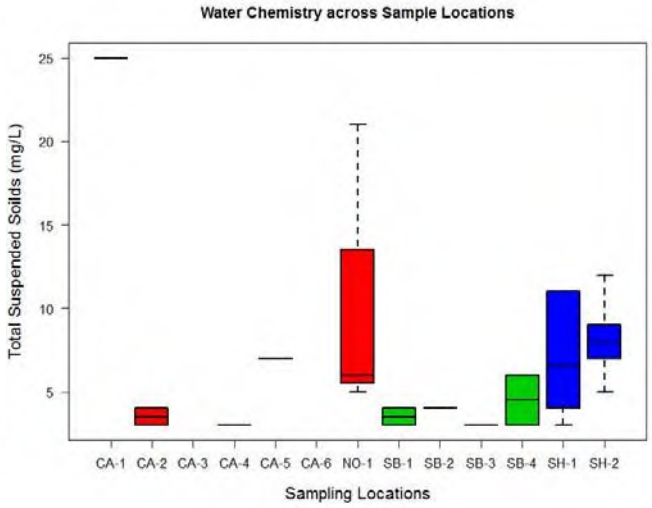
The sampling period for this study extends from June – October, however, due to delays in contracting and securing the required equipment, sampling did not begin until mid-July. Algae abundance seemed to be at a peak during the first sampling round and generally declined throughout the season. We suggest implementing the sampling protocol by June 1 and perhaps as early as May to better capture the manifestation of algal blooms and to document conditions leading to their emergence.

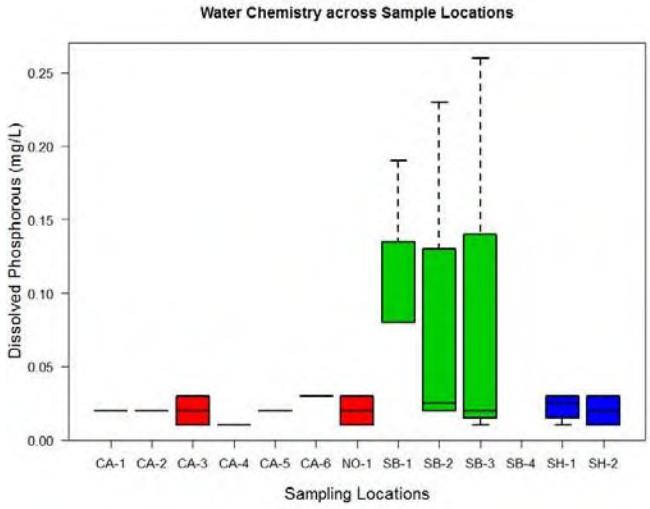
Appendix A Water Chemistry Plots across Space and Time

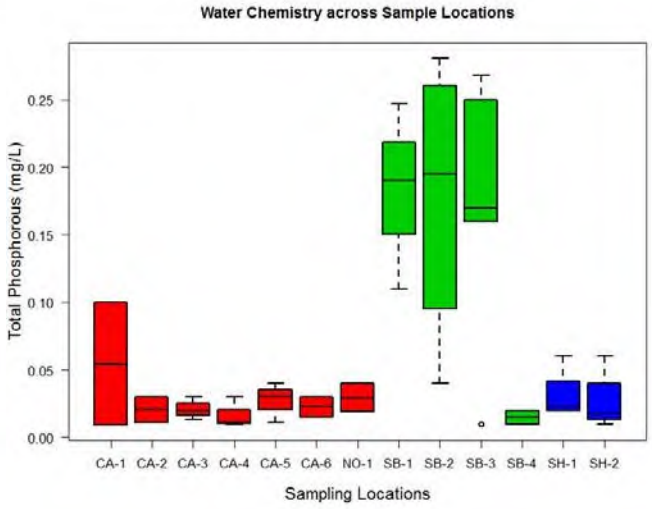


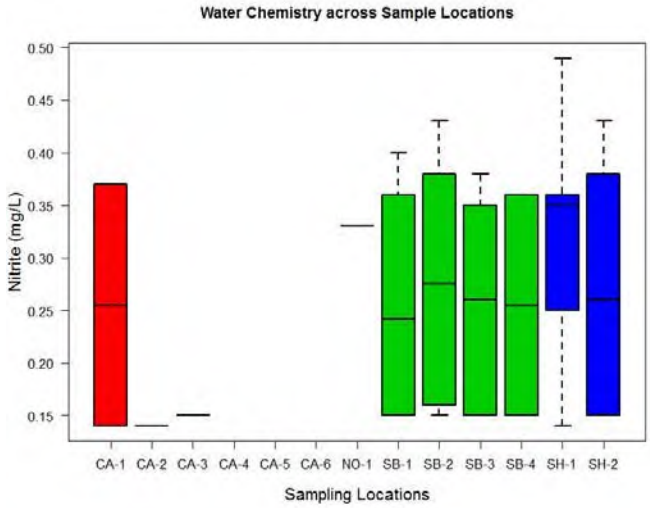


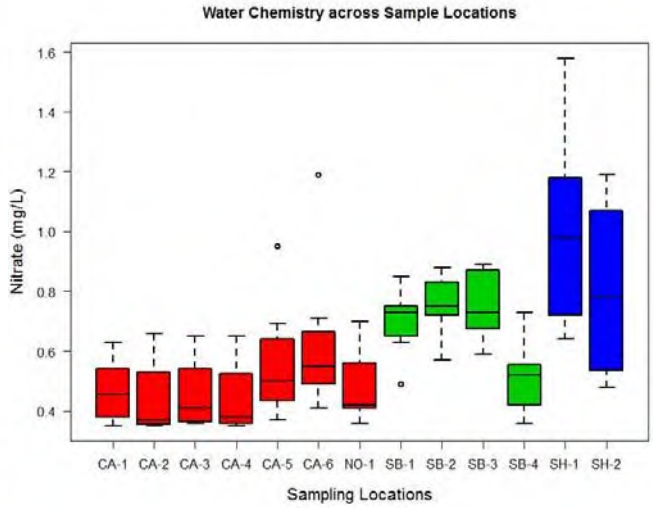


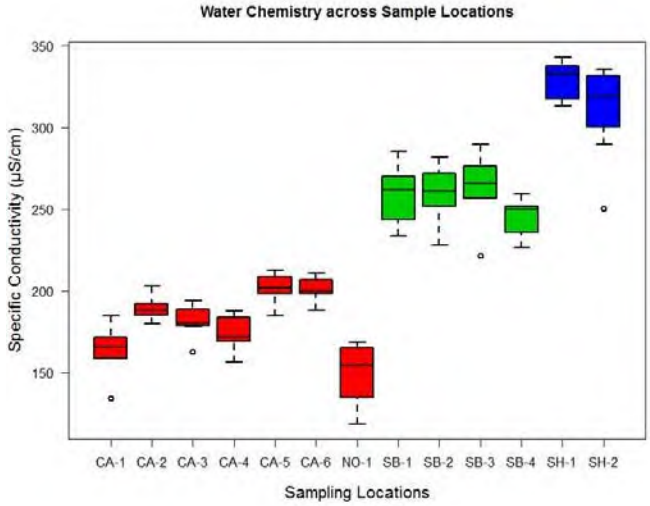


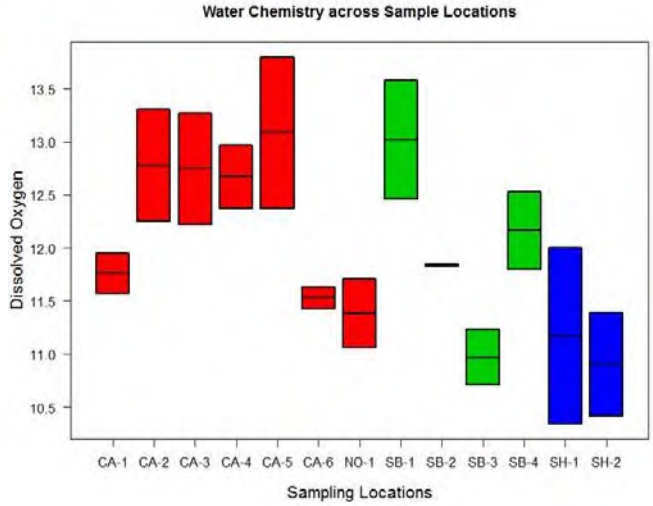


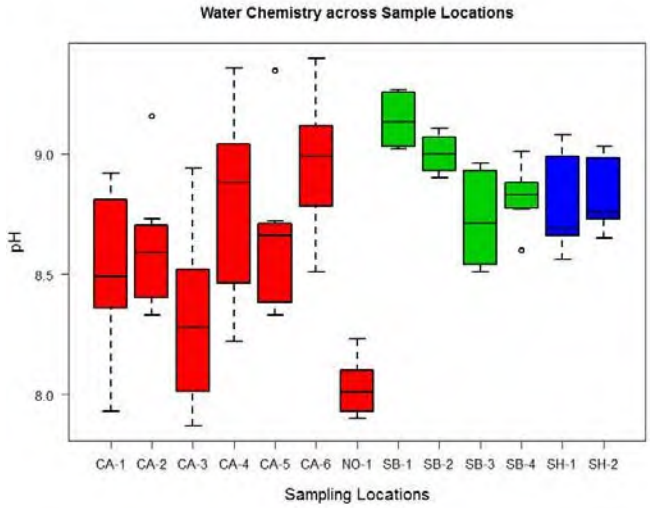


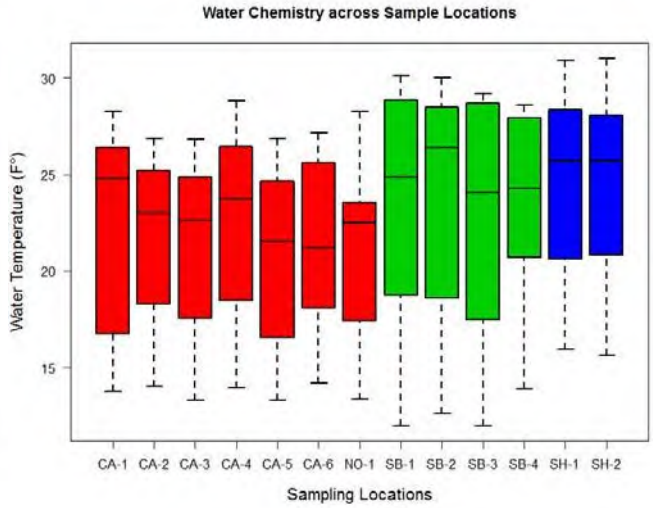

Water Chemistry across Sample Locations
Nitrite (mg/L)
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
CA-1 CA-2 CA-3 CA-4 CA-5 CA-6 NO-1 SB-1 SB-2 SB-3 SB-4 SH-1 SH-2
Sampling Locations

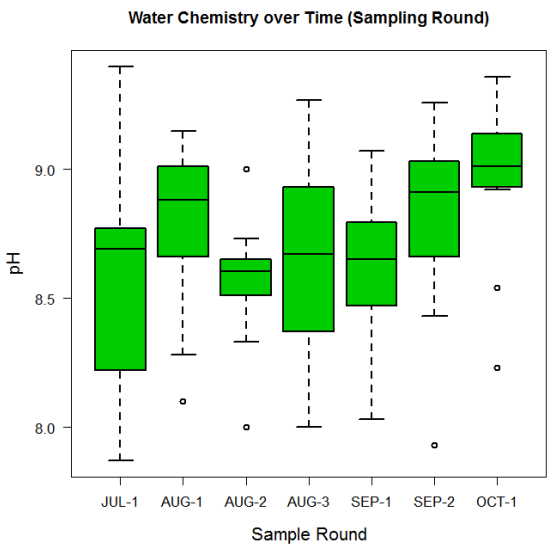

Water Chemistry across Sample Locations
Nitrate (mg/L)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
CA-1 CA-2 CA-3 CA-4 CA-5 CA-6 NO-1 SB-1 SB-2 SB-3 SB-4 SH-1 SH-2
Sampling Locations

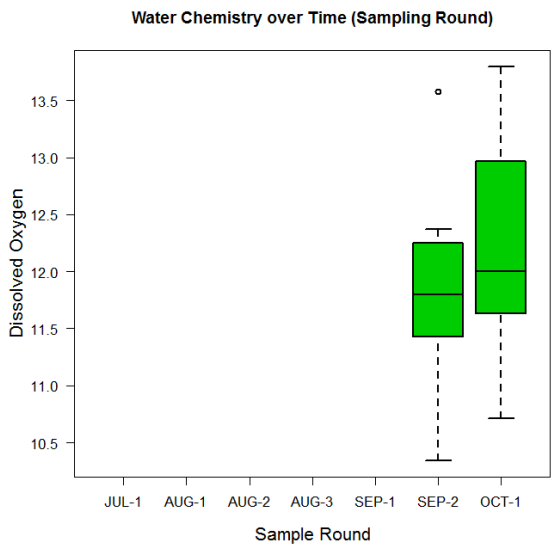

Water Chemistry across Sample Locations
Specific Conductivity (µS/cm)
350
300
250
200
150
CA-1 CA-2 CA-3 CA-4 CA-5 CA-6 NO-1 SB-1 SB-2 SB-3 SB-4 SH-1 SH-2
Sampling Locations

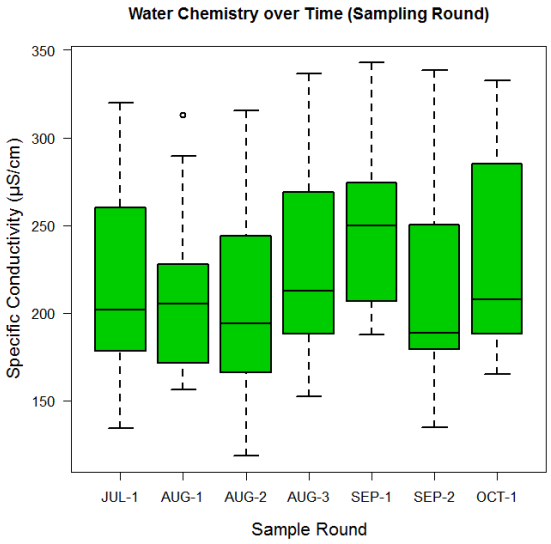

Water Chemistry across Sample Locations
Dissolved Oxygen
13.5
13.0
12.5
12.0
11.5
11.0
10.5
CA-1 CA-2 CA-3 CA-4 CA-5 CA-6 NO-1 SB-1 SB-2 SB-3 SB-4 SH-1 SH-2
Sampling Locations

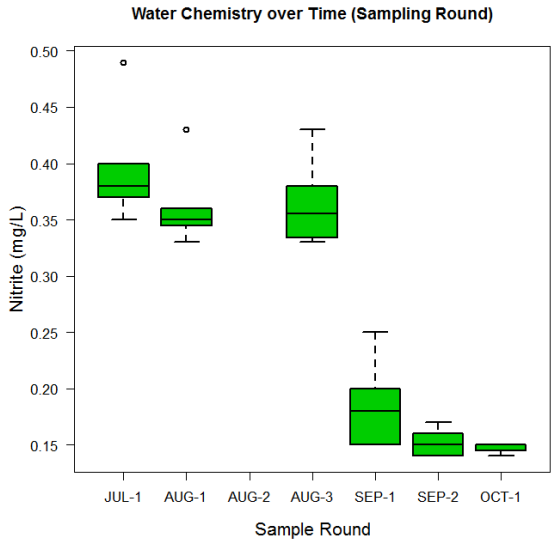

Water Chemistry across Sample Locations
pH
9.0
8.5
8.0
CA-1 CA-2 CA-3 CA-4 CA-5 CA-6 NO-1 SB-1 SB-2 SB-3 SB-4 SH-1 SH-2
Sampling Locations

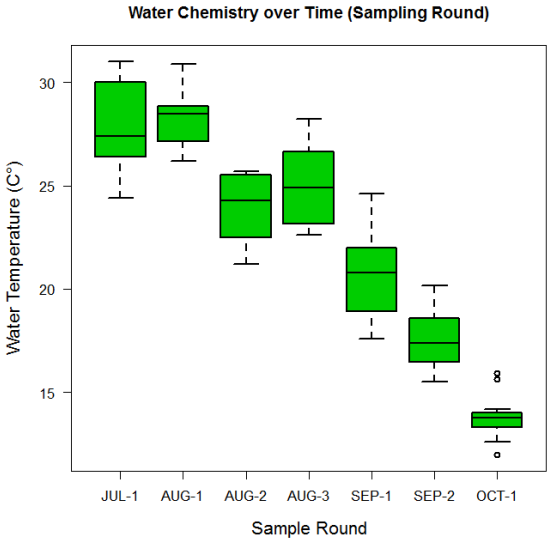

Water Chemistry across Sample Locations
Water Temperature (F°)
30
25
20
15
CA-1 CA-2 CA-3 CA-4 CA-5 CA-6 NO-1 SB-1 SB-2 SB-3 SB-4 SH-1 SH-2
Sampling Locations

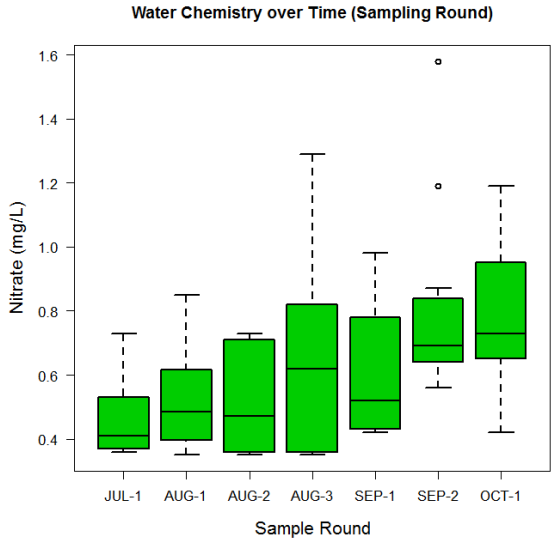

Water Chemistry over Time (Sampling Round)
pH
9.0
8.5
8.0
JUL-1
AUG-1
AUG-2
AUG-3
SEP-1
SEP-2
OCT-1
Sample Round

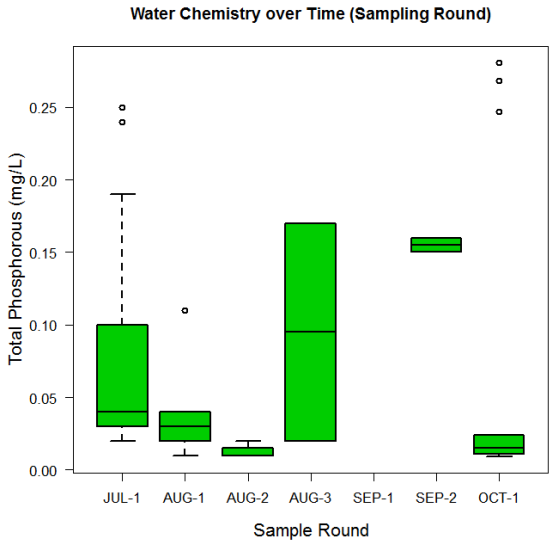

Water Chemistry over Time (Sampling Round)
Dissolved Oxygen
13.5
13.0
12.5
12.0
11.5
11.0
10.5
JUL-1
AUG-1
AUG-2
AUG-3
SEP-1
SEP-2
OCT-1
Sample Round

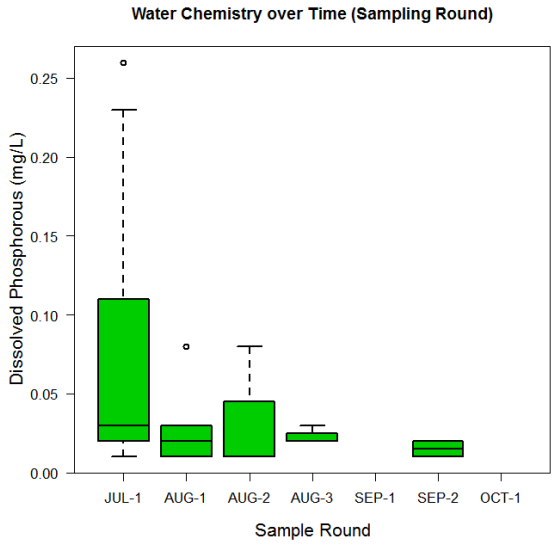

Water Chemistry over Time (Sampling Round)
Specific Conductivity (µS/cm)
350
300
250
200
150
JUL-1
AUG-1
AUG-2
AUG-3
SEP-1
SEP-2
OCT-1
Sample Round

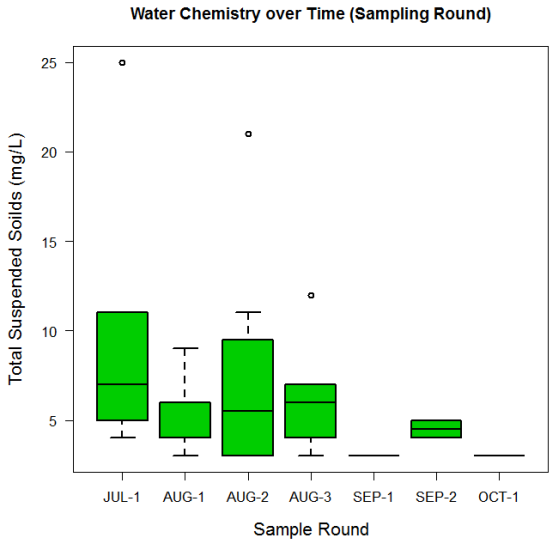

Water Chemistry over Time (Sampling Round)
Nitrite (mg/L)
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
JUL-1
AUG-1
AUG-2
AUG-3
SEP-1
SEP-2
OCT-1
Sample Round

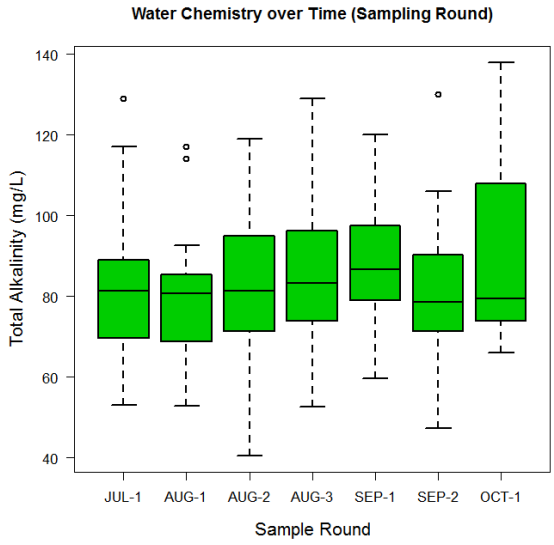

Water Chemistry over Time (Sampling Round)
Water Temperature (C°)
30
25
20
15
JUL-1
AUG-1
AUG-2
AUG-3
SEP-1
SEP-2
OCT-1
Sample Round

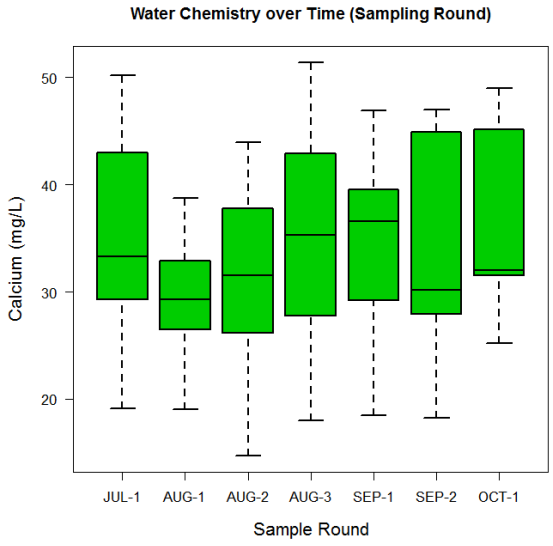

Water Chemistry over Time (Sampling Round)
Nitrate (mg/L)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
JUL-1
AUG-1
AUG-2
AUG-3
SEP-1
SEP-2
OCT-1
Sample Round

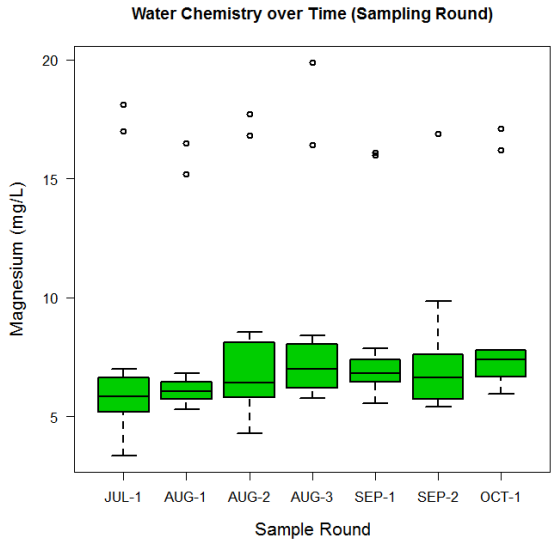

Water Chemistry over Time (Sampling Round)
Total Phosphorous (mg/L)
0.00
0.05
0.10
0.15
0.20
0.25
JUL-1
AUG-1
AUG-2
AUG-3
SEP-1
SEP-2
OCT-1
Sample Round


Water Chemistry over Time (Sampling Round)
Dissolved Phosphorous (mg/L)
0.00
0.05
0.10
0.15
0.20
0.25
JUL-1
AUG-1
AUG-2
AUG-3
SEP-1
SEP-2
OCT-1
Sample Round

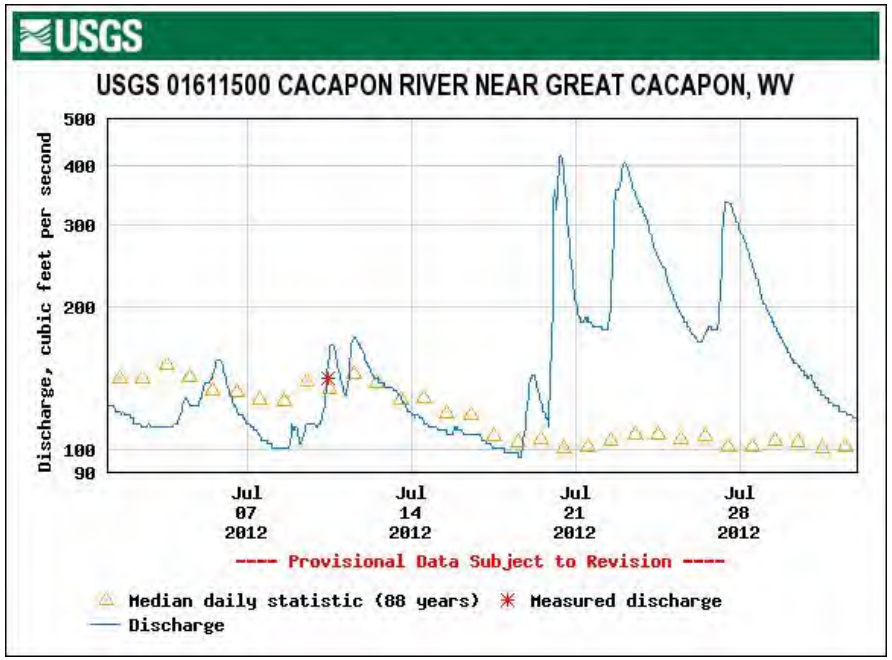

Water Chemistry over Time (Sampling Round)
Total Suspended Soilds (mg/L)
5
10
15
20
25
JUL-1
AUG-1
AUG-2
AUG-3
SEP-1
SEP-2
OCT-1
Sample Round

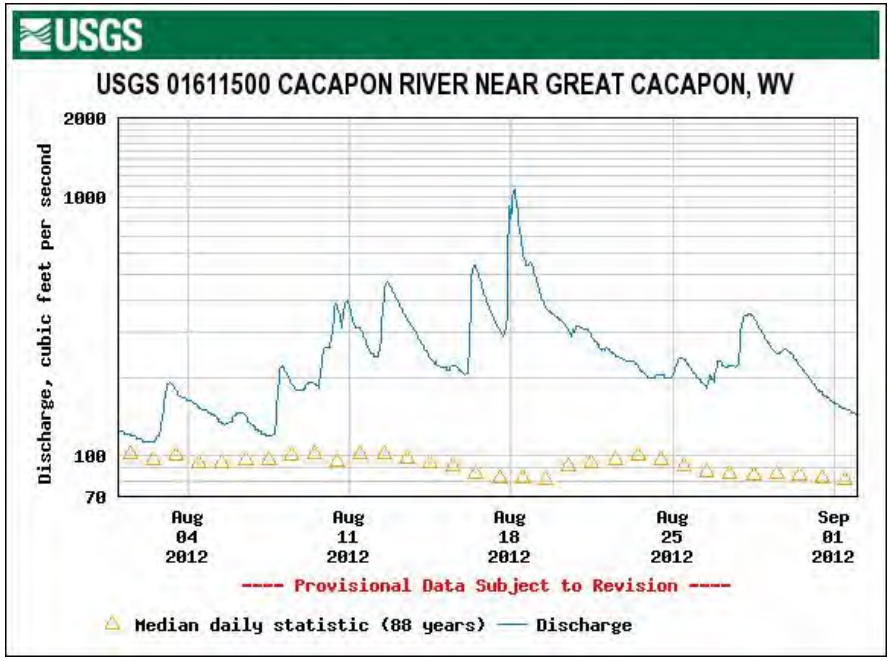

Water Chemistry over Time (Sampling Round)
Total Alkalinity (mg/L)
40
60
80
100
120
140
JUL-1
AUG-1
AUG-2
AUG-3
SEP-1
SEP-2
OCT-1
Sample Round

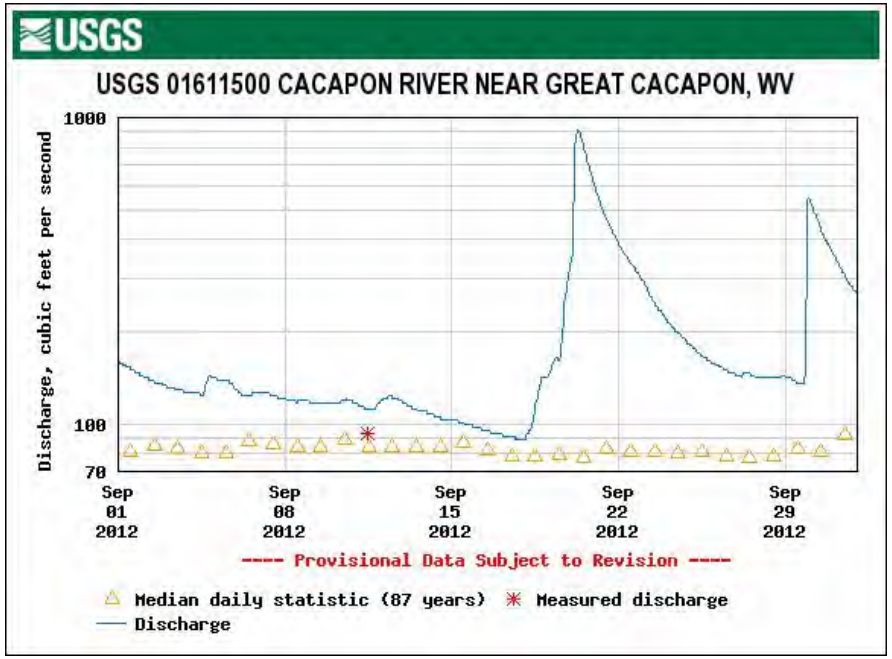

Water Chemistry over Time (Sampling Round)
Calcium (mg/L)
20
30
40
50
JUL-1
AUG-1
AUG-2
AUG-3
SEP-1
SEP-2
OCT-1
Sample Round

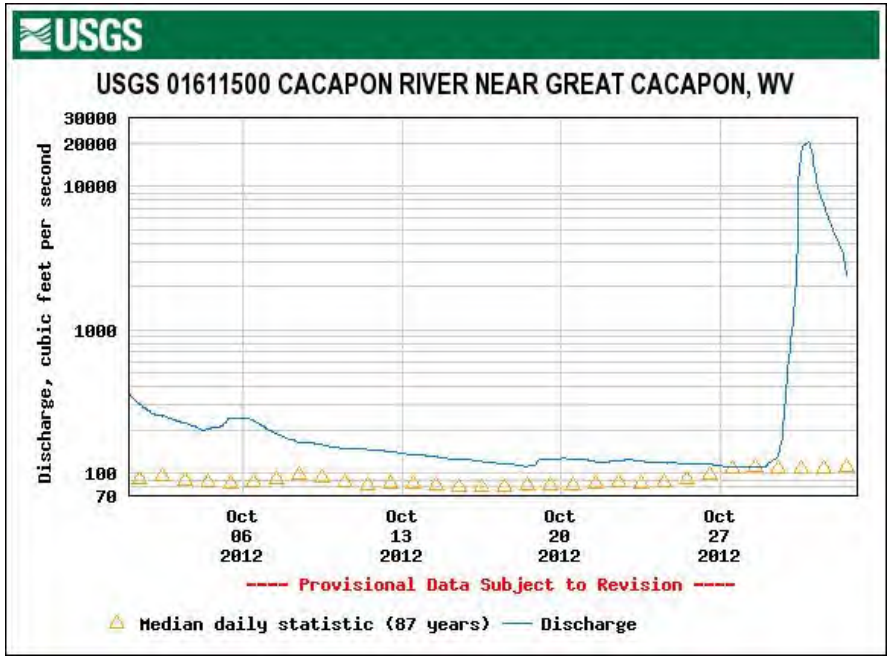

Water Chemistry over Time (Sampling Round)
Magnesium (mg/L)
5
10
15
20
JUL-1
AUG-1
AUG-2
AUG-3
SEP-1
SEP-2
OCT-1
Sample Round

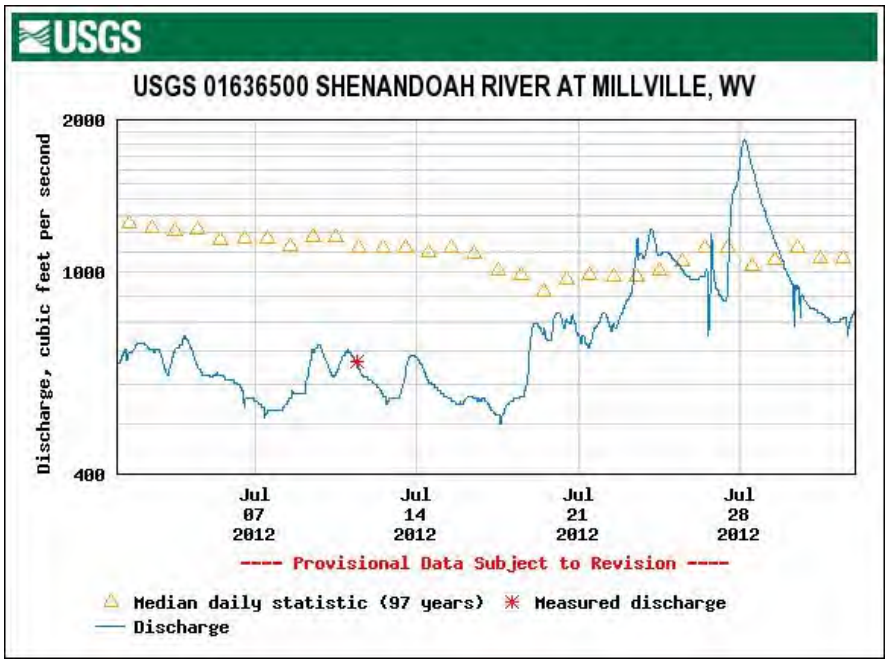
Appendix B Hydrographs of relevant USGS stream gages over the study period

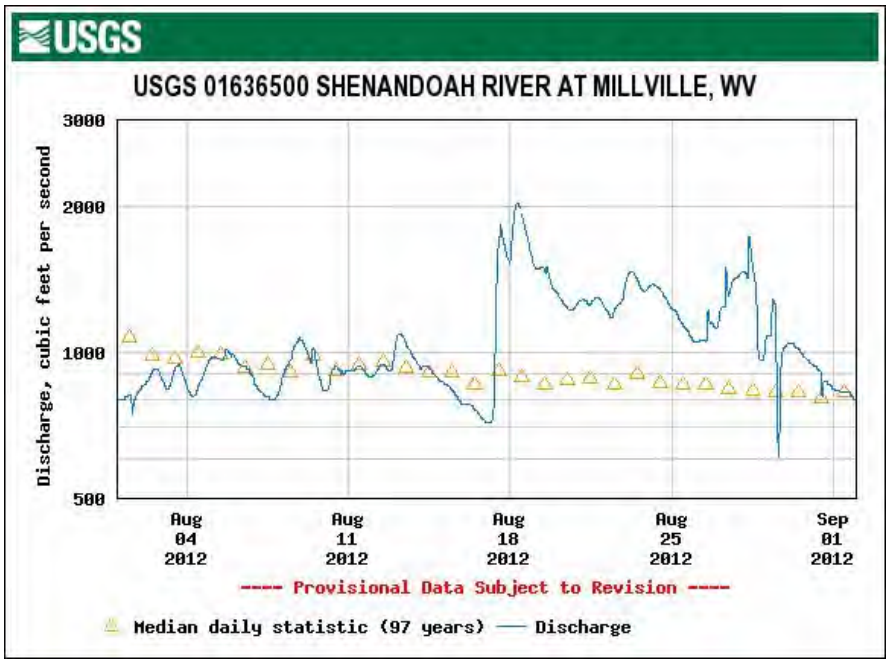


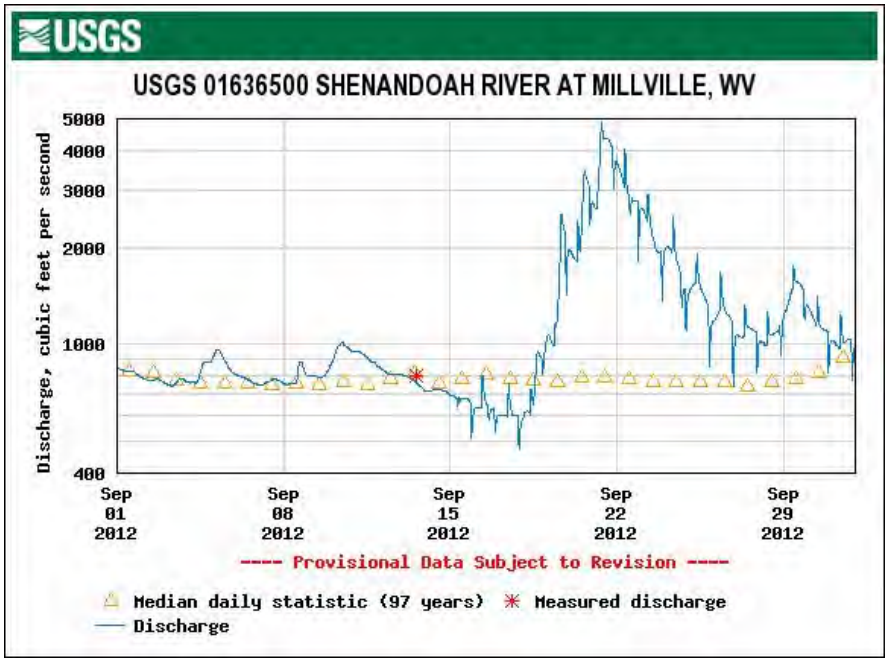


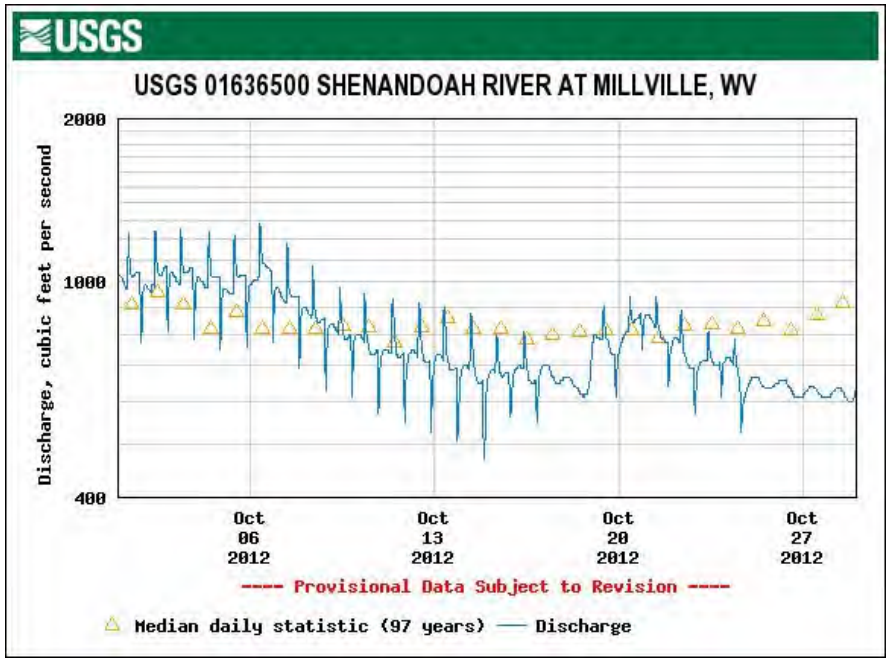


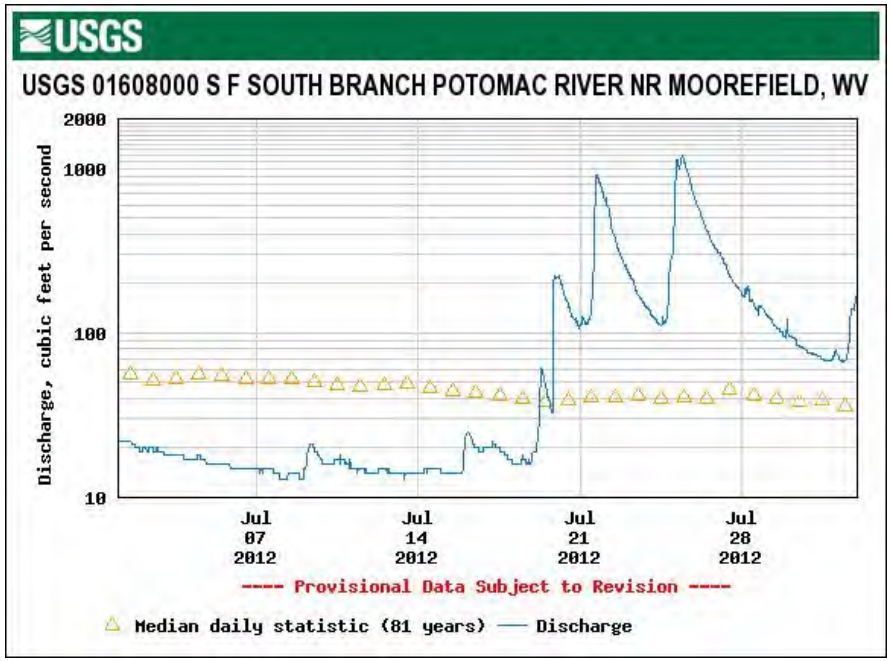


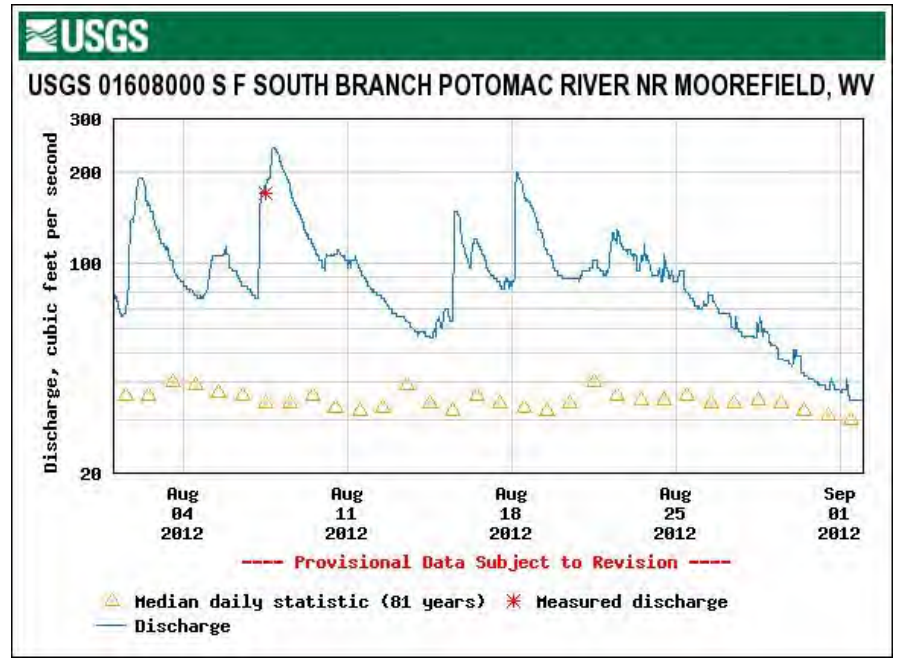

USGS
USGS 01636500 SHENANDOAH RIVER AT MILLVILLE, WV
Discharge, cubic feet per second
2000
1000
400
Jul 07 2012
Jul 14 2012
Jul 21 2012
Jul 28 2012
---- Provisional Data Subject to Revision ----
Median daily statistic (97 years)
Measured discharge
Discharge

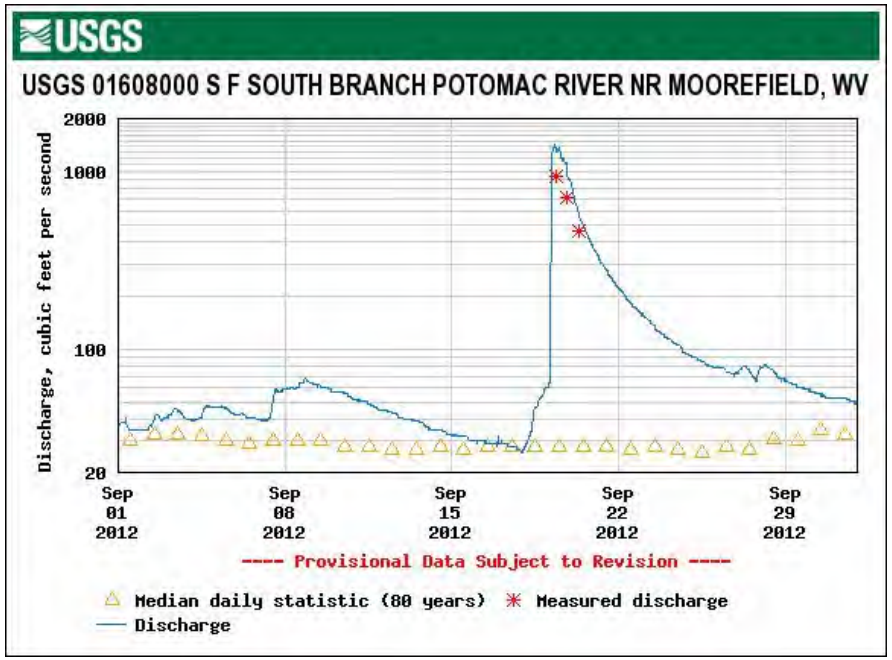

USGS
USGS 01636500 SHENANDOAH RIVER AT MILLVILLE, WV
Discharge, cubic feet per second
3000
2000
1000
500
Aug 04 2012
Aug 11 2012
Aug 18 2012
Aug 25 2012
Sep 01 2012
---- Provisional Data Subject to Revision ----
Median daily statistic (97 years)
Discharge

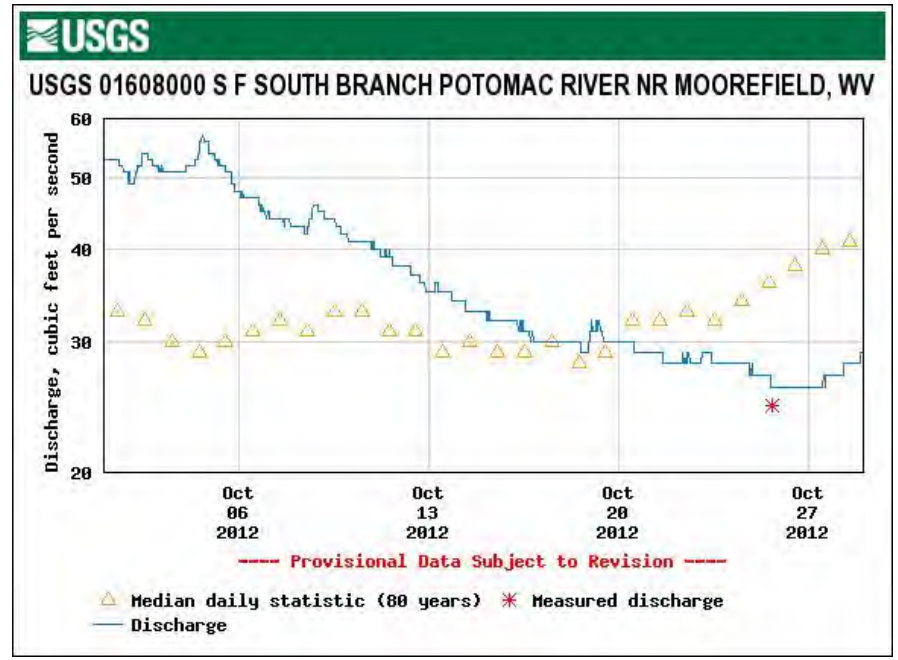

USGS
USGS 01636500 SHENANDOAH RIVER AT MILLVILLE, WV
Discharge, cubic feet per second
5000
4000
3000
2000
1000
400
Sep 01 2012
Sep 08 2012
Sep 15 2012
Sep 22 2012
Sep 29 2012
---- Provisional Data Subject to Revision ----
Median daily statistic (97 years)
Measured discharge
Discharge


USGS
USGS 01636500 SHENANDOAH RIVER AT MILLVILLE, WV
Discharge, cubic feet per second
2000
1000
400
Oct 06 2012
Oct 13 2012
Oct 20 2012
Oct 27 2012
---- Provisional Data Subject to Revision ----
Median daily statistic (97 years)
Discharge


USGS
USGS 01608000 S F SOUTH BRANCH POTOMAC RIVER NR MOOREFIELD, WV
Discharge, cubic feet per second
2000
1000
100
10
Jul 07 2012
Jul 14 2012
Jul 21 2012
Jul 28 2012
---- Provisional Data Subject to Revision ----
Median daily statistic (81 years)
Discharge


USGS
USGS 01608000 S F SOUTH BRANCH POTOMAC RIVER NR MOOREFIELD, WV
Discharge, cubic feet per second
300
200
100
20
Aug 04 2012
Aug 11 2012
Aug 18 2012
Aug 25 2012
Sep 01 2012
---- Provisional Data Subject to Revision ----
Median daily statistic (81 years)
Measured discharge
Discharge


USGS
USGS 01608000 S F SOUTH BRANCH POTOMAC RIVER NR MOOREFIELD, WV
Discharge, cubic feet per second
2000
1000
100
20
Sep 01 2012
Sep 08 2012
Sep 15 2012
Sep 22 2012
Sep 29 2012
---- Provisional Data Subject to Revision ----
Median daily statistic (80 years)
Measured discharge
Discharge


USGS
USGS 01608000 S F SOUTH BRANCH POTOMAC RIVER NR MOOREFIELD, WV
Discharge, cubic feet per second
60
50
40
30
20
Oct 06 2012
Oct 13 2012
Oct 20 2012
Oct 27 2012
---- Provisional Data Subject to Revision ----
Median daily statistic (80 years)
Measured discharge
Discharge

Appendix C. Representative Photographs and Algal Documentation of the Survey Sites

ShenandoahRiver at Rt. 340 (Lower)

Filamentous algae observed on emergent vegetation (8/5/2012)

Shenandoah River at Ann Lewis Road (Upper)

Benthic substrate devoid of algae or SAV

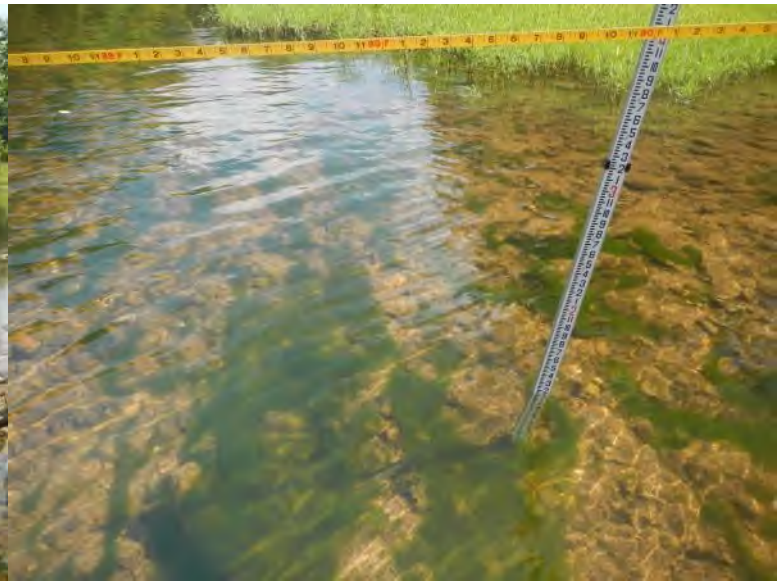
Cacapon River at Largent (Rt. 9)

Patches of *Hydrilla* at bank.

North River at Forks of Cacapon (Gaston Rd.)

The unidentified vascular plant that dominated the site.

Cacapon River at Forks of Cacapon (Rt. 127)



The peak of FGA abundance observed at the site (8/16/2012)

Cacapon River below Capon Bridge (Cold Stream Rd.)

Minor FGA observed on cobble substrate (8/16/2012).

Cacapon River at Capon Bridge (Rt. 9)

Water stargrass and cobble substrate. No FGA observed.

Cacapon River below Camp RimRock (Capon River Rd.)

Persistent benthic and water column algae (8/4/2012)

Cacapon River below Wardensville (Rt. 259)

Abundant periphyton with benthic FGA on cobble (8/4/2012)

Cacapon River above Wardensville (Rt. 48/55/259)

The peak of algae abundance observed (8/4/2012)

South Branch Potomac at Harmison's Landing

Water stargrass beds with minor attached FGA (7/18/2012)

South Branch Potomac at Trough put-in (S. Br. River Rd.)

Water stargrass and periphyton on cobble.

South Branch Potomac below Moorefield (Rt. 28/220)

Extreme SAV coverage by stargrass, milfoil, et al. (7/18/2012)

South Branch Potomac above Moorefield (Fisher Rd.)

Abundant FGA observed on first visit (7/18/2012)

WATER QUALITY AND PERIPHYTIC ALGAE COMMUNITY OF PETANI RIVER BASIN, KEDAH

by

HAZZEMAN HARIS

Thesis submitted in fulfilment of the requirements for the degree of Master of Science

JUNE 2009

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LIST OF ABBREVIATIONS

Abbreviation	Caption
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
DOE	Department of Environment
dwt	Dry weight
FSI	Fine Sediment Index
FSW	Fine Sediment Weight
ISI	Important Species Index
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
WQI	Water Quality Index

KUALITI AIR DAN KOMUNITI ALGA PERIFITIK DI LEMBANGAN SUNGAI PETANI, KEDAH

ABSTRAK

Objektif kajian ini ialah untuk menentukan status kualiti air di Lembangan Sungai Petani berdasarkan kepada klasifikasi yang digunakan oleh Jabatan Alam Sekitar Malaysia dan membandingkannya dengan ketepatan penggunaan alga perifiton sebagai penunjuk biologi bagi kualiti air. Sampel air dan alga perifiton diambil dari 6 stesen persampelan A hingga F berdasarkan pengaliran air dari hulu ke hilir Sungai Petani yang mempunyai tahap pencemaran yang berbeza di sepanjang Lembangan Sungai Petani selama 12 bulan. Oksigen terlarut (DO), permintaan oksigen biokimia (BOD), permintaan oksigen kimia (COD), jumlah pepejal terampai (TSS), pH dan ammonium diukur untuk pengiraan Indeks Kualiti Air (WQI). Parameter seperti alkaliniti, nitrit, nitrat, ortofosfat, saliniti, dan jumlah pepejal terlarut (TDS) turut ditentukan untuk mendapatkan gambaran yang lebih tepat mengenai kualiti air di Sungai Petani. Stesen B mencatatkan nilai WQI yang tertinggi (60.49), diikuti oleh Stesen F (59.56), Stesen C (57.13), Stesen D (56.92), Stesen E (55.20) dan Stesen A (55.07). Secara amnya kualiti air sungai semakin merosot apabila air mengalir dari hulu ke muara, kecuali di Stesen F. Kualiti air sungai yang melalui kawasan perumahan (Stesen B) dan air di muara sungai (Stesen F) didapati lebih bersih berbanding air sungai yang melalui kawasan perindustrian dan pusat bandar (Stesen C, D dan E). Nutrien seperti nitrit, nitrat dan ammonium adalah tinggi di sungai yang melalui kawasan perindustrian (Stesen C), manakala ortofosfat adalah lebih tinggi di sungai yang melalui kawasan perumahan dan pertanian (Stesen A). TSS pula tinggi di stesen yang mengalami hakisan (Stesen A dan Stesen F). Kajian

ini mendapati air pasang besar mempunyai kesan yang lebih jelas terhadap parameter fisiko-kimia air berbanding ketika air pasang mati. Ketika air pasang besar, peningkatan dan penurunan kandungan fisiko-kimia air berubah dengan lebih ketara sepanjang kitaran pasang-surut air berbanding ketika air pasang mati. Klorofil *a* dan berat kering tanpa abu (AFDW) digunakan untuk pengiraan Indeks Autotrofik (AI), manakala berat sedimen halus (FSW) diukur untuk pengiraan Indeks Sedimen Halus (FSI). AI yang tinggi di Stesen C menunjukkan ianya didominasi oleh organisma heterotrofik dan mempunyai kualiti air yang rendah. Keputusan ini disokong oleh hasil analisis korelasi Pearson yang mendapati kepekatan nutrien mempunyai hubungan yang positif dengan AI. Pengiraan indeks kepelbagaian Simpson, Shannon-Weiner, Margalef dan Menhinick berdasarkan kepelbagaian dan kekayaan perifiton menunjukkan Stesen E dan Stesen F (berair payau) mempunyai kepelbagaian spesies alga yang tinggi. FSW juga didapati mempunyai kesan yang positif terhadap kepelbagaian spesies. Spesies seperti *Climacosphenia moniligera, Closterium* sp. dan *Mischococcus confervicola* hanya dijumpai di Stesen C. Oleh itu spesies tersebut mempunyai potensi digunakan sebagai penunjuk kualiti air yang mempunyai kandungan nutrien yang tinggi. Pengiraan indeks saprobik (SI) menunjukkan Stesen A (1.625) sebagai stesen paling tercemar. Keputusan ini adalah sama seperti keputusan yang diperolehi melalui pengiraan WQI sekaligus membuktikan keberkesanan perifiton sebagai penunjuk kualiti air.

WATER QUALITY AND PERIPHYTIC ALGAE COMMUNITY OF PETANI RIVER BASIN, KEDAH

ABSTRACT

This study was carried to determine the status of water quality in the Petani River Basin according to the classification used by the Malaysian Department of Environment (DOE) and to evaluate the reliability of periphyton algae as a biological indicator of water quality. Water samples and periphytic algae were collected from 6 sampling stations with varying level of pollution along the Petani River Basin. Dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), pH and ammonium were measured for the calculation of Water Quality Index (WQI). Parameters such as alkalinity, nitrite, nitrate, orthophosphate, salinity and total dissolved solids (TDS) were also determined. Station B (60.49) recorded the highest WQI, followed by Station F (59.56), Station C (57.13), Station D (56.92), Station E (55.20) and Station A (55.07). This showed that the water quality decreased as it flowed downstream except in Station F. Generally the water quality at Station B where it pass through residential areas and the water in the confluence (Station F) were cleaner as compared to water which flowed through industrial and town centre (Stations C, D, and E). Nutrients such as nitrite, nitrate and ammonium were high in river which flowed through industrial area (Station C), while orthophosphates was high in river which flow through residential and agricultural area (Station A). TSS was high at stations where erosion occurred (Stations A and F). It was found that spring tide has a bigger influence on water physico-chemical properties compared to during neap tide. During spring tide, the increase and decrease of water physico-chemical

properties were more obvious throughout the tide cycle as compared to during neap tide. Chlorophyll *a* and ash-free dry weight (AFDW) were used to calculate autotrophic index (AI), while fine sediment weight (FSW) were determined for the calculation of FSI. High AI in Station C indicates that it was dominated by heterotrophic organisms and had a poor water quality. This was supported by the result of Pearson's correlation analysis which found that nutrient concentration had a positive relationship with AI. The calculation of Simpson, Shannon-Weiner, Margalef and Menhinick diversity indices had shown Station E and F (brackish) as having the highest species diversity. FSW was also observed to have a positive effect on species diversity. Species such as *Climacosphenia moniligera, Closterium* sp. and *Mischococcus confervicola* can only be found in Station C. Thus those species may have a good potential as indicators of nutrient enriched water. The calculation of saprobic index indicated that Station A (1.625) as the most polluted station. This was in agreement with the results obtained through the WQI, thus enhancing the reliability of periphyton as an indicator of water quality.

CHAPTER 1

INTRODUCTION

1.1 Background

Malaysia has an annual rainfall of 3000 mm or 990 billion m^3 of which 566 billion m^3 are surface run-off, 64 billion m^3 become groundwater recharge and 360 billion m^3 return to the atmosphere through evapo-transpiration (Azhar, 2000). Being a nation with high water consumption, freshwater resources such as streams and rivers are of paramount importance to the development of the country. They contribute up to 98% of the total water used in Malaysia and the rest are from groundwater (Abdullah and Jusoh, 1997).

As the nation develops and increases in population, a serious water crisis such as pollution due to poor planning can cause environmental degradation and a decline in beneficial use of river (Madsen *et al.,* 2002). Therefore regardless of the abundance of water, there is simply a shortage to support the consumption of the population (Madsen *et al*., 2002). FitzHugh and Richter (2004) mentioned that quenching urban thirst of growing cities and balancing the thirst against all other freshwater needs is a major challenge.

The unequal distribution of freshwater resources around the world (Flemer and Champ, 2006) also makes things worse. This can be seen throughout the world where many countries are disputing over water resources. Currently there are disputes over the Nile Basin, the Mekong Basin and the Jordan Basin (Chan and Nitivattananon, 2006).

In Malaysia for instance, the increase water demand in Penang, has force it to become dependent on the water supply from the neighbouring state Kedah. It is expected that Penang will face water shortages by 2010 when its existing water production capacities will be outstripped by population and economic growth (Chan and Nitivattananon, 2006). If this condition persists, it is possible that freshwater may over the next several decades compete with petroleum as a limiting resource to socio-economic prosperity (Flemer and Champ, 2006). Some researchers also suggested that freshwater scarcity may even be the cause of political instability (Flemer and Champ, 2006).

In order to overcome this problem and increase the water supply, the government has build dams all over Malaysia, but the problem with building dams is its high cost. For example the Beris Dam in Kedah costs RM300 million and the Teluk Bahang Dam in Penang costs RM140 million to build (Chan and Nitivattananon, 2006). Apart from that, there are limited numbers of rivers where dams can be built. The Drainage and Irrigation Department (DID) estimated that there are about 255 river basins in Malaysia that have reached its water supply capacity (Keizrul, 2002) hence no more dams can be built in these rivers.

River pollution in cities and towns around the world are caused by anthropogenic influences as well as natural process. As the urban population continues to rise, the understanding of river-base flow and surface water quality for the urban area gains greater importance (Shepherd *et al*., 2006). The contamination of rivers will impair their use for drinking, industry, agriculture, recreation and other purposes (Sánchez *et al*., 2007).

In Malaysia, domestic sewage currently contributes to almost half of the organic pollutant load in the aquatic environment. It has been reported that the main pollution source of the Sarawak River was discharges from households (NREB, 2001; Ling *et al*., 2006). In Penang, 54% of the source of pollutant load to the rivers is effluent from Indah Water Konsortium (IWK) treatment plants (JAS, 2004; Harlina *et al*., 2006). From 120 river basins monitored in 2001, 60 basins (50%) were clean, 47 (39%) were slightly polluted and 13 (11%) were polluted (DOE, 2002). 51% of the pollution in these basins were from domestic sewage facilities, 39% from manufacturing industries, 7% from pig farms and 3% from agro-based industries (DOE, 2002). However the number of river basins which were clean in 2006 had improved. Out of 146 river basins monitored, 80 river basins (55%) were clean, 59 (40%) were slightly polluted and 7 (5%) were polluted (DOE, 2006).

At present the surface and groundwater of developed nations is experiencing elevated concentrations of nitrogen and phosphorus compared to about 50 years ago (Smith *et al.*, 2003; Flemer and Champ, 2006). Over enrichment by nitrogen and phosphorus has been known to encourage the growth of undesirable amount of aquatic plant growth. Some of the effects of over-enrichment includes hypoxia resulting in fish kills, shading out of sea grasses by periphyton and phytoplankton, loss of water clarity, reduction in biotic diversity, increase in algal species of poor food web quality and an increase in algal blooms (Flemer and Champ, 2006).

In Malaysia, most river water quality monitoring has been conducted in cities and states that are fully developed or economically prominent compared to smaller developing towns. This study focuses on the water quality of the Petani River that

flows through the developing town of Sungai Petani, Kedah. The status of water quality was determined using the Water Quality Index and the river classification used by the Malaysian Department of Environment (DOE). The reliability of periphytic algal as a bioindicator of river pollution was also studied.

1.2 The Importance of Biological Monitoring

Biological monitoring methods are playing an increasingly important role in river quality monitoring, mainly due to the fact that the biota are continuous witnesses of the river's state of health and are collectively sensitive to the whole range of potential pollutants (Walley *et al*., 2001; Iliopoulou-Georgudaki *et al.*, 2003.). Apart from that, traditional approaches which depend on laboratory tests have several weaknesses including the failure to validate laboratory results under field conditions (McCormick and Cairns, 1994). So a lot of efforts have been made to characterize the cumulative impact of human activities on ecosystem more accurately by increasing the use of measures of ecological condition as an addition to chemical indicator (McCormick and Cairns, 1994).

If the full potential of biological monitoring is to be realised, much work needs to be done to improve existing methods and to develop new methods based on advanced data interpretation methods (Walley *et al*., 2001).

The usage of algae as a biological indicator has been suggested by various studies as a complement to the traditional method of monitoring (McCormick and Cairns, 1994; Knoben *et al*., 1995; Masseret *et al*., 1998; Hillebrand and Sommer, 2000; Pipan, 2000; Rauch *et al*., 2006). Biological monitoring using algae as an

indicator can help provide unique information about the ecosystem which is potentially useful as an early warning sign of deteriorating condition and its possible causes (McCormick and Cairns, 1994).

1.3 Periphytic Algae

Algae are the simplest plant without roots, stem or leave and exist in variable sizes. There are microscopic algal cells which cause water to look green, while some of the algae are macroscopic with longer and branchy structures which make them visible to the naked eye. Usually micro algae can be seen in the form of green slime. The word 'diatom' is sometimes used to refer to singular cell algae that has fibrous silicate outer layer (Hammer, 1986).

Algal identification is carried out through microscopic observation (Hammer, 1986). According to Chapman and Chapman (1990), the most important criteria for the classification of algae are the differences in pigmentation, biochemical characteristic and the organelle structure such as flagellum.

The definition of 'periphyton' is normally used in scientific writing in America to describe the micro community that are attached and submerged underwater (Weitzel, 1979). According to Cooke (1956) and Sladeckova (1962), the first groups that used this definition were Russian scientists who referred periphyton as the assemblage of microorganism that grow or live on the surface of objects or artificial substrates that are submerged under water. Cooke (1956) also mentioned that in European and Asian writings, the definition of periphyton had been widened to cover all aquatic organisms that grow or live on submerged substrates.

Young (1945) defined periphyton as organisms that live on natural and artificial substrates, excluding benthos (Young, 1945; Weitzel, 1979).

Wetzel (1964) suggested that periphyton refers to all plants that grow on submerged substrates. The submerged substrates can be sediment, rock, thrash or rubbish and living organisms (Wetzel, 1964; Vollenweider, 1969; Weitzel, 1979).

Apart from the word periphyton, there is another word that can be used to represent this kind of assemblages. The German word 'Aufwuchs' was first used to describe organisms that grow or attach on certain substrates but do not grow into or through the substrates (Weitzel, 1979). Ruttner (1953) later defined 'Aufwuchs' as all organisms that are strongly attached on a substratum but not through it.

There are several qualifying terms that are used in referring to the different types of periphytic algal community associated with different type of substrates. They are epilithic (growing on rocks), epipelic (growing on mud or sediments), epiphytic (growing on plants), epizoic (growing on animal), epidendric (growing on wood) and epipsammic (growing on sand surfaces) (Weitzel, 1979).

1.3.1 Periphytic algae as Bioindicator

Periphyton is a complex microcosm composed of living, senescent and dead autotrophic (microalgae) and heterotrophic microorganisms (bacteria, fungi, protozoa and micrometazoa), and fine particulates, enveloped in a polysaccharide matrix of biological origin (Neckles *et al*., 1994; Masseret *et al.,* 1998).

Periphytic algae were chosen as a bioindicator in this study because it can reflect the environmental condition in the recent pass (Weitzel, 1979; Masseret *et al*., 1998). Its position which is at the interface between the substrate and the water combined with the fundamental role it plays in the various biogeochemical cycles and dynamics of the aquatic ecosystem (Amblard *et al*., 1990; Hansson, 1990; Masseret *et al*., 1998) enables periphyton to be an integrated source of information by serving as an indicator of both chemical and physical stresses in aquatic ecosystem (Weber and McFarland, 1981; Masseret *et al*., 1998). Apart from that, periphyton has been recognized as being among the best biological indicator (Prygiel and Coste, 1993; Stevenson and Lowe, 1986; Hürlimann and Schanz, 1993; Masseret *et al*., 1998). The sessile nature and fast growth rate of diatoms (Stevenson and Lowe, 1986) makes it useful in studying the impact of various forms of pollution such as the discharge of wastewater, treated sewage effluents, organic and inorganic nutrients (Hürlimann and Schanz, 1993; Masseret *et al*., 1998).

Periphyton has been included in pollution monitoring in several countries such as Canada (Vis *et al*., 1998), Western Australia (Cosgrove *et al*, 2004) and Florida in the USA (Notestein *et al*., 2003). Diatoms have been used to provide an integrated measure of the effects of a variety of effluents in the natural environment (Fjerdingstad, 1964; Patrick, 1973; Stevenson and Lowe, 1986; Lowe and Pan, 1996; Vis *et al.,* 1998). Currently, European countries are developing indices to monitor eutrophication (Kelly and Whitton, 1998) and so does Malaysia (Nather Khan, 1991; Wan Maznah and Mansor, 2000, 2002), while the U.S is incorporating algal sampling into their routine monitoring program (Rosen, 1995; Charles, 1996; Hill *et al*., 2000; Leland and Porter, 2000; Fore and Grafe, 2002).

Various ways were used to determine water quality using periphyton. There are two approaches that are usually used for evaluating the impact of pollution on the periphyton community. The first one is by studying changes in the population structure by measuring a representative of the biomass (Welch *et al*, 1992; Masseret *et al*., 1998; Yamada and Nakamura, 2002; Cosgrove *et al*., 2004). The second is through the species composition by measuring the species richness or diversity of the communities (Nather Khan, 1991; Cattaneo *et al*., 1995; Kelly and Whitton, 1995; Pan *et al*., 1996; Masseret *et al*., 1998; Winter and Duthie, 2000; Soininen and Niemelä, 2002; Potapova *et al*., 2005), calculating indices based on community composition such as saprobic indec (Walley *et al*., 2001; Matsché and Kreuzinger, 2004), diatom quality index (Sgro *et al*., 2007) and etc.

However there are some problems in using periphyton as an indicator. This is because its reaction to the various physico-chemical and environmental condition may vary depending on the level of disturbance (McCormick and Cairns, 1994; Fore and Grafe, 2002).

Apart from that, as a living organism, the sensitivity of algae may vary depending on various environmental factors such as chelating agents, nutrient concentration, abiotic and biotic parameters (McCormick and Cairns, 1994). It has been known that prior exposure to environmental pollution can alter the sensitivity of periphyton (Niederlehner and Cairns, 1993; McCormick and Cairns, 1994). Foster (1982a, b), Blanck and Wängberg (1988) also mentioned that periphyton may exhibit decreased sensitivity to a stressor due to chronic exposure.

The lack of information about periphyton responses to human-induced degradation also posed a problem as compared to other works on fish or invertebrates as a monitoring tool for biological assessment of lotic waters (Rosen, 1995; Whitton and Kelly, 1995; Davis *et al*., 1996; Hill *et al*., 2000; Fore and Grafe, 2002).

This study is aimed at increasing the information concerning the effects of various anthropogenic stressors on periphyton in the natural environment using natural substrate. This is important if autoecological indices were to be used routinely for monitoring purposes in the future. Apart from that, this study also helps to increase the knowledge on the reliability of using periphyton as an indicator of water quality and its consistency under varying field conditions.

1.4 Objectives

The objectives of this study are:

1. To determine the status of water quality in the Petani River Basin based on the classification used by the Department of Environment of Malaysia.
2. To study the reliability of periphytic algal composition and community structure as a bioindicator of water quality degradation.
3. To generate a checklist of periphyton species in the Sungai Petani River Basin.
4. To study the effect of water quality and habitat suitability for periphyton species in the Sungai Petani River Basin.

CHAPTER 2

LITERATURE REVIEW

2.1 Factors Affecting the Growth and Development of Periphyton

The growth and development of periphytic algal community depends a lot on abiotic factors (Table 2.1):

Table 2.1. Abiotic factors affecting the growth and development of periphytic algal community.

Factors	References
• Type of water bodies (eg. lake, stream or river); light availability (water turbidity and clarity); • Types of substrate, the depth where the substrate is found; • Water movement, current and water velocity; • pH, alkalinity and water hardness; • The amount of nutrient (phosphorus, nitrogen and carbon)	Bothwell, 1985; Lohman *et al.,* 1991; Pan and Lowe, 1994; Dodds *et al.,* 1997; Winter and Duthie 2000; Mosisch *et al.,* 2001; Stelzer and Lamberti, 2001; Notestein at al., 2003.
• Other dissolved nutrients (calcium, sulfur and silicon); • The presence of metal and trace metal (eg. ferum, cuprum, chromium, boron, vanadium and selenium)	Sigmon *et al.,* 1977; Thomas and Seibert, 1977; Pratt *et al.,* 1987; Pratt and Bowers, 1990; Scanferlato and Cairns, 1990; McCormick and Cairns, 1994; Tang *et al*., 2003; Boivin *et al*., 2006.
• Temperature, salinity, oxygen and carbon dioxide	Walsh, 1972; Weitzel, 1979; Kosinski, 1984; Herbst and Blinn, 1998; Segal *et al*., 2006; Shun *et al*., 2008.

2.1.1 Light

One of the factors that affects the growth of periphytic algae is the presence of light. Aufwuch and periphyton are defined as micro communities that grow in the zone where the penetration of light is possible (euphotic zone) (Weitzel, 1979). Any factors that influence the amount of light reaching the surface of substrate play an

important role on the growth of periphytic algae (Weitzel, 1979; Meulemans, 1987; Paul and Duthie, 1989; Dodds, 1989; Kuhl and Jorgensen, 1994; Dodds *et al.,* 1999; Glud *et al.,* 1999; Guasch *et al.,* 2003).

A study by Evans and Stockner (1972) at Lake Winnipeg, Manitoba, found that most of the periphytic biomass accumulation occurs at a depth between 10 cm to 25 cm. The study also found that blue-green algae dominated the area at a depth of 0 cm to 10 cm. Species such as *Synedra ulna v. contracta* (Ehrenberg), *Gomphonema constrictum v. capitata* (Ehr.) Cleve, *Rhoicosphena curvata* (Kuetz.) Grunow and *Nitzschia* cf. *fonticola* (Grunow) were found at the depth between 5 cm to 25 cm, while *Navicula gracilis* (Ehrenberg) [= *N. tripunctata* (O. F. Muell.) Bory *v. tripunctata*] and *N. cryptocephala* (Kuetzing) were mostly found at a depth between 90 cm to 120 cm. Evans and Stockner (1972) concluded that the amount of light penetrating the water was an important factor that influences the growth and development of periphyton on natural or artificial substrates.

2.1.2 Substrate Suitability

Another factor that influences the growth of periphyton is the type and the availability of substrates. Blum (1956) stated that true river algae depend largely on rocky substrates and can mostly be found in fast flowing rivers. On sandy and shifting riverbeds where the turbidity was high, Nelson and Scott (1962) found that the presence of periphyton was low except on hard stable surfaces.

The abundance of periphyton in a river system depends largely on the type of substrates at that particular place. The growth of periphyton on a granite surface is

higher compared to the growth on a limestone and sandy surface (Weitzel, 1979). McConnell and Sigler (1959) found out that the surface of small pebbles support higher chlorophyll content per unit area (m^2) compared to larger rocks on the riverbed.

Larger species of periphyton are normally found on stable objects compared to epiphytic species. Other researchers also found significant differences in the type of community on different types of substrate. The same species can be found on different substrate but at a different level of abundance (Weitzel, 1979). According to Round (1964), the condition and the characteristic of the sediment influence the epipelic algal community. Potapova *et al.* (2005) mentioned that some cyanobacteria such as *Calothrix parietina, Homoeothrix janthina* and the diatom *Coconeis neodiminuta* are often associated with sandy sediments.

Epiphytic algae and diatom attached themselves on substrates using secretion in the form of jelly, while other periphyton use stalks or gelatinous branches (Round, 1964). Therefore, the different strength of attachment in the form of jelly or gelatinous method influences the presence of a particular species on a certain substrate (Weitzel, 1979).

2.1.3 Water Movement

Movement of water such as velocity, wave, current and the circulation of water in a lake influence the growth and biomass of periphyton. The movement of water act as an inhibitor or as a catalyst for periphyton growth depending on its force and direction (Weitzel, 1979). Water movement is important because flowing water

brings with it important nutrients that are needed for the growth and productivity of periphyton while flushing away the byproducts of its metabolic activities (Duffer, 1966; Blum, 1956; Hynes, 1972; McConnell and Sigler, 1959; Odum, 1956; Whitford, 1960; Weitzel, 1979). Biggs (1996) mentioned that a stable flow of water usually promotes the accumulation of algal biomass in streams. This similar pattern was also observed by Potapova *et al.,* (2005) during their study in the Boston metropolitan area. Streams that had more variable flow patterns or higher disturbance level associated with stream flashiness (rate of change in flows) would lower algal biomass and diversity and affect species composition (Biggs *et al.,* 1998). Flow variability in terms of discharges or stage variability, may affect algal assemblages at different temporal scales (Clausen and Biggs, 1997; Matthaei *et al.,* 2003; Potapova *et al.,* 2005).

The productivity of organism is influenced by water movement. Only diatoms that are attached using jelly or gelatinous stalks can endure and survive in places where the velocity of water is moderate or high (Patrick, 1948). High periphytic assemblages in rivers and streams can mostly be found in sheltered area and in ponds where the water is calm along the riverbanks. Apart from influencing the attachment ability of periphytic organism, water movement can also influence the solubility and the availability of dissolved substances such as oxygen, carbon dioxide and other nutrients. Water movement also influences the temperature, turbidity and transparency of water (Patrick, 1948; Weitzel, 1979).

Flowing water with high velocity becomes an inhibitor to the growth of periphyton due to its shearing effect even though there are a few species of

filamentous algae that require an environment where the water velocity is high (Blum, 1956; Whirford, 1960; Weitzel, 1979). It was observed that when flood occurred and water velocity in streams increased, the periphyton on the various substrates was peeled away by stream flow (Yamada and Nakamura, 2002).

The type of substrates also plays an important role in determining the influence of water velocity on the growth of periphyton. Periphyton that grows on sandy surfaces are more vulnerable to erosion compared to periphyton that grows on the surface of a rock even if they are located at a place where the water moves slowly (Duffer and Dorris, 1966; Nelson and Scott, 1962; Neel, 1968; Weitzel, 1979). McConell and Sigler (1959) observed that productivity was high in places with slow moving water. This finding is similar to an earlier research by Butcher (1932) which showed that high biomass value was obtained at sites where the water flow was slow.

2.1.4 Dissolved Substances

The role played by dissolved substances is also important. Patrick (1948) discovered that most diatoms had a high abundance when calcium carbonate concentration was 3.0 mg/liter and above, while the concentration of silicate was at 0.5 mg/L and above. This is because apart from being a buffer for pH, calcium also reacts with toxic ion and control the concentration of sulfuric acid (Weitzel, 1979). Metals and trace elements cause different reactions when exposed to different types of algae species. There are some diatoms that can tolerate copper concentration between 1.5 mg/L to 2.1 mg/L even though at this level it is toxic to many algal species (Patrick, 1948; Weitzel, 1979). A study by Patrick *et. al.* (1975) also found that the presence of several trace elements changed the community structure from

one that was dominated by diatoms to a community that was dominated by blue-green algae. They also found that diatom was dominant with higher species diversity when chromium concentration was between 40 µg/liter to 50 µg/liter. In contrast, a concentration of chromium between 95 µg/liter to 97 µg/liter lowered the diatoms diversity even though the abundance was still high. Boron also played an important part in determining the community structure of algal assemblages. A concentration of 1.0 µg/liter of boron caused a change from a community of diatom to a community of blue-green algae, while the presence of nickel at any concentration was toxic to diatoms but it was preferable for the growth of blue-green algae, such as *Stigeoclonium lubricum* (Dillw.) Kuetzing (Patrick *et. al.*, 1975; Weitzel, 1979). Potapova *et al.,* (2005) also mentioned that the concentration of dissolved solids would influence the assemblage's patterns.

Metals and trace elements are toxic to a lot of algae species. Generally diatoms are more vulnerable to the presence of trace elements compared to blue-green algae such as *Stigeoclonium lubricum* (Dillw.) Kuetzing. The effect of metals and trace elements on diatom can be seen from the reduced concentration of chlorophyll *a* (Weitzel, 1979). Apart from that, cell deformities have also been associated with contamination by heavy metals (McFarland *et al*., 1997). Studies have also found that extreme metal contamination from mining activities could decrease the number diatom taxa (Juttner *et al*., 1996; Medley and Clements, 1998; Stewart *et al*., 1999; Genter and Lehman, 2000; Verb and Vis, 2000; Fore and Grafe, 2002).

2.1.5 Nutrients

The increase of nutrients in streams and water body have been linked to the changes of autotrophic community composition, vegetative biomass and an increase of nuisance species (Wright and McDonnell, 1986a, 1986b; Notestein *et al*., 2003). This in turn, affects the community structure and alters the food web dynamic of a given system (Hershey *et al*., 1988; Peterson *et al*., 1993).

It was found that an increase in nutrient concentration would influence periphyton abundance (Notestein *et al*., 2003). An increase in nitrogen alone would stimulate periphyton growth (Stelzer and Lamberti, 2001) especially when light was not a limiting factor (Lohman *et al*., 1991; Mosisch *et al*., 2001). The addition of phosphorus whether solely or concurrently with nitrogen was also found to increase periphyton abundance (Bothwell, 1985; Pan and Lowe, 1994; Dodds *et al*., 1997; Winter and Duthie, 2000). Similar results were also obtained by Notestein *et al*., (2003) which suggested that phosphorus might be the primary nutrient limiting factor for periphyton growth in coastal stream in Florida.

McCormick *et al*., (2001) observed that two distinct periphyton assemblages developed in response to increase phosphorus loading. These assemblages were characterized by higher phosphorus content, lower N:P ratios and higher biomass-specific productivity compared to oligotrophic assemblages (McCormick *et al*., 2001). Intermediate phosphorus loads was found to change the dominance from cyanobacteria to filamentous chlorophytes, such as *Spirogyra,* while a high loading rates resulted in a direct shift from oligotrophic to eutrophic cyanobacteria (e.g. *Plectonema wollei*, *Oscillatoria princeps*) (McCormick *et al*., 2001). Similar results

were also obtained by other studies where many were enriched simultaneously by both nitrogen and phosphorus (Howard-Williams, 1981; Hillebrand, 1983; McDougal *et al*., 1997; Havens *et al*., 1999).

2.1.6 Periphyton Predation and Competition

Apart from the abiotic environment, biotic interaction such as grazing and competition also plays an important part in the colonisation of periphyton on hard substrates in freshwater (Feminella and Hawkins, 1995; McCormick, 1996) and marine habitats (Hillebrand and Sommer, 1997; Hillebrand *et al*., 2000). Hillebrand and Kahlert (2002) observed that the presence of grazer can reduce periphyton biomass by 50% in Lake Erken. Other studies revealed that up to 90% of available algal biomass was consumed by grazers and this is especially true for periphyton found on hard substrate (Feminella and Hawkins, 1995; Hillebrand *et al*., 2000). Grazers such as snails (McClatchie *et al.*, 1982), crustaceans (Hargrave, 1970; Gerdol and Hughes, 1994) and annelids (Smith *et al.*, 1996) was found to have a big influence on biomass and community structure of periphyton.

Hillebrand and Kahlert (2002) also observed that the effects of macrograzer were lesser on periphyton that grows on sediment as compared to those that grows on hard substrate. In an earlier study, Hillebrand and Kahlert (2001) noted that strong grazing pressure of epilithic benthic algae were evidence in Väddö and Lake Erken.

Earlier study done by Cattaneo and Kalff (1986) found that smaller fauna such as oligochaete and cladocerans also had similar effects on algal biomass as macrograzers. Due to this, Sunbäck *et al.* (1996) and Epstein (1997a, b) proposed

that micro and meiofauna are able to consume most of the primary production in sediments (Hillebrand and Kahlert, 2002). However, it is not clear whether small fauna can influence the algal biomass in a longer time scale (several weeks).

A close relationship between some invertebrate and periphyton were observed in Lake Iznik. An increase of nematodes resulted in the decrease of stalked and tube diatoms (Albay and Aykulu, 2002). They also found that Rotifers (mainly *Lophocharis* sp.) and Ciliates also have a negative impact on erect and *cocconeis* type diatoms. Rotifer also has a negative relation with prostrate diatoms. However Rotifer was found to have a positive relationship with Cyanophytes. Nematodes (mainly *Anonchus* sp. and *Microlaimus* sp.) on the other hand, have a negative relationship with Cyanophytes (Albay and Aykulu, 2002).

A study on the effects of snail grazing on periphyton found that grazing have a significant effect on the composition of periphyton communities (Marks and Lowe, 1989). It was observed that the relative biovolume of green algae increase to 93% from 64% on graze substrate (Marks and Lowe, 1989). Periphyton communities that were highly grazed would usually be dominated by prostrate species that adhere tightly to the substrate or by small understory species that were not grazed due to their small size (Hunter, 1980; Hunter and Russel-Hunter, 1983; Sumner and McIntire, 1982; Marks and Lowe, 1989). Grazing has been known to physiologically maintain periphyton communities in an earlier succession stage (Marks and Lowe, 1989), meaning that the development of the periphyton community into a mature state had been retarded due to grazing.

Competition for nutrient and habitat also effects periphyton growth. Brammner (1979) and Brammer and Wetzel (1984) observed that *Stratiotes* competes with phytoplankton for nutrients from the surrounding water. This competition had resulted in the decreased of phytoplankton density in the water where *Stratiotes* is dominant (Brammer, 1979; Brammer and Wetzel, 1984).

Submerged macrophytes have been found to compete with other autotrophic organisms such as algae and periphyton and limit their growth (Kufel and Ozimek, 1994; Von Donk and Van de Bund, 2002; Mulderij *et al*., 2005b). The macrophytes was also found to excrete allelophathic substances that inhibit phytoplankton growth (Gross, 2003; Mulderij *et al*., 2005b). It was reported that allelopathic activities of macrophyte such as *Chara* (Wium-Andersen *et al*., 1982; Blindow and Hootsmans, 1991; Mulderij *et al*., 2005a), *Ceratophyllum* (Jasser, 1995; Mjelde and Faafeng, 1997) and *Myriophyllum* (Jasser, 1995; Gross *et al*., 1996) could resulted in changes in phytoplankton composition and biomass (Mulderij *et al*., 2005b).

2.2 Periphytic Algal Attributes as Indicators of Aquatic Degradation

Even though physical and chemical variables are widely used in monitoring water quality due to its ability to detect changes in the environment in a quick and straightforward manner, it also has its disadvantages of not being able to reflect the changes in water quality on biological communities (Vis *et al.,* 1998). The mixture of organic and inorganic compounds in most urban effluent further complicates the monitoring process due to the high cost required for the analyses (Vis *et al.,* 1998). Therefore, the use of periphyton has several advantages compared to the traditional method of using physical and chemical variables to determine water quality. Among

the advantages of using periphyton as an indicator are; they are easy to collect, taxonomically diverse and have a short regeneration time which means that it can react quickly to changes in stream water quality (Hill *et al.,* 2000; Wan Maznah and Mansor*,* 2000). Fore and Grafe (2002) also concluded that diatoms might represent a biological alternative to fish when assessing water quality in rivers or sites that were too deep to effectively sample fish or when endangered or protected species prohibited sampling. Apart from that, where cases of chemical and biological (faunal) information disagree about a site condition, diatoms can provide clues to resolve the conflict due to their sensitive nature to water chemistry (Fore and Grafe, 2002).

Various researches have showed that major changes in water quality can influence the characteristic of periphyton. Characteristics of periphyton such as biomass (Wuhrmann and Eichenberger, 1975; Watanabe *et al.,* 1988; Biggs, 1989), diversity indices (Weitzel and Bates, 1981; Stevenson, 1984; Stewart and Robertson, 1992; Vis, 1998; Hillebrand and Sommer, 2000), biotic indices (Descy and Coste, 1991; Kelly *et al.,* 1995) and taxonomic composition (Archibald, 1972; Rott, 1991) can be used as an indicator of water quality. Some studies have also found that algal community structure (Cattaneo *et al.,* 1995; Wan Maznah and Mansor, 2002) and periphyton productivity (Ho, 1976; Anton *et al.,* 1998) can also be used to determine water quality (Wan Maznah and Mansor, 2000; Wan Maznah, 2002).

The use of periphyton as a water quality indicator has been widely accepted. Researchers have developed several schemes that classified organisms according to the amount of organic pollutants associated with their occurrence. A system that

classified water system as eutrophic, mesotrophic or oligotrophic was developed by using the correlation between the type of species that existed at different levels of pollution (Weitzel, 1979). The terms eutrophic, mesotrophic and oligotrophic refers to the level of nutrients that are high, moderate and low (Round, 1964). Eutrophication is defined as the process in which water bodies are made more eutrophic via an increase in their nutrient supply (Smith et al., 1999). Table 2.2 summarises the effects of eutrophication on stream ecosystems.

Table 2.2: Effects of eutrophication on stream ecosystems (from Smith *et al.*, 1999).

- Increased biomass and changes in species composition of suspended algae and periphyton.
- Reduced water clarity.
- Taste and odor problems.
- Blockage of intake screens and filters.
- Fouling of submerged lines and nets.
- Disruption of flocculation and chlorination processes at water treatment plants.
- Restriction of swimming and other water-based recreation.
- Harmful diel fluctuations in pH and in dissolved oxygen concentrations.
- Dense algal mats reduce habitat quality for macroinvertebrates and fish spawning.
- Increased probability of fish kills.

Many studies have been conducted to evaluate the effects of nutrient on the growth of periphytic organisms. Studies by Hutchinson (1967, 1975), Hynes (1972) and Nelson *et. al* (1973) found that nutrients such as nitrogen, phosphorus and carbon were important in determining the presence and the abundance of aquatic species. It has also been recognized that aquatic system or zone that has a different concentration or type of nutrients will create different aquatic communities (Weitzel, 1979).

The concept of using organisms as an indicator has been widely used to determine the level of pollution. Patrick and Hohn (1956) and Patrick and Reimer (1966) had discussed on developing and using indicator community to monitor pollution. Aquatic ecosystems was defined as “healthy”, “semi-healthy”, “polluted” and “severely polluted” based on the quantitative evaluation of the number of diatom species (Patrick, 1949; Patrick and Hohn, 1956; Patrick and Reimer, 1966). The scheme is based on the concept of ‘indicator organisms’ that live in different types of environment where it can be used to represent a general level of pollution, and the relative abundance of particular indicator species that are used must be significant.

Diatoms are good ecological indicators because they are found in abundance in most lotic ecosystem (Stevenson and Bahls, 1999). Since diatoms can be found in a wide range of ecological conditions, it can provide multiple indicators of environmental change.

There are also disadvantages of using periphyton as a bioindicator. Even though the changes in nutrient concentration in water had been linked to changes in periphyton community, its influence had been variable or inconsistent (Vis, 1998; Notestein *et al.,* 2003). For example, experiments on the effect of nutrient enrichment on algal density in freshwater has been contradictory. Marcus (1980) and Pringle (1990) reported an increase in algal density due to the increase in nutrient supply, but Miller *et al.* (1992) reported the opposite. Furthermore, some studies reported that nitrogen alone would stimulate periphyton growth (Stelzer and Lamberti, 2001) when light was not a limiting factor (Lohman *et al*., 1991; Mosisch *et al.,* 2001). Moreover, studies by Bothwell (1985), Pan and Lowe (1994) showed

that the addition of phosphorus and nitrogen increased periphyton abundance. Dodds *et al*., (1997) and Winter and Duthie (2000) on the other hand, observed an increase in periphyton abundance when it was concurrently enriched with phosphorus and nitrogen.

Even though some researches found that the addition of phosphorus, and nitrogen plus phosphorus resulted in greater biomass, they also noticed that the addition of nitrate alone showed not much difference in periphyton biomass from the controls (Notestein *et al*., 2003). Further analysis, showed that the differences between the treatments containing phosphorus alone, phosphorus plus nitrogen, and nitrogen alone were not significant (Notestein *et al*., 2003). Thus, making the differentiation of the effects of phosphorus, phosphorus plus nitrogen and nitrogen difficult.

Sand-Jensen (1983) highlighted several reasons why it was not possible to accurately determine when and how physico-chemical parameters affected the growth of periphytic algae. The first reason was when periphyton growth patterns were examined in a fluctuating natural habitat, it was difficult to pinpoint a single regulating factor since there were many physico-chemical parameters that influenced periphyton growth. Sand-Jensen (1983) argued that the autotrophic and heterotrophic processes that happened internally within the boundary layer would change the chemical condition and could be different from the free water phase. The change in the chemical condition within the periphytic community depended on the rate of exchange with the free water process or the balance of opposing process within the periphytic layer. Another reason was that most measurements of physico-chemical

only focused on the free-water phase above the periphytic community and not within the community where the condition was different. The growth rates of periphytic algae also were either difficult or impossible to measure directly (Sand-Jensen, 1983).

Apart from the variable response to nutrient enrichment, the viability of periphyton as an indicator is also under threat from the grazing activity of invertebrate and fishes. It was reported that the grazer would increase the grazing pressure to periphyton due to enhanced food availability and this situation could offset the effect of nutrient enrichment (Hillebrand *et al.,* 2000, 2002; Hillebrand and Kahlert, 2001; Hillebrand, 2002; Cosgrove *et al*., 2004).

The inconsistencies in the periphyton reaction to the changes in water quality are likely caused by natural variability associated with periphytic communities (Weitzel *et al,* 1979; Morin and Cattaneo, 1992; Vis, 1998) and the differences between systems under study (i.e. stream vs river, temperate vs tropical region). The complex interaction of physical, chemical and biological factors that influence periphytic communities makes it difficult to detect water quality related changes in periphyton (Vis, 1998). This can be seen in a study by Guasch *et al*., (2003) where apart from the river water quality and light, the physical structure and biotic activity in the dense biofilm also plays a significant role in determining the condition within the matrix. Their study shows that the responses of periphyton toward the concentration of organic and inorganic substances in the water are influenced by their physiological state and density (Guasch *et al*., 2003). The thickness of periphyton can causes the creation of marked gradients of light (Meulemans, 1987;

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Eutrophication and harmful algal blooms: A scientific consensus

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Eutrophication and harmful algal blooms: A scientific consensus

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ABSTRACT

In January 2003, the US Environmental Protection Agency sponsored a "roundtable discussion" to develop a consensus on the relationship between eutrophication and harmful algal blooms (HABs), specifically targeting those relationships for which management actions may be appropriate. Academic, federal, and state agency representatives were in attendance. The following seven statements were unanimously adopted by attendees based on review and analysis of current as well as pertinent previous data:

(1) Degraded water quality from increased nutrient pollution promotes the development and persistence of many HABs and is one of the reasons for their expansion in the U.S. and other nations;
(2) The composition–not just the total quantity–of the nutrient pool impacts HABs;
(3) High-biomass blooms must have exogenous nutrients to be sustained;
(4) Both chronic and episodic nutrient delivery promote HAB development;
(5) Recently developed tools and techniques are already improving the detection of some HABs, and emerging technologies are rapidly advancing toward operational status for the prediction of HABs and their toxins;
(6) Experimental studies are critical to further the understanding about the role of nutrients in HABs expression, and will strengthen prediction and mitigation of HABs; and
(7) Management of nutrient inputs to the watershed can lead to significant reduction in HABs.

Supporting evidence and pertinent examples for each consensus statement are provided herein.
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1. Introduction

This report represents the synopsis of a "roundtable discussion" on the links between water quality and eutrophication and the occurrence of harmful algal blooms (HABs). This meeting,

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sponsored by the US Environmental Protection Agency (US EPA), was held in Cambridge, Maryland, in January 2003, and included academic experts as well as federal, state and local agency representatives. The authors of this report represent all those in attendance; our colleague, John Heisler, who originated and guided development of the meeting and chaired the discussion group, is now deceased and this paper was written in his honor.

The goal of the meeting was to develop a set of consensus statements on presently understood relationships between eutrophication and HABs, with particular emphasis on implications for nutrient management programs. A broad definition (GEOHAB, 2001) of HABs was adopted for this purpose, including potentially toxic (both autotrophic and heterotrophic) species and high-biomass producers that can cause hypoxia and anoxia and indiscriminant mortalities of marine life after reaching dense concentrations, whether or not toxins are produced.

In advance of the meeting, succinct statements for discussion were provided to participants and a presentation was given on each statement by one member of the group. The presenter was asked to make a case "for" or "against" the given statement. In discussion, and through analysis of data and evidence presented, the goal was to accept, reject, or modify each statement so that a set of unanimously accepted statements was developed by the end of the meeting. Statements were specifically crafted so as to have management implications. The relevance and applicability for management of these consensus statements remain as valid at present as when they were developed.

One recommendation from the roundtable was to develop a GEOHAB Open Science Meeting on HABs and Eutrophication to broaden the discussion on this topic to a wider community of international participants. Such a meeting was held in 2005 and the scientific results from that meeting have been synthesized into a report (GEOHAB, 2006) and some of those results are further detailed in the other papers of this issue of *Harmful Algae*.

Herein, each consensus statement is summarized along with a synopsis of its rationale and examples of supporting evidence. These synopses are not intended to be thorough reviews. In keeping with the mission of the US EPA, the sponsoring agency for this roundtable, preference is given to US examples where available. It is recognized that, in some cases, significant new data have become available since the 2003 roundtable. This report generally uses examples presented in the roundtable and only provides new results where advancements in the field have been substantial.

2. The consensus statements

2.1. Degraded water quality from increased nutrient pollution promotes the development and persistence of many HABs, and is one of the reasons for their expansion in the U.S. and other nations

It is generally recognized that there have been more coastal algal blooms, often of greater geographic extent and/or longer duration, with more toxic species observed, more fisheries affected, and higher associated costs from HABs in the past decade than in previous decades (Anderson, 1989; Smayda, 1990; Hallegraeff, 1993; Anderson et al., 2002; Glibert et al., 2005a). Many possible reasons for the reported global expansion exist in addition to nutrient pollution. Species dispersal or introduction, through transport of cells or cysts, can occur via natural currents and storms or human activities such as dispersal by ballast water exchange or shellfish seeding operations by which algal cells or cysts may be transported. Both overfishing and increased aquaculture alter food webs which may, in turn, alter the community of grazers that feed on HABs. Finally, improved observation and tools for detection have increased public and scientific awareness (GEOHAB, 2001, HARRNESS, 2005). More than one cause is frequently involved, and the causes for global expansion of a given species can differ among species.

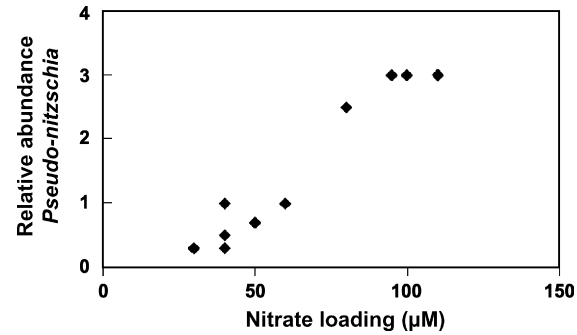

Fig. 1. Relative abundance (%) of the diatom *Pseudo-nitzschia* in the sedimentary record as a function of the nitrate loading in the northern Gulf of Mexico (redrawn from Turner and Rabalais, 1991 and Parsons et al., 2002).

There are numerous examples worldwide of increases in HABs linked to increased nutrient loading, a few of which are highlighted here. While strong relationships have been shown for many years in freshwater ecosystems between phosphorus loadings and harmful cyanobacteria blooms (Schindler, 1977; Burkholder, 2002), increasing linkages between nutrient loading and estuarine/coastal marine HABs have more recently been recognized (Smayda, 1990, 1997; Anderson et al., 2002; Glibert et al., 2005a,b; Glibert and Burkholder, 2006). For example, in the Gulf of Mexico, the sedimentary record of concentration of potentially toxic diatoms, *Pseudo-nitzcschia* spp., increased in parallel to increased nitrate loading over the past several decades (Fig. 1; Turner and Rabalais, 1991, Parsons et al., 2002). In Puget Sound, Washington, USA, a strong correlation has been reported between the increase in documented levels of paralytic shellfish toxins (PST) from toxic dinoflagellates over four decades and the increase in human population in the counties bordering Puget Sound (based on US census statistics; Fig. 2; Trainer et al., 2003). Although, as above, a statistical correlation does not establish a causal link, this relationship suggests that coastal eutrophication may be a causative agent of change (Trainer et al., 2003). Blooms off the coast of China, involving *Prorocentrum* sp., *Karenia mikimotoi* and other species, have expanded during recent years in geographic extent and duration, from km^2 to tens of km^2, and from days to months, related to the increase in fertilizer use over the past two

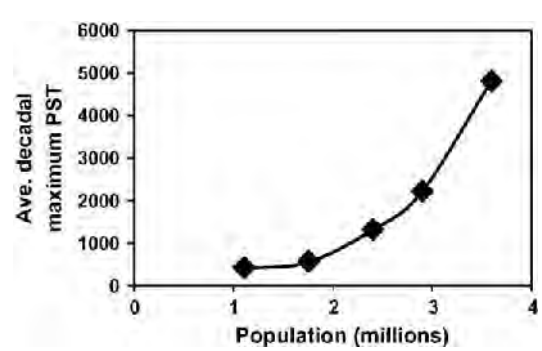

Fig. 2. Relationship between the growth in human population and the average decadal maximum paralytic shellfish toxins (PST) from dinoflagellate HABs from Puget Sound, Washington State, where continuous monitoring of paralytic shellfish poisoning has been ongoing since the mid-1950s. Human population data for the counties bordering Puget Sound for the past 40 years were derived from the U.S. census (redrawn from Trainer et al., 2003).

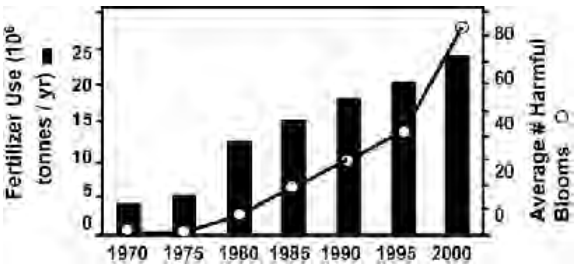

Fig. 3. Long-term trends in the use of nitrogen-based fertilizers and the number of occurrences of red tide blooms in Chinese coastal waters (modified and redrawn from Anderson et al., 2002; Zhou, 2005).

decades (Fig. 3; Anderson et al., 2002; Zhou, 2005). The Baltic Sea, Aegean Sea, Northern Adriatic, and Black Seas have all sustained increases in HABs coincident with increases in nutrient loading (e.g., Larsson et al., 1985; Bodenau, 1993; Moncheva et al., 2001). On a shorter time scale, Beman et al. (2005) reported, during a 5-year study, a strong positive relationship between nitrogen-rich agricultural runoff to the Gulf of California and the development, within days, of extensive phytoplankton blooms. In Sequim Bay, Washington, an intense bloom of the toxigenic diatom *Pseudo-nitzschia pseudodelicatissima* was found a week after elevated ammonium levels were reported in these waters (Trainer et al., 2007), and similarly, in Chesapeake Bay, blooms of *Prorocentrum minimum* have been found to follow within days of elevated levels of urea following agricultural applications (Glibert et al., 2001).

Although such field examples, supported by experimental and physiological data (e.g., Riegman, 1995; Bates et al., 1998; Imai et al., 1998; Smayda, 1998; Johansson and Granéli, 1999; Li et al., 2001; Fan et al., 2003; Glibert et al., 2006b), are increasing, recognition that degraded water quality from nutrient pollution contributes to the development and persistence of many HABs does not diminish the possibility that other factors may promote some HABs. Physical, biological, and other chemical factors may modulate harmful algal species' responses to nutrient loadings (e.g., Vadstein et al., 2004; Gobler et al., 2002; Sellner et al., 2003; Glibert et al., 2005a), and this complexity of HAB dynamics is a challenge for their management, as further described below.

2.2. The composition – not just the total quantity – of the nutrient pool impacts HABs

Specific algal species or species groups have numerous physiological adaptations that permit them to exploit nutrients differentially. Different species groups display preferences for specific nutrient regimes, including nutrient ratio or form (Smayda, 1990, 1997; Anderson et al., 2002; Smayda and Reynolds, 2001, 2003; Glibert and Burkholder, 2006). For example, diatoms require silicon for their growth, whereas certain dinoflagellates apparently have a higher phosphorus requirement than some other species groups. Thus, according to the nutrient ratio hypothesis (Tilman, 1977; Smayda, 1990, 1997), if nutrient loading leads to a proportional enrichment of phosphorus relative to silicon, then a proportional shift away from a diatom-dominated community toward flagellates might be expected over time.

The relationship between alteration in nutrient composition and the development of HABs is supported by examples in freshwaters, estuaries and marine coastal waters worldwide (reviewed by Anderson et al., 2002). Off the coast of Germany, time series analysis of nutrient concentrations over several decades showed that a fourfold increase in the N:Si ratio coincided with decreased abundance of diatoms and an increase in *Phaeocystis* blooms (Radach et al., 1990). Similarly, a nearly four-decade time series from Narragansett Bay, Rhode Island, shows a relationship between increases in the N:Si ratio and a proportional increase in flagellates (Smayda et al., 2004). Decreases in N:P ratios due to phosphorus loading have sometimes been related to increased abundance of certain harmful dino-flagellate species. For example, in Tolo Harbor, Hong Kong, where phosphorus loading increased due to human population growth in the late 1980s, a shift from diatoms to dinoflagellates was observed, coincident with a decrease in the ambient N:P ratio from ca. 20:1 to <10:1 (Hodgkiss and Ho, 1997; Hodgkiss, 2001). On shorter time scales, in Tunisian aquaculture lagoons, blooms of toxic dinoflagellates have been shown to develop when the N:P ratio drops in autumn (Romdhane et al., 1998), and along the eastern seaboard of the US, outbreaks of the toxic dinoflagellates, *Pfiesteria piscicida* and *P. shumwayae,* have been associated with low dissolved N:P ratios from high phosphate loading by effluent spills from concentrated animal operations (Burkholder et al., 1997). Blooms of *Karenia brevis* on the western Florida shelf are also found in waters with lower dissolved inorganic N:P ratios than in water directly to the south with higher N:P ratios, where diatoms tend to be more prevalent (Fig. 4; Heil et al., 2007).

The impacts of varying nutrient composition on HAB proliferation, however, are in many cases more complicated than the above examples. Some studies have shown, for example, that neither the quantity nor the ratio of inorganic nutrients alone can adequately explain sustained high biomass blooms of extended duration (e.g., Vargo et al., 2004). We now recognize that inorganic nutrients are not the only nutrients used by many HABs, and that many organic nutrients are bioavailable and dynamic in their composition (e.g., Seitzinger et al., 2002; Berman and Bronk, 2003). Many HAB genera, including dinoflagellates, cyanobacteria, and pelagophytes (e.g., brown tides), can use organic (dissolved or particulate) forms of nutrients for some or all of their nitrogen, phosphorus and/or carbon demands (e.g., Paerl, 1988; Burkholder and Glasgow, 1997; Granéli et al., 1997, 1999; Berg et al., 1997, 2002; Lewitus et al., 1999; Stoecker, 1999; Kudela and Cochlan, 2000; Berman, 2001; Glibert et al., 2001, 2006a,b, 2007; Lomas et al., 2001, 2004; Mulholland et al., 2002, 2004; Gobler et al., 2005; Glibert and Legrand, 2006; Lewitus, 2006; Herndon and Cochlan, 2007; Kudela et al., 2008; Cochlan et al., 2008).

Nutrient "packaging" can also be important. For example, many flagellate species, including various harmful dinoflagellates, are mixotrophic or heterotrophic phagotrophs, and consume pre-dominantly particulate rather than dissolved nutrients (e.g., Nygaard and Tobiesen, 1993; Stoecker, 1999; Jeong et al., 2005). Thus, the toxic, heterotrophic dinoflagellate *Pfiesteria* spp. can be indirectly stimulated by inorganic nutrient enrichment, mediated through an increase in the abundance of algal prey that may be directly stimulated by specific nutrient forms (Burkholder et al., 2001; Glibert et al., 2006a). Similarly, *Karlodinium veneficum* may be stimulated by the growth of cryptophytes, the abundance of which may be influenced by its preferred nutrient forms (Adolf et al., this issue).

Furthermore, some cyanobacteria also have the ability to convert or "fix" gaseous nitrogen into reduced nitrogen, enabling them to succeed in nitrogen-depleted conditions if other environmental conditions permit their growth (Howarth et al., 1988). The dynamics of nitrogen fixation by the cyanobacterium *Trichodesmium* have been related to increases in dissolved organic nitrogen which is released from the cyanobacteria and which may be available for subsequent uptake by dinoflagellates (e.g. Capone et al., 1994; Glibert and Bronk, 1994; Glibert and O'Neil, 1999; Lenes et al., 2001; Mulholland et al., 2004). Thus, nutrient ratios based solely on dissolved inorganic nutrients often do not yield sufficient insights regarding the influences of nutrient enrichment.

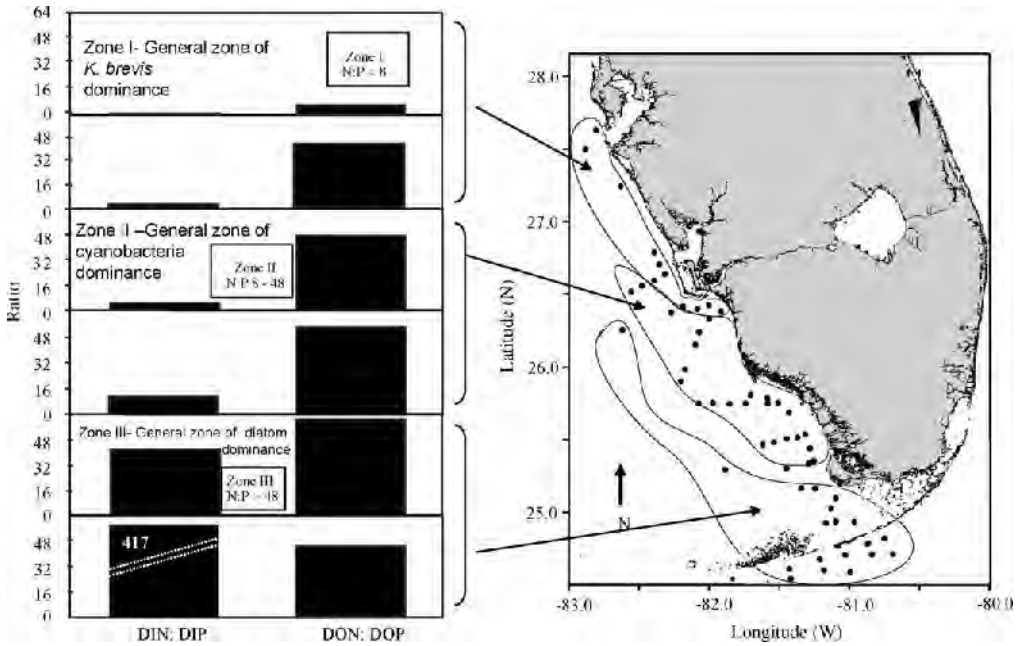

Fig. 4. Mean dissolved inorganic and organic N:P ratios at the mouths of each of the major riverine systems of western Florida shelf sampled during the dry season of May 2003. Each bar represents the means of the stations sampled along an individual riverine transect. The insert box indicates the mean particulate N:P ratio. The general zones are indicated on the companion map of southern Florida (modified from Heil et al., 2007).

Toxin production may also vary with nutrient form. For example, laboratory studies have revealed that the neurotoxin domoic acid (DA) production by the diatom *Pseudo-nitszchia* spp. can vary as a function of the nitrogen substrate being utilized for growth. For exponentially growing cultures of *P. australis*, nitrate and ammonium-grown cells produce equivalent amounts of dissolved and particulate DA, whereas DA production is enhanced in cultures growing solely on urea (Cochlan et al., 2005, Armstrong-Howard et al., 2007). However, for the smaller-celled species, *P. cuspidata*, the nitrate-grown cells are the most toxic (Auro, 2007).

In addition to macronutrients, micronutrients such as trace metals are essential for the growth of all phytoplankton and play critical roles in both photosynthesis and assimilation of essential macronutrients, and the toxicity of some HAB species (Sunda, 2006). For example, laboratory studies have shown that the toxigenic diatoms *Pseudo-nitszchia multiseries* and *P. australis* produce increasing amounts of DA as a function of iron or copper limitation (Rue and Bruland, 2001; Maldonado et al., 2002; Wells et al., 2005). Additionally, it has been seen that DA production in these diatoms increases under high copper concentrations, presumably to detoxify copper (Maldonado et al., 2002; Ladizinsky, 2003; Ladizinsky et al., 2005). Iron, besides being essential for phytoplankton growth and nitrogen assimilation, is also required for nitrogen fixation, and therefore the deposition of iron-rich dust in the Gulf of Mexico has been related to the strength of *Trichodesmium* blooms and, indirectly, to subsequent *K. brevis* blooms (e.g., Lenes et al., 2001).

In summary, nutrient preferences by different algal species groups are strongly supported by physiological evidence and by recognition of an array of adaptive mechanisms used by different species. The extent to which these adaptations are expressed depends on other environmental conditions at the time of nutrient supply, the types of nutrient supplied and other factors, including the micronutrient environment. The inability to apply a single criterion, such as the nutrient ratio, to an assessment of phytoplankton species dominance does not negate the utility of this approach; rather, it underscores the complexity of the physiology of many HAB species and the interdependence of environmental factors in the outcome of species succession (Glibert and Burkholder, 2006).

2.3. High-biomass blooms must have exogenous nutrients to be sustained

Algal proliferations, by definition, are due to increased *net* growth, and this must be accomplished either through increases in gross growth rate and/or through reductions in loss processes such as grazing (Harris, 1986). "New" nutrients from allochthonous sources can fuel biomass increases. While increased total algal biomass is often an obvious response to nutrient loading (see reviews in Cloern, 2001; Anderson et al., 2002), the overall species-specific response will depend upon the physiology of the organisms present, environmental conditions, and the form of nutrient supplied, as described above. Clear dose-response relationships between nutrient loading and biomass response (typically termed a "phase I" eutrophication model *sensu* Cloern, 2001) are more common in lakes, however, than in estuarine and marine systems.

The relationships between nutrient loading and algal proliferations are complicated by shifts in food webs, habitat changes, climate changes and other system alterations that affect the extent to which a given species may accumulate (typically termed a "phase II" model of eutrophication; Cloern, 2001). Increased nutrient enrichment can lead to a shift in plankton community composition, which in turn can afect predator–prey relationships, further altering the transfer of nutrients (e.g., Smayda, 1989; MacIntyre et al., 2004; Irigoien et al., 2005; Mitra and Flynn, 2006; Sunda et al., 2006). Incoming nutrients may be regenerated, recycled, or removed in space and time from the set of conditions that would otherwise support blooms. These links are frequently difficult to establish.

Inside blooms, ambient concentrations of inorganic nutrient forms may be reduced or depleted due to algal incorporation of the nutrients into biomass (e.g., Vargo et al., 2004; Springer et al.,

2005), leading to relationships between nutrient concentration and chlorophyll (or other biomass indicator) that may, in fact, appear to follow a saturation response, a negative relationship, or no relationship at all. Thus, the critical term is the flux of nutrient, not the absolute concentration that may be supporting the blooms.

The most complex nutrient sources to understand and regulate are non-point source inputs such as runoff, groundwater and atmospheric deposition. The relationship between increased exogenous inputs and increased algal biomass is often clearer over long time periods. Nitrogen loading, for example, in the Chesapeake Bay, which increased more than 2.5-fold from the mid-1940s to the mid-1990s, has been correlated with long-term increases in total chlorophyll, in the frequency and abundance of blooms of HAB species such as *P. minimum*, and in resulting hypoxic volume (Hagy et al., 2004; Kemp et al., 2005).

While exogenous nutrients are often necessary for high biomass blooms to be sustained, in some cases, blooms may be sustained on nutrients that are regenerated and recycled after an initial injection of new nutrients. Long-term sustained blooms, such as the bloom of *Aureoumbra lagunensis* evident in Laguna Madre for approximately 8 years in the 1990s, and the >20 month bloom of *K. brevis* that was sustained off western Florida in 2005, appear to have been fueled by an initial injection of nutrients, and then sustained on regenerated nutrients. In the former case, intense rains after years of drought led to a sequence of blooms and ecosystem conditions suitable for *Aureoumbra* (e.g., Buskey et al., 2001), while in the latter case, a series of hurricanes in preceding months may also have altered both nutrient availability and other habitat considerations (Mallin and Corbett, 2006; Neely et al., 2006). A third such example is that of the >18 months bloom of *Synechococcus* in eastern Florida Bay that followed an injection of phosphorus from two apparent sources: high freshwater discharge from Hurricanes Katrina, Rita and Wilma that impacted south Florida in 2005, and a very high organic loading from a unique situation of road construction that required mulching of significant amounts of mangroves (Madden, in press). Consequently, chlorophyll concentrations rose and were sustained at levels roughly eightfold higher than pre-bloom levels, while an increase in phosphorus concentration was only observed during the initiation stages of the bloom and then declined. A final example comes from brown tides (*Aureococcus anophagefferens*) in US mid-Atlantic estuaries (Gobler et al., 2005). These blooms are often preceded by a 'pre-bloom' of other algal species that are stimulated by nitrogen from groundwater flow (Gobler and Sañudo-Wilhelmy, 2001). When groundwater flow diminishes during summer and the initial bloom is remineralized, organic nitrogen levels increase, and *A. anophagefferens* becomes the dominant alga. Thus, nutrients may continue to serve as fuel for blooms long after the initial nutrient loading occurs.

2.4. Both chronic and episodic nutrient delivery promote HAB development

As described in some of the previous examples, the response of harmful algal species to changes in nutrient enrichment is often a chronic effect, becoming increasingly apparent only after long time intervals are examined (e.g., Lam and Ho, 1989; Smayda, 1989; Riegman et al., 1993; Yung et al., 1997; Marshall et al., 2005). Long-term, consistent monitoring records additionally can reveal relationships between harmful algal species composition and abundance versus other aspects of environmental change, such as climate variability or alterations in trophic control due to changes in dominant herbivorous species. Such long-term records are valuable in clarifying trends in nutrient loadings, and in identifying synergistic effects of changing climatic factors and nutrient enrichment (e.g., Smayda et al., 2004, Burkholder et al., 2006).

Episodic or "pulsed" nutrient inputs have also been related to outbreaks of harmful algal species on both small and large scales. Some of these, such as agricultural applications (timing and quantity), treated sewage discharge, and combined sewer outfalls, are amenable to management. As examples (reviewed in Harlin, 1993), in Boston Harbor, sea lettuce (*Ulva*) formed dense populations for many years near outfalls of poorly treated sewage. Along the intertidal area of the Baltic Sea, sewage inputs have been related to overgrowth of formerly dominant brown seaweeds (*Fucus* spp.) by opportunistic macroalgae. Episodic inputs resulting from storm events are, naturally, more difficult to manage.

Episodic storm events and climate variability affect the timing of freshwater flow, residence times, the magnitude and timing of nutrient pulses and resulting biotic responses (e.g., Miller et al., 2006; Burkholder et al., 2006; Mallin and Corbett, 2006; Paerl et al., 2006). In coastal lagoons, where riverine input is not the dominant source of nutrient delivery, climate variability can alter the input of groundwater nutrients (e.g., LaRoche et al., 1997). Hurricane Isabel in 2003 caused a large phytoplankton bloom to develop in Chesapeake Bay within days (Miller et al., 2005). Further, extensive hypoxia occurred in the lagoonal Neuse River Estuary after Hurricanes Dennis and Floyd, whereas a storm that caused less flooding, Hurricane Fran, led to more concentrated pollutants, extensive anoxia and massive fish kills (Burkholder et al., 2006). In the Choptank River subestuary of Chesapeake Bay, annual spring blooms of *P. minimum* generally follow within days of spring agricultural application of nitrogen fertilizer and its runoff from episodic spring rains (Glibert et al., 2001).

In some cases anthropogenic nutrients may not directly stimulate HABs, but may become linked to their growth and abundance following biogeochemical processing or following the stimulation of other components of the food web on which they may depend.

On a seasonal scale in Chesapeake Bay, USA, nutrient input in the spring is delivered largely in the form of nitrate and rapidly assimilated by diatoms which bloom, then sink and decompose as nutrients become depleted. Subsequently during warmer summer months, nitrogen, largely as ammonium, is released via decomposition in sedimentary processes which, in turn, supports an assemblage dominated by flagellates including potentially harmful dinoflagellates (Glibert et al., 1995; Malone et al., 1996; Kemp et al., 2005).

It is thus important in our interpretation of the relationships between nutrients and HAB occurrences that we move away from simplistic inorganic nutrient-dose-yield models and begin to incorporate nutrient loading parameters which encompass the increasing array of nutrient forms that are now recognized to be important. Furthermore, interpretations of nutrient effects on HABs as well as models that parameterize these relationships, need to incorporate indirect, time-lagged nutrient pathways, as well as the feedbacks and interactions of the effects of nutrient loads in ecosystems (Cloern, 2001).

2.5. Recently developed tools and techniques are already improving the detection of some HABs, and emerging technologies are rapidly advancing toward operational status for the prediction of HABs and their toxins

Due to the unpredictability of most episodic events, sampling programs historically have not "captured" the initiation phases of blooms. However, new *in situ* monitoring tools are beginning to permit resolution of the antecedent conditions and the time course response to some episodic events. Improved molecular probes, some

that can be incorporated into *in situ* microarrays, are allowing timely detection of cells. Molecular probes for individual HAB species and their toxins are now routinely applied for many species, making early detection possible (e.g., Scholin and Anderson, 1998; Scholin et al., 2003; Anderson et al., 2005a; Bowers et al., 2006). Optical sensors for *Karenia* species have been deployed on moorings and autonomous underwater vehicles (Schofield et al., 1999). *In situ* probes with telemetry for various key variables such as temperature, salinity, chlorophyll, turbidity, dissolved oxygen and pHs are also providing near-real time data for management applications (Glasgow et al., 2004; Springer et al., 2005). A suite of over 50 such probes in the State of Maryland are allowing managers and the public alike to monitor trends in Chesapeake Bay and rapidly respond when conditions warrant (www.eyesonthebay.net). *In situ* nutrient sensors are also advancing, with capability developing for some organic forms of nutrients as well as inorganic forms, so that relationships between pulses in nutrient delivery and alterations in salinity due to rainfall, for example, are now possible to establish (Glibert et al., 2005b, 2008).

In conjunction with the growing sophistication of tools, better citizen awareness and citizen monitoring can also be effective in capturing blooms or blooms conditions. Throughout the US and in many parts of the world, fishermen collect samples for cell and nutrient analyses, and local citizen groups and indigenous people participate in routine monitoring (e.g., Scaefer et al., 2004; Trainer and Suddleson, 2005; Whereat and Farestad, 2005; Granéli and Esplund, 2006).

Modeling is a rapidly developing tool advancing toward prediction of many HAB species and occurrences (e.g., Flynn, 2002; McGillicuddy et al., 2005; Hood et al., 2006), although for most estuarine and coastal HABs, prediction has not yet been attained (see, e.g., Franks, 1997; Cembella, 1998; Flynn, 2005). There are two general types of HAB models that are useful for management applications. The first is the development of models that predict "general likelihood of occurrence" of HAB species, whereas the second is the development of models that include "explicit" predictions of HAB occurrence in time or space. The former is useful for management in application of long-term actions to reduce the likelihood of future occurrences, i.e. prevention. The latter requires more refinement to understand the physics, biology and chemistry of the environment, but it can be of more value at the local community level. Models of a range of types are useful in predicting general likelihood of occurrence, from simple regression models that may yield correlation (without necessarily a biological foundation for cause and effect) and conceptual models that are useful in communicating general patterns (e.g., Smayda and Reynolds, 2001, 2003). In advancing toward more explicit predictions, mechanistic models that describe the physical, chemical and biological interactions are required. Spatially explicit models linked to hydrodynamic models are yielding some success in retrospectively tracking some species, such as for *Alexandrium* spp. in the Gulf of Maine and *K. brevis* in the Gulf of Mexico (McGillicuddy et al., 2005, Walsh et al., 2006). Multi-parameter physiological models that incorporate various organismal life stages and trophic interactions are also beginning to be developed (J. Anderson et al., 2003; D. Anderson et al., 2005b; Hood et al., 2006).

The development of explicit, reliable model predictions still needed for most HABs will require significant data on the species of interest as well as the environmental conditions (Flynn, 2002, 2005). Thus, for some species, "general likelihood" predictions are viable now and can be useful in guiding long-term management decisions with respect to nutrients. Furthermore, several types of real-time and near-real-time monitoring systems, coupled with conceptual, statistical, or mechanistic models, are also available now to assist in detecting and predicting some HABs in time and space. Now-cast models are being developed in the Chesapeake, Bay, for example, of the likelihood for *K. veneficum* blooms based on a statistical habitat relationship of the HAB with salinity and temperature, and a hydrodynamic model (Ramers et al., 2003; Tango et al., 2005). However, the prediction "window" for those species for which such mechanistic models are available is presently short, and considerable work will be needed to strengthen insights about the relationships between nutrient responses, physical dynamics, and climate change. Application of real-time monitoring of both environmental parameters and HABs species will continue to advance forecasting capabilities for these dynamic relationships.

2.6. Experimental studies are critical for further understanding about the role of nutrients on HABs expression, and will strengthen prediction and mitigation of HABs

Many physiological adaptations to nutrients are best examined under controlled laboratory conditions. For example, assessments of specific relationships that are required in models (such as parameters of nutrient uptake and growth kinetics) are better resolved under laboratory conditions where a culture of known physiological state can be established and where environmental conditions can be rigorously set (e.g., Lomas et al., 1996; Fan et al., 2003; Glibert et al., 2006b; Herndon and Cochlan, 2007; Cochlan et al., 2008). Laboratory culture studies of HAB species have been instrumental in resolving such species characteristics as life histories, toxigenesis, ecophysiological responses to specific nutrient forms, light and temperature regimes, and species-specific differences in rates of nutrient uptake and growth. As new insights are developed about various processes – for example, the extent of osmotrophy or mixotrophy within a species – generalizations based upon older laboratory data may have to be revisited, cross-confirmed, or assessed anew.

A critical but often overlooked consideration regarding experimental work is that major characteristics of species maintained in culture for extended periods (months to years) significantly change over time under (highly artificial) enclosed conditions. Over time in culture, highly toxic strains have lost their ability to make toxin; strains also have changed in polyploidy, expression of life history stages, and responses to environmental conditions such as salinity and nutrient regimes (reviewed in Wood and Leatham, 1992; Burkholder and Glibert, 2006). *Pfiesteria* spp., for example, have been shown to loose their toxicity when switched from a media that includes live to one with algal prey (Burkholder et al., 2001). Toxin production in a strain of *Alexandium lusitanicum* was also shown to loose toxicity after many years in culture, and although the exact reasons could not be ascertained, genetic mutations or prolonged use of antibiotics may have been the cause (Martins et al., 2004).

Many species have shown strain differences unrelated to extended period in culture. For example, Strom and Bright (2003) observed that strains of the haptophyte *Emiliania huxleyii* varied in their response to different forms of nitrogen. Whereas all four strains tested grew on nitrate and ammonium, three of the four could grow on organic nitrogen sources, while the fourth could not. The strains also showed distinct responses to various forms of organic nitrogen. Thus, in conducting laboratory studies, the history of the culture should be carefully considered (e.g., location of isolation and duration in culture). It is also important to compare multiple strains of the same species to assess whether the characteristic of concern is strain-specific, and generalizations at the species level (or higher) based upon one to a few strains should be avoided (Burkholder and Glibert, 2006; Burkholder et al., 2001, 2005).

Experiments should continue to build upon the knowledge gained from unialgal culture studies to assessment of more complex systems with multiple species, from microcosms to mesocosms, and from laboratory studies to field trials. Mesocosm studies and field experiments, if carefully designed and controlled, can enable *in situ* assessment of processes of trophodynamics and community interactions with individual species (e.g., Stoecker and Gustafson, 2002), and the importance of environmental fluctuations in light, temperature, and community-level responses to nutrient enrichment. Such approaches have significantly advanced understanding of nutrient limitation. As examples, the classic whole-lake studies undertaken decades ago in Canada yielded tremendous insights about phosphorus limitation in freshwater systems (Schindler, 1987, 2006). Analogously, much was learned about the regulation of marine phytoplankton production by iron in the multiple *in situ* iron enrichment experiments conducted in the high-nutrient-low-chlorophyll (HNLC) regions of the world (reviewed by de Baar et al., 2005). Smaller-scale mesocosms also have helped to resolve nutrient preferences by the harmful brown tide species, *A. anophagefferens* (Kana et al., 2004) and the effects of trophic structure on top-down regulation of phytoplankton biomass (Glibert, 1998; Granéli and Turner, 2002; Buskey et al., 2003; Vadstein et al., 2004).

As model sophistication and insights continue to develop about the dynamic regulation of adaptive characteristics of individual species, there will be a ongoing need to experimentally document specific processes under controlled conditions with clonal algal isolates, both as individual species (±bacteria, e.g., Bates et al., 1995; Burkholder et al., 2005) and as mixes of species in an increasingly complex but controlled environment (e.g., Flynn, 2002). Based upon the major insights gained from previous experiments, the data from such controlled conditions will surely strengthen scientists' abilities to predict and mitigate HABs.

2.7. Management of nutrient inputs to the watershed can lead to significant reductions in HABs

There are now multiple examples demonstrating both total algal biomass and HAB occurrence decreases after reduction in nutrient input. One of the classic examples involving decreases in cyanobacterial blooms is that of the removal of sewage discharges from Lake Washington within metropolitan Seattle, Washington (Edmondson, 1970). This lake had sustained noxious cyanobacteria blooms from the 1920s through the 1960s because of raw sewage inputs. An elimination of sewage discharges to Lake Washington was imposed in 1968, and the cyanobacterial blooms declined. In a much larger system, Lake Erie, the green macroalga *Cladophora* had choked much of the west basin with massive growth until improved wastewater treatment and detergent phosphate bans in the early 1980s led to significant reduction in the nuisance blooms (Ashworth, 1986). Reduced nutrient loading similarly has promoted declines in estuarine and marine coastal HABs. Sewage discharges to Mumford Cove, a shallow estuary in Connecticut, were re-routed to another waterway in the late 1980s, and within 2 years massive nuisance blooms of the macroalga, *Ulva lactuca*, were eliminated (Harlin, 1993). In the Potomac River, a tributary of Chesapeake Bay, phosphate removal from sewage in the late 1970s was related to significant reductions in the frequency and intensity of *Microcystis* blooms that had been problematic in the previous decade (Jaworski, 1990). In the Seto Inland Sea in Japan between 1965 and 1976, the number of red tide outbreaks (high biomass blooms) increased sevenfold (Okaichi, 1997), in parallel with the increase in industrial production, but in 1973, Japanese authorities instituted the Seto Inland Sea Law to reduce loadings to half of the 1974 levels over a 3-year period. The number of red tides began to

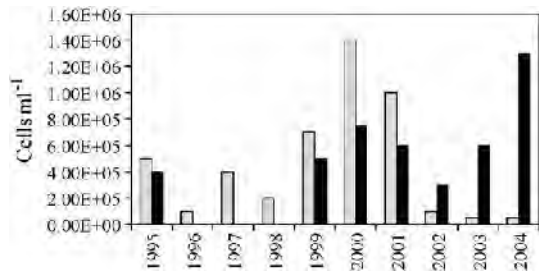

Fig. 5. Long-term trends in brown tide events in Peconic Estuary Long Island (gray bars) and in Maryland Coastal Bays (black bars; redrawn from Gobler et al., 2005; Glibert et al., 2007).

decrease in 1977, eventually falling to less than 30% of the peak frequency, which had been in excess of 300 blooms year^{-1} (reviewed by Imai et al., 2006).

A more recent example appears to be that of the Long Island brown tides which, while so common in the early 1990s, were virtually absent from the Long Island embayments through the middle of the following decade (Fig. 5; Gobler et al., 2005). Although multiple factors have been suggested to be related to the decline in bloom intensity, the recent reductions in nitrogen levels in Peconic Estuary have been correlated with these declines (Nuzzi and Waters, 2004; Gobler et al., 2005). Blooms of *A. anophagefferens* are generally reliant on organic sources of nitrogen for growth (Dzurica et al., 1989; Lomas et al., 1996; Berg et al., 1997, 2002; Mulholland et al., 2002, 2004). In the Long Island embayments, the organic sources fueling these blooms are not thought to be a direct external nutrient source, but rather derive from the internal processing of other "new" nitrogen sources, including groundwater flow, which is elevated in nitrate. This nitrate delivery supports blooms of diatoms, as well as blooms of benthic microalgae which, as they begin to dissipate, can supply the necessary organic nitrogen for the brown tide (Gobler and Sañudo-Wilhelmy, 2001; MacIntyre et al., 2004; Gobler et al., 2005). From 1995 to 2006, levels of DON declined substantially and brown tides were absent from the Peconic Bays during those years (Gobler et al., 2005). In sharp contrast, however, in Maryland embayments, where brown tide also blooms, organic nutrient concentrations have been increasing annually over the past decade, leading to more significant blooms each year over the decade from the mid-1990s to the mid-2000s (Trice et al., 2004; Glibert et al., 2007).

Although these specific examples suggest that some successes may be possible in reducing incidences of HABs by reducing nutrient inputs, for many species these relationships may be complex. Long time scales may be involved, due to long-term storage of nutrient in sediments and alteration in the biogeochemistry of systems after years of nutrient loading (e.g., Kemp et al., 2005). Furthermore, for some systems, ecosystem dynamics may be permanently altered, and nutrient reduction strategies alone will not yield original ecosystem conditions. Eutrophication stressors such as loss of benthic habitat, development of hypoxia/anoxia, and alterations in food webs (Cloern, 2001) require long time scales for restoration and may require changes at the landscape scale.

With increasing pressures on the coastal landscape, it must be underscored that prevention of large blooms through nutrient control is far preferable than attempts to eradicate HABs once they are established. It is, however, a significant challenge and extremely costly to enact such policy decisions on large-scale nutrient reductions, so there will continue to be pressure to develop bloom control strategies. Furthermore, different nutrient actions may be required for different types of HABs, and organic as well as inorganic nutrients must be considered. Effective manage-

ment is an iterative process, involving adaptive management as new knowledge is gained, and must be sustained to be effective. Lastly, timely communication of results, to stakeholders and the public, is critical. Given projected increases in human population worldwide, eutrophication pressures and increased HAB occurrences will undoubtedly continue to increase in years to come.

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COMMONWEALTH OF VIRGINIA
DEPARTMENT OF ENVIRONMENTAL QUALITY
WATER DIVISION
MELANIE D. DAVENPORT, DIRECTOR

P.O. BOX 1105 **Richmond, VA 23218**

Subject: Guidance Memo No. **14-2005**
2014 Water Quality Assessment Guidance Manual

To: Regional Directors

From: Melanie D. Davenport, Director

Date: April 21, 2014

Copies: John Kennedy, Arthur Butt, Regional Water Quality Planning Managers and staff, Office of Water Quality Programs Managers, WMA Staff

Summary:

The Environmental Protection Agency's (EPA) original 2006 Integrated Report Guidance recommends that states submit an "Integrated Report"(IR) that will satisfy Clean Water Act (CWA) requirements for both Sections 305(b) water quality reports and 303(d) impaired waters lists. According to EPA this Integrated Report should include the following information:

- delineation of water quality assessment units (AUs) based on National Hydrography Dataset (NHD);
- status of and progress toward achieving comprehensive assessments of all waters;
- Water Quality Standard attainment determination for every AU;
- additional monitoring that may be needed to determine Water Quality Standard attainment status and, if necessary, to support development of Total Maximum Daily Loads (TMDLs) for each pollutant/AU combination;
- schedules for additional monitoring planned for AUs;
- pollutant/AU combinations still requiring TMDLs;
- TMDL development schedules reflecting the priority ranking of each pollutant/AU combination; and
- Water Quality "Effluent Limited" Waters.

DEQ has incorporated the EPA Integrated Reporting guidance into the Virginia 2014 Water Quality Assessment Guidance Manual. The 2014 IR guidance is designed to integrate or combine the 305(b) overall assessment of Virginia's waters and separate out those waters impaired and needing a TMDL as per 303(d). The EPA Integrated Report Guidance and Assessment Database (ADB V2.3.1) have 5 different assessment categories in which every segment or "assessment unit" (AU) will be placed. The EPA Guidance allows the states to subdivide the federal categories in order to address state programmatic needs. Virginia's 2014 IR guidance contains the categories and subcategories Virginia has chosen for enhanced tracking and data management purposes.

The 2014 IR guidance manual contains a number of changes, all enumerated in Part II. One notable modification is the addition of Category 4D to Virginia's assessment scheme. This category allows for more precise classification of dissolved oxygen in Chesapeake Bay waters.

The data window used in the development of the Integrated Report is January 1, 2007 through December 31, 2012. The manual uses excerpts from the "EPA 2006 Integrated Report Guidance", "2008 and 2010 EPA

Integrated Report Clarification Guidance", "EPA 1997 Guidelines for the Preparation of the 1998 State Water Quality Assessment 305(b) Reports", and "Assessment Database (ADB) Systems User's Manual" published by EPA, along with other State and Federal guidelines.

Electronic Copy:

An electronic copy of this guidance in PDF format is available for staff internally on DEQNET, and for the general public on DEQ's website at: http://www.deq.virginia.gov/Programs/Water.aspx.

Contact information:

If you have any questions regarding the guidance manual, you can contact Tish Robertson, Department of Environmental Quality, P.O. Box 1105, Richmond, Virginia 23218. Telephone (804) 698-4309, FAX (804) 698-4032, or via e-mail tish.robertson@deq.virginia.gov.

Disclaimer:
This document has been developed based on Virginia's 9 VAC 25-260 Water Quality Standards, with amendments approved by the State Water Control Board January 6, 2011. It is provided as guidance and, as such, sets forth standard operating procedures for the agency. However, it does not mandate any particular method nor does it prohibit any particular method for the analysis of data, establishment of a wasteload allocation, or establishment of a permit limit. If alternative proposals are made, such proposals should be reviewed and accepted or denied based on their technical adequacy and compliance with appropriate laws and regulations.

VIRGINIA DEPARTMENT OF ENVIRONMENTAL QUALITY

WATER QUALITY ASSESSMENT GUIDANCE MANUAL for 2014 305(b)/303(d) Integrated Water Quality Report

April 2014

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PART I. REGULATORY REQUIREMENTS AND OBJECTIVES

Under the Clean Water Act, the United States Environmental Protection Agency requires that each state develop a program to monitor the quality of its surface and ground waters and prepare a report every two years describing the status of its water quality. Each state identifies waters of concern as having observed effects and schedules additional monitoring, if appropriate, to determine if designated uses are being met. EPA issues guidelines for States to use during the reporting cycle for national consistency purposes. States are encouraged to use these guidelines to prepare these water quality reports for EPA. EPA compiles the data from the State reports, summarizes them, and transmits the summaries to Congress, including an analysis of water quality nationwide. The 305(b)/303(d) Integrated process is the principal means by which the EPA, Congress, and the public evaluate current water quality, the progress made maintaining and restoring water quality and the extent of remaining work to be done. Many states, including Virginia, rely on the 305(b)/303(d) process for information needed to conduct water quality planning. The 305(b)/303(d) process is an integral part of Virginia's water quality management program, requirements for which are set forth in 40 CFR 130. The Department of Environmental Quality (DEQ) and the Department of Conservation and Recreation (DCR) are the principal state agencies charged with conducting water quality assessment and associated activities.

In 1997, the General Assembly enacted the Water Quality Monitoring, Information and Restoration Act (WQMIRA) §62.1- 44.19:4 through §62.1- 44.19:8. This legislation supplements the CWA 305(b)/303(d) federal requirements. The requirements of this legislation for State assessment procedures or processes are briefly outlined as follows:

1. The Act requires the 303(d) portion of the Integrated Report to identify geographically defined water segments as impaired if monitoring or other evidence shows:

    a. exceedences of ambient water quality standards for aquatic life or human health;
    b. fishing restrictions or advisories;
    c. shellfish consumption restrictions due to contamination;
    d. nutrient over-enrichment;
    e. significant declines in aquatic life biodiversity or populations; and/or
    f. contamination of sediments at levels which violate water quality standards or threaten aquatic life or human health.

2. Waters identified as "naturally impaired", "fully supporting but threatened" or "evaluated" (without monitoring) as impaired shall be set out in the 303(d) portion of the Integrated Report in the same format as those listed as "impaired."

3. The 303(d) portion of the Integrated Report shall include an assessment, conducted in conjunction with other appropriate state agencies, for the attribution of impairment to point and nonpoint sources. The absence of point source permit violations on or near the impaired water shall not conclusively support a determination that impairment is due to nonpoint sources. In determining the cause for impairment, the Board shall consider the cumulative impact of 1.) multiple point source discharges, 2.) individual discharges over time, and 3.) nonpoint sources.

4. The Board shall develop and publish a procedure governing its process for defining and determining impaired water segments and shall provide for public comment on the procedure.

5. The Integrated Report, inclusive of CWA sections 305(b) and 303(d) shall be produced in accordance with the schedule required by federal law and shall incorporate at least the preceding five years of data, where appropriate. Data older than five years shall be incorporated when scientifically appropriate for trend analysis or other longer term considerations.

6. The Integrated Report, inclusive of CWA sections 305(b) and 303(d), shall be developed in consultation with scientists from state universities prior to submission by the Board to EPA.

7. The Integrated Report, inclusive of CWA sections 305(b) and 303(d), shall indicate water quality trends for specific, easily identifiable, geographically defined water segments and provide summaries of the trends using available data and evaluations. This will allow the citizens of the Commonwealth to easily interpret and understand the conditions of the geographically defined water segments.

8. Based on the information in the Integrated Report, inclusive of CWA sections 303(d) and 305(b), the Board shall request the Department of Game and Inland Fisheries (DGIF) or the Virginia Marine Resources Commission (VMRC) to post notices at public access points for all "toxic" impaired waters. The notice, prepared by the Board, shall contain the basis for the impaired designation and a statement of potential health risks. The Board shall coordinate with the DGIF and VMRC to assure that adequate notice of posted waters is provided to those purchasing hunting and fishing licenses.

The Water Quality Monitoring, Information, and Restoration Act directs DEQ to develop and publish a water quality assessment guidance document governing the process for defining and determining impaired waters, and to provide an opportunity for public comment on the assessment guidance. Public comment will be solicited through February 25, 2014 on the draft version of this guidance. This document can be found on the DEQ website at http://www.deq.virginia.gov/Programs/Water/WaterQualityInformationTMDLs/WaterQualityAssessments.aspx.

The purpose of this guidance manual is to guide DEQ staff in the development and reporting of the 2014 Integrated Report (305(b) Water Quality Assessment 305(b)/303(d) Impaired Waters). It is also intended to assist the public in understanding the monitoring and assessment process.

Section 305(b) of the Clean Water Act requires each state to submit a biennial report to EPA describing the quality of its navigable waters. The 305(b) report provides DEQ's best overall assessment of water quality conditions and trends in the Commonwealth. The report is intended to be used as a tool in planning and management of waters in Virginia. The report also directs continuous planning and implementation activities in coordination with the State Water Quality Management Plan and the Continuous Planning Process (CPP).

Primary objectives of the Integrated Report are:

1. To educate and inform citizens and public officials about Virginia's overall water quality.

2. To analyze water quality data in order to determine the extent to which Virginia's waters are supporting the designated uses for all state waters and to compare the results to WQ Standards and other appropriate criteria and guidelines.

3. To determine the causes for the "failure to support" the designated uses of the State's waters.

4. To determine the nature and recognizable extent of point and nonpoint source impacts in accordance with state and federal guidelines.

Section 303(d) of the Clean Water Act and the Environmental Protection Agency's regulation 40 CFR Section 130.7 (d), promulgated in July 1992, requires each state to submit a Total Maximum Daily Load (TMDL) Priority List to EPA on April 1 of even numbered years. Category 5 signifies waters that are impaired and need a TMDL.

Impaired waters needing a TMDL are those waters that do not meet water quality standards due to a pollutant(s). *A pollutant, as defined in 40 CFR 122.2, means any dredged spoil, solid waste, incinerator residue, filter backwash, sewage, garbage, sewage sludge, munitions, chemical wastes, biological materials, radioactive materials (except those regulated under the Atomic Energy Act of 1954, as amended (42 U.S.C. 2011 et seq.)), heat, wrecked or discarded equipment, rock, sand, cellar dirt and industrial, municipal, and agricultural waste discharged into water.*

Category 4 includes waters that are "water quality effluent limited" and other waters not needing a TMDL. Waters receiving effluent from facilities with water quality-based effluent limits in their Virginia Pollution Discharge Elimination System (VPDES) permits, with schedules of compliance to meet these limits within the next reporting cycle or within the current permit cycle (5 years), are considered Subcategory 4B (impaired but not needing a TMDL) due to the control requirements and compliance schedules associated with the VPDES permit or other alternative control requirements. Waters where compliance schedules extend past the current permit cycle or into the next assessment cycle are considered part of the 303(d) impaired waters list (Subcategory 5E).

EPA's Integrated Report Guidance recommends that states submit an "Integrated Report" that will satisfy Clean Water Act (CWA) requirements for Sections 305(b) overall water quality report, 303(d) Impaired Waters List and Section 314 assessment of publicly owned lakes. This Integrated Report shows the following information:

- delineation of water quality assessment units (AUs) based on National Hydrography Dataset (NHD);
- status of and progress toward achieving comprehensive assessments of all waters;
- attainment status of water quality standards (WQS) for every AU assessed;
- additional monitoring that may be needed to determine WQS attainment status and, if necessary, to support development of TMDLs for each pollutant/AU combination;
- schedules for additional monitoring planned for AUs;
- pollutant/AU combinations still requiring TMDLs; and
- TMDL development schedules reflecting the priority ranking of each pollutant/AU combination.
- water quality "effluent limited" waters.

Virginia's biennial water quality assessment is conducted by the Department of Environmental Quality (DEQ), with the assistance of the Department of Conservation and Recreation (DCR), to determine the water quality conditions in the Commonwealth. The results of this water quality analysis are usually reported to the EPA by April 1 of even numbered years. The Integrated Report describes the aggregated water quality conditions of the State and contains the individual listing of those waters identified as "impaired" for one or more designated uses and needing a Total Maximum Daily Load (TMDL). As per EPA guidance, the former 305(b) Water Quality Assessment Report and the 303(d) Impaired Waters List are now combined into a single Integrated Report. EPA compiles the data from all State reports into a national water quality status report that is presented to Congress.

PART II. MODIFICATIONS TO PREVIOUS GUIDANCE

DEQ has incorporated EPA Integrated Reporting guidance initially developed in 2004, with all subsequent versions current to January 2014. DEQ's guidance for the 2014 Integrated Report contains the following modifications:

- The addition of two new subcategories to the assessment scheme: Category 4D and Category 5R.

  Category 4D, applicable only to segments impaired for dissolved oxygen and covered by the Chesapeake Bay TMDL, applies to waters where part(s) of a dissolved oxygen standard previously failed and currently meet, but other pertinent criteria have not been assessed due to insufficient information. Category 4D waters are considered impaired for dissolved oxygen. In past assessments, these waters were not easily differentiated from those failing Bay dissolved oxygen criteria.

  Category 5R describes waters for which there is an EPA-approved restoration plan that addresses specific impairment(s) and is expected to result in use attainment once implemented. This category is described in more detail in Appendix D-2.

- Clarification on the use of Colony Forming Units (CFU) and Most Probable Number (MPN) for recreational bacteria assessment.
- Guidance on recreational bacteria assessment using geometric means has been refined so that it is better aligned with Water Quality Standards.
- A minimum of two samples (excluding benthic evaluations) is now required for toxic-related use assessments.
- The Coastal Plain Macroinvertebrate Index has been replaced with the new Virginia Coastal Plain Macroinvertebrate Index.
- The expansion of Category 3B for estuarine benthic assessments, to include segments where assessment results have a high degree of uncertainty.
- Refinements to guidance for the nesting of Category 4A waters.
- Data collected with multi-probe meters that are reported to two decimal places should be rounded to the tenths' place. Such data have been truncated to one decimal place in previous assessments.
- Waters are no longer classified as "threatened" when analysis indicates a degrading trend in a parameter (previously specified in "Rule 11" of the General Rules section). This classification is no longer used in assessment.
- In previous guidance, a minimum duration of uninterrupted data collection (e.g, 30 *consecutive* days) was specified for continuous monitoring datasets. To allow for the inclusion of more "punctuated" datasets in assessments, this stipulation was dropped from General Rule 5.
- Uncensored values should be pulled from the Comprehensive Environmental Database System (CEDS) when evaluating toxics. (General Rule 17).

Additionally, it should be noted that reported stream mileage will expand considerably with the conversion of the National Hydrography Dataset (NHD) from 1:100K to 1:24K resolution. The increase in stream miles will reflect the incorporation of previously unmapped waterways and refinements to the representation of already mapped waters. The state's total stream mileage is expected to double in size once the conversion is completed.

Lake acreage and estuarine square mileage are not expected to change substantially.

PART III. FEDERAL AND VIRGINIA ASSESSMENT CATEGORIES

The 2014 EPA Integrated Report Guidance and Assessment Database (ADB V2.3.1) has five major categories and three subcategories which every "assessment unit" (AU) are placed based on designated use attainment. Additionally, Virginia has created several subcategories to supplement the federal categories, enabling a more precise water quality tracking and reporting mechanism.

Below are the US EPA-defined categories and associated Virginia-defined subcategories**:**

FULLY SUPPORTING - Waters are supporting one or more designated uses

- **EPA Category 1 -** Attaining all associated designated uses and no designated use is threatened

  Va. Category 1A - waters are attaining all uses and a TMDL has been developed for one or more uses.

- **EPA Category 2 –** Available data and/or other information indicate that some, but not all of the designated uses are supported.

  Va. Category 2A - waters are supporting all of the uses for which they are monitored.

  Va. Category 2B - waters are of concern to the state but no Water Quality Standard exists for a specific pollutant, or the water exceeds a state screening value or toxicity test.

  Va. Category 2C - waters are now attaining the use(s) for which they were originally 303(d) listed and the TMDL is EPA approved but other applicable use(s) were not monitored and assessed.

INDETERMINATE - Waters needing additional information

- **EPA Category 3 -** Insufficient data and/or information to determine whether any designated uses are met.

  Va. Category 3A - no data are available within the data window of the current assessment to determine if any designated use is attained and the water was not previously listed as impaired.

  Va. Category 3B - some data exist but are insufficient to determine support of designated uses. Such waters will be prioritized for follow up monitoring, as needed.

  Va. Category 3C- data collected by a citizen monitoring or another organization indicating water quality problems may exist but the methodology and/or data quality has not been approved for a determination of support of designated use(s). These waters are considered as having insufficient data with observed effects. Such waters will be prioritized by DEQ for follow up monitoring.

  Va. Category 3D - data collected by a citizen monitoring or other organization indicating

designated use(s) are being attained but the methodology and/or data quality has not been approved for such a determination.

IMPAIRED - Waters are impaired or threatened but a TMDL is not required.

- **EPA Category 4A –** water is impaired or threatened for one or more designated uses but does not require a TMDL. In the case of a nested water, a new TMDL is not necessary to address the newly impaired water if the nesting procedure is followed (see Part VII, Rule 3).

- **EPA Category 4B -** water is impaired or threatened for one or more designated uses but does not require the development of a TMDL because other pollution control requirements (such as VPDES limits under a compliance schedule) are reasonably expected to result in attainment of the Water Quality Standard by the next reporting period or permit cycle.

- **EPA Category 4C -** water is impaired or threatened for one or more designated uses but does not require a TMDL because the impairment is not caused by a pollutant and/or is determined to be caused by natural conditions.

  Va. Category 4D – part(s) of a water quality standard is attained for a pollutant with a TMDL, but the remaining criteria for the standard were not assessed due to insufficient information. (Only to be applied to dissolved oxygen in tidal waters of the Chesapeake Bay).

IMPAIRED - Waters are impaired or threatened and require a TMDL

- **EPA Category 5 - Waters are impaired or threatened and a TMDL is needed.**

  Va. Category 5A - a Water Quality Standard is not attained. The water is impaired or threatened for one or more designated uses (excluding shellfish use) by a pollutant(s) and requires a TMDL (303d list).

  Va. Category 5B - the Water Quality Standard for shellfish use is not attained. One or more pollutants causing impairment require TMDL development.

  Va. Category 5C - the Water Quality Standard is not attained due to "suspected" natural conditions. The water is impaired for one or more designated uses by a pollutant(s) and may require a TMDL (303d list). WQ Standards for these waters may be re-evaluated due to the presence of natural conditions.

  Va. Category 5D - the Water Quality Standard is not attained where TMDLs for a pollutant(s) have been developed but one or more pollutants are still causing impairment requiring additional TMDL development.

  Va. Category 5E - effluent limited facilities are not expected to meet compliance schedules by next permit cycle or reporting period.

  Va. Category 5F - the Water Quality Standard is attained for a pollutant(s) with a TMDL and

303(d) delisting approved but the water remains impaired for additional pollutant(s) requiring TMDL development.

Va. Category 5R - the Water Quality Standard is not attained and the water is impaired, and implementation of an EPA-approved restoration plan is expected to result in attainment. A status update will be provided each 303(d) cycle to evaluate progress.

- **EPA Category 5M** – the Water Quality Standard is not attained for mercury primarily due to atmospheric deposition.

PART IV. GENERAL RULES OF WATER QUALITY ASSESSMENT

305(b)/303(d) assessments seek to characterize surface waters under typical, ambient conditions. For this reason, water quality assessments are based on data that are representative of normal conditions. The assessment begins by analyzing all QA/QC approved data from DEQ ambient water quality monitoring stations, biological, sediment and fish tissue monitoring, special studies and/or other non-DEQ water quality data collected during the six-year assessment period. This interval of time works in concert with the ambient rotating watershed monitoring program. Assessment data are compared to both numeric and narrative criteria established for Virginia's designated uses and promulgated in its water quality standards (WQS). Listing decisions will not be based on datasets that are solely targeted or biased.

The following list of rules is to be applied uniformly, only to be modified after internal review or directive from EPA. Specific assessment procedures are outlined later in this document.

Rule 1

Impaired waters are defined as those with exceedences of recurring or human health-related water quality standards as documented by QA/QC-approved monitoring data. Predictive data generally refers to computer-generated modeling data and may be used for assessment purposes on a case-by-case basis. Impairments are generally determined from exceedences of the numeric/narrative water quality standards, using the guidelines described in Part V of this manual.

Previous EPA guidance recommends the use of an exceedence rate of >10.5% of the total samples analyzed to establish impairment for conventional parameters (i.e, dissolved oxygen, pH, temperature, and bacteria). This "allowable" exceedence rate takes into account equipment failure and/or human error and only applies to acute or instantaneous criteria. Single samples (n = 1) will be considered insufficient information for these assessments. A single exceedence of the WQS for conventional parameters is also considered insufficient justification for 303(d) listing (though sufficient for "observed effects" categorization). At least two exceedences and > 10.5% of the total samples are required for a water to be listed as impaired. Temperature in tidal waters up to the fall line will not be assessed due to the lack of standards for temperature in these waters.

Rule 2

Waters where restrictions are placed on shellfishing and fish consumption uses by the Virginia Department of Health (VDH) are in violation of narrative water quality standards (9 VAC 25–260–10 A.) and determined to be impaired, unless the designated use has been administratively removed. Uses are administratively removed in the presence of a permitted discharge outfall and any associated VDH safety zone, salinity regimes are not conducive for productive harvest, or a consumption advisory not restricting the designated use has been issued.

Rule 3

Escherichia coli (freshwater) and enterococci (saltwater and transition zone) data will be assessed for the recreation designated use. These indicators replaced fecal coliform bacteria in 2006. Any waters

previously listed for fecal coliform will remain as impaired until appropriate bacteria data are available and assessed.

The *E. coli*/enterococci instantaneous maximum standard of 235 per 100 ml (*E. coli* in freshwater) and 104 per 100 ml (enterococci in saltwater and transition zone) applies when a minimum of four weekly samples per month are not available to calculate a geometric mean. Where data are not sufficient to calculate a monthly geometric mean, at least two exceedences and >10.5% of the total samples taken during the assessment period exceeding the instantaneous maximum bacteria standard for primary contact recreation is impaired.

When appropriate, the monthly geometric mean standard of 126 per 100 ml (*E. coli*) for freshwater and 35 per 100 ml (enterococci) for saltwater and transition zone applies when a minimum of four weekly samples are collected during any calendar month. See 9 VAC 25-260-140-C for freshwater, saltwater, and transition zone delineation. One geometric mean exceedence in the assessment window constitutes an impairment. However, beaches under surveillance by VDH and characterized by weekly monitoring during warm weather months (May to September) are allowed a single exceedence of the geometric mean provided that it does not occur within the most recent two years of the assessment window and it can be attributed to an unusually intense wet-weather event such as a hurricane or tropical storm.

Bacteria densities reported as both Colony Forming Units (CFU) and Most Probable Number (MPN) shall be assessed against the numeric values in 9VAC25-260-170-A, pursuant EPA's approval of the methods specified in 40 CFR Part136.3. Approved test methods that report either unit shall be used for assessment.

Rule 4

Conventional parameter data generated by probabilistic monitoring (ProbMon) networks will be used to create a general overview of those waters and to direct targeted monitoring in the future. For most probabilistic monitoring stations, only one data point per parameter will be available, providing insufficient information for determination of impairment. A single "grab sample" exceedence of human health or aquatic life toxic criteria is assessed as fully supporting with an observed effect and follow-up monitoring should be conducted within a three-year period to determine if the water is impaired. A single chronic or acute exceedence of a 30-day semi-permeable membrane device (SPMD) sample for a toxic parameter associated with aquatic life and wildlife use is considered fully supporting with an observed effect. A single fish tissue, 30-day SPMD sample, or sediment sample with no exceedence is considered fully supporting the associated use because these types of samples are associated with longer-term water quality conditions. For probabilistic stations with two data points for conventional parameters, assessment will be the same as any station with two or more data points. Benthic data will be compared to the Virginia Stream Condition Index (VSCI) or Virginia Coastal Plain Macroinvertebrate Index (VCPMI) and assessed accordingly.

Rule 5

To be eligible for assessment, a continuous monitoring dataset must cover at least 30 days (consecutive or otherwise), except in the assessment of maximum hourly temperature change criteria, which may be assessed on a dataset spanning no less than 15 days. The continuous monitoring dataset will have undergone rigorous and standardized QA/QC screening before analysis. If a continuous monitoring

dataset is used to place a water on the 303(d) Impaired Waters List, then an additional continuous monitoring dataset, collected during a subsequent year, during the same month(s) as the listing dataset, must be used to delist it. See Section 5.12 for detailed assessment methodology.

Rule 6

When data are insufficient for the determination of use attainment but indicate possible impairment, additional monitoring should be considered. "Observed effects" are indications in the form of single sample WQS exceedences, observed pollutants or signs of water quality degradation (i.e., fish kills) lacking specific standards, or lower quality data that point to possible impairment (e.g., high bacteria counts on a Coliscan® plate). This rule applies to conventional and toxic parameters (water column, sediment, nutrient, and fish tissue) as well as biological monitoring.

Rule 7

Waters that are suspected to be impaired due to naturally occurring, non-anthropogenic conditions will be classified as Category 5C (possibly needing a TMDL) of the Integrated Report. Examples of natural impairments include low DO and/or pH in slow-flowing Class VII (swamp) waters or high temperature from thermal springs. If natural conditions are shown to be responsible for the impairment, the water will then be listed in Category 4C (impaired but not needing a TMDL). For waters in Category 5C or 4C, the water quality standards will be reviewed and possibly updated during the next triennial review to reflect variations caused by natural conditions for these waters. Once appropriate water quality standards are in place, data will be reviewed again to determine whether these waters meet or exceed designated uses. It may be necessary to conduct a TMDL study or Use Attainability Analysis prior to standards modification in order to determine and/or verify the appropriate criteria based on natural pollutant loadings.

Until such time that a study has been conducted that establishes the natural dissolved oxygen level(s) for a specific Class VII water, and monitoring confirms that there are no exceedences, such water may not be classified as in attainment. However, such a study is not required until either nutrient levels, land use changes, new point sources, or other potential anthropogenic causes and/or sources are occurring that could affect water quality. Once a dissolved oxygen criterion is established for a Class VII swamp water, adequate monitoring will be conducted to determine attainment or non-attainment of the criterion and the water may be reclassified in the next water quality assessment.

Rule 8

Waters that have been assessed as fully supporting (Category 2) or impaired will continue to be tracked in the Assessment Database (ADB) and monitoring station list, whether they have current monitoring data or not. Waters will retain the results of the previous assessment for all designated uses. Waters previously listed as impaired will carry this designation until a TMDL is developed or additional monitoring data reveal the waters are no longer impaired and needing a TMDL. (Justification must be provided to EPA before removing an impaired water from the 303(d) Impaired Waters List.) In contrast, Category 2 waters can retain their assessment status for only two additional reporting cycles with no new data. After two reporting cycles with no additional data, the water will be assessed as having insufficient information and will remain as such until new data is collected and assessed.

Rule 9

For effluent limited waters, if the VPDES permit has been issued with a scheduled compliance date that extends beyond the next 303(d) listing or permit cycle, the water would be listed as Category 5E. If the compliance date falls within the next listing cycle or within the current permit cycle whichever is longer, the water would be listed in Category 4B. See Part VII for additional information.

Rule 10

Duplicate and/or split samples collected for QA/QC purposes will not be used in the assessment. The primary sample (S1) will be assessed against the appropriate standard and the duplicate/split sample (S2) will be used only to document lab analysis quality control.

Rule 11

Sampling stations that happen to be located within a permitted mixing zone, primarily via probabilistic monitoring, will not be individually assessed for aquatic life use. They will be included with the overall probabilistic assessment. Any other stations that inadvertently were located in mixing zones will not be assessed individually for aquatic life use as the use is exempt in mixing zones.

Rule 12

A review of stockable and some natural trout waters currently listed as impaired has revealed that many of these impairments are due to erroneous segment boundaries or natural conditions. Both issues were addressed as part of Virginia's most recent review of its water quality standards. For the 2014 assessment, these waters will be categorized as Category 2A if specifically addressed via standards and currently meeting new criteria or 4C (impaired due to natural conditions and not needing a TMDL) as long as supporting documentation is provided by the Virginia Department of Game and Inland Fisheries (VDGIF) and endangered species are not being adversely affected. This should prevent and/or correct the misclassification of these segments, pending any amendments during Triennial Review. Once these standard reviews are completed and EPA approved, these waters will be re-evaluated and classified accordingly.

Rule 13

Data associated with multi-probe meters are to be rounded to one decimal place.

Rule 14

Nested impairments are those waters that are included ("nested") in a pre-existing TMDL watershed. A TMDL requires a specific loading reduction for all waters within the watershed whether they currently meet the standard or not. Once the TMDL is approved by EPA, any waters within the watershed that are subsequently assessed as impaired for the parameter targeted by the TMDL will be considered Category

4A (impaired but has a TMDL). Nested impairments are normally bacteria related to recreation and shellfish consumption uses, but may also include benthic impairments (aquatic life use) depending on the stressors involved.

Rule 15

Division of Consolidated Laboratories (DCLS) has determined that total dissolved ammonia and total ammonia are essentially the same thing. Thus, where only dissolved ammonia data are available, these will be used to assess against the total ammonia criteria. Where both data are available, total ammonia should be used to assess the criteria.

Rule 16

Shellfish waters where restrictions or prohibitions are due solely to a discharge outfall and associated buffer zone or where the use is deemed too limited to harvest due to low salinity or other natural reasons—and not due to water quality exceedences—will not be included in the 303(d) list. In these cases, monitoring should not be conducted as the shellfish designated use has been administratively removed through the issuance of a discharge permit or prohibition on harvesting.

Rule 17

Uncensored values should be pulled from the Comprehensive Environmental Database System (CEDS) when evaluating toxics.

PART V. ASSESSMENT METHODOLOGY

5.1 Monitoring Station Siting and Delineation

DEQ has a vast network of active Ambient Water Quality Monitoring (AWQM) stations and a number of biological stations statewide. The AWQM stations are generally monitored bimonthly while the biological stations are normally monitored twice a year (usually in the spring and fall). Monitoring programs can be designed based on a "targeted" (conventional) approach or a "probability based" approach or a combination of the two. Each monitoring program design has its advantages and disadvantages. Historically, most of DEQ's monitoring strategy has been based on the conventional approach. Many of the stations were located in proximity to (above and below) Virginia Pollutant Discharge Elimination System (VPDES) facility outfalls. During this reporting cycle, DEQ has continued to use a rotating watershed approach where stations are sited for two years of bimonthly sampling. The number of stations per watershed is based on the drainage area of the watershed and the Department of Conservation and Recreation (DCR) "nonpoint source potential" rating of the watershed. In order to provide consistency between the regional planning staff and to get an accurate number of assessed stream miles in Virginia, the following stream delineation guidelines are the primary considerations used in the assessment unit (segment) size decisions. However, in certain cases, best professional judgment of the regional staff may be used if the delineation results are contrary to these guidelines. Where appropriate, documentation of these decisions should be included in the segment narrative.

1. Typically, no more than 10 miles of free-flowing stream should be assessed by conventional pollutant data from one ambient monitoring station. Miles assessed for a toxic pollutant or biological impairment may vary from the miles assessed for conventional parameters.

2. One monitoring station should not be used to assess an entire watershed unless land use, source, and habitat are relatively homogeneous.

3. When determining the miles assessed for a free-flowing monitoring station, the following items need to be considered:

   a) WQ Standards use designations (i.e. classes and/or special standards)

   b) point and/or nonpoint source input to the stream or its tributaries,

   c) watershed characteristics such as land use,

   d) local habitat characteristics such riparian vegetation, stream banks, substrate, slope, or channel morphology,

   e) entry of a large tributary or diversion, or

   f) hydrologic features such as channelization or dams.

4. For non-Bay program tidal and estuarine stations, EPA guidance suggests using a 4-mile radius for open water stations; a 2-mile radius for sheltered bay stations, and a 0.5 mile radius for highly sheltered bay stations.

5. Segment delineation will be performed using the USGS National Hydrography Dataset (NHD) coverage or other appropriate GIS dataset.

6. Spatial coverage for estuarine probabilistic monitoring stations should be identified in conjunction with the development of the monitoring plan and coordinated by regional monitoring and assessment staff and/or the Chesapeake Bay Program monitoring coordinator and Bay monitoring staff. Estuarine B-IBI data will be assessed according to the methodology described in Appendix G.

7. If the mixing zone of a VPDES-permitted facility exists in an impaired segment, the parameter-specific length of the mixing zone is specifically understood to be separate from the impaired segment, even though the boundaries of the segment and/or its description may show the impairment as continuous.

8. Probabilistic stations in free-flowing waters will not be delineated into 303(d) segments unless they are characterized by toxics data, biological data, or more than one measurement of a conventional parameter.

5.2 Evaluation of Designated Uses

The 305(b) process assesses a total of six primary designated uses, as appropriate for a particular waterbody, based on WQS: wildlife, aquatic life, recreation, fish consumption, shellfishing, and public water supply uses.

The Chesapeake Bay criteria, adopted in 2005, have sub-divided the aquatic life use into several distinct sub-uses. Following are details relating to the assessment of the six designated uses of Virginia's waters.

1. Wildlife Use:

Wildlife use includes the propagation, growth, and protection of a balanced, indigenous population of wildlife.

Support of wildlife use is determined by assessing the toxic standards for aquatic life found in 9 VAC-25-260-140 B. These criteria were developed to protect aquatic life as well as wildlife. For toxic pollutant assessment in free-flowing streams, waters where there are two or more samples and no exceedences of aquatic life criteria within a running 3-year period, using grab samples or SPMD data, are considered fully supporting for wildlife use. A single exceedence of aquatic life criteria within a 3-year period, using grab samples or SPMD data, is considered fully supporting but having an observed effect. For toxic pollutant assessment in free-flowing streams, waters where there are two or more exceedences of the same WQS acute aquatic life toxic criteria in a running 3-year period using grab samples or SPMD data are considered impaired for wildlife use.

2. Aquatic Life Use:

Aquatic life use includes the propagation, growth, and protection of a balanced indigenous population of aquatic life (including game and marketable fish) which may be expected to inhabit the waters.

Support of aquatic life use can be determined by the assessment of conventional parameters (dissolved oxygen, pH and temperature), toxic pollutants in the water column (relative to acute water quality standards), toxic pollutant analysis of sediments, toxicity testing, nutrient analysis and/or the biological assessment of benthic communities. All available data, relative to aquatic life use, shall be considered to determine if the aquatic life use is being supported. This assessment includes the sub-categories of aquatic life use associated with the Chesapeake Bay criteria. The maximum temperature will not be assessed for aquatic life in tidal waters since no maximum temperature standard is applicable.

Conventional parameters are assessed using the "Percent Method". A 10.5% exceedence threshold is used for determining full support or impairment for conventional pollutants. An exceedence rate that is > 10.5% with at least two exceedences is normally considered impaired. An exceedence rate ≤ 10.5% is considered fully supporting. A single exceedence in a small dataset (2-9 samples) is considered insufficient to indicate fully supporting or impaired.

For dissolved oxygen, the instantaneous minimum standard is used to assess exceedences unless continuous monitoring data are available to assess the daily average. See Section 5.12 for assessment methodology for continuous monitoring. Dissolved oxygen in the Chesapeake Bay and its tributaries is assessed according to the method outlined in Section 5.3.

For free-flowing stream benthic macroinvertebrate assessments, data for the overall assessment period is rated as non-impaired when the Virginia Stream Condition Index (VSCI) or the Virginia Coastal Plain Macroinvertebrate Index (VCPMI) scores are at or above their respective impairment thresholds. (60 for the VSCI or 40 for the VCPMI).

A project to refine the estuarine biological assessment methodology (B-IBI) was completed in 2006 and approved for use by EPA. The same methodology will be used again for 2014. See Section 5.3 and Appendix G for more information.

For toxic pollutant assessment in free-flowing streams, waters where there are two or more samples and no exceedences of aquatic life criteria within a running 3-year period, using grab samples or SPMD data, are considered fully supporting for aquatic life. A single exceedence of aquatic life criteria within a 3-year period, using grab samples or SPMD data, is considered fully supporting but having an observed effect. For toxic pollutant assessment in free-flowing streams, waters where there are two or more exceedences of the same WQS acute aquatic life toxic criteria in a running 3-year period using grab samples or SPMD data are considered impaired for aquatic life use.

3. Fish Consumption Use:

Fish consumption use includes the propagation, growth and protection of a balanced population of aquatic life including game and marketable fish. Human health is also a primary consideration with regard to fish consumption use. Support of this use is determined using three separate criteria.

First, support or lack thereof, is based on human health related advisories and/or restrictions issued by the Virginia Department of Health (VDH). The fish consumption use is determined to be impaired when the public is advised by VDH that fish consumption is prohibited for the general population or an advisory that certain fish species should not be consumed by the general population or sub-populations at greater risk, such as children and/or pregnant women.

Second, the assessment methodology used for fish consumption use is a comparison of fish tissue data to WQS criterion-based tissue values (TVs) and tissue screening values (TSVs) for toxic pollutants. Any single observation above the TV or TSV results in the water being assessed as fully supporting but having an observed effect. Two or more exceedences of a particular TV listed in Appendix E-1 results in an impaired assessment of the water for the fish consumption designated use.

Third, support of the fish consumption use is determined by comparison of water column or semi-permeable membrane device analytes to the human health criteria in public water supplies and other surface waters, as listed in the WQS (9 VAC-25-260-140 B).

4. Shellfishing Use:

Shellfishing use includes the propagation, growth and protection of a balanced population of aquatic life including marketable shellfish.

Use support is based on the determination of restrictions or condemnations on the harvesting and marketability of shellfish resources made by the VDH-Division of Shellfish Sanitation (DSS) as of the most recent condemnation list (January 2013) associated with the reporting period. DSS has the statutory authority to determine shellfish harvesting and marketability status. DSS uses four classifications for describing the status of shellfish waters:

Approved area:	Growing areas from which shellfish may be taken for direct marketing at all times.
Conditionally Approved:	Growing areas where the water quality may be affected by seasonal or sporadic use of boat docks or harbor facilities are considered conditionally

approved. Normally, this would occur during the boating season (April 30 through October 31).

Restricted Area:	Growing areas where a sanitary survey indicates a limited degree of pollution which makes it unsafe to market shellfish for direct marketing. Shellfish from such areas may be marketed after purifying or relaying activities in accordance with certain VDH-DSS requirements.
Prohibited Area:	Growing areas where the sanitary survey indicates dangerous numbers pathogenic microorganisms or other contaminants that might reach that area. The harvesting of shellfish from these areas for direct marketing, relaying, or depuration is prohibited. Additionally, prohibited areas due to administrative closures.

Specific information regarding DSS assessment methodology and the listing/delisting flowchart for shellfish waters can be found in Appendix C of this guidance document. For the 305(b)/303(d) Integrated Report, listing and delisting will be based on data assessed for the reporting period. However, as the TMDL begins development, if new or more recent data shows the shellfish water is no longer impaired, a petition for delisting will be crafted and submitted to EPA for their approval by the Watershed Program (TMDL) staff.

5. Recreation/Swimming Use:

Recreation use assessment includes swimming and other primary and secondary water contact recreation uses such as water skiing and pleasure boating.

Normally, support or lack thereof of this use is determined based on a comparison of *E. coli* (freshwater) or Enterococci (saltwater) bacteria data to the instantaneous criterion and applying the > 10.5% assessment rule. However, if a special study, designed to collect at least 4 weekly bacteria data points within a calendar month, is conducted, such as in VDH's BEACH (Beaches Environmental and Coastal Health) program, then these results should be compared to the appropriate geometric mean criterion described in 9 VAC-25-260-170. A water is considered impaired for the recreation use under the following conditions: more than 10.5% of bacteria samples exceed the instantaneous criterion, there is a single geometric mean exceedence, or VDH has issued one or more beach closures of at least one-week duration due to contamination or two or more swimming advisories of at least one week-duration due to contamination—based on QA/QC-approved data within the assessment cycle with a medium to high probability that the closure/advisory will recur.

For bacteria monitoring in lakes/reservoirs, including the monitoring of freshwater beaches, data from multiple stations should be aggregated unless there is reason to believe stations represent disparate environments (e.g., isolated coves).

6. Public Water Supply Use:

Waters that are used for public drinking water supply are identified in the WQS and are protected by additional health related standards that are applicable only to these waters. Support or lack thereof of this use is based on VDH closures or advisories due to excessive pollutant(s) and/or a comparison of water column data to applicable public water supply criteria. Impairment is determined if one or more VDH public water supply

source closures due to contamination are issued within the assessment cycle, with a medium to high probability that the contamination will recur.

Table 1 and 2 summarize the kind of information required to establish designated use support.

Table 1.

DESIGNATED USE	USE DESCRIPTION/INDICATORS
Aquatic Life Use, Chesapeake Bay sub-uses	Description: The propagation, growth, and protection of a balanced indigenous population of aquatic life that may be expected to inhabit a waterbody
	Indicators: Dissolved oxygen, pH, temperature, chlorophyll a*, nutrients*, water column and sediment toxics, toxicity tests, benthics, submerged aquatic vegetation
Fish Consumption Use	Description: Game and marketable fish species that are safe for human health
	Indicators: VDH notices, fish tissue toxics, water column toxics
Shellfishing Use	Description: Marketable shellfish (clams, oysters, mussels) that are safe for human health
	Indicators: VDH notices
Recreation (Swimming) Use	Description: Swimming, boating, and other recreational activities
	Indicators: VDH notices, bacteria
Public Water Supply Use	Description: Drinking water safe for human health
	Indicators: VDH notices, water column toxics
Wildlife Use	Description: The propagation, growth, and protection of associated wildlife
	Indicators: Water column toxics

*Chlorophyll *a* and nutrients (total phosphorus) are assessed only in the lakes listed in Section 187 of the WQS. Chlorophyll *a* is also assessed in the tidal James River.

Table 2.

Designated Use	Parameter/Data Type	Fully Supporting	Observed Effects (either as supporting or with insufficient data)	Impaired
Aquatic Life **Wildlife (toxics only)**	Conventional[1]	• n ≥ 2, exceedence rate ≤ 10.5% for field parameters • Median lacustrine TP[2] below criterion • 90th percentile lacustrine chlorophyll a below criterion	• Level II[3] data with an exceedence rate > 10.5% • Single exceedence in small dataset (n <10)	• n ≥ 2, exceedence rate > 10.5% for field parameters • Two exceedences in small dataset • Median lacustrine TP above criterion • 90th percentile lacustrine chlorophyll a above criterion
	Biological	Benthic index scores ≥ impairment threshold	• Level II[3] data suggest degraded community • Benthic index score conflicts with biologist's best professional judgment	Benthic index score < impairment threshold
	Toxics	n ≥ 2, no exceedences	• Single water column grab or SPMD[4] sample exceedence of acute aquatic life use criteria • Single water column grab or SPMD sample exceedence of chronic aquatic life use criteria in a 3-year period • Single sediment toxicity test or screening value exceedence (aquatic life only)	Two or more grab or SPMD exceedences of the same acute aquatic life criteria in a 3-year period
Recreation	Bacteria	• No geometric mean exceedence • n ≥ 2, exceedence rate ≤ 10.5%	• Level II[3] data with exceedence rate > 10.5% • Single exceedence in small dataset (n <10)	• One or more geometric mean exceedence(s) • n ≥ 2, instantaneous exceedence rate > 10.5% • Two instantaneous exceedences in small dataset
	VDH notice	No swimming advisory	A single short-term (< 1 week) VDH closure/advisory with low probability of recurrence, based on bacteria data	One or more closure(s) and/or two or more advisories > 1 week duration with medium or high probability of recurrence, based on bacteria data
Shellfishing	VDH notice	Approved shellfish harvest waters	Area classified as "conditionally approved"	Areas classified as "restricted" or "prohibited"—excluding VPDES[5] outfalls and administrations closures where no data are available
Fish Consumption	Toxics	No exceedences of fish tissue criteria	• Single exceedence of a human health criterion using grab sample or SPMD data • Single exceedence of a tissue value or tissue screening value	• Two or more exceedences of a human health criterion using grab sample or SPMD data • Two or more exceedences of a tissue value

Designated Use	Parameter/Data Type	Fully Supporting	Observed Effects (either as supporting or with insufficient data)	Impaired
	VDH notice	No advisories	A VDH advisory which does not limit consumption is in effect	A VDH advisory or restriction limiting or prohibiting consumption
Public Water Supply	Toxics	n ≥ 2, no exceedences	A single exceedence of human health criteria using grab or SPMD data	Two or more exceedences of the same human health criteria using grab samples or SPMD data
	VDH notice	No closures	A single VDH closure with low probability of recurrence	One or more closures with medium or high probability of recurrence

[1]*Refer to Section 5.3 for methodology specific to Chesapeake Bay criteria. Refer to Section 5.12 for methodology specific to continuous monitoring data.*
[2]*TP = total phosphorus concentration. Along with lacustrine chlorophyll* a, *only data from the most recent two years are aggregated. See Section 5.7 for methodology specific to lakes/reservoirs.*
[3] *Level II data are lower-quality data submitted to DEQ from other sources. See Part VI for more information.*
[4]*SPMD = semi-permeable membrane device (an instrument that passively samples ambient toxics over some length of time)*
[5]*VPDES=Virginia Pollution Discharge Elimination System*

5.3 Chesapeake Bay Assessment

In addition to assessment of criteria for state-wide aquatic life designated uses as described elsewhere in this document, the Chesapeake Bay and its tidal tributaries will be assessed for: 1) sub-categories of aquatic life use specific to the Chesapeake Bay estuarine system, and 2) the general narrative standard for aquatic life use through assessment of benthic invertebrate community condition. The following describes the aquatic life use sub-categories, applicable criteria, assessment process, segmentation issues, as well as Assessment Database (ADB) and Integrated Reporting issues. Bay-specific criteria for dissolved oxygen, chlorophyll *a*, and submerged aquatic vegetation/water clarity are detailed in 9 VAC25-260-185.

- **Migratory Fish Spawning and Nursery Designated Use**: waters in the Chesapeake Bay and its tidal tributaries that protect the survival, growth and propagation of the early life stages of a balanced, indigenous population of anadromous, semi-anadromous, catadromous and tidal-fresh resident fish species inhabiting spawning and nursery grounds. This designated use extends from the end of tidal waters to the downriver end of spawning and nursery habitats that have been determined through a composite of all targeted anadromous and semi-anadromous fish species' spawning and nursery habitats (see boundaries in U.S. Environmental Protection Agency. 2004. *Technical Support Document for Identification of Chesapeake Bay Designated Uses and Attainability 2004 Addendum*. Chesapeake Bay Program Office, Annapolis, Maryland. This designated use extends horizontally from the shoreline of the body of water to the adjacent shoreline and extends down through the water column to the bottom water-sediment interface. This use applies February 1 through May 31 and applies in addition to the open-water use described in this subsection.

- **Shallow-Water Submerged Aquatic Vegetation Designated Use**: waters in the Chesapeake Bay and its tidal tributaries that support the survival, growth and propagation of submerged aquatic vegetation (rooted, underwater bay grasses). This use applies April 1 through October 31 in tidal-fresh, oligohaline and mesohaline Chesapeake Bay Program segments, and March 1 through November 30 in polyhaline Chesapeake Bay Program segments and applies in addition to the open-water use described in this subsection.

- **Open-Water Aquatic Life Designated Use:** waters in the Chesapeake Bay and its tidal tributaries that protect the survival, growth and propagation of a balanced, indigenous population of aquatic life inhabiting open-water habitats. This designated use applies year-round but the vertical boundaries change seasonally. October 1 - May 31, the open water aquatic life use extends horizontally from the shoreline at mean low water, to the adjacent shoreline, and extending through the water column to the bottom water-sediment interface. June 1 - September 30, if a pycnocline is present and, in combination with bottom bathymetry and water column circulation patterns, presents a barrier to oxygen replenishment of deeper waters, this designated use extends down into the water column only as far as the upper boundary of the pycnocline. June 1- September 30, if a pycnocline is present but other physical circulation patterns (such as influx of oxygen rich oceanic bottom waters) provide for oxygen replenishment of deeper waters, the open-water aquatic life designated use extends down into the bottom water-sediment interface (see boundaries in U.S. Environmental Protection Agency. 2004. *Technical Support Document for Identification of Chesapeake Bay Designated Uses and Attainability 2004 Addendum.* Chesapeake Bay Program Office, Annapolis, Maryland. This designated use includes the migratory fish spawning and nursery and shallow-water submerged aquatic vegetation uses.

- **Deep-Water Aquatic Life Designated Use**: waters in the Chesapeake Bay and its tidal tributaries that protect the survival, growth and propagation of a balanced, indigenous population of aquatic life inhabiting deep-water habitats. This designated use extends to the tidally influenced waters located between the upper and lower boundaries of the pycnocline where, in combination with bottom bathymetry (depth, contour & shape) and water circulation patterns, a pycnocline is present and presents a barrier to oxygen replenishment of deeper waters. In some areas, the deep-water designated use extends from the upper boundary of the pycnocline down to the bottom water-sediment interface (see boundaries in U.S. Environmental Protection Agency. 2004. *Technical Support Document for Identification of Chesapeake Bay Designated Uses and Attainability 2004 Addendum.* Chesapeake Bay Program Office, Annapolis, Maryland.) This use applies June 1 - September 30.
- **Deep-Channel Seasonal Refuge Designated Use**: waters in the Chesapeake Bay and its tidal tributaries that protect the survival of a balanced, indigenous population of aquatic life inhabiting deep-channel habitats. This designated use extends to the tidally influenced waters at depths greater than the lower boundary of the pycnocline in areas where, in combination with bottom bathymetry and water circulation patterns, the pycnocline presents a barrier to oxygen replenishment of deeper waters (see boundaries in U.S. Environmental Protection Agency. 2004. *Technical Support Document for Identification of Chesapeake Bay Designated Uses and Attainability 2004 Addendum.* Chesapeake Bay Program Office, Annapolis, Maryland.) This use applies June 1 through September 30.

Assessment Process

Full details of the assessment processes are described in USEPA, *Ambient Water Quality Criteria for Dissolved Oxygen, Water Clarity, and* Chlorophyll a *for the Chesapeake Bay and Tidal Tributaries*, EPA 903-R-03-002, April 2003 and the 2004 (EPA 903-R-002 October 2004) and 2007 (CBP/TRS 285-07, EPA 903-R-07-003), 2007 (CBP/TRS 288/07, EPA 903-R-07-005), 2008 (CBP/TRS 290-08, EPA 903-R-08-001), and 2010 (CBP/TRS 301-10, EPA 903-R-10-002) addenda. A very general summarization of key aspects of the process follows.

The assessment period for DO, water clarity and chlorophyll *a* criteria shall be the most recent three consecutive years within the data window. When three consecutive years of data are not available, three years within the most recent data assessment window must be available and used for the assessment.

Attainment of the dissolved oxygen and numeric chlorophyll *a* criteria shall be assessed through comparison of a cumulative frequency distribution of criteria exceedences to the applicable criteria reference curve for each designated use. A first step in the process involves spatial interpolation and extrapolation of data collected at individual fixed locations to project water quality conditions throughout the segment. A subsequent step involves development of cumulative frequency distribution (CFD) of criteria exceedences combining both spatial and temporal domains for each segment-designated use combination. A final step is to compare this CFD of criteria exceedences against a reference CFD of allowable exceedences to determine if the criteria are attained.

The revised methodology for DO and chlorophyll *a* criteria assessment is described in the 2010 addendum. For DO, the algorithm used for the calculation of the pycnocline has been adjusted, and a 10% reference curve is now being used for the assessment of the Open Water and Deep Channel designated uses. Additionally, a geometric mean, rather than an arithmetic mean, has been explicitly specified for use in the assessment of seasonal chlorophyll a criteria.

For the Shallow Water Submerged Aquatic Vegetation use criteria, if the submerged aquatic vegetation (SAV) acres are met in any individual Chesapeake Bay Program segment, then the shallow-water submerged aquatic vegetation use is met in that segment. If the SAV acres are not met, then the water clarity criteria shall be examined with either a CFD methodology or a "water clarity acres" methodology. If sufficient water clarity is available to support SAV growth through either of these alternatives, then the Shallow Water Submerged Aquatic Vegetation use is met regardless of the number of acres of SAV in that segment.

The assessment of criteria for Chesapeake Bay continues to undergo refinements. We anticipate that if data of sufficient quantity and quality are available we may eventually be able to use the "spectral analysis" procedure described in *USEPA, Ambient Water Quality Criteria for Dissolved Oxygen, Water Clarity, and Chlorophyll a for the Chesapeake Bay and Tidal Tributaries,* EPA 903-R-03-002.

Assessment Units

The Chesapeake Bay program segmentation scheme (*Chesapeake Bay Program Analytical Segmentation Scheme-Revisions, Decisions and Rationales: 1983 -2003,* CBP/TRS 268/04. Chesapeake Bay Program, Annapolis, Maryland) shall be used as the assessment unit to determine attainment of the criteria in this section for each designated use. The spatial boundaries of each aquatic life use subcategory within each of these CBP segment are described in the *Technical Support Document for Identification of Chesapeake Bay Designated Uses and Attainability 2004 Addendum.* Chesapeake Bay Program Office, Annapolis, Maryland. Assessment results for each CBP segment/designated use will determine the Integrated Report listing category of all waterbodies (i.e. all ADB assessment Units) geographically within that CBP segment/designated use. For example, the listing category of all tidal Onancock Creek assessment units will be determined by the appropriate designated use attainment of CBP segment CB7PH. In this example, it is likely that only open water and shallow water uses of CB7PH extend into Onancock Creek.

Assessment Database (ADB) Reporting Units

The Assessment Database (ADB) is used to track assessment data for all designated uses in distinct geographically defined waterbodies across the state. ADB assessment unit spatial boundaries are defined by many factors including the spatial distribution of available data to assess for designated uses. There may be several ADB assessment units included in each Chesapeake Bay Program segment.

ADB can only accept estuarine assessment units defined by surface areas (i.e. square miles). The complete water column within that assessment unit is assigned to a single overall aquatic life use attainment. Each individual Bay segment assessment unit may have deep channel, deep water, and open water sub-categories of aquatic life designated use (that may only account for a portion of the total volume/area of the ADB assessment unit).

Each ADB assessment reporting unit will be designated as having the aquatic life use and sub-use status according to the appropriate CBP segment/Aquatic life sub-designated use assessment. The rules to be applied are:

a) Open water designated extends from "shoreline to shoreline" within each CBP segment and thus all ADB reporting units located within each CBP segment is reported as having "open water" aquatic life use attainment consistent with the CBP segment attainment of open water criteria.
b) Deep water and deep channel designated use spatial boundaries within each CBP segment are spatially constrained as smaller areas within the larger CBP segments (see *Technical Support Document for*

Identification of Chesapeake Bay Designated Uses and Attainability 2004 Addendum. Chesapeake Bay Program Office, Annapolis, Maryland. Thus the deep water or deep channel designated use status for each CBP segment will apply only to ADB reporting units which contain a "deep water" designated use area. The two-dimensional (i.e. square miles) size of each deep channel and deep water area will be measured and reported as the actual impaired area (i.e. the whole ADB unit area is not considered impaired if only a smaller deep water or deep channel area was impaired).

c) The Shallow Water Submerged Aquatic vegetation designated use applies only out to a maximum of 2 meter contour. Each ADB unit having this sub-use present in some portion will designate the sub-use as attained or not. However, the actual size of the impaired use will be tracked outside of the ADB system and reported in the Integrated Report as being only the size of area within the two meter contour.

d) The general standard aquatic life use status of the ADB unit will be assigned to the "worst case" status of aquatic life sub-use within that ADB assessment unit (e.g. an ADB reporting unit containing an open water use which meets its associated criteria and a deep water use which fails its associated criteria will be categorized as failing the general aquatic life use). Other criteria applicable to the general standard for aquatic life use (e.g. for benthic communities, toxics, or "weight of evidence" etc…) will also determine the overall aquatic life use attainment. If the general aquatic life use is impaired only due to a smaller area of aquatic life sub-use, then only the area (i.e. square mileage) of the sub-use is reported as impaired for general aquatic life use.

Impact of Chesapeake Bay TMDL

The Chesapeake Bay TMDL, developed by EPA in cooperation with Bay state partners, was approved by EPA on December 31, 2010. This TMDL focuses on reductions to nutrients (nitrogen and phosphorus) and sediment inputs into Bay waters (including major tributaries). Improvements in DO and water clarity, which are indicators for the Aquatic Life and Shallow Water Submerged Aquatic Vegetation designated uses, are anticipated after the implementation of this TMDL.

All Bay waters that are on the 303(d) list for dissolved oxygen, SAV, and chlorophyll *a* impairments should be in Category 4, and any waters newly impaired for these parameters should also get this designation. Chesapeake Bay and tidal tributary assessment units, as described in 9 VAC 25-260-185 (d), that have been previously listed as not supporting the aquatic life use and associated subcategories of aquatic life use due to DO criteria failure will continue to remain in Category 4 until all applicable criteria are attained (e.g. any 7-day mean or instantaneous criteria must be assessed and attained as well as the 30-day criteria).

Category 4D should be used to classify waters listed for dissolved oxygen by EPA in the 1999 consent decree that are found to be meeting all *assessed* dissolved oxygen criteria. For instance, if a water meets the 30-day mean criterion for the Open Water sub-use, but the 7-day mean and instantaneous minimum criteria were not assessed, the Open Water sub-use should be categorized as "4D" in the Assessment Database. In the absence of other aquatic life use impairments (pH, benthics, etc.), the aquatic life use for this water would be assessed as "4D" as well. Refer to Part III for the full description of Category 4D.

5.4 *Biological Assessments*

Biological monitoring of streams and rivers using benthic macroinvertebrates is an integral component of the water quality monitoring program in the Commonwealth of Virginia. Biological monitoring allows the Virginia DEQ to assess the ecological condition of streams and rivers. Benthic macroinvertebrate surveys are used to determine if the waterbodies meet their designated aquatic life uses.

The Virginia Stream Condition Index (VSCI)

In 2000, the United States Environmental Protection Agency (USEPA) contracted TetraTech to develop a multimetric macroinvertebrate index for Virginia. This index contains eight core metrics that when calculated into one number is known as the Virginia Stream Condition Index (VSCI). TetraTech developed the VSCI using Virginia's existing biomonitoring database, which contained a significant amount of upstream (reference) control sites for use with the USEPA's Rapid Bioassessment Protocols.

Using an independent probabilistic database (sample n=350) with data collected from 2001-2004, Virginia has validated the VSCI using a spatially diverse (ecoregionally and stream size) data set free of pseudoreplication. These probabilistic data sets have allowed DEQ to narrow data gaps and test the proposed VSCI against many classification variables, which include season, stream size, ecoregion, bioregion, river basin, regional office, and sampling technique. The VSCI validation study was designed to incorporate suggestions provided through public comment from the Academic Advisory Committee (AAC), the USEPA and the regulated community.

The validation study using probabilistic biological data has confirmed that the VSCI works well to discriminate between sites with acceptable water quality and habitat versus sites with degraded water quality and habitat. A VSCI impairment threshold score of 60 was determined from statistical analyses of the original TetraTech report and the DEQ validation study. The VSCI validation study and the aquatic life use assessment guidance using the VSCI has been reviewed and approved by the USEPA. The validation study "Using Probabilistic Monitoring Data to Validate the Non-Coastal Virginia Stream Condition Index" can be found at http://www.deq.virginia.gov/Portals/0/DEQ/Water/WaterQualityMonitoring/ProbabilisticMonitoring/scival.pdf.

The Virginia Coastal Plain Macroinvertebrate Index (VCPMI)

In the late 1990s, the United States Environmental Protection Agency (USEPA) coordinated a six-state monitoring effort to develop a multimetric macroinvertebrate index that included Virginia's coastal plain. This index contained five metrics that when calculated into one number is known as the Coastal Plain Macroinvertebrate Index (CPMI). This index was adopted by DEQ in the early 2000's to make aquatic life use impairment determinations in the coastal plain of Virginia. Virginia biologists recommended validation of the index and initiated a special study.

Over the past decade DEQ compiled a new database of coastal plain macroinvertabrate data, which includes significantly more Virginia reference samples than the original CPMI study. Virginia has created the new VCPMI using a spatially diverse (ecoregionally and stream size) dataset free of pseudoreplication. The VCPMI replaces metrics that did not work well in Virginia's coastal plain and has correctly calibrated each metric's best standard values. The VCPMI study has confirmed that the VCPMI works well to discriminate between sites with acceptable water quality and habitat versus sites with degraded water quality and habitat. The impairment threshold score of 40 was determined from statistical analyses conducted during the VCPMI study. The VCPMI study and the aquatic life use assessment guidance using the VCPMI have been reviewed and approved by the USEPA. The VCPMI technical report, "The Virginia Coastal Plain Macroinvertebrate Index", can be found at:
http://www.deq.virginia.gov/Portals/0/DEQ/Water/WaterQualityMonitoring/ProbabilisticMonitoring/vcpmi.pdf
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Free-flowing Aquatic Life Use Determination

The DEQ uses the VSCI for non-coastal streams for biological assessment as well as the Virginia Coastal Plain Macroinvertebrate Index (VCPMI) for coastal plain streams. Assessment rankings, based on a single VSCI or VCPMI bioassessment, are the result of the data evaluation and reduction of numerous measurements and observations conducted during the biomonitoring survey. Bioassessment measures the response of the biological community to all perturbations it has experienced. A single, properly conducted VSCI or VCPMI bioassessment is not a "single data-point" analogous to a single dissolved oxygen (DO) measurement or bacteria sample. Non-coastal streams with VSCI scores ≥ 60 or coastal plain streams with VCPMI scores ≥40 will be assessed as "fully supporting for aquatic life use". VSCI scores < 60 and CPMI scores < 40 will result in streams being listed as "impaired".

If the biologist has observed natural conditions, such as recent drought or flooding, etc, that could be responsible for a ranking below the impairment threshold, they should note the lack of confidence in the survey and the stream will be listed as "fully supporting but having observed effects for aquatic life use" until further analysis can be conducted.

The regional biologists should review the biological assessments for the assessment cycle and they should make a final biological assessment ranking based on these data. Since biomonitoring surveys are records of the condition of the community at the time of the survey, the most recent bioassessment should be the most accurate indicator of stream ecological health. An attempt to average the data would weaken the ability to accurately predict current conditions. In cases where biological assessment rankings fall above and below the impairment threshold over multiple sampling events, more weight should be given to the most recent bioassessment. In cases where only one biomonitoring survey was conducted, a stream may be assessed for aquatic life use based on a single VSCI or VCPMI score. A standardized fact sheet, as found in Appendix B of this manual, has been developed to help the regional biologists review and assess the data for the assessment cycle. The fact sheet allows for consideration of supplemental information about the watershed that is important in making the final assessment decision.

Estuarine Aquatic Life Assessment

In cooperation with EPA Region III and the State of Maryland, DEQ has developed an assessment methodology for estuarine benthic community biological (B-IBI) data. This methodology assures Bay-wide consistency in determinations of estuarine benthic impairments and requires a sample size >= 10 for statistical purposes. In order to assist with meeting the sample size requirement, a six-year data window is used. This corresponds with the data window used for the assessment of other non-Chesapeake Bay criteria data.

The methodology incorporates uncertainty in the reference condition and is based on the confidence limit and bootstrap simulation concept described in Alden et al. (2002). Bootstrap simulation (Efron and Tibshirani 1998) will be applied to incorporate uncertainty in reference conditions as well as sampling variability in the assessment data. For each habitat, a threshold based on percentiles in an unimpaired reference data set will be applied (i.e. 5th percentile). This threshold is not intended to serve as criteria for classifying individual B-IBI scores, rather it will be used to categorize the segment as impaired or not based on the proportion of samples below the threshold and the variance associated with this estimate.

The impairment assessment for each segment is based on the proportion of samples below the threshold with the variance in this proportion estimated by simulation. In each simulation run, a subset of the reference "unimpaired" data for each habitat is selected at random, and the threshold is determined (i.e., the B-IBI score at the 5th percentile of the un-impaired dataset). A random subset of the assessment data is compared to the threshold value to estimate the proportion of sites below the threshold. By repeating this process over and over again (2000 runs) we estimate the variance in the proportion of sites below the threshold from the bootstrap estimates. For this analysis, it is assumed that each reference 'un-impaired" data set (by habitat) is a representative sample from a "super population" of reference sites.

The assessment result for each benthic segment (i.e. % of area with IBI score below 5th percentile threshold) is then statistically compared ($p<0.05$) with the percentage that would be expected even if the segment is unimpaired. This percentage under "un-impaired" conditions is assumed to be 5%.

A benthic segment will be classified as having insufficient information (Category 3B) when the number of sites sampled during the six-year data window is less than 10. A segment will also be classified as Category 3B when the analysis suggests non-impairment but the difference between the upper and lower 95% confidence limits equals or exceeds 0.5 and the average BIBI score is less than 2.7. This is a new rule adopted for the 2014 assessment.

In addition to an assessment of impairment, a discriminant analysis tool (benthic diagnostic tool) has been developed that can be used to identify sources of stress affecting benthic community condition in the Chesapeake Bay (Dauer et al. 2002). The results can distinguish stress due to contaminants versus stress due to other factors (e.g., low dissolved oxygen, or unknown). This tool will be used to identify which impaired segments have high probability of sediment contamination. Separately from the discriminant tool, the B-IBI metric scoring will also be used to identify (1) insufficient abundance patterns consistent with a low dissolved oxygen effect and (2) excessive abundance patterns consistent with eutrophication effects in the absence of low dissolved oxygen events. The combined use of these causal analyses will be used to assign causes for benthic impairments to either 1) Sediment chemical contaminants 2) Low dissolved oxygen 3) Eutrophication or 4) Unknown.

The spatial assessment unit for determining attainment of the general standard for aquatic life use using benthic community data will be the same as used in the 2008 assessment report. These criteria assessment units are described in "*Chesapeake Bay Program Analytical Segmentation Scheme-Revisions, Decisions and Rationales: 1983 -2003,* CBP/TRS 268/04. Chesapeake Bay Program, Annapolis, Maryland" with the additional caveat that minor tidal tributaries are considered separate benthic assessment segments.

Assignment of aquatic life use status, as determined by benthic assessments to ADB reporting waterbodies, will be the same as described previously for the Bay criteria assessments found in Section 5.3. Each ADB reporting unit will be assigned the general aquatic life use status of the benthic assessment segment in which it is geographically located.

References:

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Report submitted to the USEPA Chesapeake Bay Program Office, Annapolis, Maryland, by Old Dominion University Department of Biological Sciences, Norfolk, Virginia. 65 pp.
Efron, B. and R. Tibshirani. 1998. An Introduction to the Bootstrap. Chapman & Hall/CRC.
Llansó, R.J., J.H. Vølstad, and D.M. Dauer. 2003. Decision Process for Identification of Estuarine Benthic Impairments. Final Report submitted to Maryland Department of Natural Resources, Tidewater Ecosystem Assessments, Annapolis, Maryland, by Versar, Inc., Columbia, Maryland.

5.5 Toxics Assessment

Fish Tissue (Consumption) Use

The Fish Tissue Monitoring Program (FTM) collects fish tissue samples from designated monitoring stations for contaminant analysis. FTM staff identifies the results of any analysis that exceeds the WQS criterion-based tissue value (TV) or tissue screening value (TSV) found in Appendix E-1 and E-2 respectively, for the toxic contaminants and provides the data to water quality assessment staff. Older fish tissue data may be included where deemed appropriate.

Fish tissue data collected at stations during routine monitoring throughout Virginia represent Tier 1 monitoring data. Tier 1 monitoring data are meant to identify sites where concentrations of contaminants in the edible portions of commonly consumed fish indicate a potential health risk to humans. Usually, three fish tissue composite samples are analyzed for chemical contaminants at each Tier 1 station. Each is a composite of edible fillets for one species of fish from a top-level predator, a mid-level predator, and a bottom feeder. If Tier 1 results reveal potential problems, a more intensive Tier 2 study is initiated by the FTM staff to determine the magnitude, geographical extent, and potential sources of contamination in the fish. The need for a more intensive Tier 2 study takes into consideration the severity of the potential concern and is initiated as soon after the discovery of a potential problem as resources allow. Generally, if additional information is requested by the Virginia Department of Health (VDH) for determining the need for fish consumption advisories, a follow-up monitoring effort is initiated the year after the discovery of the potential problem. If limited resources prevent this, the water body will be sampled more intensely as soon as resources allow and/or during the next scheduled monitoring event in the affected river basin.

Currently, most fish tissue monitoring is focused on the development of PCB TMDLs throughout the Commonwealth.

Analytical results for fish tissue are expressed in wet-weight and are compared to WQS TVs and TSVs for the toxic pollutants using EPA risk assessment techniques for non-carcinogen and carcinogen effects. WQS human health calculations use the 10^{-5} risk level adopted by the State Water Control Board in 1992, an average human body weight of 70 kg and a lifetime average fish consumption rate of 17.5 grams per day (general U.S. population adopted in 2008). These same values are used to calculate the human health water quality criteria found in 9 VAC 25-260-140 B. Also included in the calculation are toxicological data pertinent to human health effects. A reference dose (RfD) is used for non-carcinogen toxic effects and a cancer oral slope factor is used for carcinogen effects. TVs are based on the same toxicological data (and body weight, fish consumption, and RfD or cancer risk level) that form the basis for the water quality criteria listed in 9 VAC 25-260-140 B, under the column labeled "Human Health, All Other Surface Waters". These water quality criteria are water column concentrations that are based on a specific fish tissue concentration, which were calculated to represent a safe or acceptable minimal human health risk level. The water quality criteria are designed to prevent the fish from bioconcentrating the toxic contaminants to levels greater than these fish tissue concentrations. The TV concentrations listed in

Appendix E-1 represent the same fish tissue concentrations that are the basis for the water quality criteria listed in 9 VAC-25-260-140 B and may be considered the fish tissue concentration equivalent of those water quality criteria. Appendix E-1 contains TVs for all chemicals for which Virginia has adopted water quality criteria. However, many of the TVs listed in Appendix E-1 do not bioaccumulate and are not often found in fish tissue and have been included for completeness. All TVs are rounded to two significant digits.

Appendix E-2 also lists TSVs for additional toxic chemicals for which Virginia has not adopted water quality criteria that are based on fish tissue concentrations (those criteria listed under " Human Health, All Other Surface Waters" in 9 VAC 25-260-140.B). It includes chemicals recommended for monitoring by EPA or of special interest to DEQ as well as some chemicals that are based on recent changes to toxicological data and/or exposure assumptions that are different from those used to calculate the water quality criteria found in 9 VAC 25-260-140 B. The TSVs are updated using available data from the EPA IRIS database and/or recommendations from EPA or the VDH before each assessment effort.

If a fish tissue composite sample exceeds a single WQS TV or TSV, the water body should be assessed as fully supporting but having an observed effect for the fish consumption use (Category 2B). If the TV for the same toxic pollutant is exceeded in two or more samples from the same site, the water is considered impaired. For example, both of the following situations would qualify as impaired under this criterion: two different fish samples from different species during one sampling event or two or more different samples of the same or different species from different sampling events. Data from all Tier 1 and Tier 2 monitoring studies are evaluated by DEQ as well as provided to the VDH for their consideration of the need for establishing fish consumption advisories. DEQ and VDH have signed a Memorandum of Agreement (MOA) that describes how the agencies exchange information regarding the results of all Tier 1 and Tier 2 fish tissue monitoring. If VDH issues a fishing ban or advisory, limiting consumption, the segment should be designated impaired for fish consumption use based on the advisory. The results of the Tier 2 study should be clearly communicated in the Integrated Report narrative.

Sediment (aquatic life use)

Similar to the sediment monitoring and analysis conducted by FTM, the regional offices will assess the AWQM sediment data. For freshwater sediments above the fall-line and tidal fresh zones, as described in the WQS, the consensus-based Probable Effects Concentrations (PEC; MacDonald et al. 2000) should be applied. Estuarine sediment contaminant data collected during scheduled AWQM monitoring should be compared to National Oceanic and Atmospheric Administration (NOAA *Screening Quick Reference Tables* (SQuiRT) Tables 1999) for effects-range-median (ER-M) SVs for sediment. Transition zones should be assessed against the more stringent of the two screening values. One or more exceedences of an ER-M/PEC value results in a fully supporting but having observed effects status for aquatic life use support. In these cases, additional biological monitoring should be scheduled to assess actual aquatic life use support. For National Coastal Assessment, a "weight of evidence" approach using sediment toxicity and sediment chemistry will be used to determine aquatic life designated use. See Appendix G for additional information. All metals contaminant screening values found in Appendix F have been converted to parts per million (ppm) for consistency.

Freshwater Toxics Evaluation (Water Column)

For overall freshwater toxics evaluation, DEQ uses the Virginia WQS for human health in surface waters, other than public water supplies (9 VAC 25-260-140 B). These same values are used to assess the fish consumption use in public water supplies as well as all other surface waters. (Please note the criteria for human health in public water supplies will be used to assess the drinking water use in PWSs only). For metals assessment, only

dissolved metals data will be used. In conformance with water quality management plans and VPDES permitting procedures, water column toxicant data collected up to six years prior to the current 305(b) period should be assessed along with current data if they reflect current conditions. When assessing the aquatic life and wildlife use support for toxic contaminants, compliance should be based on meeting the aquatic life WQS found in 9 VAC 25-260-140 B. See Section 5.2 for additional information.

Virginia will declare waters impaired for aquatic life use and included in Category 5A if a 30-day semi-permeable membrane device (SPMD) sampled parameter exceeds a water quality criteria two or more times within a 3-year period. As in the past, DEQ will identify other waters of concern as having observed effects and will schedule additional monitoring, if appropriate, to determine if the designated uses are being met.

Weight-of-Evidence Aquatic Life Use Assessment in Estuarine Waters

The "Weight-of-Evidence" (WOE) approach that DEQ currently uses for its general evaluation and assessment of the designated Aquatic Life Use (ALU) for estuarine benthic communities has evolved from a previously more limited application of the "Sediment Quality Triad" concept (SQT – Figure 1). The SQT concept was originally conceived and applied for the evaluation of the presence and effects of toxic contaminants in marine sediments (Long and Chapman, 1985). It was further applied by Chapman et al. (1986, 1987), and has continued to be one of the preferred approaches for the evaluation of toxics in marine and estuarine benthic environments (Chapman, 1992; Chapman et al., 1997; McGee et al., 2001). The Interstate Chesapeake Bay Program (CBP) employed SQT evaluations along with other methods to produce a Bay-wide toxics characterization in 1999 (US EPA, 1999) that identified (1) "Regions of Concern – areas with probable adverse effects," (2) "Areas of Emphasis – areas with potential adverse effects," (3) "Areas with Low Probability for Adverse Effects", and (4) "Areas with Insufficient or Inconclusive Data" relative to toxics contamination in Bay waters. Maps of more recent characterizations (2006, 2008, and 2009) can be found at: http://www.chesapeakebay.net/maps.aspx?menuitem=15230.

Appendix G describes the SQT in more detail.

5.6 Naturally Low DO and pH Evaluation in Swamp Waters

Virginia's list of impaired waters currently identifies many waters as not supporting the aquatic life use due to exceedences of pH and/or DO criteria that are designed to protect aquatic life in Class III waters. However, there is reason to believe that some of these streams or stream segments have been misclassified and should more appropriately be classified as Class VII, Swamp Waters. A procedure for assessing if natural conditions are the cause of the low pH and/or low DO levels in a given stream or stream segment has been developed.

The level of dissolved oxygen (DO) in a water body is dependent on the balance of oxygen-depleting processes (*e.g.*, decomposition and respiration) and oxygen-generating processes (*e.g.*, aeration and photosynthesis). Certain natural conditions promote a situation where the latter are not sufficient to overcome the former. The level of acidity as indicated by pH is dependent on the balance between the production of organic acids via decay processes and the inherent buffering capacity of the system.

Conditions in a stream that would typically be associated with naturally low DO and/or naturally low pH include slow-moving, ripple-less waters. In such waters, the decay of organic matter depletes DO at a faster rate than it can be replenished and produces organic acids (tannins, humic and fulvic substances). These

situations can be compounded by anthropogenic activities that contribute excessive nutrients or readily available organic matter to these systems.

Waters that are shown to have naturally low DO and pH levels will be re-classified as Class VII, Swamp Waters, with the associated pH criterion of 3.7 to 8.0 SU. An associated DO criterion is currently being reviewed by the Academic Advisory Committee. A TMDL is not needed for these natural Class VII waters. An assessment category of 4C will be assigned until the waterbody has been re-classified and then re-assessed against the Class VII criteria.

Procedure

Following a description of the watershed (including geology, soils, climate, and land use), a description of the DO and/or pH water quality problem (including a data summary, time series and monthly data distributions), and a description of the water quality criteria that were the basis for the impairment determination, the available information should be evaluated in four steps:

Step 1. Determine appearance and flow/slope.
Streams or stream segments that have naturally low DO (< 4 mg/L) and low pH (< 6 SU) are characterized by very low slopes and low velocity flows (flat water with low re-aeration rates). Decaying vegetation in such swampy waters provides large inputs of plant material that consumes oxygen as it decays. The decaying vegetation in swamp water also produces acids and decreases pH. Plant materials contain polyphenols such as tannin and lignin. Polyphenols and partially degraded polyphenols build up in the form of tannic acids, humic acids, and fulvic acids that are highly colored. The trees of swamps have higher polyphenolic content than the soft-stemmed vegetation of marshes. Swamp streams (blackwater) are therefore more highly colored and more acidic than marsh streams.

Appearance and flow velocity (or slope if flow velocity is not available) must be identified for each stream or stream segment to be assessed for natural conditions and potential re-classification as Class VII "swamp water". This can be done through maps, photos, field measurements or other appropriate means.

Step 2. Determine nutrient levels.
Excessive nutrients can cause a decrease in DO in relatively slow moving systems, where aeration is low. High nutrient levels are an indication of anthropogenic inputs of nitrogen, phosphorus, and possibly organic matter. Nutrient input can stimulate plant growth, and the resulting die-off and decay of excessive plankton or macrophytes can decrease DO levels.

USGS (1999) estimated national background nutrient concentrations in streams and groundwater from undeveloped areas. Average nitrate background concentrations are less than 0.6 mg/L for streams, average total nitrogen (TN) background concentrations are less than 1.0 mg/L, and average background concentrations of total phosphorus (TP) are less than 0.1 mg/L.

Nutrient levels must be documented for each stream or stream segment to be assessed for natural conditions and potential re-classification as Class VII swamp water. Streams with average concentrations of nutrients greater than the national background concentrations should be further evaluated for potential impacts from anthropogenic sources.

Step 3. Determine degree of seasonal fluctuation (for DO only).
Anthropogenic impacts on DO will likely disrupt the typical seasonal fluctuation seen in the DO concentrations of wetland streams. Seasonal analyses should be conducted for each potential Class VII stream or stream segment to verify that DO is depressed in the summer months and recovers during the winter, as would be expected in natural systems. A weak seasonal pattern could indicate that human inputs from point or nonpoint sources are impacting the seasonal cycle.

Step 4. Determine anthropogenic impacts.
Every effort should be made to identify human impacts that could exacerbate the naturally low DO and/or pH. For example, point sources should be identified and DMR data analyzed to determine if there is any impact on the stream DO or pH concentrations. Land use analysis can also be a valuable tool for identifying potential human impacts. Lastly, a discussion of acid rain impacts should be included for low pH waters.

7Q10 Data Screen
If the data warrant it, a data screen should be performed to ensure that the impairment was identified based on valid data. All DO, temperature or pH data that violate WQ Standards should be screened for flows less than the 7Q10. Data collected on days when flow was < 7Q10 should be eliminated from the data set and the violation rate recalculated accordingly. Only those waters with violation rates determined to be from days with flows ≥ 7Q10 flows should be classified as impaired.

In some cases, data were collected when flow was 0 cfs. If the 7Q10 is identified as 0 cfs as well, all data collected at or above 0 cfs flow would need to be considered in the water quality assessment. In those cases, the impairment should normally be classified as 4C, "impaired due to natural conditions", no TMDL needed. However, a reclassification to Class VII may not always be appropriate.

Natural Condition Conclusion Matrix

The following decision process should be applied for determining whether low pH and/or low DO values are due to natural conditions and justify a reclassification of a stream or stream segment as Class VII, Swamp Water.

If velocity is low or if slope is low (<0.50%) AND
If wetlands are present along stream reach AND
If no point sources or only point sources with minimal impact on DO and pH AND
If nutrients are < typical background
- ❖ average (= assessment period mean) nitrate less than 0.6 mg/L
- ❖ average total nitrogen (TN) less than 1.0 mg/L, and
- ❖ average total phosphorus (TP) are less than 0.1 mg/L AND

For DO: If seasonal fluctuation is normal AND
For pH: If nearby streams without wetlands meet pH criteria OR if no correlation between in-stream pH and rain pH,

THEN determine as impaired due to natural condition
→ assess as Category 4C in next assessment
→ initiate WQ Standards reclassification to Class VII Swamp Water
→ get credit under consent decree

The analysis must state the extent of the natural condition based on the criteria outlined above. A map showing land use, point sources, water quality stations and, if necessary, the delineated segment to be classified as swamp water should be included.

In cases where not all of these criteria apply, a case by case argument must be made based on the specific conditions in the watershed.

Example Analysis – pH

Following a description of the watershed (including geology, soils, climate, and land use); a description of the DO and/or pH water quality problem (including a data summary, time series and monthly data distributions); and a description of the water quality criteria that were the basis for the impairment determination, the available information should be evaluated as follows:

- Step 1: Are there low velocities or low slope? Are there large inputs of decaying vegetation in a wetland that produce acids and lower pH as they decay?
- Step 2: Are there excessive nutrients instream that can indicate human activity?
- Step 3: Does evidence of human impact through discharges or land use warrant a TMDL?

Example Stream: White Oak Swamp

APPEARANCE/FLOW or SLOPE:
Visual inspection upstream and downstream of bridges at Rt. 156 and Poplar Springs Rd, revealed very swampy conditions usually with standing water in woods on either side of the channel (provide photos and map of area).

The hydrologic slope from the 110 ft topographic contour at rivermile 6.60 downstream to the 50 ft contour at rivermile 1.12 is estimated at 0.21%, considered low slope.

NUTRIENTS:
- Total Phosphorus Av. 0.047 mg/l (n=78)
- Orthophosphorus Av. 0.024 mg/l (n=70)
- Total Kjeldahl Nitrogen Av. 0.61 mg/l (n=78)
- Ammonia as N Av. 0.03 mg/l (n=78)
- Nitrite + Nitrate as N Av. 0.10 mg/l (n=6)

→ Below USGS Average Backgrounds

HUMAN IMPACTS:
- Capital Regional Airport Commission (VA090301) reported pH twice per year for 2000 - 2003 at pH 7.19, 5.10, 6.56, 6.89, 6.44, and 8.44. One pH 4.20 in Aug 2001 during no flow period. Max flow 1357 cfs at Beulah Rd. stormwater outfall during Nov. 2001 to Apr 2002.
- Henrico MS4, 3 General Ind. Minors and 5 Ind. Stormwaters have no pH reporting requirements.
- High Intensity Commercial / Industrial land use comprised 9.0 % of watershed (1586 ac), however only 6.7% pH violations at Beulah Rd, with highest pH values.
- Watershed predominately forested (57.3 percent), with 9.2 percent wetlands and open water.
- Human E. coli impairment at 22% of annual load, therefore it is possible that human activities impact watershed in headwaters.
- Acid rain impact analysis

- White Oak Swamp is located east of the fall line and an acid rain impact analysis developed for the nearby Mechumps Creek can be applied
- 10 stations within 17 miles of Mechumps Creek have 2 to 15 years of pH data.
- If acid rain is an impact, all stations should have low pH impairment, however:
- 5 stations within 13 miles to the west above the Fall line have higher pH and no impairment (mean pH 6.63 - 7.01); Little, Newfound, and South Anna Rivers, Falling Creek, Stony Run.
- 5 stations within 17 miles to the east below the Fall line have low pH and natural impairment (mean pH 5.89 - 6.44); Hornquarter, Herring, Totopotomoy, Monquin, and Matadequin Creeks.

CONCLUSIONS:

- Low slope, with predominantly wetlands, not indicative of human impact.
- Low nutrients, not indicative of human impact.
- Human activity above Beulah Rd. can affect pH in headwaters, but there was no observed pH impact downstream at Rt. 156 attributed to the headwaters commercial / industrial land use.
- Low pH is more related to swamp water from low slope swamps below the Fall Line than to acid rain.

→ White Oak Swamp and its tributaries exhibit low pH due to natural conditions and should be re-classified as Class VII, Swamp Water, with the associated pH criterion range of 3.7 to 8 SU. An associated DO criterion is currently being developed from swamp water data. A TMDL is not needed for these waterbodies. An assessment category of 4C will be assigned until the waterbody has been re-classified and then re-assessed.

EXAMPLE ANALYSES - DO

Following a description of the watershed (including geology, soils, climate, and land use); a description of the DO and/or pH water quality problem (including a data summary, time series and monthly data distributions); and a description of the water quality criteria that were the basis for the impairment determination, the available information should be evaluated as follows:

- Step 1: Are there low velocities or low slope? Are there large inputs of decaying vegetation in a wetland that produce acids and lower DO as they decay?
- Step 2: Are there excessive nutrients in-stream that can indicate human activity?
- Step 3: Do seasonal changes lower DO in summer and raise it in winter?
- Step 4: Does evidence of human impact through discharge or land use warrant a TMDL?

<u>Example Stream: Tuckahoe Creek</u>

APPEARANCE/FLOW or SLOPE:
Visual inspection at bridges on Rt. 6 and Rt.650 revealed very swampy conditions. A large wetland named Big Swamp exists for 4 miles above Rt. 6. There are wetlands noted on the land use map along Tuckahoe Creek and Little Tuckahoe Creek from just below Rt. 250 downstream approximately 8 miles to below Rt. 650. Wetlands promote input of decaying vegetation throughout this 8 mile segment, which causes low DO from bacterial decomposition (provide photos and map of area).

The hydrologic slope from the 150 ft topographic contour at river mile 10.55 below Rt. 50 downstream to the 120 ft contour at river mile 2.59 above the old railroad grade below Rt. 650 is estimated at 0.07%, considered very low slope.

NUTRIENTS:

- Total Phosphorus Av. 0.074 mg/l (n=226)
- Orthophosphorus Av. 0.043 mg/l (n=218)
- Total Kjeldahl Nitrogen Av. 0.64 mg/l (n=224)
- Ammonia as N Av. 0.067 mg/l (n=226)
- Nitrite + Nitrate as N Av. 0.31 mg/l (n=23)

→Below USGS average backgrounds, and below background levels in a permitted livestock study by DEQ.

SEASONAL FLUCTUATIONS:
Seasonal fluctuations of DO values were within normal ranges.

HUMAN IMPACTS:

- Henrico Water Treatment Plant (VA0091197) is not required to report DO or CBOD.
- Henrico County MS4 (VA0088617) is not required to report DO or CBOD.
- Two general stormwater permittees, Henrico WTP and Short Pump Town Center, are not required to report DO or CBOD.
- High Intensity Residential, Commercial / Industrial land use comprise 21 % of watershed (8647 ac), located in the eastern portion of the watershed.
- Watershed is predominately forested (52 percent), with 5 percent wetlands / open water.
- Human E. coli impairment is at 12% of annual load, the lowest among three watersheds in Henrico County receiving bacterial TMDLs. However it is still possible that human activities impact watershed.

CONCLUSIONS:

- Low slope, predominantly wetland in impaired segment, not indicative of human impact.
- Low nutrients, not indicative of human impact.
- Normal seasonal DO fluctuation.
- Unknown if DO impact observed at Rt. 6 can be attributed to human activity. Henrico WTP has little impact on DO. The Henrico MS4 has an unknown impact on DO, but discharges following rain events with high velocity, promoting elevated DO from reaeration, and unknown BOD loads. Residential, Commercial / Industrial land use (21%) has suspected effect on watershed.

The impaired segment of Tuckahoe Creek exhibits low DO due to natural conditions and should be re-classified as Class VII, Swamp Water, with the associated pH criterion range of 3.7 to 8 SU. An associated DO criterion is currently being developed from swamp water data. A TMDL is not needed for this waterbody. An assessment category of 4C will be assigned until the waterbody has been re-classified and then re-assessed against Class VII criteria.

Low DO values in the two tributaries appear to be at least partially due to anthropogenic inputs. However, 7Q10 analysis resulted in changed violation rates for two tributaries from 12.1% to 8.9% (Little Tuckahoe Creek) and from 15.9% to 9.6% (Deep Run).

5.7 Lakes/Reservoirs Assessment

The current agency guidance on the monitoring and assessment of targeted lakes and reservoirs is found in the Department Guidance Memo No. 09-2005 “Monitoring and Assessment of Lakes and Reservoirs" (April 2009).

Section 5.6 provides summary guidance on how to prioritize and evaluate the many lakes and reservoirs in the Commonwealth for monitoring. This prioritization allows the Department to focus on the most important lakes as they relate to designated uses.

GM09-2005 defines "significant lakes and reservoirs":

"A significant lake/reservoir is defined as: a publicly accessible lake/reservoir that is a public water supply and/or 100 acres or more in size ***and*** *is included in Section 187 list of reservoirs with nutrient criteria."*

A list of the current "significant lakes" is included in Appendix G of this document. Since 2007, these are the 121 man-made lakes and reservoirs identified under the nutrient standards for lakes and reservoirs (9 VAC 25-260-187), and the two natural lakes, Mountain Lake and Lake Drummond, which have been assigned special standards for nutrients (9 VAC 25-260-310).

Publicly accessible means direct access to the water from public property during normal work hours.

The significant lakes designation includes the federally owned lakes which meet these criteria, but all other federally owned lakes would be excluded from the agency lakes monitoring program.

There are additional lakes/reservoirs that should also be considered, as resources allow, for monitoring and assessment that are not in §187 but do meet one of the other two characteristics above. Although nutrient criteria do not apply to these, other criteria should be assessed as available data will allow.

At least one of these two criteria must be met for a lake or reservoir to be assessed as impaired:

1. A lake/reservoir has exceedences of numerical WQS, as observed during multiple sampling events, or
2. There is a loss of a designated use as evidenced in ancillary data, such as records documenting conditions unconducive for swimming and/or boating, recurrent fish kills, and other QA/QC approved non-agency studies or reports, etc. This applies even if there is no water quality standard for the parameter(s) in question.

This section incorporates summary guidance from Guidance Memo 09-2005 that documents how nutrients and dissolved oxygen data collected from the man-made lakes and reservoirs listed in §187 and the two natural lakes listed in the special WQS section (9 VAC 25-260-310) will be assessed by DEQ for the 2014 Integrated Report. Nutrient criteria will not be applicable to lakes/reservoirs not included in §187; these waterbodies will only be evaluated if low DO concentrations have been documented.

The assessor should provide a complete narrative documenting assessment decisions. If uses are impacted, document those uses impacted and how they are impacted. Name causes and sources where possible, (e.g. nuisance algal blooms preventing swimming during summer months, numerous complaints on file or aquatic weed growth preventing free navigation of lake and/or expensive mechanical or chemical clearing, etc).

Nutrient Evaluation of §187 Reservoirs development

Both nutrient (chlorophyll *a* and total phosphorus if there is documented use of algaecides any time during the Department's seven month monitoring period from April through October) and dissolved oxygen/pH data are

assessed for aquatic life use. Bacteria data are used to assess recreational use. Observations regarding nuisance algal, plant growth, or discolored water are assessed using the general standard; the recent criteria amendments for lakes and reservoirs did not modify these existing criteria.

Since the 2010 assessment, the Trophic State Index (TSI) evaluation for nutrient impacts in §187 lakes has been replaced by nutrient criteria. The TSI evaluation will continue to be used in those lakes that are not included in §187.

- Assessment for aquatic life (fishery) use of §187 lakes/reservoirs for chlorophyll *a* and total phosphorus (if there is documented algaecide use):

This assessment procedure for nutrients in §187 lakes replaces the combined TP/DO TSI approach used in 2006 for nutrient assessment related to assessing natural low DO conditions. However, the TSI approach will be used to determine natural conditions for other non-§187 lakes if DO problems have been documented. The nutrient criteria for the man-made lakes and reservoirs listed in §187 of the WQS only apply in the top 1 meter of the lacustrine zone. "Lacustrine" means the zone within a lake or reservoir that corresponds to non-flowing lake-like conditions within reservoirs that are deeper than 3 meters (10 feet). The other two zones within a deeper reservoir are riverine (flowing, river-like conditions) and transitional (transition from river to lake conditions). If total phosphorus or chlorophyll *a* data are collected outside the lacustrine zone in the riverine or transitional zone, the data from these two zones will not be used in the assessment for lake or reservoir impairment due to nutrients. As previously stated, the nutrient criteria cannot be used for assessment of lakes and reservoirs that are not listed in §187 of the WQ Standards. For lakes and reservoirs without defined nutrient criteria, but with DO problems, the TSI approach may still be used to determine if those problems are natural.

The regional office staff will base their determination of algaecide use on discussions with the lake owner regarding use of algaecides during the monitoring period and/or DEQ monitoring staff observations of algaecide applications during their monitoring runs on the lake or reservoir. (The intent is to use both chlorophyll *a* and total phosphorus when algaecides are applied within any zone of the reservoir.)

The 90th percentile of chlorophyll data collected at one meter or less within the lacustrine portion of the man-made lake or reservoir between April 1 and October 31 (considered a lake monitoring year) shall not exceed the chlorophyll *a* criterion for that waterbody in *each* of the two most recent monitoring years within the assessment window. For a waterbody that received algaecide treatment, the median of the total phosphorus data collected at one meter or less within the lacustrine portion of the man-made lake or reservoir between April 1 and October 31 shall not exceed the total phosphorus criterion in each of the two most recent years that total phosphorus data are available. The aquatic life (fishery) use of any lake (not just the lacustrine zone but rather the entire lake/reservoir) listed in §187 is considered impaired for nutrients if the criterion for either chlorophyll *a* or total phosphorus is exceeded. For each nutrient criterion, chlorophyll *a* and total phosphorus (if documented algaecide use), the assessor will pool all data collected at one meter or less for all months and all stations within the lacustrine portion collected between April and October. Each year must have valid data for 6 of the 7 months of required monitoring to be considered a valid year.

- Assessment for aquatic life (fishery) use for nutrients in the two natural lakes:

  Assessments of the two natural lakes in the special standards section will follow the guidelines above for chlorophyll *a* and total phosphorus except that orthophosphate-P rather than total phosphorus applies to Mountain Lake.

- Use of citizen and other external data:

  In order to use citizen data in assessments for nutrient impairments, the collector must provide documentation that the data meet QA/QC requirements for chlorophyll *a* and total phosphorus (orthophosphate-P for Mountain Lake) and that the location of the sampling was within the lacustrine portion of the reservoir and outside the littoral (near shore) zone and corresponds with the lake monitoring year requirements.

Dissolved Oxygen Evaluation

The dissolved oxygen criteria are based on the appropriate criteria established for that class of waters in Section 9 VAC 25-260-50. Dissolved oxygen information is used for assessment of aquatic life use.

- Assessment for aquatic life use of lakes and reservoirs for the dissolved oxygen criterion:

  The 10.5% rule is applicable to assessments for the minimum dissolved oxygen criterion in all assessed lakes and reservoirs for each monitoring year. For §187 lakes/reservoirs, dissolved oxygen samples taken for all months within the monitoring year, at all stations within a given lake or reservoir, are assessed only in the epilimnion if the water body is thermally stratified. If not stratified, dissolved oxygen should be assessed throughout the water column. A lake or reservoir is considered stratified if there is a difference of 1ºC /meter. If the differential is < 1ºC /meter, the lake is not considered stratified. Two or more exceedences and >10.5% exceedence of total samples are required before a water body is listed as impaired for the minimum dissolved oxygen criterion (4 mg/l for most freshwater lakes and reservoirs) under § 62.1-44.19:5 and 7 of the Code of Virginia.

pH Evaluation

The pH criteria are based on the appropriate criteria established for that class of waters in section 9 VAC 25-260-50. pH information is used for assessment of aquatic life use.

The 10.5% rule is applicable to assessments for the pH criterion range in all lakes and reservoirs sampled during the lake monitoring year, not just the significant man-made lakes and reservoirs and two natural lakes included in the WQS.

All pH monitoring observations collected during all months in a monitoring year within the assessment period at all stations within the lake/reservoir are assessed against the pH criterion range. Two or more exceedences and >10.5% exceedences are required before a water body is listed as impaired for pH. In cases where the applicable nutrient criteria are met for the man-made lakes/reservoirs listed in §187 but the maximum pH criterion is exceeded, the lake or reservoir should be classified as Category 4C and recommended for a WQS review due to natural pH fluctuations. In lakes that are not in §187, the waterbody would be listed as impaired (Category 5A). See lakes/reservoir assessment flowchart below.

Trophic State Index (for non-significant/non-**§187 lakes)**

Trophic state index equations for secchi depth (SD), chlorophyll *a* (CA), and total phosphorus (TP) will be calculated in stratified lakes using aggregated station data in the epilimnion from mid-June through mid-

September (at 0.3 m for TP and CA) and will be used to determine if DO problems in non §187 lakes and reservoirs.

A trophic state index value of 60 or greater for any one of the 3 indices will indicate that nutrient enrichment from anthropogenic sources are adversely interfering, directly or indirectly, with the designated uses. A TSI value of 60 corresponds to a CA concentration of 20 ug/l, a SD of 1 meter, and a TP concentration of 48 ug/l.

The TSI equations:

$$TSI(SD) = 10(6 - (\ln SD / \ln 2))$$
$$TSI(CA) = 10(6 - ((2.04 - 0.68 \ln CA) / (\ln 2)))$$
$$TSI(TP) = 10(6 - ((\ln (48 / TP)) / (\ln 2)))$$

SD = meters
CA = ug/
TP = ug/l

The following rules apply:

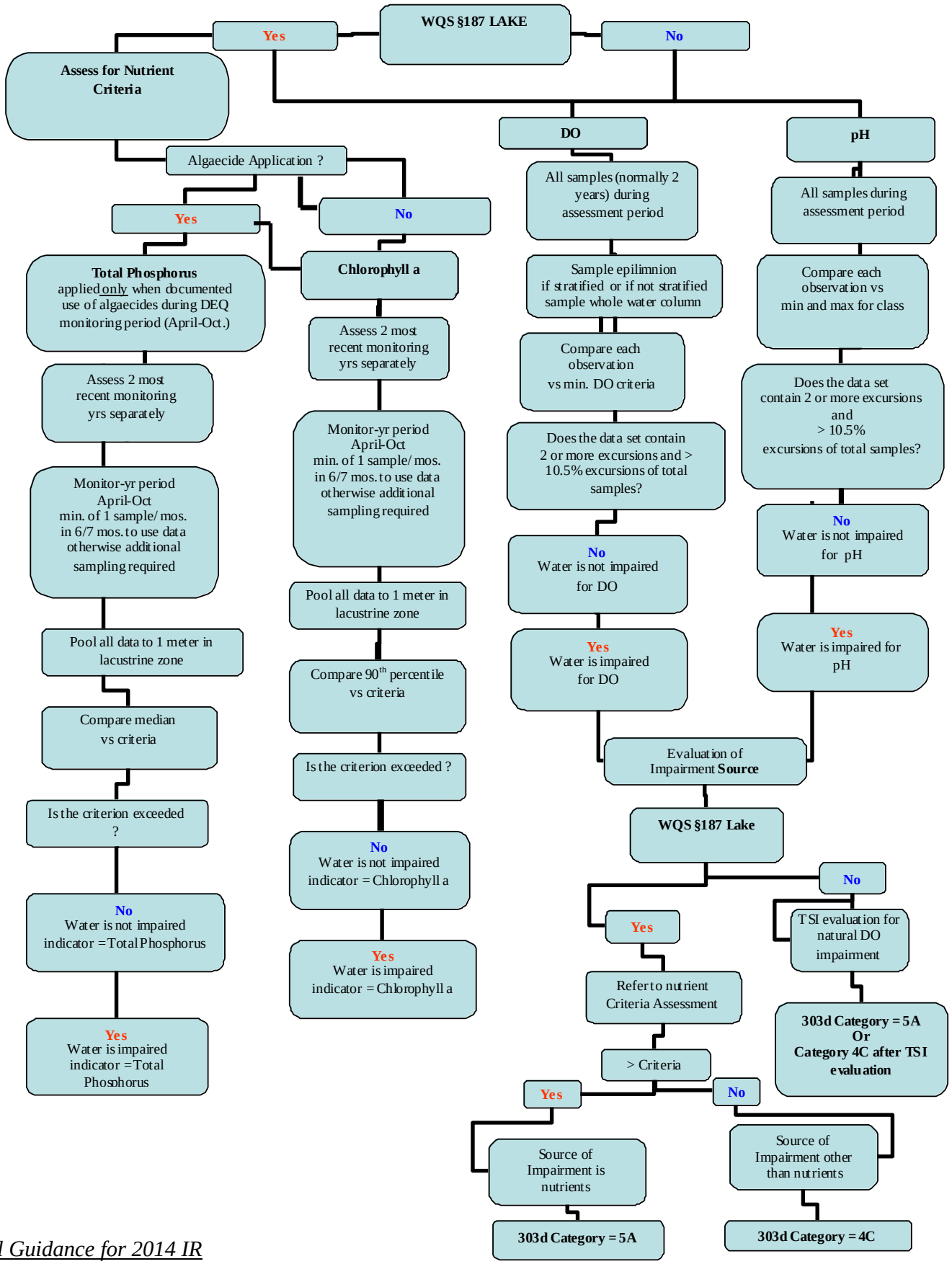
1. Do not calculate a chlorophyll *a* TSI in lakes that are treated with algaecides.
2. The chlorophyll *a* TSI will normally be the preferred indicator in untreated lakes.
3. Assume that typical Virginia freshwater lakes and reservoirs are phosphorus limited.
4. Do not use the secchi depth index in the assessment if it is much larger than the CA and TP indices in the same assessment unit (this indicates prevalence of inorganic matter).
5. The appropriate TSIs should be calculated based on all summer sample data collected in the segment using the spreadsheet that has been developed for easier data processing.

For each monitoring station, if one or more of the TSIs ≥ 60*, the non-§187 lake/reservoir will be assessed as impaired partially due to one or more pollutants from anthropogenic sources. The assessment unit or entire lake/reservoir will be placed in Category 5A for TMDL development.

For each monitoring station, if each of the TSIs < 60, the lake/reservoir will be assessed as impaired due to pollution from natural sources and placed in Category 4C. A TMDL is not needed for the assessment unit represented by the monitoring station(s) and appropriate DO criteria will be developed for the hypolimnion.

The TSIs will be used to determine trophic status in the Assessment Database (ADB):

Trophic Index Trophic State	**Carlson Trophic State Index**	**ADB Category**
Hypereutrophic	80 – 100	5A
Eutrophic	60 – Less than 80	5A
Mesotrophic	40 – Less than 60	4C
Oligotrophic	0 – Less than 40	4C
Unknown	Insufficient Data	3A


WQS §187 LAKE
Yes
No
Assess for Nutrient Criteria
DO
pH
Algaecide Application ?
Yes
No
All samples (normally 2 years) during assessment period
All samples during assessment period
Total Phosphorus applied only when documented use of algaecides during DEQ monitoring period (April-Oct.)
Chlorophyll a
Sample epilimnion if stratified or if not stratified sample whole water column
Compare each observation vs min and max for class
Assess 2 most recent monitoring yrs separately
Assess 2 most recent monitoring yrs separately
Compare each observation vs min. DO criteria
Does the data set contain 2 or more excursions and > 10.5% excursions of total samples?
Monitor-yr period April-Oct min. of 1 sample/ mos. in 6/7 mos. to use data otherwise additional sampling required
Monitor-yr period April-Oct min. of 1 sample/ mos. in 6/7 mos. to use data otherwise additional sampling required
Does the data set contain 2 or more excursions and > 10.5% excursions of total samples?
No
Water is not impaired for pH
No
Water is not impaired for DO
Pool all data to 1 meter in lacustrine zone
Pool all data to 1 meter in lacustrine zone
Yes
Water is impaired for pH
Yes
Water is impaired for DO
Compare median vs criteria
Compare 90th percentile vs criteria
Evaluation of Impairment Source
Is the criterion exceeded ?
Is the criterion exceeded ?
WQS §187 Lake
No
Water is not impaired indicator = Chlorophyll a
No
Water is not impaired indicator = Total Phosphorus
Yes
TSI evaluation for natural DO impairment
Yes
Water is impaired indicator = Chlorophyll a
Refer to nutrient Criteria Assessment
Yes
Water is impaired indicator = Total Phosphorus
303d Category = 5A Or Category 4C after TSI evaluation
> Criteria
Yes
No
Source of Impairment is nutrients
Source of Impairment other than nutrients
303d Category = 5A
303d Category = 4C

5.8 Nonpoint Source (NPS) Assessment

Nonpoint Source (NPS) Assessment

The 2014 nonpoint source pollution (NPS) assessment will be performed by the Virginia Department of Conservation and Recreation (DCR) at the 6th order hydrologic units of the National Watershed Boundary Dataset. This assessment will consist of calculations of net loadings of the NPS pollutants - nitrogen, phosphorous, and sediment - per hydrologic unit as well as evaluations of NPS-related measures in these units.

Gross loadings of NPS pollutants are determined via a modeling process that closely approximates the results of the Chesapeake Bay Program water quality model in regards to loadings in the Bay watersheds, thereby diminishing the uncertainty of having significantly conflicting assessment results for that portion of the state. This model, as deployed, also calculates similar values for non-Bay watersheds to develop consistent statewide loadings. Inputs to this modeling process include:

- A DCR modified land use / land cover layer
- A DCR developed confined animal data set
- Census of Agriculture animal numbers by jurisdiction
- Virginia Department of Forestry (VDOF) forest harvesting data by jurisdiction
- Virginia Department of Mines, Minerals, and Energy (VDMME) extraction data
- The USDA's Natural Resources Inventory
- Chesapeake Bay Program Watershed Model output
- USDA statewide and jurisdiction level soil surveys
- VALUES based pasture yields
- A DCR developed table of dominant crop types by modeled hydrologic unit
- National Weather Service weather records for a multi state area
- USGS stream flows from gage stations
- Census of Population and Housing indicators of non-sewered population by block group
- Slopes developed from USGS Digital Elevation Models (DEMs)
- A DCR developed indicator of stream density by modeled hydrologic unit
- A DCR developed manure application schedule by manure type by region

Improvements will be made to several of these inputs to better represent model year conditions. As loadings are significantly influenced by land use/land cover changes, improving this input layer is a priority. An evaluation of all available recent sources of this data will occur and establish the model year. Farm animal related uses such as pasture-cattle grazed, manure acres, etc. are also noteworthy load contributors. Thus this input will also be updated using various sources, including the US Census of Agriculture, the DCR Animal Feeding Operation (AFO) database, and DEQ's Virginia Pollution Abatement permits for significant AFOs.

Net loadings are formed by subtracting the reductions in nitrogen, phosphorous, and sediment that are realized from both best management practice (BMP) installations and relevant grant projects from calculated gross loads. This includes BMPs funded and installed through DCR, VDOF, and the USDA. Updated BMP datasets will be used for this purpose. Results will produce NPS pollution load rankings per pollutant by categorized land use of the modeled hydrologic units.

In contrast to modeled potential nutrient loadings, the NPS related portions of the most current available list of water quality limited waters (from the 303(d) report) will be assessed by modeled hydrologic unit. This will produce rankings of hydrologic units from monitoring the impaired waters by water regime of the modeled hydrologic units.

Aside from the NPS loadings described above, two variables used in the past NPS assessments for prioritizing watershed protection efforts for biological health will also be recalculated and ranked by modeled hydrologic unit in 2014 – an aquatic Index of Biological Integrity (IBI) and a public source water protection need.

A modified aquatic IBI score, calculated by the Center for Environmental Studies at Virginia Commonwealth University (VCU), will be used to indicate modeled hydrologic units in need of aquatic species health protection. The IBI score will be developed from the most recent aquatic species data collected by DCR, the Virginia Department of Game and Inland Fisheries (VDGIF), and VCU.

As an indication of human health protection concerns, a public surface source water protection variable will be calculated by hydrologic unit. This variable will reflect the area in each hydrologic unit that is within a Zone 1 protection level of public source water intakes as defined by the Virginia Department of Health (VDH), weighted by the population served by each intake.

DCR rates modeled hydrologic units as high, medium, or low for potential NPS problems as indicated by the NPS assessment. This categorization is performed so that approximately the highest 20% of the net loadings by unit are assigned the high rank. The next highest 30% of the net loading values are assigned the medium rank. All other units are assigned a low NPS rank. Rather than make a hard and true category split at these percentages, the category breaks are made where the larger net loading differences occur nearest to the stated percentages.

Impaired riverine and lacustrine waters, as well as the biological indicators, are ranked based on the clustering and spread of values. Impaired estuarine waters are not evaluated at all at this time due to the difficulty of associating their impairment sources with the surrounding land activities.

No single NPS ranking will be produced from the rankings of the various pollutant loadings, biological indicators, and NPS-impaired water regimes. Each user's total ranking needs can be met by deciding which of the ranked categories are pertinent to their program's cause and creating customized rankings using only those categories. DCR will, however, be flagging units with significant combinations of measures from this assessment, such as those with high aquatic biological diversity and/or public water supply protection need, and those with this same condition but with high NPS pollutant threats.

Other NPS reduction activities and results will be summarized. This will include agricultural BMP installations and NPS TMDLs.

5.9 Coastal Assessment

Virginia has 120 miles of Atlantic Ocean coastline and approximately 2,500 square miles of estuary. This resource has a prominent place in Virginia's history and culture. It is valued for its commercial fishing, wildlife, sporting, and recreational opportunities, as well as its commercial values in shipping and industry. In the 1970's adverse trends in water quality and living resources were noted and prompted creation of the Federal-Interstate Chesapeake Bay Program (CBP). The coastal assessment is conducted in the same manner as the estuarine assessments previously. Additionally, the federal BEACH program, implemented by VDH, has enabled the collection of recreational use data during the swimming season. Assessment of this data has been incorporated into the Integrated Report.

5.10 Wetlands Assessment

Background

Impacts to tidal wetlands, including vegetated tidal wetlands and non-vegetated shoreline between mean low and mean high water, are regulated under the Virginia Tidal Wetlands Act (Title 28.2, Chapter 13 of the Code of Virginia) enacted in 1972 and revised in 1982. The Virginia Marine Resources Commission (VMRC) is the regulating authority for the tidal wetlands laws while localities in Tidewater Virginia have the option to regulate their own tidal wetlands through citizen Wetlands Boards with oversight from VMRC. The Virginia Department of Environmental Quality (DEQ) is responsible for providing Section 401 Certification of Clean Water Act for Section 404 federal permits for tidal and nontidal wetlands and water withdrawals, through the Virginia Water Protection Permit (VWPP) Program, first developed in 1992.

The VWP permit program is administered by DEQ's Office of Wetlands & Stream Protection, and derives its regulatory authority from both the Clean Water Act (§401) and State Water Control Law (§62.1-44.20 of the Code of Virginia), found at http://water.epa.gov/lawsregs/guidance/wetlands/sec401.cfm and http://leg1.state.va.us/cgi-bin/legp504.exe?000+cod+62.1-44.15C20, respectively

Before July 1, 2000, applicants seeking a Clean Water Act § 404 permit (http://water.epa.gov/lawsregs/guidance/wetlands/sec404.cfm) from the U.S. Army Corps of Engineers (the Corps) for the discharge of dredged or fill materials in wetlands or waters of the United States were also required to submit an application to DEQ for a permit or waiver under § 401 Certification. In 2000, Virginia passed a Nontidal Wetlands Act that amended Title 62.1 of the Code of Virginia relating to wetlands. The Nontidal Wetlands Act mandates that the Commonwealth implement a nontidal wetlands regulatory program to achieve no net loss of existing wetland acreage and function, and to develop voluntary and incentive based programs to achieve a net resource gain in wetlands. Amendments to the Virginia Water Protection (VWP) permit program, fully implemented in October 2001, provided additional state jurisdiction and required a state permit for the following activities in a wetland: excavation, filling or dumping, activities in a wetland that cause drainage or otherwise significantly alter or degrade existing wetland acreage or function, and permanent flooding or impounding.

Amendments to the VWPP program in July 2007 provided clarifications of state jurisdiction of small water withdrawals, incorporated several provisions of the Local and Regional Water Supply Planning regulation, and addressed water supply permitting and surface water withdrawal concerns. Further amendments to the VWPP program in December 2008 provided exclusion of certain in-stream fills for water supply on agricultural properties.

Further reducing duplication of permitting between State and Federal agencies while ensuring minimal individual and cumulative consequences to wetland and stream resources, the Corps issued a State Program General Permit (SPGP), and suspended a few of the Nationwide Permits. The State Program General Permit (SPGP-01) was granted to the Commonwealth of Virginia by the Norfolk District Corps of Engineers (Corps) for the discharge of dredged and/or fill material in nontidal wetlands and waters associated with residential, commercial, and institutional developments and linear transportation projects within the Commonwealth. Corps general permits are authorizations issued on a nationwide or regional basis by the Army Corps of Engineers for categories of activities that have minimal environmental impacts. Programmatic general permits may be issued in situations where a state, regional, or local authority has a regulatory program in place that provides a similar level of review as the Corps.

The SPGP-01 became effective on November 1, 2002, and included additional activities normally permitted by Corps' nationwide permits. The Corps issued modified SPGPs in June 2007 and June 2012. The Norfolk District Corps (Corps) revised and expanded the State Program General Permit (12-SPGP-01), which became effective on June 1, 2012. A project must meet the wetland and stream impact thresholds and all other limitations and conditions of the SPGP to be used.

The permit process for both tidal and nontidal wetland activities relies on a Joint Permit Application (JPA) which receives independent and concurrent review by local wetlands boards, VMRC, DEQ and the U.S. Army Corps of Engineers (Corps), as appropriate.

By statute and by regulation, Virginia adopted the same definition of wetlands as the federal definition, and requires that wetlands be defined in the field using the Corps' 1987 Manual. Specifically, wetlands are defined as "those areas that are inundated or saturated by surface or groundwater at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions. Wetlands generally include swamps, marshes, bogs, and similar areas." Wetlands are part of state waters, which are defined as "all water, on the surface and under the ground, wholly or partially within or bordering the Commonwealth or within its jurisdiction, including wetlands." The Corps has created Regional Supplements in an effort to address regional wetland characteristics and improve the accuracy and efficiency of wetland-delineation procedures. The Regional Supplements that apply to Virginia are: U.S. Army Corps of Engineers (2010) "Interim Regional Supplement to the Corps of Engineers Wetland Delineation Manual: Eastern Mountains and Piedmont Region," ERDC/EL TR-10-9, U.S. Army Engineer Research and Development Center, Vicksburg, MS and U.S. Army Corps of Engineers (2010). "Regional Supplement to the Corps of Engineers Wetland Delineation Manual - Atlantic and Gulf Coastal Plain Region (Version 2.0)," ERDC/EL TR-10-20, U.S. Army Engineer Research and Development Center, Vicksburg, MS.

Tidal wetlands are defined to include tidally influenced areas within Tidewater Virginia contiguous to mean low water extending landward to an elevation 1 1/2 times the mean tide range at a site and upon which is growing certain listed plant species. They also include "nonvegetated wetlands" which include unvegetated lands between mean low water and mean high water tides.

Section 62.1-44.15:21 of the Code of Virginia specifies that the state utilize the Corps' Wetlands Delineation Manual (Technical Report Y-87-1, January 1987, Final Report) as the approved method for delineating wetlands, and that the state shall adopt appropriate guidance and regulations to ensure consistency with the Corps' implementation of delineation practices.

Purpose

A monitoring and assessment program is defined as the establishment and operation of appropriate devices, methods, systems and procedures necessary to monitor, compile, and analyze data on the condition of wetlands (adapted from the United States Environmental Protection (EPA) Agency's "Elements of a State Water Monitoring and Assessment Program", March 2003). Monitoring is the systematic observation and recording of current and changing conditions, while assessment is the use of that data to evaluate or appraise wetlands to support decision-making and planning processes. Wetlands can be characterized both by their condition and by functions. Wetland condition is the current state as compared to reference standards for physical, chemical, and biological characteristics, while functions represent the processes that characterize wetland ecosystems.

The overarching goal of Virginia's wetland monitoring and assessment strategy was to develop a long-term implementation plan for a wetland monitoring and assessment program that protects the physical, chemical, and biological integrity of the Commonwealth's water resources, including wetlands. In order to accomplish this goal, it is critical to first know the status of wetland resources in Virginia, in terms of location and extent of wetlands in each watershed, and have a general knowledge of the quality of these wetland resources. Secondly, the functions of wetland resources impacted through VWP permitting program must be accurately evaluated to determine those functions to be replaced through compensatory mitigation. It is also important to assess the degree to which the required compensatory mitigation is performing in relation to those impacted functions.

Since 2003, the overall wetland monitoring and assessment strategy has been to establish baseline conditions in various broad contexts, such as land use, watershed, and wetland type. This information can then be used to guide management decisions regarding wetland restoration efforts, programmatic compensatory mitigation, and integration with overall WQ Standards. This strategy provides the ultimate framework for an ongoing assessment of the status of the Commonwealth's wetland resources and the success of both wetland regulatory and voluntary programs. The wetlands monitoring strategy will be coordinated with Virginia's comprehensive water quality monitoring program strategy. The monitoring objectives are designed to support regulatory decision-making, allow reporting of wetland conditions, and provide information for policy development.

The wetland monitoring program will also meet the Clean Water Act objectives for water monitoring programs by addressing the quality of the Commonwealth's wetlands and their condition as part of the overall condition assessment of state waters.

Wetlands Assessment Approach

Virginia has developed a three-tiered approach to wetland sampling and analysis. Comprehensive coverage of all mapped wetlands is achieved with a GIS based analysis of remotely sensed information (Level I analysis). These data are summarized on the basis of small watersheds or hydrologic units. It provides a first order evaluation of the condition and functional capacity of wetlands based on their landscape position.

The second level assessment (Level II) is intended for use in a statistically selected sub-sample of the watershed wetland population and involves a more sophisticated analysis of remotely sensed information and a site visit for verification and additional data collection. The third level assessment (Level III) involves very detailed analysis of wetland performance of specific functions (i.e., habitat and water quality). This involves extensive sampling of a limited number of sites, specifically chosen to allow validation of the conceptual model of wetland function that underlies the Level I and Level II assessments.

A critical part of the overall monitoring and assessment strategy is effective validation and calibration of the underlying models. The Level III assessments are designed to specifically evaluate performance of functions in wetlands under varying degrees of stress, as indicated by the Levels I and II protocols.

Monitoring Program Development

The DEQ wetlands program, in coordination with the overall DEQ water quality monitoring program, has developed a ten-year plan for wetland monitoring and assessment in Virginia. This work is being accomplished as work products under EPA State Wetland Development Grants CD-983380-01, CD 983815-01, BG 983924-4, and BG-983925-01, BG-98392502 and BG-98392503 to the Department of Environmental Quality. The development of this strategy follows the EPA October 2002 draft document "Elements of a Wetland Monitoring and Assessment Program Checklist," EPA May 2006 "Application of Elements of a State Water Monitoring and

Assessment Program for Wetlands" (a supplement to the 2003 EPA document) and includes discussion of the following 'Ten Essential Elements of a State Water Monitoring and Assessment Program' (USEPA, March 2003):

1. Monitoring Program Strategy
2. Monitoring Objectives
   Information derived from monitoring will be used to:

   - Report ambient wetland conditions in Virginia's Clean Water Act (CWA) Section 305(b) reports;
   - Assist in the evaluation of environmental impacts of proposed impacts to wetlands during permit review as part of Virginia's regulatory program;
   - Evaluate the performance of wetland restoration and compensatory wetland mitigation in replacing wetland acreage and function; and
   - Evaluate the cumulative impacts of wetland loss and restoration in watersheds relative to ambient ecological conditions.
3. Monitoring Design
4. Core and Supplemental Water Quality Indicators
5. Quality Assurance
6. Data Management
7. Data Analysis/Assessment
   Examples of different wetland quality data analyses may include:

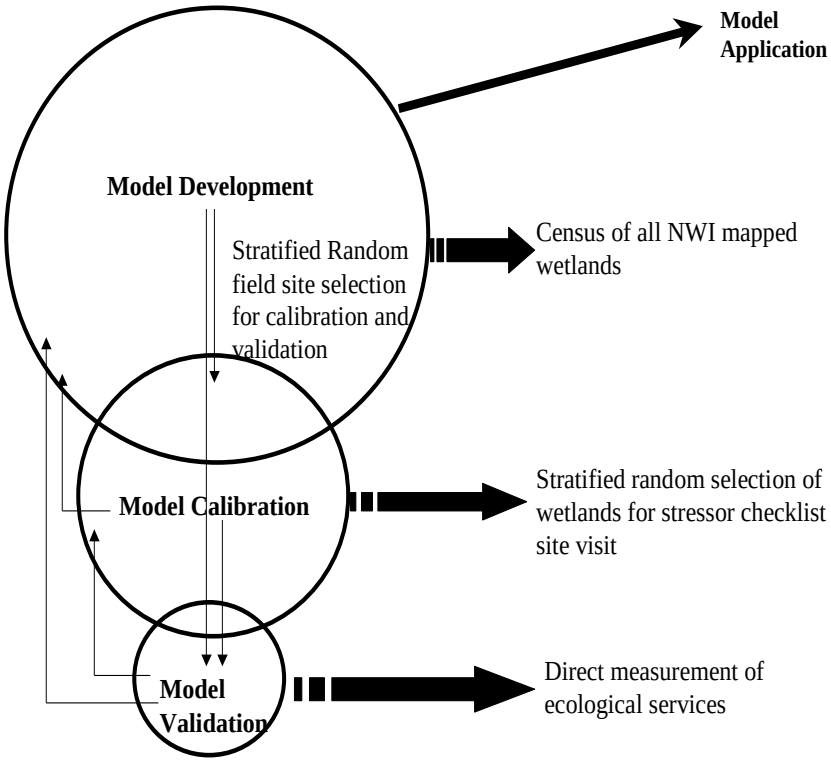
   - Comparison of wetland quality within a watershed and between watersheds
   - Comparison of wetland quality within a locality and between different localities
   - Comparison of wetland quality within a watershed or locality over time
   - Comparison of wetland quality between wetland types
   - Correlation of wetland type and specific stressor
   - Comparison of wetland quality within and between hydrogeomorphic (HGM) classes
   - Comparison of wetland quality within a specific wetland over time
8. Reporting
9. Programmatic Evaluation
10. General Support and Infrastructure Planning

Virginia's wetland monitoring and assessment program is being implemented through a cooperative agreement between DEQ and the Center for Coastal Resources Management at the Virginia Institute of Marine Science (CCRM) using funds awarded through EPA's Wetland Program Development Grants to continue these efforts. DEQ has received seven grant awards from EPA over the past eight years for this initiative, and Virginia is recognized as one of five states leading this initiative nationally.

Parameters used in the assessment reflect information from published literature, with consideration of on-going work being conducted through the Mid-Atlantic Wetland Workgroup (MAWWG), regarding each parameter's validity, usefulness, and utility for field data collection.

The protocol for the wetland monitoring and assessment developed in Virginia consists of a multi-tiered sampling design coupled with methods for regulatory updates and field office data delivery (see Figure 2 below). Each assessment level informs the other levels, and is essential in development of the final assessment protocol.

Figure 2. Multi-tiered sampling design.



The elements of Virginia's wetland monitoring and assessment program are listed in Table 3 below.

Table 3 - Wetland Monitoring and Assessment Program Elements	
Monitoring Strategy	Establish baseline condition of nontidal wetlands by broad category scalable from individual wetland to small watershed to physiographic province to entire State. Guide management decisions regarding restoration, compensation, and regulation of wetlands.
Monitoring Objectives	Support regulatory decision-making. Report wetland condition. Guide policy development. Evaluate cumulative impacts of wetland loss.

	Evaluate wetland restoration and compensatory mitigation effectiveness.
Survey Design	Three-Tiered: Sample Frame = all NWI wetlands Enhanced GIS analysis (census) – Level I. Probability-based sampling for field assessment of anthropogenic stressors – Level II. Intensive study of biological endpoints (birds, amphibians, water quality) along stressor gradient – Level III+
Assessment Indicators and Methods	Level I: land use adjacent, within 200m, and within 1000m of wetland, wetland size, type, hydroperiod, proximity to other wetlands, road type, road density, and road alignment. Level II: Field assessment of anthropogenic stressors within 30m of wetland assessment point and within 100m of wetland assessment point. Level III: Population and community structure metrics for birds and amphibians. Water quality modification metrics.
Quality Assurance	An EPA-approved Quality Management Plan coupled with the Center Quality Assurance Plan used to prevent random and systematic errors. Techniques include direct electronic field data assimilation to prevent transcription error as well as random return site visits and redundant QA assessment loops.

The strategy continues to develop a complete wetland monitoring and quality assessment in Virginia's Coastal Plain, Piedmont, and Ridge and Valley physiographic provinces in Virginia. The long-term field assessment strategy is outlined in Table 4 below. The strategy developed in Phase 1 provides the framework for the ongoing assessment of the status of the Commonwealth's wetland resources and performance measures for both the wetland regulatory and voluntary programs. Products from this strategy directly support Goal 4 of EPA's Strategic Plan to provide *"…additional focus on assessment of wetland condition"* and the National Priority of "*wetlands monitoring and assessment."*

Table 4: Long-term wetlands field assessment strategy for Virginia

Phase 1	Oct. 2003	Begin Level I assessment for Virginia.	Complete
	Dec. 2004	Begin Level II site assessment of Coastal Plain wetlands.	Complete
	Dec. 2005	Complete Level I assessment of Virginia, Complete Level II site assessment of Coastal Plain, Develop protocol for Level III assessment for Coastal Plain physiographic province.	Complete

Phase 2	Dec. 2005	Begin Level II site assessment of Piedmont physiographic province.	Complete
	Sept. 2007	Complete Level II site assessment of Piedmont. Begin Level III sampling for coastal plain sites.	Complete
Phase 3	Oct. 2007 – Sept. 2008	Complete enhanced wetland site selection for Ridge and Valley Level II site assessment using a protocol for probable wetlands location. Complete Level II site assessment for Ridge and Valley physiographic provinces. Continue Level III sampling for Coastal Plain.	Complete
Phase 4	Oct. 2008 – Sept. 2010	Begin Level III (model validation) sampling for Piedmont, and Ridge and Valley. Begin Level II re-sample coastal plain subset for calibration.	Complete
Phase 5	Oct. 2010	Begin Level I re-sample of Virginia for trends analysis.	Complete
Phase 6	Oct. 2011	Development of a Wetland Program Comprehensive Plan, refinement of our environmental database, and continued development of the wetlands monitoring and assessment program.	Complete
Phase 7	Jan. 2012 – Dec. 2014	Collaborate with VDOT to incorporate linear transportation projects into the wetland data viewer, review and update the monitoring and assessment strategy to incorporate completed tasks and re-evaluate the direction of the strategy.	In Progress

The level I assessment, which has been completed for all wetlands in Virginia, is based on wetland type and surrounding landscape. The Level II and Level III sampling are intended to calibrate and validate the model that is applied at the Level I (model development) stage. The data collections are not designed to operate independently. The method characterizes the capacity of the wetland to provide water quality and habitat services using remotely sensed data. The underlying models are based on existing research. They specify the combination of landscape level parameters that are most likely predictive of these capacities. The model application produces a relative score for each wetland for each service. The scores are then refined and calibrated by site visits to randomly selected wetlands. The relationship between structure and function is validated by intensive study of ecological service endpoints.

The assessment was done using existing data sets from the National Wetlands Inventory (NWI), Landsat Thematic Mapper (TM) satellite, protocols developed by the Coastal Change Analysis Program (CCAP) of the National Oceanic and Atmospheric Administration (NOAA), U.S. Geologic Survey National Elevation Dataset (NED), and Digital OrthoPhoto Quads. The parameters chosen for Virginia's Level I assessment wetland quality score include: (i) wetland size, (ii) wetland type, (iii) wetland hydroperiod; (iv) proximity to other wetlands; (v) proximity to roads and highways, (vi) density of roads and highways; and (vii) percent land cover (immediately adjacent to the study wetland, at a 200 meter radius from the study wetland, and at 200-1000 meter radius from the study wetland). The data set will be updated periodically, when resources allow, as revised land cover and NWI maps are updated.

The level I (model development) analysis, combined with validation and calibration from the level II and level III assessments, will provide an evaluation of the condition of wetlands based on their position in the landscape. This information is directly applicable to status and trends reporting under Clean Water Act Section 305(b), and can be utilized in permitting programs to assess cumulative impacts to wetlands within watersheds.

Level II and III assessments have proceeded by physiographic province from the coastal plain to piedmont to the ridge and valley with a sampling effort succeeded by model validation. Re-calibration of the stressors by landcover to verify the correlation of stressor type to landcover and validate the use landcover for condition assessment scoring has been completed.

Resampling of NWI mapped wetlands in the Coastal Plain and the Piedmont to investigate possible changes between surrounding land use and wetland stressors has been completed. This information is critical in the Virginia assessment protocol as the foundation of the stressor prediction algorithm in the Level I assessment model. It is essential to revisit the relationship between land use practices and stressors impacting wetlands as the pattern of development changes. Evolving best management practices in agriculture, and changing stormwater and site development regulations in suburban communities alter the probable occurrence of selected stressors. Since the Level I protocol uses remotely sensed land cover information to predict stressor occurrence, it is critical to periodically reassess the prediction algorithms. This task involved re-sampling the Piedmont region with the Level II protocol. Sixty sites, $1/10^{th}$ of the original sample number, were randomly sampled to detect potential significant changes in the relationships established in the original sample set. The major stressors found within wetlands remained similar between sample periods with mowing, brush cutting, roads, eroding banks, and unfenced livestock predominating. There was an uptick in the ditch/drain stressor in the 2011 sample and a downtick in the presence of potential nonpoint discharge.

A critical part of the overall monitoring and assessment strategy is effective validation and calibration of the underlying models. The level III assessments are designed to specifically evaluate performance of functions in wetlands under varying degrees of stress, as indicated by the level I and level II protocols. This project completed Level III validation within in the Piedmont and Ridge and Valley and began the wetlands condition status and trends analysis for the Coastal Plain. The Coastal Plain analysis provided a direct measurement of the selected sites' performance of habitat (avian and amphibian) functions to allow testing for correlations between ecological service and stressor levels.

One of the potential advantages of the Virginia protocol for monitoring and assessment of nontidal wetlands is the opportunity to develop a comprehensive assessment of the functional condition of all mapped wetlands whenever there is updated land cover information. This information is particularly useful for evaluating the performance of the regulatory program. It is also useful for indicating cumulative impacts to wetland resources arising from development activities that do not directly impact wetlands. This information can help to raise awareness of consequences and motivate essential change in general land use management and planning that affects lands outside wetland jurisdictional boundaries. Linking decisions in these areas to wetlands policy will be essential to attainment of the no net loss goal.

This task took advantage of the recently updated coastal plain assessment protocol, and the newly available land cover information from the NOAA Coastal Change Analysis Program. The recent update of the land cover classification for the coastal plain of Virginia provides a 2006 land cover that can be used in conjunction with the 1996 and 2001 land cover data set to assess change. All three land cover data sets were analyzed using the Level 1 assessment model. CCRM then summarized the changes in wetland condition output by the model. This represents the first comprehensive assessment of trends in wetland condition over a relatively modern time

interval. Analysis of wetland water quality condition and habitat condition scores by 12-digit hydrologic unit code showed some changes in average water quality and average habitat condition over time.

Using the analysis of wetland condition change, the water quality data was analyzed for Virginia's coastal plain. By developing catchment areas for the various water quality monitoring stations, the primary objective of this task was to search for relationships between water quality condition recorded at DEQ water quality stations and the condition of wetlands in the contributing drainage.

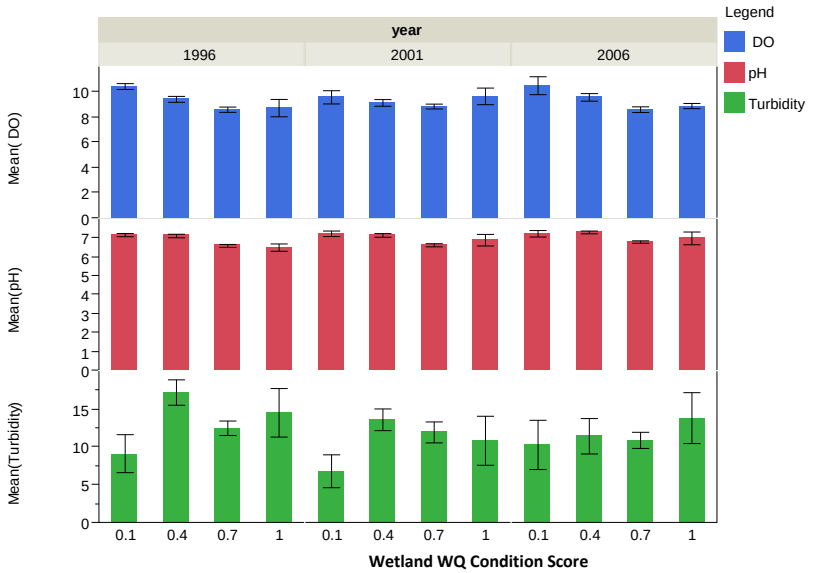
To test wetland water quality condition scores, Virginia Department of Environmental Quality coastal plain water quality stations (n=99) were used to determine possible trends between wetland water quality condition scores and in-stream water quality metrics (E. coli, fecal coliform, total nitrate nitrogen, DO, pH, and turbidity). Contributing drainage areas were developed for water quality stations using the same protocol for development of individual wetland drainage areas (Figure 4). Water quality station data was compared to contributing drainage wetland water quality condition scores for multiple years (1996, 2001, and 2006).

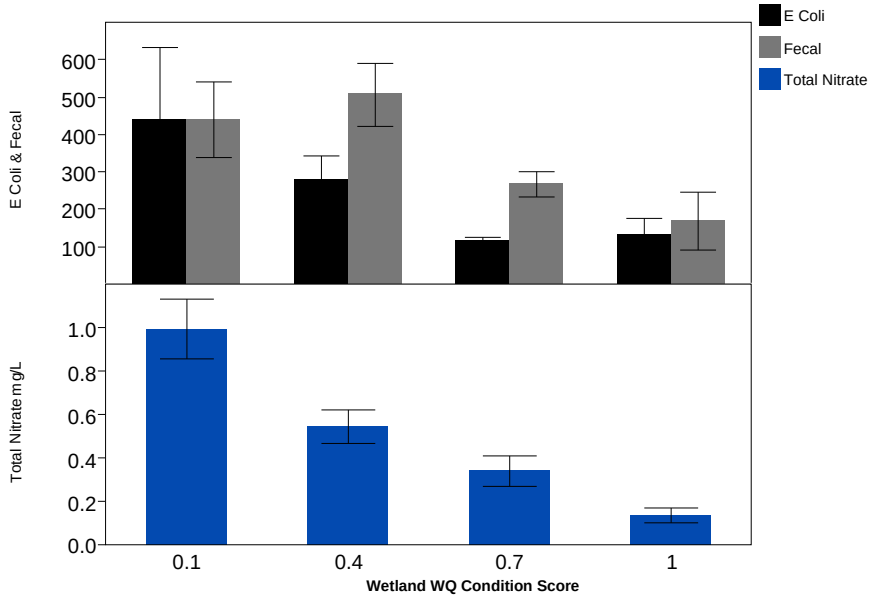
Figure 3. Wetland water quality stress condition within the contributing drainage to a Virginia Department of Environmental Quality water quality station.

While there were no obvious trends between wetland water quality condition score and average DO, pH, and turbidity, there were trends in total nitrate nitrogen, fecal coliform levels, and E. coli levels. As shown in Figure 5, the higher the wetland water quality condition score in the contributing drainage the lower the levels of

nitrate, fecal coliforms, and E. coli suggesting a relationship between those water quality parameters and wetland condition.

Figure 4. Comparison of wetland water quality condition scores (mode) and in-stream water quality parameters (mean).





Coordination with VIMS and DEQ staff to design and implement procedures to facilitate the routine application of inventory and monitoring data for regulatory decisions on wetland permits is ongoing. The data collected has been compiled into a wetland data viewer created by CCRM with substantial input from DEQ. The goal is to automate the processing of database information through GIS necessary to support DEQ's regulatory decision-making, allow reporting of wetland condition, and provide information for policy development.

The additions of data sets and GIS layers will allow Virginia to continue to develop a GIS-based wetland data viewer for use by regulatory agencies and the general public (see Figure 5). Our success will be measured by an increasing trend in the statistically-reliable Level I protocol and a decreasing trend in cumulative wetland impacts. By having a statistically-validated tool that measures wetland quality as a function of habitat and water quality parameters, our permit staff will be able to make better permit decisions relative to potential cumulative impacts. Further, we will also be able to measure how well we are protecting the function of our more vulnerable wetlands (i.e. isolated wetlands, vernal pools, Atlantic white cedar swamps), by comparing the condition of wetland habitat and water quality parameters, as a function of the assessment scoring over time.

Figure 5. Nontidal Wetlands Data Viewer

The wetland data viewer illustrated above is currently under design modifications and testing, and is not expected to be available for general use until late 2013 or early winter of 2014. The overall outcome of this continued focus on wetland monitoring and assessment will be better protection of wetlands and more definitive and defensible information on wetland condition over time and documentation of how we are achieving no net loss of wetland acreage and function in Virginia.

5.11 Freshwater Probabilistic Assessment

The freshwater probabilistic monitoring program is designed to allow Virginia DEQ make estimates of water quality with known confidence for 100% of Virginia's freshwater stream miles. Freshwater probabilistic monitoring is not designed to make segment/assessment unit decisions. However, a small number of parameters collected at probabilistic sites can be used to determine use support for that site.

The following parameters will be used for assessment unit decisions:

1) Dissolved Oxygen (if 2 out of 2 violate standard for stream class)
2) pH (if 2 out of 2 violate standard for stream class)
3) Temperature (if 2 out of 2 violate standard for stream class)
4) Virginia Stream Condition Index – Using the guidance set forth in the freshwater benthic assessment guidance.

5.12 Continuous Monitoring Assessment Methodology

Continuous monitoring, in which multiple observations are collected during a 24-hour period at a relatively high frequency, can provide for a more comprehensive assessment of water quality than what more traditional discrete or "grab sample" monitoring provides because it generates more accurate descriptive statistics and can reveal daily, weekly, monthly, and seasonal variability. High-frequency data collection allows for a more accurate calculation of the frequency and duration of violations as well. This is especially true for conventional field parameters (i.e., dissolved oxygen concentration and saturation, conductance, pH, temperature, and turbidity). Traditional sampling regimes (semi-monthly, monthly, bimonthly, or quarterly) can only provide a snap-shot of parameters, only allowing evaluation of parameter magnitudes and a very rough estimate of violation frequencies. Another advantage of continuous monitoring is that it monitors environmental conditions at times when field staff rarely sample, such as during nighttime or early morning hours.

Although these are significant benefits of continuous monitoring, the large datasets generated by such monitoring can be a challenge for assessment. It is considered appropriate to apply a 10.5% rule to grab sample datasets, which tend to be relatively small, but applying that rule to a continuous monitoring dataset, which can contain as many as tens of thousands of observations, could result in a water being assessed as attaining the standard for a parameter that it may be actually impaired for. Thus, using continuous monitoring data for listing and delisting waters requires caution and thoughtfulness. The following rules were crafted with this in mind:

<u>Rule 1</u>

A continuous monitoring dataset that is eligible for assessment must cover at least thirty 24-hour periods (with the exception of data being assessed for maximum temperature violations, which must cover at least fifteen 24-hour periods). This allows for an informative characterization of a water during the critical period (May to September) when violations of conventional field parameters are most expected.

Rule 2

The continuous monitoring dataset will have undergone rigorous and standardized QA/QC screening before analysis. Every 24-hour period with at least 75% of its observations deemed as valid should be assessed and counted as a single sample. Grab samples must be collected during the run that a continuous monitor is deployed.

Rule 3

Daily averages are the mean of all valid observations (including grab samples from the same station) collected during a 24-hour period. A violating DO daily average is defined as a mean calculated from all valid data collected during a 24-hour period that is below the appropriate daily average criterion for a given water. To count two violating daily means as separate violations, they must not be contained within the same four-day interval. This is consistent with 4-day experimental tests conducted by USEPA during the development of chronic DO criteria.

Rule 4

A 24-hour period violates minimum and maximum criteria when > 10.5% of its observations violate the criteria. Any two such days, even if consecutive, would count as two separate violations. Water temperature should be evaluated for violating increases as described in Section 9VAC25-260-60 of the Water Quality Standards. The "natural temperature" for a site should be determined upstream from a point-source discharge prior to assessment. Violations recorded during the continuous monitoring run should be combined with grab samples within the assessment data window. A 10.5% rule should then be applied to the combined data set.

Rule 5

For water temperature standards specifying a maximum hourly change (9VAC25-260-70), a 10.5% rule should be applied to the total number of monitored hours where data meet QA/QC (including hours of the first and last days of deployment.) An additional continuous monitoring dataset, collected during a subsequent year, during the same month(s) as the listing dataset, must be used to delist it.

Rule 6

If a continuous monitoring dataset is used to list a water on the 303(d) Impaired Waters List, then an additional continuous monitoring dataset, collected during a subsequent year, during the same month(s) as the listing dataset, must be used to delist it. A water that was previously listed using grab samples may be delisted using continuous monitoring data collected for at least 30 days, during a subsequent year and during the same month(s) when violations were previously found.

SCENARIO # 1:

A monitor was deployed July 31 at noon and run continuously through September 1 (noon) at a station. Five grab samples were collected at that station during the same year as the monitor's deployment (during February, April, June, July, and November); no other data exist in the assessment window for this station. No violation of the minimum DO criterion is detected in the grab samples, while four 24-hour periods in the continuous monitoring dataset have >10.5% of their total observations in violation of the minimum DO criterion.

Assessment

- The sample size is 36 (31 continuous monitor "samples" + 5 grab samples). The first and last 24-hour periods observed by the monitor should not be used for assessment, since at least 75% of the diurnal cycle was not recorded by the monitor on these two days.
- The violation rate is 11.1% and is therefore excessive. Accordingly, the water fails to meet the water quality standard for DO and should be placed on the 303(d) Impaired Waters list for this parameter.
- To delist this water, a continuous monitor must be set up for the same length of time as the original run, during the same month (August). Grab samples should be collected during other months of the year to maintain "temporal representativeness".

SCENARIO #2:

A monitor was deployed April 1 at noon and run continuously through August 31 (noon). Three grab samples were collected at that station during the same year as the monitor's deployment (February, October, December), and ten were collected two years previously. None of the newer grab samples violate any standard, but twelve 24-hour periods, the majority clustered in the summer months, have >10.5% of their observations in violation of the minimum DO criterion. The older dataset contained 2 violations of the DO minimum criterion, and these violations were also found during the summer. The water had therefore been placed on the 303(d) Impaired Waters list during the previous cycle.

Assessment

- The sample size is 164 (151 continuous monitoring samples + 3 new grab samples + 10 older grab samples).
- The violation rate is not technically excessive (8.5%), as defined by the 10.5% rule. However, there is evidence that the water experiences hypoxia during the summer. Before considering to delist the water, the assessor should address the following questions:

  a) Do the violations observed in the continuous monitoring dataset correspond temporally to those found in the older dataset used to list the water?
  b) What is the average duration of the violations? It would not be wise to delist a water characterized by long durations of violations—particularly for violations of the DO minimum.
  c) What is the temporal frequency of the violations? Are the violating 24-hour periods mostly consecutive, or are they spaced relatively far apart (potentially allowing for aquatic life recovery if the excursions are not too severe)?
  d) Were hydrological and/or weather conditions similar between the current dataset and the older dataset?
  e) Were there specific documented practices put into place that have improved water quality over the two-year period? (refer to Appendix D for more details)

f) Are violations observed in the grab samples collected during the continuous monitoring run?

Note that this is not an exhaustive list of considerations. To resolve situations such as the one described above, the assessor may need to rely on best professional judgment rather than following a strict interpretation of the 10.5% rule.

PART VI. PROCEDURES FOR CITIZEN AND NON-AGENCY DATA

For the purposes of this guidance document, a citizen water quality monitoring program, or "citizen monitoring," is defined as water quality monitoring which uses volunteers to collect the data. Some of these programs are run by local governments, Soil and Water Conservation Districts, citizen organizations, community organizations or colleges. Generally, K-12 school monitoring is conducted for educational purposes and does not fall under citizen monitoring unless working in cooperation with existing citizen monitoring efforts. Citizen monitoring is not defined as monitoring conducted by all entities external to DEQ, such as colleges and local governments, unless volunteers are used in their efforts.

DEQ does routinely receive water quality data from non-citizen volunteer sources such as local governments, universities, and other non-state or federal sources. The review and assessment of non-agency data is done using the same QA/QC review as with citizen monitoring data.

In 1997, Water Quality Monitoring, Information and Restoration Act (WQMIRA) was passed by the Virginia General Assembly. This bill charged DEQ with monitoring and assessing all the waters within the Commonwealth. During this same General Assembly session, the position of citizen monitoring coordinator was added into the operating budget of DEQ. The primary duties of the citizen monitoring coordinator were providing guidance and support to citizen water quality monitoring groups in the development of monitoring programs and quality assurance project plans. In addition, the citizen monitoring coordinator facilitated communication among citizen groups and other state agencies, sponsoring citizen monitoring seminars, promoting the use of citizen water quality data in a manner consistent with the data use goals of the organization and encouraging additional citizen monitoring efforts. In 2002, the Virginia General Assembly passed legislation that established the Virginia Citizen Water Quality Monitoring Program in the Code of Virginia (§62.1-44.19:11).

In 2004, the citizen monitoring coordinator position evolved into the role of water quality data liaison. This was done to centralize the task of requesting any and all available data collected outside of DEQ for inclusion into water quality assessment reports and follow up monitoring by DEQ. The duties and responsibilities of the former position regarding citizen monitoring data submissions and working with the citizen monitoring community have been maintained and expanded to include all other non-DEQ potential sources of water quality data.

Assessment Process:
The process of assessing water quality data submitted to DEQ involves staff from both the central office headquarters and the regional offices. In order to include any citizen or non-agency monitoring data in the biennial 305(b)/303(d) Integrated Water Quality Assessment Report (Integrated Report), it must be received and evaluated by the agency. By adhering to the tasks outlined below, the agency can ensure that all qualifying monitoring data is properly assessed.

Submitting Data for Evaluation:

1. All water quality data provided to DEQ from citizen and non-agency organizations should be sent to the water quality data liaison at DEQ. The liaison and the appropriate QA/QC staff in the Water Monitoring and Assessment (WMA) Office will review all standard operating procedures (SOPs), QA/QC plans or Quality Assurance Project Plans (QAPPs) for each citizen/non-agency monitoring group submitting chemical data.

For citizen/non-agency chemical and bacteria monitoring programs, the liaison will work with the WMA quality assurance (QA) coordinator. The liaison, QA coordinator, and the Biological Monitoring Program Coordinator will review all supporting documentation for benthic macroinvertebrate citizen/non-agency monitoring programs. Based upon the review of all procedures, the appropriate use of the data will be determined based on a three-tiered system.

2. The designation of DEQ tiered uses of data will be determined based upon the review of all procedures in conjunction with the organization submitting the water quality data. Any changes in QA/QC and/or SOP methods and/or any additions or deletions of current monitoring sites should be brought to the attention of the WQDL.

   Since 2007, DEQ has provided a data use authorization form to monitoring groups. Because not all non-DEQ organizations may wish to have their data used for water quality assessment reports, this form allows DEQ to meet their wishes. This authorization form cannot be used to upgrade the use of lower tiered data for a higher tiered purpose. Such an example would be a data submitter requesting DEQ to assess their data for Level III (use for 303(d) listing/delisting of impaired waters) based on Level II or Level I quality data.

Central Office Assessment Tasks:

1. The QA coordinator, with the help of the liaison, will provide a copy of all Level II and III citizen and non-agency monitoring data received during a given assessment cycle to the regions. The format of the data provided to the regions will be as follows:

   a. Data will be in electronic spreadsheet format compatible with programs used by the regional assessors.

   b. Level II and III data will be on separate spreadsheets denoting their QA status and assessment use.

   c. All data not meeting QA/QC requirements or otherwise not relevant for assessment will be omitted by the QA Coordinator. However, an unedited master copy of all data submitted will be maintained.

   d. At a minimum, all citizen and non-agency monitoring sites submitted to the regions for assessment will contain the following metadata:

      i. Name of waterbody monitored
      ii. Latitude and Longitude information
      iii. Physical description of the site (i.e. At Route 646 bridge crossing)

2. The liaison and QA coordinator will review data collected without SOPs and QAPPs plans. This data will be acknowledged in the applicable river basin evaluation as appropriate.

3. Citizen and non-agency monitoring groups that provided data for the assessment will have a summary of their results placed in a separate Citizen Monitoring/Non-Agency section of the Integrated Report.

4. The QA coordinator, with the help of the liaison, will coordinate with each regional office regarding the final assessment of the citizen and non-agency monitored data. In coordination with the liaison and the assessment coordinator, each regional office should provide any appropriate final editing of the citizen and non-agency monitoring assessment.

5. After the release of the final biennial Integrated Report, regional DEQ planning and monitoring staff will receive a list of all stations where monitoring results indicate possible water quality impairments. This list will identify waters based on the probability of impairment ranked from low to high. The regional monitoring staff should review the station list results and consider including monitoring sites as appropriate to their regional monitoring plan for future monitoring.

6. With the help of the liaison, the QA coordinator will provide all data approved by DEQ for use in the Integrated Report in basic data tables. The tables will be posted on the DEQ website along with the final Integrated Report. At a minimum, these data tables should include each individual sample period.

Regional Office Assessment Tasks:

1. All approved conventional parameter data should be summarized by major watershed and characterized according to the procedures and considerations in Part V of this manual.

2. For benthic macroinvertebrate monitoring programs used by citizen and non-agency monitoring organizations, data will be assessed based on the criteria outlined in *Guidance Memo No. 06-2010, Guidelines for DEQ Review and Approval of Biological Monitoring QAPPs*.

    a. For organizations that complete the requirements outlined in the guidance memo for Level III, DEQ staff will assess the data for the purposes of 305(b) water quality assessment and 303(d) listing and delisting of impaired waters. If a validation study showed inconclusive correlation with DEQ benthic protocols, the corresponding scores showing inconclusive correlation will not be assessed as Level III. These 'gray zone' scores may be used to characterize waters with or without observed effects (Category 3C or 3D).

    b. For all other methods not validated by DEQ or using DEQ protocols, biological monitoring sites characterized by citizen and non-agency organizations as "excellent," "good" or "acceptable" should be designated as "Area of low probability for adverse conditions" (Category 3D). Biological sites periodically characterized as "fair," "poor," "unacceptable" or "moderate" should be designated as "Area of medium probability for adverse conditions" and listed as insufficient data with observed effects and prioritized for follow-up monitoring (Category 3C). Likewise, biological sites that are consistently "poor" or "unacceptable" should be characterized as "Area of high probability for adverse conditions" and listed as insufficient data with observed effects with DEQ follow up monitoring to be prioritized (Category 3C).

3. Segment lengths represented by a monitoring site should be determined using the mileage delineation guidance found in Section 5.1. Each monitoring site used in the assessment should have a unique station ID using a system similar to the DEQ station ID system. The regional office staff assigns this station ID to each citizen/non-agency monitoring site and relays the station ID to the QA Coordinator.

4. Level III data collected at sites that complement and are comparable (i.e. chemical to chemical comparisons and biological to biological comparisons) to DEQ monitoring sites, should be included in

the major basin report. However, the final assessment of that segment will be made using the DEQ monitoring data (found in the appropriate section of the Integrated Report). In this case, the data collected by the monitoring organization would be used as supplemental data.

5. Level III data collected at sites that do not complement or compare (i.e. benthic to chemical comparisons) to DEQ monitored sites, should be included in the major basin report. The final assessment of the segment should be primarily assessed using the non-DEQ monitoring data. For example, Level III citizen benthic macroinvertebrate data shows impairment while a nearby DEQ chemical monitoring station does not directly show benthic impairment.

6. Level II ambient and bacteria data collected at sites will undergo the similar evaluation process as used for Level III and DEQ results. Since Level II data may have some variation in quality assurance, corresponding waterbodies that indicate poor water quality will be listed as insufficient data with observed effects and prioritized for follow-up monitoring (Category 3C). Waterbodies that have Level II data indicating good water quality will be listed as insufficient data with low probability for adverse conditions (Category 3D).

7. If during the regional review, a discrepancy between data from DEQ monitoring stations and data from nearby citizen/non-agency monitoring stations is believed to be suspect, the QA coordinator should be notified and effort made to rectify the discrepancy.

   The QA coordinator and liaison will evaluate the potential causes for the data disparity and/or review the QAPP and the monitoring techniques of the data submitting group. After this evaluation is complete and a problem is confirmed, appropriate corrective actions will be recommended to the monitoring group for inclusion in the citizen/non-agency monitoring organization's QAPP and/or SOPs.

   Until the discrepancies with the data and/or methods are fully evaluated by DEQ, the data (either for the parameter(s) of concern or for all observations) should not be used in agency assessments. If the citizen or non-agency monitoring group does not initiate corrective action, the QAPP for that parameter and/or for the group as a whole may no longer be considered valid by DEQ, and the data will not be considered for statewide water quality assessments.

Other State and Federal Water Quality Data

After review and approval of monitoring and QA/QC protocols, DEQ will consider data generated by other State and Federal monitoring programs for use in the Integrated Report. DEQ has established a water quality data sharing agreement with several state and federal agencies that includes the Virginia Department of Health, Tennessee Valley Authority, National Park Service, United States Forest Service, and the United States Geological Survey.

Virginia Department of Health (VDH) - DEQ receives and lists areas closed by VDH for shellfish harvesting due to high bacteria levels. All Enterococcus bacteria results provided by VDH are also used along with any DEQ water quality data in assessing water quality. Any other water quality data collected by VDH and shared with DEQ will be used at the latter agency's discretion.

Tennessee Valley Authority (TVA) - The TVA routinely monitors for *E. coli* bacteria along TVA reservoirs in Virginia. These data are considered acceptable for assessing water quality in Virginia.

National Park Service - The National Park Service has several long-term monitoring programs in place at many of the national parks in Virginia. Many of the parks monitor for chemical and benthic macroinvertebrate parameters using varying methodologies or procedures. Because of this, the liaison and QA coordinator provide guidance to the regional office assessment staff in assessing data received from the parks.

United States Forest Service (USFS) - The USFS program collected macroinvertebrate data from numerous monitoring stations within the George Washington and Jefferson National Forests. Sampling for macroinvertebrates is conducted utilizing the same collection methodology (Plafkin et al 1989) that DEQ biologists use in the ambient biomonitoring program. Therefore, the raw data collected by the USFS should be highly comparable with DEQ data. The USFS has used the Macroinvertebrate Aggregated Index for Streams (MAIS) to assess this raw data and make an initial water quality interpretation.

The DEQ regional biologists and planners may use the data, provided to DEQ by the USFS, in the Integrated Report if they find it acceptable for assessment purposes. If the regional biologists or planners have information that conflicts with the initial USFS assessment, or for any other reason questions the USFS stream assessment, they may elect to disregard the USFS assessment results until further verification can be obtained. If the initial assessment is not used, documentation relating to this decision will need to be provided. The regional biologists may elect to reevaluate the raw data using the Virginia Stream Condition Index (VSCI) metrics to confirm consistent assessment methodology and conclusions. If differences become apparent, the regional biologists may decide not to use the assessment data in the Integrated Report until an on-site stream visit can be performed and conditions verified. Final assessment results of the USFS data should be consistent with the ambient biological assessment criteria described in Section 5.4 of this guidance. Any non-approved data will not be used directly in the assessment.

United States Geological Survey (USGS) - The USGS monitors several water quality stations throughout Virginia. Data collected by the USGS is considered Level III by DEQ and is used in assessing water quality including 303(d) impairment listings and delistings. Water quality parameters for which there are no established numerical criteria in Virginia's water quality standards are not used for the purposes of 303(d) impairment listing, but can be used to assess waters for observed effects (Category 3C/3D).

PART VII 303(d) LISTING/DELISTING and TMDL PRIORITY RANKING

Effluent Limited and Alternative Control Waters (Category 4B/5E)

Rule 1

When reviewing waters receiving effluent from facilities with water quality-based effluent limits in VPDES permits, the following should be considered in developing the 303(d) list:

1. If the permit has been issued with no compliance schedule and the limits are to be met upon permit issuance, then listing is not necessary.

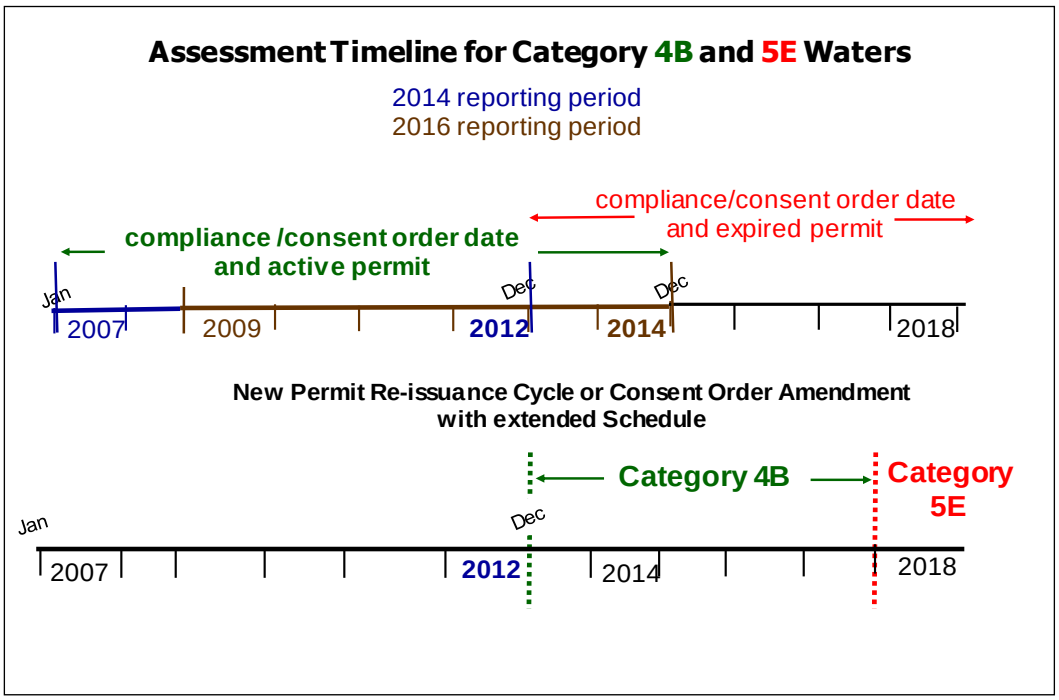
2. If the permit for a previously listed water has since been issued with no compliance schedule and the limits are required to be met upon permit issuance, then the facility should be delisted. EPA must be provided a verification package for delisting waters (see Section 5.2 Rule 2).

3. If achievement with the existing permit compliance schedule or consent order has not occurred by the end of the 2014 reporting period (12/31/2012) but is anticipated to meet the schedule by the end of the 2016 reporting period (12/31/2014) AND the permit is still in effect, it is Category 4B.

4. If the existing permit expiration date is before the end of the 2014 reporting period (12/31/2012) and the compliance schedule or consent order compliance date is after the 2014 reporting period ending (12/31/2012), it is Category 5E.

5. If a permit re-issuance occurs with a new compliance/consent order schedule date between 12/31/2012 to 12/31/2017 (reflecting a five-year permitting cycle) and compliance with the previous permit compliance or consent order schedule was not achieved, the water is Category 4B.

   However, if a staged or phased permit compliance schedule (greater than the permit five-year cycle) or consent order extends beyond 12/31/2017, then the water is Category 5E**.**



Rule 2

The verification process for removing or delisting effluent-limited waters must consider the following:

- The removal or delisting process applies only to waters impacted by a single point source discharge. TMDLs will have to be developed and approved by EPA prior to delisting waters impacted by multiple discharges or a single point source with a significant nonpoint source "load allocation" component. A water listed in Part II for NH3-N discharging into a segment listed for nonpoint source fecal coliform bacteria could be removed since the bacteria problem is unrelated to the NH3-N.

- If compliance with the water quality-based effluent limits is not met by the compliance date, the waters should not be removed from the list or should be relisted in Category 4B if previously removed and a new compliance schedule requiring compliance by the end of the next reporting period is in place. If a new compliance schedule has not been negotiated or extends past the next reporting period, the water should be listed as Category 5E. If post-operational water quality data shows that WQS are not being met, the water should remain on the list or be relisted in Category 5A.

If the above conditions are met, the following information should be submitted to EPA for delisting those waters identified in Category 4B of the 2014 303(d) Report. Waters that do not meet the above conditions should be listed or remain in Category 4B of the 2014 303(d) Report.

Verification Packet for VPDES Permits:
Hydrologic Unit Code (HUC), Watershed Identity Number, Stream Name, Parameter, and VPDES Permit Number, Owner/Facility Name and recent DMRs showing compliance.

- A statement identifying the basis for delisting the water. The statement should confirm that water quality based effluent limits were in place by the compliance date, and these effluent controls are sufficient to attain or maintain WQS. If the facility will meet the water quality-based effluent limits within the listing cycle required by federal law and WQS are expected to be attained or maintained, the verification should describe the facility's progress in meeting the effluent requirements and the expectation that the compliance date in the permit will be met.

- Copy of water quality analysis modeling conducted as part of permit development that shows the level of controls necessary to implement WQ Standards.

- Copy of permit page (and/or any State compliance order and associated interim limits and schedule to achieve the final limit) that contains the required control levels.

- Copy of permit page that provides the compliance date for water quality based controls.

Rule 3

Category 4B – Alternative Control

EPA's 2006 IR Guidance acknowledged that the most effective method for achieving water quality standards for some water quality impaired segments may be through controls developed and implemented prior to the TMDL development and/or implementation (referred to as a "4B alternative"). DEQ requests EPA to evaluate, on a case-by-case basis, the Commonwealth's decisions to exclude or delist certain segment/pollutant combinations from Category 5 based on the 4B alternative. A 4B rationale will be provided to EPA in the submission of the 2014 IR which supports the Commonwealth's conclusion that there are "other pollution control requirements" sufficiently stringent to achieve applicable water quality standards within a reasonable period of time.

Required elements of the 4B rationale:
Specifically, this rationale should include:
(1) a statement of the problem causing the impairment,

(2) a description of the proposed implementation strategy and supporting pollution controls necessary to achieve water quality standards, including the identification of point and non-point source loadings that when implemented assure the attainment of all applicable water quality standards,
(3) an estimate or projection of the time when water quality standards will be met,
(4) a reasonable schedule for implementing the necessary pollution controls,
(5) a description of, and schedule for, monitoring milestones for tracking and reporting progress to EPA on the implementation of the pollution controls, and
(6) a commitment to revise, as necessary, the implementation strategy and corresponding pollution controls if progress towards meeting water quality standards is not being shown.

Delisting Rules

Rule 1

Waters listed as impaired and needing a TMDL in the Integrated Report will remain on the list and tracked in subsequent Integrated Reports until:

- An EPA approved TMDL is developed for all pollutants causing impairment

OR

A subsequent assessment of new monitoring data (or in special cases, modeling data) results show that the water is no longer impaired and EPA approves the delisting of the water.

Rule 2

Documentation required by EPA for delisting previously listed impaired waters that are now restored:

Scenario # 1: when new data demonstrates a previously impaired waterbody is currently attaining WQS, DEQ should submit the following documents to justify the delisting of this segment from the 303(d) list.

- Hydrologic Unit Code (HUC), Federal TMDL ID (if available), Cause Group Code, Watershed Identity Number, Stream Name and Listed Parameter
- Rationale for the decision to remove the previously impaired segment from the 303(d) list
- Copies of the data that are being used to justify the removal of the segment
- Copies of the previous data which were used to list the segment
- Any differences between the sampling techniques should be documented and submitted
- A description of the water including but not limited to: stream name, impaired miles (acres or sq. mi.), beginning and ending river miles, impairment, watershed identification code and HUC

Scenario # 2: when new water quality modeling determines the stream is now attaining WQS, DEQ should submit the following documents to justify the removal of this segment from the 303(d) list.

- Hydrologic Unit Code (HUC), Federal TMDL ID (if available), Cause Group Code, Watershed Identity Number, Stream Name and Listed Parameter.

- Rationale for the decision to remove the previously impaired segment from the 303(d) list
- Submission of any new data that were used in the modeling
- A copy of the EPA approved model that was used. A summary of the differences between the new and the old models. The reasons why the stream attains WQS under the new model opposed to the former model (data, modeling assumptions, modeling applications, etc)
- A description of the water including but not limited to: stream name, impaired miles (acres or sq. mi.), beginning and ending river miles, impairment, watershed identification code and HUC

Scenario # 3: when new management practices from point and/or nonpoint sources lead to the attainment of WQS, DEQ should submit the following documents to justify the removal of this segment from the 303(d) list.

- Hydrologic Unit Code (HUC), Federal TMDL ID (if available), Cause Group Code, Watershed Identity Number, Stream Name and Listed Parameter.
- Rationale for the decision to remove the previously impaired segment from the 303(d) list.
- Submission of the most recent 2 years of water quality data that indicate the water is a candidate for delisting and
- A description of the new management practices and other changes that have occurred in the watershed to explain the change in water quality.
- A description of the water including but not limited to: stream name, impaired miles (acres or sq. mi.), beginning and ending river miles, impairment, watershed identification code and HUC.

The TMDL staff should apply the Proactive Approach, as appropriate, any time a TMDL is scheduled for development. Appendix D contains additional procedural information on this approach.

Scenario # 4: when errors are detected in the rationale for the initial listing of the segment or WQ Standards have been modified and the segment is attaining WQ Standards, DEQ should submit the following documents to justify the removal of this segment from the 303(d) list.

- Hydrologic Unit Code (HUC), Federal TMDL ID (if available), Cause Group Code, Watershed Identity Number, Stream Name and Listed Parameter.
- Rationale for the decision to remove the previously impaired segment from the 303(d) list
- Documentation of the errors in the initial listing
- A copy of the data and/or modeling that demonstrates the segment attains WQS at least 90% of the time
- A description of the water including but not limited to, stream name, impaired miles (acres or sq. mi.), beginning and ending river miles, impairment, watershed identification code and HUC

In certain cases EPA may request additional documentation to justify the removal of the segment from the 303(d) list.

Rule 3

A new impairment is "nested" when it is determined that the impairment has the same source/cause as a previously listed impairment within an existing TMDL. In such a case, it is assumed the new impairment is adequately addressed by the pre-existing TMDL and should thus be classified as Category 4A. Assessors should coordinate with TMDL staff to review nesting guidance for specific qualifications for nesting, procedural requirements, and appropriate documentation.

Bacteria impairments within the existing TMDL watershed or within the "tidal range" of the existing TMDL boundary can be immediately nested when land uses in the existing TMDL and newly impaired segment are comparable and all existing sources are accounted for in the TMDL.

Nesting non-bacterial impairments may be appropriate if the existing TMDL(s) addresses all appropriate stressor(s) for benthic impairments or all source(s) for other non-bacterial impairments. It is not appropriate if new applicable stressor(s) or source(s) exist.

A rationale memo describing the TMDL, the watershed, and the relevant assessments unit(s) as well as justification for the nesting should be submitted to EPA before nesting impairment(s) under the following conditions:

- Non-bacteria impairment (e.g., nesting a pH impairment under a TMDL originally addressing DO and nutrients)
- Bacteria impairment outside the boundary of a TMDL watershed or not within the "tidal range" of the existing TMDL boundary.

For newly nested segments, the following should be entered in the Assessment Database:

- Change Impaired Category code to 4A
- Enter Nested Year: Federal TMDL ID, EPA approval date
- In Cause Comment field enter "Proposed nested because addressed by [name of TMDL]". E.g. "Proposed nested because addressed by Rivanna River Sediment TMDL."

Rule 4

Section 303(d) requires States to "establish a priority ranking" for the waters it identifies on the impaired waters list, taking into account the severity of the pollution and the uses to be made of such waters, and to establish TMDLs "in accordance with the priority ranking." Federal regulations provide that "schedules for submissions of TMDLs shall be determined by the Regional Administrator and the State" (40 CFR 130.7(d)(1)). Other reasonable factors such as the State's use of a rotating basin approach or commitments specified in court orders or consent decrees may also be considered when States develop priorities and schedules.

For the waters covered by the June 1999 Consent Decree pertaining to Virginia's TMDL program, DEQ has developed a TMDL development schedule that ended on May 1, 2010. For waters listed as impaired subsequent to the Consent Decree, TMDLs are expected to be completed within 12 years of the first listing date. Outside of the specific TMDL development schedule, TMDL due dates assigned to each water body reflect the date when a TMDL must be established. However, if subsequently listed waters are within a Consent Decree watershed, every effort will be made to address the impairments at the same time. This may result in TMDL

development much sooner than the 12 years generally anticipated. Also, in response to concerns raised by the United States Fish and Wildlife Service during the development of the 2002 303(d) List, impaired waters this agency determines are of concern may have accelerated TMDL development dates.

In preparing the TMDL development schedule, Virginia does not specifically identify each TMDL as high, medium or low priority. Instead, DEQ uses the TMDL schedule itself to reflect Virginia's priority ranking. The CWA does not prescribe a particular method of expressing a priority ranking, and DEQ believes a TMDL schedule is a reasonable, efficient way to demonstrate priority ranking.

In scheduling TMDLs for development, every effort should be made to address all related impairments in a watershed at the same time. If endangered species are affected by an impairment listing, TMDL development should be scheduled as expeditiously as possible. If a public water supply is affected by an impairment listing, TMDL development should be scheduled as expeditiously as possible. In the absence of impacts to public water supplies or endangered species, a watershed approach should be used for TMDL development scheduling. Other factors that may impact TMDL scheduling include public interest and support, locally available funding to implement controls, or coordinating TMDL development efforts with an adjoining state.

After the TMDL schedule has been developed, the order in which TMDLs are established might be subject to some modifications to accommodate logistical efficiencies or data availability. The process is a dynamic process and any priority ranking may be changed if substantial factors change or become apparent during the scheduling process.

APPENDIX A

Clean Water Act Sections

SEC. 305. WATER QUALITY INVENTORY

(b) (1) Each State shall prepare and submit to the Administrator by April 1, 1975, and shall bring up to date by April 1, 1976, and biennially thereafter, a report that shall include—

(A) a description of the water quality of all navigable waters in such State during the preceding year, with appropriate supplemental descriptions as shall be required to take into account seasonal, tidal, and other variations, correlated with the quality of water required by the objective of this Act (as identified by the Administrator pursuant to criteria published under section 304(a) of this Act) and the water quality described in subparagraph (B) of this paragraph;

(B) an analysis of the extent to which all navigable waters of such State provide for the protection and propagation of a balanced population of shellfish, fish, and wildlife, and allow recreational activities in and on the water;

(C) an analysis of the extent to which the elimination of the discharge of pollutants and a level of water quality which provides for the protection and propagation of a balanced population of shellfish, fish, and wildlife and allows recreational activities in and on the water, have been or will be achieved by the requirements of this Act, together with recommendations as to additional action necessary to achieve such objectives and for what water such additional action is necessary;

(D) an estimate of (1) the environmental impact, (ii) the economic and social costs necessary to achieve the objective of this Act in such State, (iii) the economic and social benefits of such achievement, and (iv) an estimate of the date of such achievement; and

(E) a description of the nature and extent of nonpoint sources of pollutants, and recommendations as to the programs which must be undertaken to control each category of such sources, including an estimate of the costs of implementing such programs. (2) The Administrator shall transmit such State reports, together with an analysis thereof, to Congress on or before October 1, 1975, and October 1, 1976, and biennially thereafter.

GRANTS FOR SEC. 106. POLLUTION CONTROL PROGRAM

(e) Beginning in fiscal year 1974 the Administrator shall not make any grant under this section to any State which has not provided or is not carrying out as a part of its program—

(1) the establishment and operation of appropriate devices, methods, systems, and procedures necessary to monitor, and to compile and analyze data on (including classification according to eutrophic condition), the quality of navigable waters and to the extent practicable, ground waters including biological monitoring; and provision for annually updating such data and including it in the report required under section 305 of this Act;

SEC. 204 LIMITATION AND CONDITIONS

(a) Before approving grants for any projection for any treatment works under section 201(g)(1) the Administrator shall determine—

"that (A) the State in which the project is to be located (1) is implementing any required plan under section 303(e) of this Act and the proposed treatment works are in conformity with such plan, or (ii) is developing such a plan and the proposed treatment works will be in conformity with such plan, and (b) such State is in compliance with section 305(b) of this Act;"

SEC. 314. CLEAN LAKES

(a) Each State shall prepare or establish, and submit to the Administrator for his approval—

"(A) an identification and classification according to eutrophic condition of all publicly owned lakes in such State;
"(B) a description of procedures, processes, and methods (including land use requirements), to control sources of pollution of such lakes;
"(C) a description of methods and procedures, in conjunction with appropriate Federal agencies, to restore the quality of such lakes;
"(D) methods and procedures to mitigate the harmful effects of high acidity, including innovative methods of neutralizing and restoring buffering capacity of lakes and methods of removing from lakes toxic metals and other toxic substances mobilized by high acidity;
"(E) a list and description of those publicly owned lakes in such State for which uses are known to be impaired, including those lakes which are known not to meet applicable WQ Standards or which require implementation of control programs to maintain compliance with applicable standards and those lakes in which water quality has deteriorated as a result of high acidity that may reasonably be due to acid deposition; and
"(F) an assessment of the status and trends of water quality in lakes in such State, including but not limited to, the nature and extent of pollution loading from point and nonpoint sources and the extent to which the uses of lakes is impaired as a result of such pollution, particularly with respect to toxic pollution.

"(2) SUBMISSION AS PART OF 305(b) (1) REPORT. – The information required under paragraph (1) shall be included in the report required under section 305(b) (1) of this Act, beginning with the report required under such section by April 1, 1988".

APPENDIX B

Virginia Department of Environmental Quality
Biological Monitoring Program
305(b) Assessment Fact Sheet

Regional Office:
Regional Biologist's Signature: _____
Review Date:
River Basin:
Stream Name and Site Location:
Station ID #:
Reference Station ID #:
Assessment Method:
VSCI
Coastal Plain (MACS)

Biological Assessments for the Last Six Years

Year	Spring score	Spring assessment	Fall score	Fall assessment
2007				
2008				
2009				
2010				
2011				
2012	0.0		0.0	
Seasonal avg 6-yrs	0.0		0.0	
Seasonal avg last 2-yrs	0.0		0.0	
Final 6-yr average	0.0		0.0	
Final 6-yr average	0.0		0.0	

Note, because of the long, six-year time frame covered by this review and for a variety of reasons, some sites may not have been sampled during every year or season and/or an assessment ranking or score may not be available for every "cell" in the above table. The above table is intended to be a convenient method to summarize and review all the data available for the reporting period. The final assessment ranking for each site should be based on a review of all the available rankings shown in the above table and any pertinent supplemental data described below. For the purpose of Integrated Report preparation, if more recent bioassessment rankings differ significantly from earlier rankings, primary consideration should be given to the more recent assessed data. This is described in more detail of Section 5.4 of the Integrated Report Guidance Manual.

Supplemental Information (if applicable):

Are any seasonal differences noted?

Summary of any comments associated with assessments.

Have any factors been observed in watershed that may be affecting the benthic community? Have there been any recent changes in activity in the watershed that may have affected the more recent bioassessments. Are these changes likely to affect the benthic community for a short or long term basis?

Final Assessment Rating:

APPENDIX C

Classification of Virginia's Shellfish Growing Areas
Robert E. Croonenberghs, PhD

The Division of Shellfish Sanitation (DSS) follows the requirements of the National Shellfish Sanitation Program (NSSP), which is regulated by the U.S. Food and Drug Administration. The NSSP classification uses the shoreline survey as its primary tool for classifying shellfish growing waters. Fecal coliform concentrations in seawater samples collected in the immediate vicinity of the shellfish beds function to verify the findings of the shoreline surveys, and to define the border between approved and condemned (unapproved) waters.

DSS uses the shoreline survey to locate as many sources of pollution as possible on the watersheds of shellfish growing areas. DSS conducts a property-by-property inspection of the onsite sanitary waste disposal facilities of many properties on un-sewered sections of watersheds, and investigates other sources of pollution such as wastewater treatment facilities (WWTF), marinas, livestock operations, landfills, etc. The information is compiled into a written report with a map showing the location of the sources of real or potential pollution found, and sends it to the various state agencies that are responsible for regulating these concerns and the city or county. The local health departments (LHDs) of the Virginia Department of Health (VDH) play a major role in the process by obtaining correction of the onsite sanitary waste disposal problems. Most of the Division's shoreline survey effort is focused on locating potential fecal contamination, and in this manner we prevent significant amounts of human pathogens from getting into shellfish waters. We believe that this is a primary reason why we have not had a confirmed shellfish-borne disease outbreak due to Virginia-grown shellfish since the early 1960's. VDH is reducing the input of these pathogens to back yards, waterways, unofficial swimming areas and shellfish waters. The shoreline survey work is the foundation of the shellfish growing area classification program.

In addition to the shoreline survey, the NSSP requires that DSS collect seawater samples in the growing areas as part of the classification procedure. States must use the most recent 30 samples, collected randomly with respect to weather (scheduled one month in advance), to classify a station. The two-part standard for fecal coliforms in waters for direct shellfish harvest to market is a geometric mean no greater than 14 MPN fecal coliforms/100 ml and an estimated 90th percentile no greater than 31. Exceeding either number requires closure of that station.

To a lesser degree, the Division collects shellfish samples from sentinel growing areas and has them analyzed for heavy metals and chlorinated hydrocarbons (pesticides and PCBs). Such toxic substances in shellfish are not a public health threat in Virginia's waters, with the potential exception of the Southern Branch of the Elizabeth River and perhaps Little Creek, both of which are located in the Hampton Roads area.

Thus, classification based on fecal pollution is a multi-layered and multi-step process. Initially one uses the shoreline survey to determine if there are any actual or potential sources of fresh fecal pollution to the growing area. If so, then the area cannot be used for the direct harvest of shellfish for marketing. Hampton Roads is an example. Most of Hampton Roads is permanently closed, due to the tremendous amount of shipping and the concern of contamination from treated sewage outfalls and runoff from the urban watershed. However, microbiological results are generally acceptable.

Another example of actual or potential pollution that requires closure is a discharge, such as from a WWTF or the potential discharge from boats in marinas. DSS uses relatively simple computer models developed by VIMS, which employ fairly sophisticated mathematics, to determine the size of buffer zones around these sources. These models use inputs of fecal coliforms (estimated from sewage treatment facility outfall volumes or factors related to the number and size of boats in marinas), die-off factors, and readily available tidal current and channel configuration information. Buffer zones around marinas are typically only in effect during the warmer boating months (April 1 -

October 31), whereas those around WWTF are in effect all year. Once these buffer zones are determined, they do not change in size unless the capacity of the WWTF or the marina changes.

Our third layer of classification, and our most common in Virginia, consists of evaluating areas that are not affected by urban runoff or significant wastewater discharges. One must evaluate the watershed for the potential impacts of known failing onsite sanitary waste facilities to estimate whether their input could be of such a magnitude as to require closure, even if the water quality data is acceptable. If the impact from these failing systems does not appear to pose an undue threat, then the water quality data can be used to verify whether the waters should be classified as approved or not.

Since DSS collects approximately 9-10 seawater samples annually, this means that our geometric mean typically incorporates data reaching back 2.5 to 3 years. Heavy rainfall or very high tides due to winds or moon phase can wash unusually high concentrations of fecal coliforms into shellfish growing areas that can increase the geometric mean or the 90th percentile beyond the allowed standard. As more data is collected and the unusually high concentrations fall off the trailing end of the data set, the water quality then appears to improve. This is one of the factors that can cause a continual fluctuation in the classification of the water quality at the interface between impacted upstream waters and the relatively unaffected downstream water body.

Since DSS is not a research organization, we cannot do much to determine the cause of water quality deterioration in areas. However, the Division has tried over the years to do so, and we have encouraged the Commonwealth to put resources into determining those causes. The Division has rarely found an association between obviously failing septic systems adjacent to growing areas and deteriorating water quality in large bodies of water. We have seen areas where impacts on fecal coliform concentrations in smaller bodies of water occur due to failing onsite sanitary waste disposal systems, but these seem to be rare. This should not be taken to downplay the concern from such failing onsite sanitary waste disposal systems, since even small inputs of fecal coliforms from these systems are quite likely to contain significant concentrations of human pathogens. Indeed, failing onsite sanitary waste disposal systems are one of the types of pollution sources of greatest concern with regard to the consumption of bivalve molluscan shellfish. Drainfields located in seasonally high water tables may contribute significant numbers of fecal coliforms to impact water quality, and research into this potential source is needed.

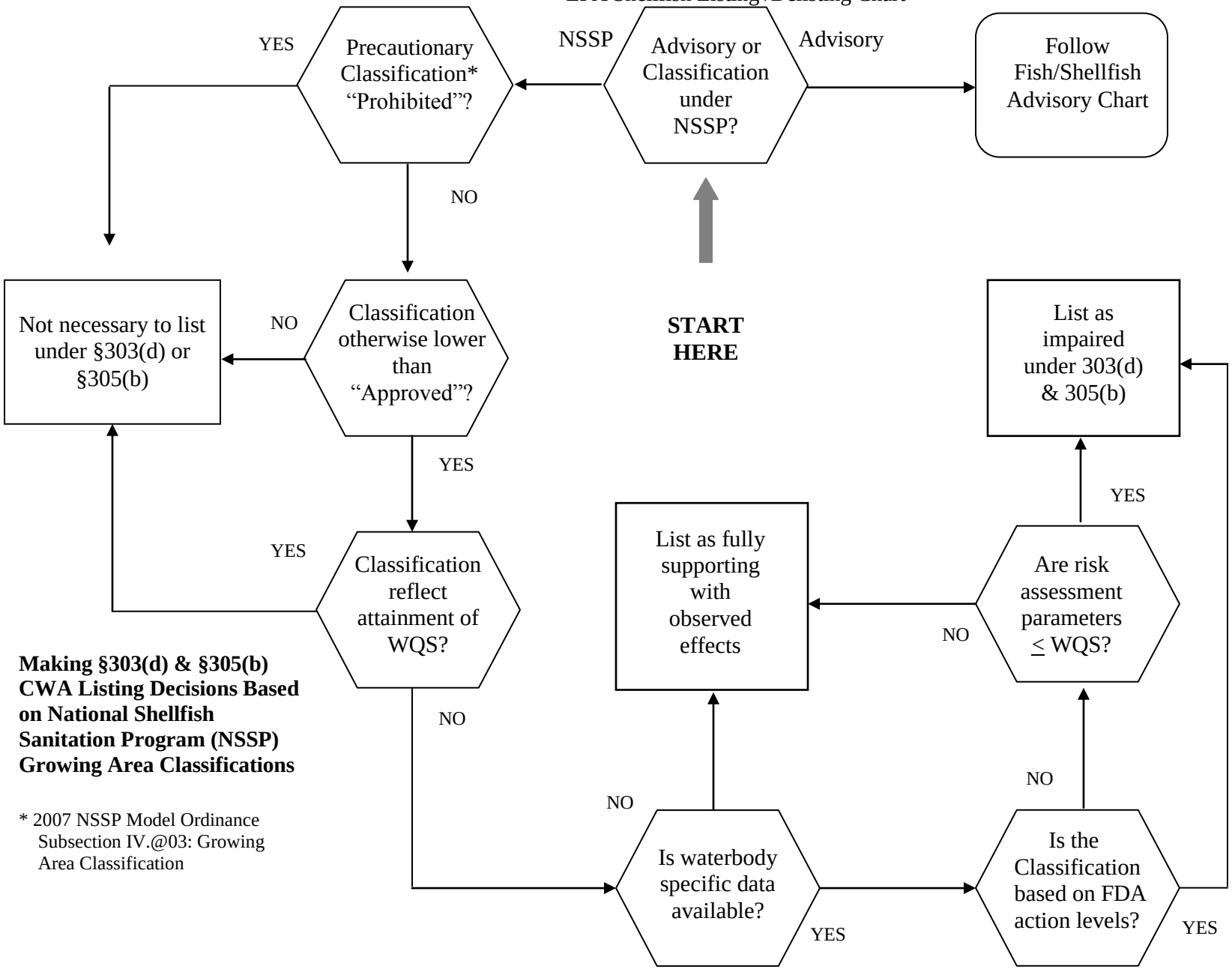
Virginia's urban suburban watersheds like the Lynnhaven River in Virginia Beach are clearly impacted by the flushing action of rapid runoff from storm drains. Other areas are much less predictable. Sometimes heavy rainfalls cause elevated counts in rural areas and sometimes they do not. While the Division used to depend upon rain gauges at airports and other widely scattered locations, it now uses NOAA Doppler predicted rainfall, which provides much improved data during spotty summertime thunderstorms. We may find that with this new data, that some areas respond more predictably to rainfall events than was apparent in the past.

In more rural areas the wildlife component of fecal coliform inputs is significant, as can be the human input. Wildlife, such as raccoons, muskrats and deer, living near the intertidal zone, can have dramatic local impacts on fecal coliform concentrations in the adjacent shellfish waters, with the attendant possibility of introducing human pathogens. New data indicates that wildfowl can have significant impacts on water quality too. Wildlife inputs of fecal material are basically accounted for by the seawater sampling data.

The Division is not seeing a steady increase in the number of acres of condemned waters in the state. Instead, what we see are fluctuations in the location of the border between acceptable and unacceptable water quality measurements moving up and down tributaries over time. Again, these fluctuations seem to be due largely to changing factors on the watershed, chance weather events (rain, high tides), changes in wildlife populations near shore or unknown factors (perhaps movement of livestock from one field to another, migratory bird flocks, or runoff from recently plowed fields that later contribute little when crops stabilize the soil).

Man does directly impact the fecal coliform counts in the waters. The headwaters of smaller streams are impacted by development due to the loss of the filtering and detention of runoff waters through upland swamps and other slow moving water areas. These natural detention areas provide the extended time element so that predators (*e.g.*, rotifers and ciliates) and sunlight can reduce the numbers of fecal coliforms and pathogenic human bacteria and viruses. When these are replaced with drainage systems the fecal coliforms and potentially present human pathogens are directly discharged into the shellfish waters.

EPA Shellfish Listing /Delisting Chart



Making §303(d) & §305(b) CWA Listing Decisions Based on National Shellfish Sanitation Program (NSSP) Growing Area Classifications

* 2007 NSSP Model Ordinance Subsection IV.@03: Growing Area Classification

APPENDIX D-1

Incorporating the *Proactive Approach* to delisting 303(d) listed segments into the 2014 Water Quality Assessment

For the 1998 assessment cycle, EPA changed the data analysis period for the 305(b) assessment from two to five years. Virginia's water quality assessments and the subsequent 303(d) list have since been based on a 5-year data window. In 2008 the assessment data window was expanded to six years to coincide with the two-year ambient watershed rotation monitoring schedule. The data window for 2014 is January 1, 2007 through December 31, 2012.

In August 2001, the Office of Water Quality Programs negotiated with EPA an approach, termed the *Proactive Approach*, which results in the proposed delisting of waters on the Section 303(d) list through assessment of less than six years of data. Correspondence and information related to the issue is attached to this memorandum. In short, EPA Region III has consented that Virginia can delist a segment on the 303(d) list if the following requirements are met:

1) For conventional parameters, no more than one of twelve samples taken over a two-year period exceeds the water quality criteria ($\geq$ 10.5 percent exceedence for larger data sets).
2) For biological impairment, a minimum of 2 consecutive samples, taken over a one to two year period, show attainment of the applicable standard.
3) The samples are taken at the same location (monitoring station) which demonstrated the impairment.
4) A rationale document is submitted to EPA justifying why the State believes the waters are achieving WQ Standards. This rationale document can consist of a description of measures taken in the watershed which are considered to be responsible for improvement of the water quality.

Eligibility and Water Quality Assessment

The following procedure is to be used to consider the eligibility of, and to subsequently assess, any particular waterbody segment submitted for consideration for delisting under the *Proactive Approach*.

Locations where proactive measures are being taken to improve water quality through the TMDL or Water Quality Management Plan program such that the *Proactive Approach* is eligible for consideration are to be provided by the DEQ TMDL program. Assessment staff can recommend segments for consideration, but only those locations provided by the DEQ TMDL program as candidates for the *Proactive Approach* are to be considered for assessment under the *Proactive Approach*. Notification must be made in writing through memorandum to the affected regional assessment manager, copied to the DEQ 305(b) coordinator, and must include the required documentation supporting consideration of the *Proactive Approach*. At a minimum, this is to include documentation of those implementation measures considered to be responsible for improvement in water quality and subsequent achievement of WQ Standards.

Regional assessment staff members are responsible for assessment of water quality in their respective regions and for the defense of their assessments. Therefore, the decision for delisting consideration is to be made by regional assessment staff based on the analysis of the proactive measures being taken, available monitoring data, any ancillary information collected, and their professional knowledge of site specific influences on water quality in the affected segment.

Where there is agreement between TMDL program and assessment staff that it is appropriate to pursue delisting based on implementation of the *Proactive Approach*, the assessment must be performed based on the requirements outlined in 1, 2 and 3 above. For a scheduled 305(b)/303(d) assessment, only the last two years of the assessment window are to be used for assessment of eligible segments. For delisting assessment at any other time, all years of the assessment window are to be used.

Assessment Documentation and Delisting Procedure

ADB Database	A segment meeting the above criteria is considered monitored, fully supporting. The assessment comments section should include the phrase *Proactive Approach Assessment*. The *Proactive Approach* data window used must be specifically identified.
Delisting Documentation	Documentation must include the information provided by the TMDL program related to control measures implemented using the *Proactive Approach* (requirement 4, above), and the results of data analysis related to requirements 1, 2, and 3 above.
EPA Review, Approval and Public Participation	Fulfillment of EPA review and approval requirements, and fulfillment of public participation requirements for removal of waterbody segments (delisting) at EPA required 303(d) list submittal dates, is the responsibility of the Monitoring and Assessments Program. At other times, fulfillment of these requirements in an effort to delist waters not needing TMDLs is the responsibility of the TMDL program. Final documentation for segments delisted by the TMDL program staff must be provided to the regional assessment manager and copied to the DEQ 305(b) coordinator at least five months prior to any EPA required 303(d) list submittal date, if time permits.

APPENDIX D-2

Requirements for Category 5R Waters

EPA specifically recommends that the 5R documentation describe the following six minimum elements:

a) *The identification of the point and nonpoint sources.* For point sources, an analysis should be included to document whether they are causing or contributing to the water quality impairments. If it is determined that the point sources are causing or contributing, then a Water Quality Based Effluent Limitation (WQBEL) or Best Management Practices Approach[1] should be developed and implemented through NPDES permits.

b) *The point source and nonpoint source water quality restoration activities that are expected to result in water quality improvements and restoration.* Where applicable, describe any authorities that may require water quality controls to be implemented (e.g., state or local regulations, permits, contracts and grant/funding agreements).

c) *Cost estimates and funding commitments to implement the water quality restoration activities.* In order to provide assurance that water quality restoration can occur through the implementation of water quality restoration activities, cost estimates and secured funding sources that will be used to implement these activities should be identified.

d) *An anticipated schedule for implementing the water quality restoration activities, including the anticipated completion date and the estimated pollutant load reductions necessary to meet water quality standards.* The schedule should outline specific activities and include a timeline of when each phase will be implemented and accomplished. The schedule can be revised and updated at each 303(d) listing cycle.

e) *A water quality monitoring component to evaluate and track the effectiveness of the scheduled water quality restoration activities at each 303(d) listing cycle.* Baseline water quality conditions should be established in order to accurately measure water quality progress. At each 2-year 303(d) listing cycle, performance measurements, whether environmental, programmatic, or social, should be provided for each implemented water quality restoration activity to measure progress. It is understood that each water restoration activity may not result in improved water quality; however the combined restoration activities should result in improved water quality at each 303(d) listing cycle.

f) *An anticipated date for achieving water quality standards.* Projects are expected to follow adaptive management allowing critical milestones to be adjusted as project plans and goals may change as implementation occurs. Once water quality standards have been met, the State may determine that the waterbody is appropriate to be included in category 1 or 2. If the project does not meet water quality standards by the estimated completion date, sufficient trends toward improved water quality must be shown in order to continue in the 5R program and an updated implementation schedule including revised critical milestones should be submitted to EPA. The project will continue to be reviewed every 2-year 303(d) listing cycle until water quality standards are met.

[1] EPA currently recommends point sources be addressed with WQBEL, but DEQ intends to explore how BMPs can also be effectively employed.

APPENDIX E-1

FISH TISSUE VALUES (TV)*		NON CARCINOGEN	CARCINOGEN
		CRITERION BASED TISSUE VALUE (TV)	CRITERION BASED TISSUE VALUE (TV)
COMPOUND	CAS #	PPB (wet–weight)	PPB (wet-weight)
Acenaphthene	83-32-9	240,000.00	
Acrolein	107-02-8	2,000.00	
Acrylonitrile	107-13-1		74
Aldrin	309-00-2		2.40
Anthracene	120-12-7	12,000,000	
Antimony	7440-36-0	1,600	
Benzene	71-43-2		2,700
Benzidine	92-87-5		0.17
Benzo(a)anthracene	56-55-3		5.50
Benzo(b)fluoranthene	205-99-2		5.50
Benzo(k)fluoranthene	207-08-9		5.50
Benzo(a)pyrene	50-32-8		5.50
Bis2-chloroethyl ether	111-44-4		36
Bis2- chloroisoproply ether	108-60-1	160,000	
Bis2- ethylhexyl Phthalate	117-81-7	2,900	
Bromoform	75-25-2		5,100
Butyl benzyl phthalate	85-68-7	800,000	
Carbon tetrachloride	56-23-5		310
Total Chlordane	57-74-9		110
Chlorobenzene	108-90-7	16,000	
Chlorodibromomethane	124-48-1		480
2-Chloronaphthalene	91-58-7	320,000	
Chloroform	67-66-3		40,000
2-Chlorophenol	95-57-8	20,000	
Chrysene	218-01-9		5.50
Cyanide	57-12-5	80,000	
DDD	72-54-8		170
DDE	72-55-9		120
Total DDT	50-29-3		120
Dibenz(a,h)anthracene	53-70-3		5.50
1,2-Dichlorobenzene	95-50-1	72,000	
1,3-Dichlorobenzene	541-73-1	54,000	
1,4-Dichlorobenzene	106-46-7	11,000	
3,3-Dichlorobenzidine	91-94-1		89
Dichlorobromomethane	75-27-4		650
1,2-Dichloroethane	107-06-2		440
1,1-Dichloroethylene	75-35-4	40,000	
1,2-Trans-dichloroethylene	156-60-5	16,000	
2,4-Dichlorophenol	120-83-2	12,000	
1,2-Dichloropropane	78-87-5		600

1,3-Dichloropropene	542-75-6	400	
Dieldrin	60-57-1		2.50
Diethyl phthalate	84-66-2	3,200,000	
2,4-Dimethylphenol	105-67-9	80,000	
Dimethyl Phyhlate	131-11-3	40,000,000	
Di-n-butyl phthalate	84-74-2	400,000	
2,4-Dinitrophenol	51-28-5	8,000	
2-Methyl-4,6-dinitrophenol	534-52-1	1,600	
2,4-Dinitrotoluene	121-14-2		130
Dioxin	1746-01-6		0.00026
1,2-Diphenylhydrazine	122-66-7		50
Endosulfan (I and II)	115-29-7	24,000	
Endosulfan sulphate	1031-79-8	24,000	
Endrin	72-20-8	240	
Endrin aldehyde	7421-93-4	1,200	
Ethylbenzene	100-41-4	80,000	
Fluoranthene	206-44-0	160,000	
Fluorene	86-73-7	160,000	
Heptachlor	76-44-8		8.90
Heptachlor epoxide	1024-57-3		4.40
Hexachlorobenzene	118-74-1		25
Hexachlorobutadiene	87-68-3		510
Hexachlorocyclohexane (alpha-BHC)	319-84-6		6.30
Hexachlorocyclohexane (beta -BHC)	319-85-7		22
Hexachlorocyclohexane (gamma-BHC) (lindane)	58-89-9		240
Hexachlorocyclopentadiene	77-47-4	4,800	
Hexachloroethane	67-72-1		2,900
Indeno(1,2,3-cd)pyrene	193-39-5		5.5
Isophrone	78-59-1		42,000
Mercury (Methyl) **	22967-92-6	300	
Methyl Bromide	74-83-9	5,600	
Methylene Chloride	75-09-2		5,300
Nickel	744-00-2	220,000	
Nitrobenzine	98-95-3	2,000	
N-nitrosodimethylamine	62-75-9		0.78
N-nitrosodiphenylamine	86-30-6		8,200
N-nitrosodi-n-propylamine	621-64-7		5.70
PCB Total/congeners	1336-36-3		20
Pentachlorophenol	87-86-5		330
Phenol	108-95-2	1,200,000	
Pyrene	129-00-0	120,000	
Selenium	7782-49-2	20,000	
1,1,2,2-Terachloroethane	79-34-5		200
Tetracholoethylene	127-18-4		1,000
Thalium	7440-28-0	54	
Toluene	108-88-3	64,000	
Toxaphene	8001-35-2		36
1,2,4-Trichlorobenzene	120-82-1	8,000	
1,1,2-Trichloroethane	79-00-5		700

Trichloroethylene	79-01-6		3,200
2,4,6-Trichlorophenol	88-06-2		3,600
Vinyl Chloride	75-01-4		29
Zinc	7440-66-6		1,200,000

*These fish tissue values have been calculated based on the Water Quality Standards that are associated with the latest Triennial Review criteria proposals as adopted by the State Water Control Board in October 2008.

**The fish tissue criterion for methylmercury applies to fish species commonly eaten in the local waterbody and applies to most fish species in the DEQ database except bowfin or longnose gar because fish consumption surveys show that these species are rarely consumed in Virginia. Total mercury concentrations in fish tissue are assumed to equal methylmercury concentrations.

APPENDIX E-2

RISK-BASED TISSUE SCREENING VALUE (TSVs) FOR FISH TISSUE UPDATED FROM INTEGRATED RISK INFORMATION SYSTEM (IRIS) FOR GENERAL POPULATION (ADULT)

BODY WEIGHT (KG) 70
RISK LEVEL 10^{-5}
CONSUMPTION RATE (KG/DAY) 0.0175

Fish Tissue Screening Values (TSV)		**NON CARCINOGEN**	**CARCINOGEN**
		TISSUE SCREENING VALUE (TSV)	**TISSUE SCREENING VALUE (TSV)**
COMPOUND	CAS #	PPB (wet-weight)	PPB (wet-weight)
Arsenic (inorganic)	7440-38-2		270*
Barium	7440-39-3	800,000	
BHC isomers	608-93-1		0
Brominated Diphenyl ethers (BDEs)			5000 (VDH)**
Cadmium	7440-43-9	4,000	
Decabromdiphenyl ether	1163-19-5		28,000
Hexabromodiphenyl ether	36483-60-0		800
Pentabromodiphenyl ether	32534-81-9		8,000
Chromium III	16065-83-1	6,000,000	
Chromium VI	18540-29-9	12,000	
Chlorpyrifos	2921-88-2	12,000	
Diazinon	333-41-5	3600	
Disulfoton	298-04-4	160	
Ethion	563-12-2	2,000	
Kepone	143-50-0		300 (VDH)**
Methoxychlor	72-43-5	20,000	
Mirex	2385-85-5	8,000	
Oxyfluorfen	42874-03-3	12,000	
PAHs (sum PEC) ***			15
Terbufos	13071-79-9	100	
Tributyltin	56-35-9	1,200	

*The screening value for arsenic applies to inorganic arsenic only. Organic forms of arsenic are not carcinogenic and are relatively nontoxic. There is a general consensus that 85 to 90% of arsenic found in fish tissue is organic arsenic. The screening value of 270 ug/kg total arsenic is based on the estimate that 10% of total arsenic detected in fish tissue is inorganic arsenic.

** These values are based on recent changes to the toxicological data used to calculate the screening values, or recent recommendations from U.S. EPA or the Virginia Department of Health. These screening values are not based on the same toxicological data that were used to develop the existing water quality criteria.

*** Mixtures of seven polynuclear aromatic hydrocarbons (PAHs) that are classed as probable human carcinogens were assessed based on a screening value concentration of 15 ppb calculated as a sum potency equivalency concentration (PEC) using methods described in EPA's Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Vol. 1, (EPA 823-R-95-007) and Vol. 2 (EPA 823 B-00-008) using the following equation;

$$PEC = \sum_{i} (RPi \times Ci)$$

where; RPi = relative potency for the ith PAH
Ci = concentration of the ith PAH in fish tissue)

The relative potency estimates used for these PAHs were:

Benzo(a)pyrene	1.0
Benzo(a)anthracene	0.145
Benzo(b) fluoranthene	0.167
Benzo(k)fluoranthene	0.020
Chrysene	0.0044
Dibenz(a,h)anthracene	1.11
Indeno(1,2,3-cd)pyrene	0.055

APPENDIX F

Freshwater Consensus- Based Sediment Screening Values (SVs)

Analyte (Metals)	**Consensus PEC (ppm) dry weight**
Arsenic	33
Cadmium	4.98
Chromium	111
Copper	149
Lead	128
Mercury	1.06
Nickel	48.6
Silver	NA
Zinc	459
Analyte (Organics/Pesticides)	**Consensus PEC (ppb) dry weight**
Acenaphthene	NA
Acenaphthylene	NA
Anthracene	845
Benzo-a-pyrene	1,450
Benz(a)Anthracene	1,050
Chrysene	1,290
Dibenz[a,h]Anthracene	NA
Fluoranthene	2230
Fluorene	536
Methylnaphthalene, 2-	NA
Naphthalene	561
Phenanthrene	1,170
Pyrene	1,520
LMW PAHs	NA
HMW PAHs	NA
Total PAHs ** (see footnote)	22,800
Chlordane	17.6
DDD	28
DDE	31.3
DDT	62.9
DDT, total	572
Dieldrin	61.8
Total PCBs	676
Endrin	207
Heptachlor Epoxide	16
Lindane	4.99
NA = Not Available	

Estuarine NOAA-based ER-M Sediment Screening Values (SVs)

Trace Elements (Metals)	ER-M Value ppm (dry weight)
Antimony (Sb)	NA
Arsenic (As)	70
Beryllium	NA
Cadmium (Cd)	9.6
Chromium (Cr)	370
Copper (Cu)	270
Lead (Pb)	218
Manganese (Mn)	NA
Mercury (Hg)	0.71
Nickel (Ni)	51.6
Selenium (Se)	NA
Silver (Ag)	3.7
Thallium	NA
Zinc (Zn)	410

Pesticides and Other Organic Substances –parts per billion dry weight

CAS #	Substance	ER-M Value(dry weight) (ppb)	
336363	Polychlorinated Biphenyls (PCBs)	180	
309002	Aldrin	NA	
57749	Chlordane	6	
NA	total DDT (include metabolites)	46.1	
72548	DDD	20	
50293	DDT	7	
72559	DDE	27	
60571	Dieldrin (EPA proposed criteria)	8	
72208	Endrin	NA	
76448	Heptachlor	NA	
1024573	Heptachlor epoxide	NA	
118741	Hexachlorobenzene	NA	
608731	Hexachlorocyclohexane	NA	
58899	Lindane	NA	
2385855	Mirex	NA	
108952	Phenol	NA	
117817	Di (2-Ethylhexyl) Phthalate	NA	
84742	N-Butyl Phthalate	NA	
83329	Acenapthene	500	LMW P AH
208968	Acenapthylene	640	LMW PAH
120127	Anthracene	1100	LMW PAH
50328	Benzo-A-Pyrene	1600	HMW PAH
191242	Benzo [GHI] Perylene	NA	HMW PAH
56553	Benz[A] Anthracene	1600	HMW PAH
218019	Chrysene	2800	HMW PAH
53703	Dibenz [A,H] Anthracene	260	HMW PAH
206440	Fluoranthene	5100	HMW PAH
86737	Fluorene	540	LMW PAH
193395	Indeno (1,2,3-CD)Pyrene	NA	HMW PAH

91576	Methylnaphthalene, 2	670	LMW PAH
91203	Naphthalene	2100	LMW PAH
85018	Phenanthrene	1500	LMW PAH
129000	Pyrene	2600	HMW PAH
NA	Low Molecular Weight (LMW)PAHs		3160
NA	High Molecular Weight (HMW) PAHs		600
NA	Total PAHs **(see footnote)		44,792

*Changes or updates to any of the ER-M or PEC screening values should be updated in the assessment spreadsheet used to calculate the estuarine weight of evidence.
**sum of 24 Polyaromatic hydrocarbons used in previous reports, also polynuclear aromatic hydrocarbons (PNAs)

DEQ acknowledges the use of the ER-M or PEC may be limited (for several reasons) in their ability to accurately predict biological effects. Given that DEQ continues to employ the collection of bulk sediment with chemical analysis as a cost-effective way to monitor a great number of sediment sites, these thresholds are an appropriate tool for assessing sediment data relative to its potential harm to aquatic life.

Citation:

Freshwater PECs: MacDonald, D.D., C.G. Ingersoll, T.A. Berger. 2000. Development and Evaluation of Consensus-Based Sediment Quality Guidelines for Freshwater Ecosystems. *Arch. Environ. Contam. Toxicol.* 39:20-31.

Estuarine ER-Ms: Buchanan, M.F. 1999 National Oceanic and Atmospheric Administration *Screening Quick Reference Tables,* NOAA HAZMAT Report 99-1 Seattle, WA, Hazardous Materials Response and Assessment Division, 12 pages.

APPENDIX G

Weight-of-Evidence Aquatic Life Use Assessment in Estuarine Waters

The "Weight-of-Evidence" (WOE) approach that DEQ currently uses for its general evaluation and assessment of the designated Aquatic Life Use (ALU) for estuarine benthic communities has evolved from a previously more limited application of the "Sediment Quality Triad" concept (SQT – Figure 1). The SQT concept was originally conceived and applied for the evaluation of the presence and effects of toxic contaminants in marine sediments (Long and Chapman, 1985). It was further applied by Chapman et al. (1986, 1987), and has continued to be one of the preferred approaches for the evaluation of toxics in marine and estuarine benthic environments (Chapman, 1992; Chapman et al., 1997; McGee et al., 2001). The Interstate Chesapeake Bay Program (CBP) employed SQT evaluations along with other methods to produce a Bay-wide toxics characterization in 1999 (US EPA, 1999) that identified (1) "Regions of Concern – areas with probable adverse effects," (2) "Areas of Emphasis – areas with potential adverse effects," (3) "Areas with Low Probability for Adverse Effects", and (4) "Areas with Insufficient or Inconclusive Data" relative to toxics contamination in Bay waters. Maps of more recent characterizations (2006, 2008, 2009, and 2010) can be found at: http://www.chesapeakebay.net/maps.aspx?menuitem=15230.

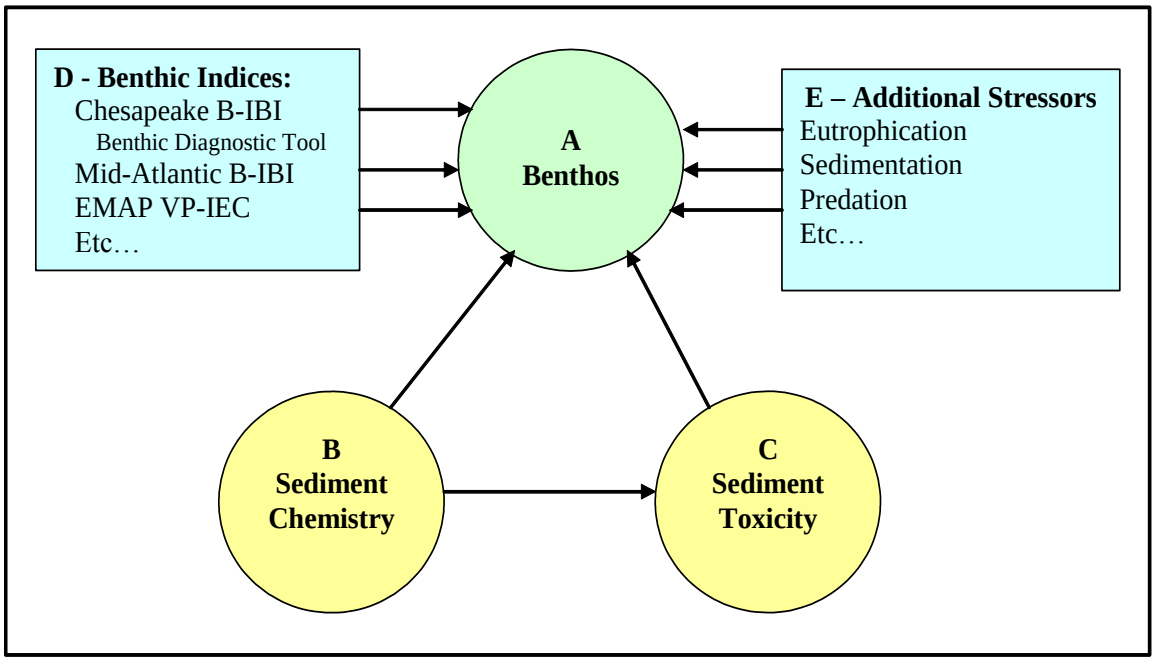


Figure 1 - The Sediment Quality Triad (SQT = triangle A.B.C.) as originally conceived for the identification and characterization of potential toxics-induced stressors. The apex of the triangle, Circle A or "Benthos," represents the condition of the benthic community, which is the primary objective of the "Aquatic Life Use" assessment, while B - "Sediment Chemistry" and C - "Sediment Toxicity" provide two lines of evidence for the evaluation of possible causes of stress due to toxic contamination. Tools for the evaluation of benthic condition (D – "Benthic Indices") and "Additional Stressors" (E), as well as Sediment Chemistry (B), and Sediment Toxicity (C), are discussed in the text.

Subsequent to the 1999 characterization, DEQ, in conjunction with researchers from the Virginia Institute of Marine Science (VIMS), used the SQT for the characterization of those Virginia Bay waters that had been identified as Class 4 (Insufficient or Inconclusive Data) in the previous CBP study, namely the tidal fresh regions of the James River, the tidal York River drainage, and Mobjack Bay (Roberts et al., 2002a, 2002b,

2003). A new report on toxics throughout the Chesapeake Basin was published in December 2012 (EPA, *et al*., 2012).

The original objective of such ambient toxics monitoring was primarily to perform a quick screening of the medium of interest (water, sediment, fish tissue, etc.) to determine whether toxic pollutants were present and could potentially have a negative impact on aquatic life or human health. In addition to the evaluation of potential *causes* of impact (based on Sediment Chemistry – element B), potential toxic *effects* on the biota (including individual survival, growth and/or reproduction) could be evaluated based on the results of toxicity tests (Sediment Toxicity – element C), most commonly conducted in the laboratory but at times carried out with test species maintained *in situ* (*e.g.*, Roberts et al., 2002a, 2002b, 2003). The general welfare of the benthic community in the field (Benthos – element A) was evaluated as a manifestation of elements B and C (if they were positive), i.e. actual *observed effects* of sediment contamination. Although the potential effects of other stressors were acknowledged, they did not play a significant role in the earlier SQT evaluations.

The Virginia DEQ began to apply a modified, more formal Weight-of-Evidence assessment procedure employing the Sediment Quality Triad in its 2006 Integrated 305(b)/303(d) Water Quality Assessment Report. DEQ's assessment procedure, however, goes beyond the original SQT toxics-related evaluations and includes tools for the tentative evaluation of some of the additional potential stressors (E – "Additional Stressors" of Figure 1) effecting estuarine benthic communities. WOE assessment is carried out on data collected within DEQ's Estuarine Probabilistic Monitoring Program (ProbMon) and, periodically, the National Coastal Condition Assessment (NCCA) surveys which sample the coastal Delmarva region, the Back Bay / North Landing River region, and the tidal tributaries, embayments, and mainstem of the Chesapeake Bay drainage. Because all three elements of the SQT are collected and water quality and additional sediment analyses are carried out simultaneously, the WOE procedure is able to provide an <u>integrated assessment for individual sampling sites.</u> General guidance for the delineation of the area represented by each site is provided in Section 6.2.1, "Monitoring Station Delineation", Rule 4, of this Assessment Guidance Manual. It should be pointed out here that, within the tidal portions of the Chesapeake Bay basin, the weight-of-evidence assessment discussed in this section complements the probabilistic benthic assessments carried out by Virginia's Chesapeake Bay Program (CBP). The probabilistic benthic monitoring carried out by the CBP collects benthic samples and a few measures of bottom conditions at each site (sediment type and TOC content, salinity, dissolved oxygen, etc), but does not carry out chemical analyses or toxicity tests of sediment. Consequently, the results of CBP benthic characterizations are spatially integrated and assessments are performed only on pre-designated Bay segments that have a sufficiently large sample size ($N \geq 10$).

The SQT is an effects-based approach that describes the condition of the sediment and associated benthic infaunal communities relative to toxic pollutants and their effects. The three main data components that were integrated into the original "weight-of-evidence" (WOE) SQT analysis included: (1) sediment bulk chemical concentrations, (2) sediment toxicity test results, and (3) an evaluation of benthic infaunal community condition. Rather than considering each type of characterization individually, the complementary methods integrate biological responses with chemical data (Chapman, 1992) for a more scientifically defensible assessment process. Chapman (1992) provided eight possible scenarios from which conclusions could be drawn with the SQT approach. It was this concept that first served as the foundation required to implement the "weight of evidence" assessment of triad data as summarized in the evaluation matrix of Figure 2, below.

The objective of this guidance is to provide orientation for interpreting data generated by the traditional SQT approach, as complemented by additional lines of evidence, with added insight on how to consider "weighting" of each component. This is not to suggest that sound scientific interpretation and best professional judgment are unnecessary, but does provide some degree of standardization for the process. Conceptually, this is similar to the approach used by the Chesapeake Bay Program and its partners for its Toxics Characterization of the Bay

(EPA 903-R-00-010, June 1999). The use of this guidance will provide assistance in applying "weights" to the different triad components, which are then inserted into the classic SQT matrix. The bulk chemistry results, for example, can receive additional weighting based on the magnitude of exceedence of the applicable Sediment Quality Guideline (SQG). For toxicity tests, greater weight is applied with two or more statistically significant sediment toxicity tests than is applied to a single significant test, for the effected endpoint. The type of toxicity test endpoints that exhibit statistical significance must also be given consideration, since the acute ecological consequence of not surviving would be greater on the population of a species than the rate of growth of individuals. Test results based on survival/mortality would therefore receive a greater weight.

All of the data contributing to each line of evidence, the intermediate results, the tentative conclusions, and the final integrated WOE assessment are aggregated into a single "Weight of Evidence Assessment (Excel ®) Workbook" for each site. An example of the basic template used for the Weight-of-Evidence Assessment Workbooks is provided here for reference. An example of a completed workbook will be provided later in this section.

	Scenario	Chemistry	Toxicity	Benthic Community Alteration	Total Score (Sum)	Tentative/Possible Conclusions	Listing Category (Weight dependent)
Observed Scores	>>>	-	-	-	0	???	???
Hypothetical Scenarios	1	Score > 0	Score > 0	Score > 0	3-9	If "3" in all three categories, strong evidence for chemical contaminant-induced degradation. (Benthic "Diagnostic Tool" results?)	VA Category 5A (Cause = Toxics) or 3B
	2	0	0	0	0	Strong evidence for absence of chemical contaminant-induced degradation.	VA Category 2A
	3	Score > 0	0	0	1-3	Chemical contaminants are not bioavailable.	VA Category 2A (or 2B)
	4	0	Score > 0	0	1-3	Unmeasured chemical contaminants or conditions may exist that have the potential to cause degradation.	VA Category 2A, 2B (or 3B)
	5	0	0	Score > 0	1-3	Alteration is probably not due to chemical contaminants. Bay waters - moderate to severe benthic degradation => 3B (Benthic "Diagnostic Tool" results?); Elsewhere, severe benthic degradation =>5A when corroborated by two or more indices.	VA Category 3B or 5A (Cause = Water Quality)
	6	Score > 0	Score > 0	0	2-6	Chemical contaminants are likely stressing the system.	VA Category 3B (or 2B)
	7	0	Score > 0	Score > 0	2-6	Unmeasured chemical contaminants are causing degradation. Slight or moderate benthic degradation => 3B (or 2B); severe benthic degradation => 5A. (Benthic "Diagnostic Tool" results?)	VA Category 3B (or 2B); or 5A
	8	Score > 0	0	Score > 0	2-6	Chemical contaminants are not bioavailable or benthic alteration is not due to chemical contaminants. (Benthic "Diagnostic Tool" results?)	VA Category 3B or 5A (Cause = Water Quality)

Figure 2 - The SQT Evaluation Matrix summarizing the eight scenarios originally described by Chapman (1992). Refer to the "SQT Evaluation Matrix" Tab of the Weight-of-Evidence Assessment Workbook. This matrix has been adapted from the original in order to incorporate additional lines of evidence and to provide numerical scores for the three classes of characterization: Chemistry, Toxicity and Benthic Community Alteration. The penultimate column summarizes Chapman's descriptions of the eight possible scenarios with the addition of some comments on possible assessment classifications, and the last column lists the specific listing categories that may be assigned for Virginia's 305(b)/303(d) Integrated Water Quality Assessment Report. Further discussion of the matrix is provided in the text sections below related to the Microsoft Excel® "Weight-of-Evidence Assessment Workbook."

The complete Weight-of-Evidence Workbook consists of 12 individual worksheets that fully document the location of the monitoring site, the complete analytical results of sediment chemical analyses, of sediment toxicity tests, and of benthic identifications and enumerations, the interpretation of those results, and the integration of all lines of evidence into a final assessment:

Page No.	Title
1.	Orientation - Read Me First
2.	Summary Sheet
3.	SQT Evaluation Matrix
4.	(1) Sediment Chemistry
5.	(2) Interstitial Dissolved PAH Evaluations
6.	(3) Sediment Toxicity
7.	(4) Benthic Infauna
8.	SedChem Data
9.	Chemistry QA Codes
10.	SedTox Data
11.	Benthic Data
12.	Site Map

In keeping with DEQ's continual planning process, the WOE assessment procedure and the format of the Weight-of-Evidence Assessment workbook continue to evolve as additional lines of evidence and new assessment tools become available. The following discussions will describe the various lines of evidence considered and summarize the characterization procedures as currently employed (April 2013). More details and explicit instructions relative to each step are included in the Weight-of-Evidence Workbook.

Workbook Summary Sheet - Final Assessment and Comments:
Descriptive information identifying the specific sampling site is entered into this workbook Tab prior to adding additional results of field measurements and laboratory analyses here or elsewhere in the file. As on other tabs throughout the workbook, information should be typed or pasted into fields that are highlighted in pastel green. Fields that are highlighted in pale yellow should not be altered in any way. Yellow fields are populated automatically by embedded calculations or direct transfers from other fields within the workbook. Entering observations or comments in undesignated fields should be avoided, since many fields contain hidden reference values for populating other cells of the workbook.

Benthic Community Characterization: Workbook Tab 4

Because the WOE assessment is applied strictly for the designated "Aquatic Life Use" (ALU), evaluation of the structure and function of the benthic community is the ultimate, most heavily weighted indicator for site characterizations. The condition of the benthic community constitutes an integrated ***observed effect*** of the existing environmental stressors, whether the individual stressors are identified or not. If the benthic community is found to be severely degraded, a site may be assessed as "Impaired" for ALU even if evidence for a specific cause is lacking. Additional, conformational sampling would be required, however, prior to initiating TMDL development. In the opposing case, if the benthic community was found to be in good condition ("non-degraded" or "meeting goals"), a classification of "(5A) Impaired" would be unlikely unless chemical and/or toxicity results were exceptionally extreme.

The general objective of the weight-of-evidence (WOE) assessment methodology is to integrate multiple lines of evidence, based primarily on sediment analyses, to provide a standardized, objective evaluation of the severity and probable cause(s) of benthic degradation. Individual benthic index scores are subject to sampling error, which results from the great heterogeneity of biological communities as well as from methodological variations in sample collection, handling and analysis. When additional lines of evidence such as significant chemical contamination and/or significant acute or chronic toxic effects corroborate low benthic IBI scores, they serve to identify probable causes of degradation, and consequently to confirm the validity of low benthic scores and to justify an assessment classification of "Impaired" (5A). When low benthic scores are not corroborated by integrative chemical or toxicological measures, additional lines of evidence (e.g., low DO, high nutrient concentrations, evidence of sedimentation, or other habitat characterizations) may contribute to their interpretation. These alternative lines of evidence are of limited value, however, in the case of single-visit probabilistic sampling.

Within tidal Chesapeake Bay waters the natural variability of benthic communities, both within and among habitat types, is recognized and included in a formal statistically-based procedure which integrates multiple ($N \geq 10$) CBP probabilistic benthic IBI scores for ALU assessment of pre-defined tidal water segments. The benthic IBI scores from the Estuarine Probabilistic Monitoring Program (ProbMon) are included in this procedure. Consequently, within Bay waters and in the absence of corroborating evidence from chemical or toxicological measures, benthic IBI scores from this ProbMon program are integrated into the CBP assessment and are not further evaluated using the WOE approach. In non-Bay tidal waters, however, where no other benthic community evaluation is carried out, the WOE assessment places greater weight on alternative lines of evidence from the probabilistic sample, including the degree of consensus among the three benthic indices generally calculated. In coastal Delmarva waters and in the Albemarle Sound drainage (Back Bay, North Landing River), greatest weight is given to the Middle Atlantic Benthic IBI. In these waters, if the CBP Benthic IBI and/or the EMAP Virginia Province Index of Estuarine Condition corroborate evidence of severe benthic degradation, an assessment of impaired ALU may result even in the absence of supporting chemical or toxicological evidence.

The number of different benthic taxa present in a standardized sample[2], their relative abundances, and knowledge of their specific ecological/functional roles provide the information for calculating numerous measures or metrics of community structure and function. Several of these metrics are used individually for a preliminary, general characterization of the benthic community while many of them are subsequently integrated into various, more objective multi-metric indices of biological integrity (Benthic IBIs) or of estuarine condition (IEC). Raw taxonomic data and intermediate results are provided on the "Benthic Data" Tab of the WOE Workbook, while the integrated evaluation is summarized on Tab (4) – Benthic Infauna.

The values of seven individual metrics derived from the taxonomic results provide an initial qualitative evaluation of benthic condition during the WOE process. The first five are measures of taxonomic abundance and diversity, while the last two are the abundances of two stress-tolerant taxa that are also used in calculating an IEC, to be discussed below.

[2] The standardized benthic sample for the Estuarine Probabilistic Monitoring Program consists of a composite of two separate sediment grabs using a 6-inch Petite Ponar sediment sampler, … continued on following page … representing a total bottom area of approximately 0.046 m^2. The contents of each grab must conform to quality assurance criteria specified in the National Coastal Condition Assessment program QAPP (U.S. EPA., 2009b) and Field Operations Manual (U.S. EPA., 2009c) and must include at least 7.0 cm of sediment. The B-IBI results with this sampling protocol have been shown not to differ significantly from standardized samples collected with a single 8" Young sampler (Dauer and Lane, 2005).

1. Total Abundance - The total number of individuals in a benthic sample; generally symbolized as "N".
2. Total Taxa - The total number of taxa that are identified from a sample. Depending upon the group of organisms, an individual taxon may represent a species, a genus, a family, or a higher level of identification. Usually symbolized as "S" for number of species, but "S" is maintained here as the number of taxa.
3. Shannon H' - The Shannon-Weiner Species Diversity Index: $H' = -\Sigma_{i = 1, S} (p_i \ln p_i)$. This index is calculated here as was originally expressed, using $\log_2$. Elsewhere, however, it is often calculated using natural logs (ln) or occasionally using decimal logs ($\log_{10}$). The use of "S" as defined here would make H' an index of taxonomic diversity rather than of species diversity.
4. Gleason-D - Gleason's Diversity Index: $D = S / \ln N$
5. Pielou-J' - Pielou's Index of Equitability (or Evenness): $J' = H' / H'_{max}$, where H'_{max} is the theoretical maximum diversity with "N" individuals divided among "S" taxa. The value of J' can vary from 0.0 to 1.0; both H' and H'_{max} must be calculated to the same logarithmic base; in this case $H'_{max} = \log_2 (S)$
6. Tubificidae - The numeric abundance of the family Tubificidae (Annelida, Oligochaete), a stress-tolerant taxon.
7. Spionidae - The numeric abundance of the family Spionidae (Annelida, Polychaete), another stress-tolerant taxon.

Although the abundance of individuals and the diversity of taxa vary among habitat types - muddy vs. sandy sediment, in combination with salinity regime - within a specific habitat type higher values of metrics 1 through 5 are generally indicative of more healthy, less degraded benthic communities. A high abundance (metric 1) with few taxa (metric 2) may indicate a degraded benthic community, especially if the abundant taxa are stress-tolerant as are those of metrics 6 and 7. This would result in relatively low values for metrics 3, 4 and 5. An excessive abundance of tubificids (6) and/or spionids (7) generally indicates a highly stressed and probably degraded benthic community.

Benthic community alteration is also evaluated by integrating various individual metric scores into a single Benthic Index of Biotic Integrity (B-IBI) based on previously established and verified thresholds (e.g., Chesapeake Bay B-IBI; Weisberg et al., 1997), and then comparing the overall site B-IBI score with the defined ranges characteristic of specific habitat types (e.g., Llansó et al., 2003 for the Chesapeake Bay). Weighting the overall benthic community condition with this CBP B-IBI is straight-forward, since there are four previously established categories ranging from good to severely degraded (Please refer to **Table 1** below). It is the preferred and most appropriate index for use within the Chesapeake Bay drainage. An alternate B-IBI, developed for estuaries of the Middle Atlantic Region (Llansó et al., 2002a, 2002b), is used for assessment in the Atlantic coastal estuarine waters of the Delmarva Peninsula. Index values < 3.0 for this B-IBI are considered to be indicative of stressed benthic assemblages and degraded conditions (SQT Matrix Score = 3 or 2), while scores ≥ 3.0 indicate that benthic goals are met (Matrix Score = 0).

Table 1 - Chesapeake Bay B-IBI Ranges and Benthic Community Condition

CBP B-IBI Score	Benthic Community Condition	SQT Matrix Score
≥ 3.0	Meets Goal	0
2.7-2.9	Marginal	1
2.1-2.6	Degraded	2
≤ 2.0	Severely Degraded	3

When one or more measurements essential for the calculation of either of these B-IBIs is lacking, or if they are considered geographically inappropriate, a third alternative is available. Paul et al. (2001) developed a benthic-based "Index of Estuarine Condition" (VP-IEC) for the Virginian Biogeographic Province (from Cape Cod to the mouth of Chesapeake Bay), based on the 1990-1993 results of EPA's Middle Atlantic Integrated Assessment (MAIA) Program. This index is given minimum weight when either of the B-IBIs is available and more appropriate, but is more heavily weighted when neither of the B-IBIs is available. In the original publication of the VP-IEC, calculated as a linear discriminant function, final values greater than zero (> 0.000) were interpreted as an indication of non-degraded conditions and values less than zero (< 0.000) were interpreted as an indication of degraded sites. No indeterminate "gray zone" was specified. For the purpose of weight-of-evidence assessment, discriminant scores of this index between -0.1 and +0.1 are considered "marginal." Although no systematic salinity-induced bias has been demonstrated for any of these indices, it should be noted that all three are notably less reliable in low-salinity habitats, *i.e.*, oligohaline and tidal fresh waters (salinity < 5.0 ppt). Approximately 20% of Virginia's estuarine probabilistic sites sampled between 2001 and 2012 were within this salinity range.

Back Bay and the North Landing River, in southeastern coastal Virginia, fall within the Carolinian Biogeographic Province. They constitute a unique tidal freshwater/oligohaline region that is so isolated from Albemarle Sound and the Atlantic Ocean that none of the previously described benthic indices may be completely appropriate. Most of the benthos in this region is more characteristic of freshwater than of tidal estuarine waters. For the purpose of assessment in this region, all three benthic indices are compared and a tentative characterization is based on the relative degree of concordance among them and other individual metrics of species abundance, taxonomic richness, and diversity.

Since the summer of 2005, the separation, taxonomic identification and enumeration of all benthic samples collected within the Estuarine ProbMon Program has been carried out at the Benthic Ecology Laboratory (BEL) at Old Dominion University (ODU) under the auspices of Dr. Dan Dauer. Dr. Dauer is the principal investigator responsible for Virginia's portion of the Chesapeake Bay Program's Probabilistic Benthic Monitoring Program. In addition to providing a complete list of all benthic taxa and their abundances (in terms of numbers of individuals and biomass), Dr. Mike Lane (ODU, BEL) uses the BEL database to calculate all of the individual metrics required and the final score for each of the benthic and estuarine indices discussed above. In practice, all three benthic indices are calculated and evaluated for all benthic samples. The greatest weight is given to the results of the most appropriate index, but the degree of concordance (or disagreement) among the three is also considered for the final characterization.

Tab (4) "Benthic Infauna" of the WOE Assessment Workbook summarizes the integrated scoring and weighting for the three benthic and condition indices, along with a number of associated habitat and sediment characteristics that contribute to the final characterization of the benthic community. Several of these complementary characteristics (*e.g.*, bottom DO, bottom temperature, sediment TOC, and habitat type – salinity regime and mud or sand substrate) are helpful in identifying potential causes of any observed benthic degradation. Comments and the final Matrix Score recorded on Tab (4) are subsequently transferred to the SQT Evaluation Matrix Tab for integration into the final assessment.

Under special conditions (*i.e.*, when the CBP B-IBI indicates significant degradation of the benthic community at sites within the Chesapeake Bay drainage) an additional "Benthic Diagnostic Tool", developed for the Chesapeake Bay Program by Dr. Dan Dauer et al. (2002), may be utilized to tentatively identify the potential cause(s). These analyses are carried out by the CBP Program on a biennial basis for the Integrated 305(b)/303(d) Water Quality Assessment Report. The procedure is described in more detail elsewhere in this

Assessment Guidance Manual. When the results become available they are added to Tab (4) – "Benthic Infauna" of the WOE Assessment Workbook as one additional line of evidence for determining probable causes of benthic degradation. The *a posteriori* inclusion of the diagnostic tool results does not influence the final WOE assessment classification in any way.

Sediment Characterization: Workbook Tabs (1), (2), and (3)

Sediment Chemistry: Workbook Tab (1)

At the present time, EPA has not yet established specific criteria for toxic contaminants in sediment, and Virginia has not established sediment quality standards against which to assess sediment contamination. Consequently, a site is rarely assessed as impaired based on sediment chemistry alone. However, numerous empirical studies carried out over the past 15 to 20 years have provided "Sediment Quality Guidelines" (SQGs) or "Screening Values" (SVs) that serve to tentatively identify the range of concentrations of specific contaminants or classes of contaminants that are likely to cause adverse effects in benthic communities. Virginia currently employs two sets of screening values to characterize sediments: consensus-based Probable Effects Concentrations (PECs - MacDonald et al., 2000) for freshwater sediments and Effects Range Median (ER-M - Long et al., 1995) concentrations for estuarine and marine sediments. **APPENDIX F** of this Assessment Guidance Manual lists the "Consensus Based and ERM Sediment Screening Values" currently applied in Virginia. They also appear on Tab (1) - "Sediment Chemistry" of the WOE Assessment Workbook, where they are compared to observed sediment contaminant concentrations. Virginia's Water Quality Standards ("WQS" - 9 VAC 25-260) provide guidelines for the application of these screening values and indicate that in transitional Class II (oligohaline) waters the "more stringent of either the freshwater or saltwater criteria apply." Section 9 VAC 25-260-140, Subsection C, of the WQS defines specific, fixed zones of transitional Class II waters for Virginia's major tidal tributaries (Potomac, Rappahannock, York, and James Rivers) and Back Bay. (Transitional or oligohaline waters vary in salinity from 0.5 ppt. to 5.0 ppt.) Fixed transition zones within the Chesapeake Bay drainage correspond with pre-established Chesapeake Bay Program assessment segments. However, at any specific estuarine site the salinity, the sediment chemistry, and the resultant toxicity of contaminants vary temporally. As a consequence, the bottom salinity observed at the time of sampling is used to define habitat classes for benthic IBI evaluations and for the selection of PEC *vs.* ER-M screening values for WOE assessment. To assure maximum protection of the aquatic life community during WOE assessment, the concept of applying the "more stringent of either the freshwater or saltwater criteria" is also extended to include tidal fresh waters. An exceedence of these screening values raises a red flag of warning, but does not in itself result in an "Impaired" assessment. The final assessment classification - impaired, observed *potential* effects, or fully supporting of ALU, ultimately depends upon the *observed effects* on the benthic community and not upon *potential causes* identified with the use of screening values.

When the appropriate SVs are exceeded for one or more contaminants, and no ancillary biological data are available to corroborate significant degradation, the site is still considered fully supporting but having *observed (potential) effects* status for aquatic life use support (Virginia Assessment Category 3B). In such cases, additional biological monitoring should be scheduled to assess actual aquatic life use support. In practice, for WOE assessment, each SV is evaluated based upon its Sediment Quality Guideline Quotient (SQGQ, sometimes abbreviated as "Q"), which is calculated as the ratio between the observed concentration in the sediment and the screening value: SQGQ = observed concentration / SV. A ratio of 1.0 or greater indicates that the screening value was exceeded. A ratio of 2.0 indicates that the observed concentration was twice the screening value, etc. In the WOE assessment, the magnitude of each exceedence, abbreviated as "Q", is considered and weighted in scoring the degree of chemical contamination. A summary of the SQT Matrix Scoring Guidelines for sediment contamination can be found at the top of Tab (1) - "Sediment Chemistry" of the WOE Assessment Workbook.

The use of such screening values for assessment suffers several limitations. First, although they are available for most of the trace toxics metals, they are only available for a very limited number of organic contaminants. Secondly, each screening value reflects the potential effects of a single contaminant and does not consider possible interactions with other contaminants in the same sediment matrix. The often significant effects of additivity, antagonism, and synergism are not considered. A number of efforts have been made in recent years to integrate SQG quotients across multiple contaminants (see Long et al., 2006 for a critical review). The most successful and commonly applied integrated measure is the mean SQG quotient (mSQGQ). In a study of southeastern estuaries Hyland et al. (1999), applying the methods of Long et al. (1998), demonstrated that sites with mean SQG quotients as low as 0.1 had relatively high probabilities of significant degradation of their benthic communities. Applying the mean ER-M quotient of eight trace metals (excluding Ni), 13 PAHs (excluding total PAHs), total PCBs, plus 4,4'-DDE and total DDT, they found that when the mean ER-M quotient exceeded 0.1, the probability of adverse effects on the benthic community was ≥ 0.75. Similar results were observed when using mean quotients for another set of sediment quality guidelines, the Probable Effects Concentration (PEC). For the purpose of WOE assessment, therefore, when the mean SQG quotient for the selected contaminants exceeds 0.1 a positive chemical score is reported, whether an individual screening value is exceeded or not.

<u>Equilibrium Partitioning Sediment Benchmarks (ESBs) - PAHs:</u> Workbook Tab (2) - Dissolved PAH Evaluations

The concentration of dissolved contaminants in the interstitial water of sediment may also stress benthic infauna. The interstitial water in sediment is difficult to collect and analyze accurately and this is not commonly carried out during normal monitoring programs. However, the concentrations of dissolved contaminants in interstitial water can be estimated from the concentrations in the sediment itself using their **equilibrium partitioning coefficients** and their integrated effects can be predicted by applying procedures similar to those applied for integrating sediment quality guidelines. EPA has published procedures for the derivation of equilibrium partitioning sediment benchmarks (ESBs) for the protection of benthic organisms from several classes of contaminants (US EPA - 2001, 2003a, 2003b, 2003c, 2005, 2008). The guidance manual "Procedures for the Derivation of Equilibrium Partitioning Sediment Benchmarks (ESBs) for the Protection of Benthic Organisms: PAH Mixtures" (EPA/600/R-02/013, November 2003) provides orientation for calculating an integrated ESB for a suite of 34 common PAHs that have been included in various intensive sediment studies, as well as conversion factors to be applied for smaller subsets of 23 and 13 PAHs analyzed in other studies. The suite of PAHs analyzed in DEQ's Estuarine ProbMon Program includes all 23 PAHs of the 23-analyte subset for which conversion factors have been provided. Tab (2) - "Dissolved PAH Evaluations" performs the necessary calculations and conversion based on the concentrations of PAHs and total organic carbon (TOC) measured in the sediment. When the converted sum of the 22 individual benchmarks reaches or exceeds 1.0, there is a high probability of adverse chronic effects due to the toxicity of dissolved PAHs. Observations and comments from Tab (2) are copied to the SQT Evaluation Matrix where they contribute to the final weighting of the SQT Matrix score given for sediment chemistry from Tab (1).

An additional tool for the identification of potential sources of PAHs is the ratio between the members of each of two pairs of compounds (Neff et al., 2005). Depending upon the value of the ratio (see **Table 2**, below), the source may be identified as probably pyrogenic as opposed to petrogenic in origin. Petrogenic PAHs are found in nature, usually at low concentrations; they may be associated with petroleum spills. Pyrogenic PAHs are combustion byproducts, and usually result from the combustion of petroleum products.

Table 2 – The Identification of Pyrogenic vs. Petrogenic Sources of PAH Contaminants based on the ratio of concentrations of Phenanthrene / Anthracene and Fluoranthene / Pyrene (Neff et al., 2005)

Ratio	Value	Probable Source of PAHs
Phenanthrene / Anthracene Ratio	**If <7.0**	**Probably Pyrogenic**
	If >10.0	**Probably Petrogenic**
Fluoranthene / Pyrene Ratio	**If <0.9**	**Possibly Petrogenic**
	If >1.0	**Possibly Pyrogenic**

The Fluoranthene / Pyrene Ratio is much more variable among pyrogenic and petrogenic sources of PAHs than is the Phenanthrene / Anthracene Ratio. Consequently the resultant classification is considered a possible rather than a highly probable source.

Sediment Total Organic Carbon (TOC):

The concentration of total organic carbon in the sediment influences the availability and route of uptake of toxic contaminants by benthic organisms. Organic carbon absorbs or sequesters many organic and inorganic contaminants, and many benthic infauna organisms actively feed on the organic detritus where these contaminants concentrate. Elevated amounts of TOC are consequently considered undesirable for benthic infauna that ingests sediment particulates. Sediment quality indices published in a series of National Coastal Condition Reports (US EPA, 2001, 2005, 2008) classify sediments with more than 5% TOC as being of poor quality. Conversely, because of the equilibrium partitioning of contaminants between sediment carbon and interstitial water, high TOC concentrations in the sediment tend to lower their ESBs and reduce the risk from dissolved toxics that would diffuse across gills and other semi-permeable membranes. Higher TOC concentrations would be beneficial in reducing toxic effects through this route of uptake. Sediment TOC concentration is consequently maintained as an ancillary line of evidence for the interpretation of sediment contamination and is used in the calculation of ESBs on Tab (2).

Sediment Toxicity: Workbook Tab (3)

The magnitude of effects observed during sediment toxicity tests can be applied for weighting this line of evidence. The survival of test organisms, expressed by the percent control-corrected survival or control-corrected mortality endpoint, is generally associated with the acute effects of higher levels of toxicants (although chemical additivity, antagonism and synergism can also play a role). Sub-lethal test endpoints that provide a measure of chronic exposure effects at an increased level of sensitivity, with lower toxicant concentrations, include organism growth (expressed in weight), reburial (amphipods), reproductive rate, etc. In relative terms, the ecological significance of these endpoints is not likely to be as critical as the measure of survival. Therefore, less weight is applied in cases where only these endpoints show effects. In situations where the survival endpoint yields statistically significant effects by one or more species, greater weight would be applied accordingly. It is important to factor the number of test species, their taxonomic identifications and the associated sensitivities of each test species into the weighting. During the tests, attention must also be applied to artificial toxicity such as in the case of naturally occurring sediment ammonia. Another complication is salinity adjustment, which can geo-chemically alter the sediment, thus leading to changes in chemical bioavailability and ultimately affecting sediment toxicity (Roberts et al., 2002). In the field, indigenous predators can also

significantly alter the test outcome. Most of these factors are considered and controlled during the performance of toxicity tests in the laboratory.

Toxicity tests performed in compliance with the QAPP of the National Coastal Assessment Program (US EPA, 2009b) and continued within the DEQ Estuarine Probabilistic Monitoring Program are currently limited to a single test format with a single test species: ten-day static acute toxicity tests with an estuarine amphipod, conducted in accordance with standard ASTM guidelines and EPA methods. (Prior to 2010, *Ampelisca abdita* was the amphipod of choice. Beginning in 2010, the NCCA Program as well as DEQ's Estuarine Probabilistic Monitoring Program switched from *Ampelisca abdita* to the amphipod *Leptocheirus plumulosus* as a test organism.) The specified end-point is amphipod survival. The results of these tests are provided on the "SedTox Data" Tab of the WOE Assessment Workbook and the final evaluation and scoring are carried out on Tab (3) "Sediment Toxicity." Both statistical significance and ecological significance of the results are considered. The statistical significance of test results is tested at a significance level of $\alpha = 0.05$ ($\geq$ 95% confidence that differences from control are real); ecological significance is assumed only if control-corrected survivorship is < 80%. On rare occasion, results may be statistically significant but not ecologically significant, or *vice versa*. In such cases a score of 1 (marginal toxicity) is awarded for evaluation in the SQT matrix.

Ancillary Parameter Values and the Identification of Additional Potential Stressors

Near Bottom Salinity (‰) and Percent Sand (%): The near-bottom salinity class observed at the time of sample collection, and the percent sand in the sediment sample are used in several ancillary evaluations. On the "Summary Sheet" Tab of the WOE Assessment Workbook these two measurements are automatically integrated into a "Habitat Type" characterization for application of the CBP B-IBI on Tab "(4) Benthic Fauna". This integration is also performed by the BEL benthic database at Old Dominion University during the calculation of the CBP Benthic IBI.

In addition, the relative proportions of sand *vs.* fines (silt/clay) in the sediment ("sand" vs. "mud" substrate) can be used to differentiate high energy from low energy benthic environments. Sandy, high energy environments include shallow waters where wave action is more prevalent or deeper channels where significant currents are present. Both are areas where the substrate is at least periodically in movement and fine particulates tend to be washed away. Filter feeders may predominate in such areas, while deposit feeders may predominate in low energy areas where fine particulates accumulate. Substrate type may also serve as an indication of the relative risk of chemical contamination. Contaminants are more readily absorbed, transported and deposited by fine particles (silt/clay) and associated organic detritus than by sand.

Near Bottom Dissolved Oxygen (DO - mg/L) and Depth: Low dissolved oxygen presents a direct stress on benthic fauna. This may result from natural thermal and/or saline stratification that inhibits mixing in deep channels, from nutrient enrichment and eutrophication, or from a combination of both. In warmer, shallower waters low DO concentrations (mg/L) may result simply from its lower solubility at higher temperatures. In either case, the observation of a single low near-bottom DO concentration at a probabilistic site is not sufficient to result in an impaired assessment. It is evaluated only as one among various potential causes for any benthic degradation that is observed.

Bottom Temperature: (^{o}C): Higher water temperature may itself be a significant stressor, in addition to its indirect effect via DO depression.

TOC: As indicated above, the concentration of Total Organic Carbon in the sediment can influence the degree of exposure to and the uptake route of chemical contaminants by benthic organisms. In association with other

water quality characteristics, it may also provide insight into the degree of eutrophication present in the estuarine system.

While potential scenarios based on these integrated lines of evidence are too numerous to list, for many sites the conclusion should be obvious. For example, chemical contaminants are commonly detected but at concentrations below their respective SQGs. If the biological results from the same samples indicate a lack of effects, as demonstrated by lack of significant sediment toxicity and the presence of a healthy benthic community, the resultant listing would be VA Category 2A (fully supporting designated use). On the opposite end of the spectrum, all three components of the triad may show extreme effects and the site would consequently be listed in the 5A category (impaired for toxics - needing a TMDL).

Ancillary Indices: Beginning in 2011, several indices used for site evaluation by the National Coastal Condition Assessment (NCCA) Reports were added to the Summary sheets of WOE Assessment workbooks. A Sediment Quality Index (SQI) integrates the results of sediment toxicity, sediment contamination, and sediment TOC content into a rating of "Good", "Fair" or "Poor." A Water Quality Index (WQI) integrates near surface dissolved inorganic nutrients (nitrogen and phosphorus), chlorophyll-a, water clarity, and near-bottom dissolved oxygen into a similar rating scale. At the present time (April 2013), a national workgroup is reevaluating the thresholds for classification of the individual components of these indices for the fifth National Coastal Condition Report (U.S. EPA, NCCA V in prep.), and water clarity may be removed from the WQI. Once a final consensus is reached for the national report, the thresholds in the WOE workbooks will be adjusted accordingly. A résumé of the three types of benthic evaluations (CBP B-IBI, Mid Atlantic B-IBI, EMAP VP-IEC) applied in the WOE Assessment has also been included on the Summary tab to facilitate site characterizations.

An example of a completed WOE workbook is included here to illustrate the final product and provide additional information to help with the Weight-of-Evidence assessment process.

It is preferred that this WOE approach only be applied when all three sediment data components are available from a particular site. It is still possible, however, to implement this process if only two elements are available, as long as data on the condition of the resident benthic community is included (e.g., sediment chemistry and benthic IBI or sediment toxicity and benthic IBI). If both lines of evidence are in agreement as to the condition of the site (e.g., degraded or severely degraded), a corresponding assessment may be attained (i.e., Category 5A with toxics as a potential cause). If such a conclusion is suggested based solely on sediment chemistry and sediment toxicity data, follow-up monitoring should be scheduled (Virginia Assessment Category 3B), even if both chemical and toxicological results are in agreement on the potential existence of a toxic condition. For those instances where the conclusions are not obvious, it will be necessary to obtain consensual agreement between Central Office and the Regional Office responsible for the assessment of that water body. If agreement cannot be attained, advice should be sought from DEQ's Academic Advisory Committee.

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APPENDIX H

SIGNIFICANT LAKES/RESERVOIRS BY REGION

Northern Regional Office – 17 Reservoirs/Lakes

Abel Lake	Stafford Co.	174 (Acres)	PWS
Aquia Reservoir (Smith Lake)	Stafford Co.	131	PWS
Beaverdam Reservoir	Loudoun Co.	301	PWS
Breckenridge Reservoir	Prince William Co.	47	PWS
Burke Lake	Fairfax Co.	208	VDGIF
Curtis Lake	Stafford Co.	58	
Goose Creek Reservoir	Loudoun Co.	40	PWS
Hunting Run Reservoir	Spotsylvania Co.	440	PWS
Lake Anna	Louisa, Spotsylvania, Orange	9,595	
Lake Manassas	Prince William Co.	675	PWS
Lake Pelham	Culpeper Co.	250	PWS
Lunga Reservoir	Prince William Co.	477	PWS
Motts Run Reservoir	Spotsylvania Co.	137	PWS
Mountain Run Lake	Culpeper Co.	73	PWS
Ni Reservoir	Spotsylvania Co.	408	PWS
Northeast Creek Reservoir	Louisa Co.	178	PWS
Occoquan Reservoir	Fairfax, Prince William Co.	1,333	PWS

Piedmont Regional Office – 15 Reservoirs/Lakes

Amelia Lake	Amelia Co.	98	VDGIF
Brunswick Lake	Brunswick Co.	138	VDGIF
Lake Chesdin	Chesterfield Co.	3,164	PWS
Chickahominy Lake	Charles City Co.	1,049	PWS
Diascund Creek Reservoir	New Kent Co.	1,055	PWS
Emporia Lake	Greensville Co.	290	PWS
Falling Creek Reservoir	Chesterfield Co.	88	
Great Creek Reservoir	Lawrenceville	219	
Harrison Lake	Charles City Co.	60	
Lake Nottoway	Nottoway Co.	161	PWS
Lakeview Reservoir	Chesterfield Co.	43	
Little Creek Reservoir	James City Co**.**	926	PWS
Powhatan Lake (U & L)	Powhatan Co.	61	
Swift Creek Lake	Chesterfield Co.	102	
Swift Creek Reservoir	Chesterfield Co.	1,581	PWS

Blue Ridge Regional Office - Lynchburg – 21 Reservoirs/Lakes

Briery Creek Lake	Prince Edward Co.	825	VDGIF
Cherrystone Reservoir	Pittsylvania Co.	104	PWS
Georges Creek Reservoir	Pittsylvania Co.	8	PWS
Graham Creek Reservoir	Amherst Co.	40	PWS

Holiday Lake	Appomattox Co.	113	
Kerr Reservoir (Va.'s portion)	Halifax Co.	33,300	ACOE/PWS
Keysville Reservoir	Charlotte Co.	36	PWS
Lake Conner	Halifax Co.	98	VDGIF
Lake Gordon	Mecklenburg Co.	115	VDGIF
Lake Gaston (Va.'s portion)	Brunswick Co.	5,614	PWS
Lunenburg Beach Lake	Town of Victoria	12	PWS
Mill Creek Reservoir	Amherst Co.	190	
Modest Creek Reservoir	Town of Victoria	20	PWS
Fort Pickett Reservoir	Nottoway Co.	319	
Pedlar Lake	Amherst Co.	118	PWS
Phelps Creek Reservoir	Campbell Co.	19	PWS
Roaring Fork Reservoir	Pittsylvania Co.	19	PWS
Sandy River Reservoir	Prince Edward Co.	718	
Stonehouse Creek Reservoir	Amherst Co.	34	
Thrashers Creek Reservoir	Amherst Co.	32	
Troublesome Creek Reservoir (SCS Impoundment #2)	Buckingham Co.	53	PWS

Southwest Regional Office – 11 Reservoirs

Bark Camp Lake	Scott Co.	29	USFS
Big Cherry Lake	Wise Co.	103	PWS
Hidden Valley Lake	Russell Co.	58	VDGIF
Hungry Mother Lake	Smyth Co.	100	DCR
J. W. Flannagan Reservoir	Dickenson Co.	1,177	ACOE/PWS
Lake Keokee	Lee Co.	97	VDGIF
Laurel Bed Lake	Russell Co.	312	VDGIF
North Fork Pound Reservoir	Wise Co.	116	ACOE/PWS
Rural Retreat Lake	Wythe Co.	85	VDGIF
South Holston Reservoir	Washington Co.	1,699	TVA/PWS
Wise Reservoir	Wise Co.	46	WISE/PWS

Tidewater Regional Office – 21 Reservoirs/Lakes

Airfield Pond	Sussex Co.	120	VDGIF
Harwood Mills Reservoir	York Co.	258	PWS
Lake Burnt Mills	Isle of Wight Co.	638	PWS
Lake Cohoon	Suffolk City	454	PWS
Lake Drummond	Suffolk City	3,242	
Lake Kilby	Suffolk City	200	PWS
Lake Lawson	Virginia Beach	75	
Lake Meade	Suffolk City	490	PWS
Lake Prince	Suffolk City	709	PWS
Lake Smith	Norfolk City	185	PWS
Lake Whitehurst	Norfolk City	495	PWS
Lake Wright	Norfolk City	12	
Lee Hall Reservoir	Newport News	290	PWS
Little Creek Reservoir	Norfolk City	200	PWS
Lone Star Lake F	Suffolk City	19	PWS
Lone Star Lake G	Suffolk City	90	PWS

Lone Star Lake I	Suffolk City	33	PWS
Speights Run Lake	Suffolk City	118	PWS
Stumpy Lake	Virginia Beach	263	
Waller Mill Reservoir	York Co.	288	PWS
Western Branch Reservoir	Norfolk City	1,205	PWS

Valley Regional Office – 21 Reservoirs/Lakes

Beaver Creek Reservoir	Albemarle Co.	96	PWS
Chris Green Lake	Albemarle Co	57	
Coles Run Reservoir	Augusta Co.	11	USFS/PWS
Douthat Lake	Bath Co.	47	
Elkhorn Lake	Augusta Co.	51	USFS/PWS
Fluvanna Ruritan Lake	Fluvanna Co.	51	
Lake Albemarle	Albemarle Co.	37	
Lake Arrowhead	Page Co.	36	
Lake Frederick	Frederick Co.	67	VDGIF
Lake Nelson	Nelson Co.	41	
Lake Robertson	Rockbridge Co.	24	
Mount Jackson Reservoir	Shenandoah Co.	1	
Ragged Mountain Reservoir	Albemarle Co.	71	PWS
Rivanna Reservoir	Albemarle Co.	399	PWS
Shenandoah Lake	Rockingham Co.	36	
Silver Lake	Rockingham Co.	11	PWS
Staunton Dam Lake	Augusta Co.	21	PWS
Strasburg Reservoir	Shenandoah Co.	5	
Switzer Lake	Rockingham Co.	99	USFS/PWS
Sugar Hollow Reservoir	Albemarle Co.	47	PWS
Totier Creek Reservoir	Albemarle Co.	37	PWS

Blue Ridge Regional Office - Roanoke – 17 Reservoirs/Lakes

Beaverdam Creek Reservoir	Bedford Co.	70	PWS
Bedford (Stony Cr.) Reservoir	Bedford Co.	28	PWS
Carvin Cove Reservoir	Botetourt Co.	632	PWS
Claytor Lake	Pulaski Co.	4,287	PWS
Clifton Forge (Smith Cr.) Reservoir	Alleghany Co.	10	PWS
Fairystone Lake	Henry Co.	127	
Gatewood Reservoir	Pulaski Co.	176	PWS
Hogan Lake	Pulaski Co.	36	PWS
Leesville Reservoir	Bedford Co.	2,630	PWS
Little River Reservoir	Montgomery Co.	60	PWS
Martinsville Reservoir	Henry Co.	181	PWS
Lake Moomaw	Bath Co.	2,389	ACOE
Philpott Reservoir	Henry Co.	2,813	ACOE
Smith Mountain Lake	Bedford Co.	19,820	PWS
Spring Hollow Reservoir	Roanoke Co.	113	PWS
Talbott Reservoir	Patrick Co.	141	
Townes Reservoir	Patrick Co.	28	

Total 123= Significant Reservoirs/Lakes statewide
PWS = Public Water Supply

VDGIF = Virginia Department of Game and Inland Fisheries
ACOE = Army Corps of Engineers

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The influence of nutrient loading, dissolved inorganic carbon and higher trophic levels on the interaction between submerged plants and periphyton

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Summary

1 We studied the growth of a submerged aquatic plant in relation to periphytic and planktonic algae over a range of nutrient and dissolved inorganic carbon (DIC) availabilities.

2 In consecutive years two factorial experiments were conducted in 48 artificial ponds (each 3.14 m^3), comprising four concentrations of DIC (1.5, 2.5, 3.5 or 4.5 mM) each crossed with three separate nutrient loadings (10 μg L^{-1} P and 0.2 mg L N, 50 μg L^{-1} P and 1 mg L N, or 200 μg L^{-1} P and 4 mg L N). The second experiment differed by the inclusion of fish in the ponds.

3 In the first year DIC had no effect on plant growth, but nutrient loading did. Plants failed to grow in treatments where phytoplankton density was high (> 100 μg L^{-1}). Where phytoplankton was low, high numbers of invertebrates colonized the ponds, and periphyton abundance on the plants was low. In the second year, where phytoplankton never achieved the densities of the previous year, there was a significant effect of DIC concentration on plant growth but not of nutrients. Invertebrate abundance was lower and periphyton on the plants correspondingly higher.

4 In both years increased nutrient loading had no effect on the abundance of periphyton growing on the surface of the plants. Periphyton abundance was determined by the density of grazing invertebrates in the ponds.

5 There was a negative relationship between periphyton density and final plant density, which became significantly less steep with increasing DIC, indicating that periphyton and plants were competing for carbon.

6 DIC concentration has the potential to influence community structure in shallow lakes, altering competitive interactions between periphyton and plants and rendering low DIC lakes more prone to loss of plants when nutrient loading increases. However, the expression of this competition between periphyton and plants will depend on the density of grazing invertebrates present, which is itself influenced by the intensity of fish predation on those invertebrates.

Key-words: alternative stable states, benthic invertebrates, macrophytes, phytoplankton, shallow lakes

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Introduction

Since theoreticians predicted the possibility of alternative equilibria in communities (Noy-Meir 1975; Gilpin & Case 1976; May 1977) there has been increasing evidence to indicate that they exist in nature (Sousa 1979; Sousa & Connell 1985; Laycock 1991; Knowlton 1992). Positive feedback mechanisms maintain the dominance of either one or the other state, in systems where initial environmental conditions were identical (Wilson & Agnew 1992).

In the case of shallow lakes, communities can be dominated either by aquatic plants, with clear water, or by high concentrations of phytoplankton, with sparse plants (Canfield *et al.* 1984; Jeppesen *et al.* 1990). Aquatic plants stabilize the water column and

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increase habitat heterogeneity (Jeppesen *et al.* 1998a). This in turn affects the interactions of many organisms both in vegetated areas and in the open water (Jeppesen *et al.* 1998b). Thus, aquatic plants provide zooplankton with refuge from fish (Timms & Moss 1984), deplete dissolved nutrients (Howard-Williams 1981; van Donk *et al.* 1993) and increase settlement and reduce resuspension of particulates (Peticrew & Kalff 1992). The outcome is to maintain clear water with low phytoplankton populations, to the ultimate benefit of aquatic plants. There is a corresponding alternative suite of positive feedback mechanisms that can maintain the alternative state of phytoplankton dominance and turbid water (Scheffer *et al.* 1993).

Such positive feedbacks may explain the maintenance of the two alternative equilibria in shallow lakes. However, the mechanisms that sustain these alternative equilibria are not necessarily the same as those involved in their origin, a dichotomy which has been recognized in other habitats for some time (Wilson & Agnew 1992; Petraitis & Latham 1999). Alternative assemblages occur under identical environmental conditions where positive feedback mechanisms have exaggerated and reinforced an initial chance imbalance in species abundance. For a switch between equilibria to occur, some event must cause a change in species abundance that perturbs one assemblage sufficiently from its equilibrium to come within the basin of attraction of the equilibrium of the alternative assemblage (Scheffer *et al.* 1993). Thus, stochastic physical disturbances cause switches in rocky shore and heathland–forest communities (Petraitis & Latham 1999). In shallow lakes such disturbance-driven switches have been described (Mitchell 1989; Blindow *et al.* 1993), but are infrequent and unlikely to explain the change of community from plants to phytoplankton that typically accompanies eutrophication (Mason & Bryant 1975; Jeppesen *et al.* 1991). But, phytoplankton and plants appear to be largely incompatible, each suppressing the abundance of the other (Howard-Williams 1981; Canfield *et al.* 1984; Balls *et al.* 1989). As such, it has been suggested that a nutrient-mediated increase in the abundance of periphyton (attached algae growing on the plants' surfaces) is responsible for the suppression of plant growth, facilitating the replacement of plants by phytoplankton (Phillips *et al.* 1978; Hough *et al.* 1989; Daldorph & Thomas 1995). Clearly, the inclusion of a deterministic process in such a pivotal role contradicts the stochastic requirements of the theory of alternative equilibria. Put simply, if high nutrient availability inevitably leads to increased periphyton, reduced plant growth and phytoplankton dominance, then there is no alternative.

Evidence in support of a deterministic, periphyton-based suppression of plants comes from various quarters. Palaeolimnological records from eutrophic lakes show that an increase in periphytic algae occurs before any increase in phytoplankton (Osborne & Moss 1977; Phillips *et al.* 1978). In controlled laboratory experiments, increased nutrient loading encourages periphyton growth (Eminson & Phillips 1978; Jones *et al.* 1999), and a negative relationship between periphyton abundance and plant performance has been shown, particularly for isoetid plants (Sand-Jensen 1977; Sand-Jensen & Søndergaard 1981; Sand-Jensen & Borum 1984). Conversely, reductions in periphyton abundance, due to experimental (Brönmark 1985; Underwood 1991; Jones *et al.* 1999) or fortuitous (Irvine *et al.* 1993) increases in the number of grazing invertebrates, result in increased plant growth.

The rosette-forming isoetid plants, which have been repeatedly described as showing a negative interaction between periphyton and plants (Sand-Jensen & Søndergaard 1981; Sand-Jensen & Borum 1984; Sand-Jensen 1990), obtain carbon dioxide from the sediment (Wium-Andersen 1971) and the negative effect of periphyton can only be one of reduced availability of light. Taller, caulescent species more typical of mesotrophic and eutrophic lakes, on the other hand, depend on the water column for their supply of carbon (Madsen & Sand-Jensen 1991; Jones *et al.* 2000a), and their suppression by periphyton may be through interception of carbon (Jones *et al.* 2000b) as well as light. Carbon availability affects the distribution of aquatic plants (Kadono 1982; Hough & Fornwall 1988) and is one of the major constraints on photosynthesis in the field (Jones *et al.* 1996). Due to differences in geology, the pool of dissolved inorganic carbon (DIC) in lakes varies between catchments. This affects not only the total availability of carbon, but also the buffering capacity of the water (Stumm & Morgan 1981), both of which are likely to influence the outcome of competition for DIC between plants and periphyton (Jones *et al.* 2000b). Hence, the relationship between nutrient loading and plant growth is likely to differ between lakes with respect to a hitherto largely unmeasured parameter, the concentration of DIC. If this is the case, the community assemblages described from field data (e.g. Canfield *et al.* 1984; Jeppesen *et al.* 1990) may not be true alternatives, but the outcome of a deterministic relationship between plants and periphyton in lakes varying in DIC concentration.

Experiments were conducted in a series of artificial ponds to test the effect of increasing nutrient availability on the interactions between phytoplankton, periphyton and plants in waters of differing DIC concentration. The experiments were intended to test if the influence of a nutrient-mediated increase in periphyton on plant growth is important in structuring shallow lake communities. As the final outcome potentially may depend upon both resource and biotic interactions, the influence of zooplankton, macroinvertebrates and fish on both algae and plants were taken into consideration also. We predicted that periphytic algae would suppress plant growth more at low DIC availability, but that the final outcome for the plants would depend upon many interacting variables,

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including the control of periphyton density by invertebrate grazers and the abundance of phytoplankton.

Methods

The experimental facility at the University of Liverpool's Experimental and Horticultural Research Station at Ness Gardens, Cheshire, UK (53°16′ N, 3°2′ W) consisted of 48 cylindrical, fibreglass ponds, each 2 m diameter and 1 m depth. Artificial sediment, comprising one part grass loam to seven parts sand, was spread in a 5-cm thick layer over the base of each pond. Water from a borehole was passed through an industrial deioniser (reversible ion exchange column, Permutit TS150, output < 30 μS) and mixed with raw borehole water to yield a DIC of 1.5 mM, Total Dissolved Phosphorus < 1 μM and Total Dissolved Inorganic Nitrogen of 0.2 mg/L. The borehole water contained no particulates.

When all the ponds had been filled with water, five large-mesh net sacks each filled with 50 g wet weight of *Elodea nuttallii* (Planch.) St. John and weighted with stones, were added to each. The plants were collected with a grapnel from the Manchester, Bolton and Bury Canal, Lancashire, UK (53°33′ N, 2°33′ W). A 100-mL inoculum of mixed periphytic algae, obtained by shaking a collection of several plant species from water bodies varying in both nutrient and DIC availability, was then added to each pond, together with zooplankton, comprising approximately 300 *Daphnia magna* Straus and 150 *Cyclops strenuus* (Fisher).

Once all the tanks had been stocked, a single addition of $NaHCO_3$ was made to produce one of four concentrations of DIC (1.5, 2.5, 3.5 and 4.5 mM). Each pond also received a weekly nutrient loading of (i) 10 μg L^{-1} P, 0.2 mg/L N; (ii) 50 μg L^{-1} P, 1 mg/L N; or (iii) 200 μg L^{-1} P, 4 mg/L N; hereafter referred to as low, medium and high nutrients. P was added as NaH_2PO_4 and N as $Ca(NO_3)_2$. To ensure that diatoms had a supply of silicate, $Na_2SiO_3{\cdot}5H_2O$ was added weekly to all the ponds to create a concentration of 2 mg/L Si. These concentrations approximately correspond to those found under nutrient-poor to very nutrient-rich conditions (Moss 1998). All treatments were replicated in four randomized blocks of 12 ponds. To fill all the ponds with water took a total of 28 days. To collect, sort and add the plants and periphytic algae, add zooplankton and, finally, add $NaHCO_3$ and nutrients, took a further 4 days. All procedures were carried out blockwise and as quickly as possible. The experiments then ran for a further 16 weeks from May to September in both 1995 and 1996.

The 1996 experiment differed from that in 1995 in two ways. In the first year phytoplankton developed substantial populations in the first ponds to be filled whilst the remainder were being set-up, prior to the addition of plants, periphytic algae, zooplankton, DIC and nutrients. Hence, in the second year, the ponds were covered with black polythene to prevent any algal growth during this initial period whilst the ponds were being filled. The covers were removed when plants were added but before any experimental manipulation. Substantial populations of invertebrates colonized the tanks in 1995, and a single rainbow trout (*Oncorhynchus mykiss* (Walbaum)) was therefore added to each pond in 1996 at a density of 50 g/m^2. Trout were chosen as they are generalist predators of large gape. Such fish are known to have an impact on the abundance of benthic invertebrates (Diehl & Kornijów 1997).

INTERIM VALUES

Weekly determinations of pH and conductivity, using portable field meters, and alkalinity, by titration, were made for all ponds. Any losses of water to evaporation were topped up with deionized water.

Zooplankton numbers were determined (every 6 weeks in year 1 and every 4 weeks in year 2) from three whole-water-column samples (total volume, 9 L) taken at random positions in each pond and passed through a zooplankton net (mesh size 250 μm). For estimation of phytoplankton populations (every 6 weeks in year 1 and every 2 weeks in year 2), three further whole-water-column samples (total volume, 9 L) were taken and mixed in a bucket. From this mixed sample, subsamples were taken and filtered (GF/C) for subsequent chlorophyll and dry weight determination. A further 50 mL subsample was preserved with Lugol's iodine for counting.

Every 5 weeks the abundance of periphytic algae attached to the plants was determined from four randomly selected 20 cm shoots taken from each pond. These shoots were shaken separately by hand for 90 s, sufficient to remove all the algae from their surfaces, in 150 mL of algae-free borehole water (Zimba & Hopson 1997). The shoot was removed from the washings and dried to constant mass. The resultant algal suspension was divided into three 50 mL aliquots, treated as above. The surface area of the plants was calculated from the dry weight using an empirically derived relationship (Jones *et al.* 1999), and used to standardize measures of periphyton.

To determine the abundance of periphytic algae attached to the side of the ponds, every 5 weeks a perspex chamber with neoprene edges was used to isolate randomly selected areas of pond wall (5 × 8 cm). All the pond water was removed from the chamber with a syringe and replaced with 150 mL of fresh algae-free borehole water. The algae were then removed from the pond wall by vigorous scrubbing with a small brush to give a suspension for determination of chlorophyll and dry mass, and for counting.

FINAL HARVEST

At the end of the experiment all the plants were removed from the ponds using a rake. The plants were

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Table 1 Statistical significance of treatment effects and their interactions following repeated measures ANOVA of the parameters quantified in the experimental ponds over two years, year 1 without fish, year 2 with fish. Only significant values shown

	DIC	Nutrients	DIC × Nutrients	Time	Time × DIC	Time × Nutrients	Time × DIC × Nutrients
Year 1							
pH	0.002	0.007		0.0001	0.0001	0.0001	0.004
CO_2	0.0001	0.0001		0.0001	0.0001	0.0001	0.0001
Plants		0.0001		NA	NA	NA	NA
Phytoplankton		0.0001				0.002	
Daphnia magna		0.0001					
Cyclops strenuus		0.024					
Periphyton on plants							
Year 2							
pH	0.047	0.003		0.0001	0.004	0.003	0.013
CO_2	0.0001	0.0003		0.0001	0.0001	0.0001	0.0001
Plants	0.019			NA	NA	NA	NA
Phytoplankton				0.0001	0.006	0.009	
Daphnia magna	0.049	0.0012		0.009			
Cyclops strenuus				0.0001			
Chydorus sphaericus		0.002		0.0001			
Heterocypris incongruens				0.001			
Cypridopsis vidua				0.008			
Periphyton on plants							
Side periphyton	0.0005	0.0001		0.018		0.014	

NA, analysis of time not applicable for plants (only measured at final harvest).

washed in a bucket and the washings returned to the pond. *Elodea nuttallii* was separated from any filamentous macroalgae (*Cladophora glomerata* Kuetz.), and both dried at 60 °C to constant mass.

After allowing 30 min for the pond to settle, invertebrates were sampled using a dip net (mesh size = 0.33 mm). All habitats were sampled by means of four vertical pulls up the sides of the pond, one scrape across the sediment surface, and 90 s of sweeping through the water column. The proportion of each habitat sampled relative to the total per pond was determined and used to calculate densities. If fish were present they were netted, killed, and frozen for subsequent gut analysis.

Interim measures were analysed using repeated measures ANOVA and a fully factorial, blocked design, and for plant-associated periphyton a nested, mixed model, using SAS. Final measures were analysed by ANOVA using a fully factorial, blocked design using SAS. Regression analysis and ANCOVA were conducted using SAS and type I or type II models as appropriate. Fish gut contents were analysed by canonical correspondence analysis (CCA) using CANOCO (ter Braak 1987).

Results

YEAR 1

The additions of $NaHCO_3$ were sufficient to create the four stated concentrations of DIC (1.5, 2.5, 3.5 and 4.5 mM) which were consistently and significantly different throughout the experiment ($P = 0.0001$). Since DIC forms the major component of the buffering system of the water, the DIC concentration had a significant influence on the pH of the ponds (Table 1), the pH generally being lower in ponds with higher DIC concentration. Nutrient loading also had a significant influence on the pH of the water, but there was no significant interaction between DIC and nutrients (Table 1). There was an increase in pH with time, with significant interactions between time × DIC, time × nutrients, time × nutrients × DIC. DIC concentration influenced free CO_2 availability both directly and through an effect on pH (Table 1); free CO_2 concentration increased with rising DIC concentration. Nutrient loading also influenced free CO_2, as did time and all interactions with time.

The final standing crop of *E. nuttallii* was not influenced by DIC concentration (Fig. 1) but in this year was negatively affected by nutrient loading ($P = 0.0001$, Table 1), with plants virtually absent from the highest nutrient loading (Fig. 1). During the 32-day set-up period (see methods) phytoplankton began to develop in the otherwise unoccupied ponds, possibly thriving on nutrients leached from the sediment. A significant effect of nutrient loading, and of the interaction between nutrient loading and time (time × nutrients), on phytoplankton concentration was found (Table 1). The phytoplankton concentration responded very strongly to high nutrient loading, increasing with time to give a final mean in excess of 100 μg chl L^{-1} (Fig. 2). A further analysis where time of filling of the tanks was included in the model found a significant effect ($P = 0.008$).

Zooplankton was only affected by nutrients (Table 1), with numbers of both species lower under the highest nutrient loading on all occasions (Fig. 3a,b).

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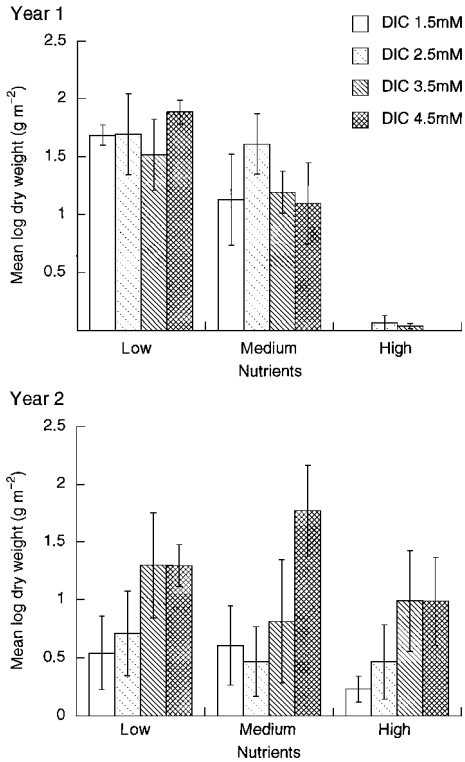

Fig. 1 Effect of DIC and nutrient loading (see text for details) on the mean final standing crop of *Elodea nuttallii* (as dry mass m^{-2} ± SE) in the experimental ponds. Year 1, in the absence of fish. Year 2, in the presence of fish.

The abundance of periphyton on the plants was not affected by any variable (Table 1). However, the density of algae was very low under all treatments (Fig. 4): mean = 0.987 µg chl cm^{-2} plant surface. A similar result was found when periphyton was measured as dry weight per unit plant surface area (DIC, nutrients, time, and interactions, $P > 0.25$).

A large number of invertebrate species were found in the ponds, including several which feed upon periphyton (Table 2). Some may have been introduced with the plants (e.g. Crustacea, Mollusca), but many arrived independently from surrounding populations and remained as adults (e.g. Hemiptera-Heteroptera, Coleoptera) or laid eggs which developed into larvae (e.g. Ephemeroptera, Trichoptera, Coleoptera, Diptera). Due to past agricultural practices, the Wirral is an area with an exceptionally high density of ponds, which will have provided a ready source of colonists.

YEAR 2

Additions of $NaHCO_3$ were again sufficient to create and maintain differences in DIC ($P = 0.0001$), and DIC concentration and nutrient loading therefore had similar effects on pH and free CO_2 (Table 1).

In marked contrast to the previous year, however, there was no effect of nutrient loading on the standing crop of *E. nuttallii*, but a strong effect of DIC concentration (Fig. 1, Table 1). Phytoplankton concentrations were not affected by either of the significant factors in 1995, nutrient loading and DIC concentration, but increased over time (Fig. 2) with both two-way interactions involving time being significant (Table 1). Phytoplankton abundance increased more rapidly with increasing nutrients, and with increasing DIC concentration. The abundance of phytoplankton was generally low, and did not reach the concentrations seen in the

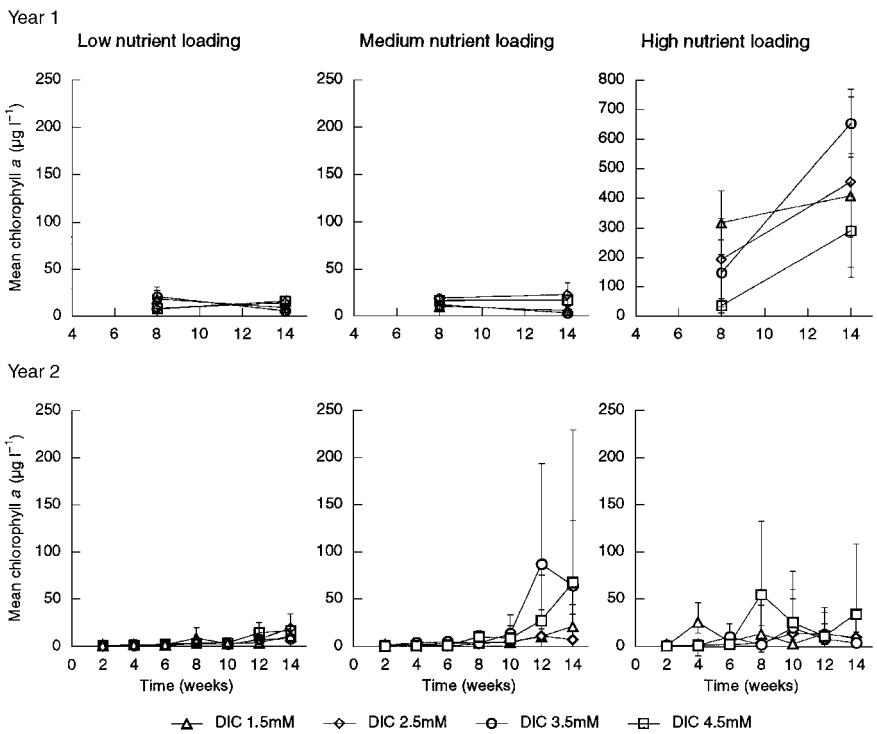

Fig. 2 Effect of DIC and nutrient loading on the mean density of phytoplankton (as µg chlorophyll L^{-1} ± SE) in the experimental ponds. Note differences in scales between years.

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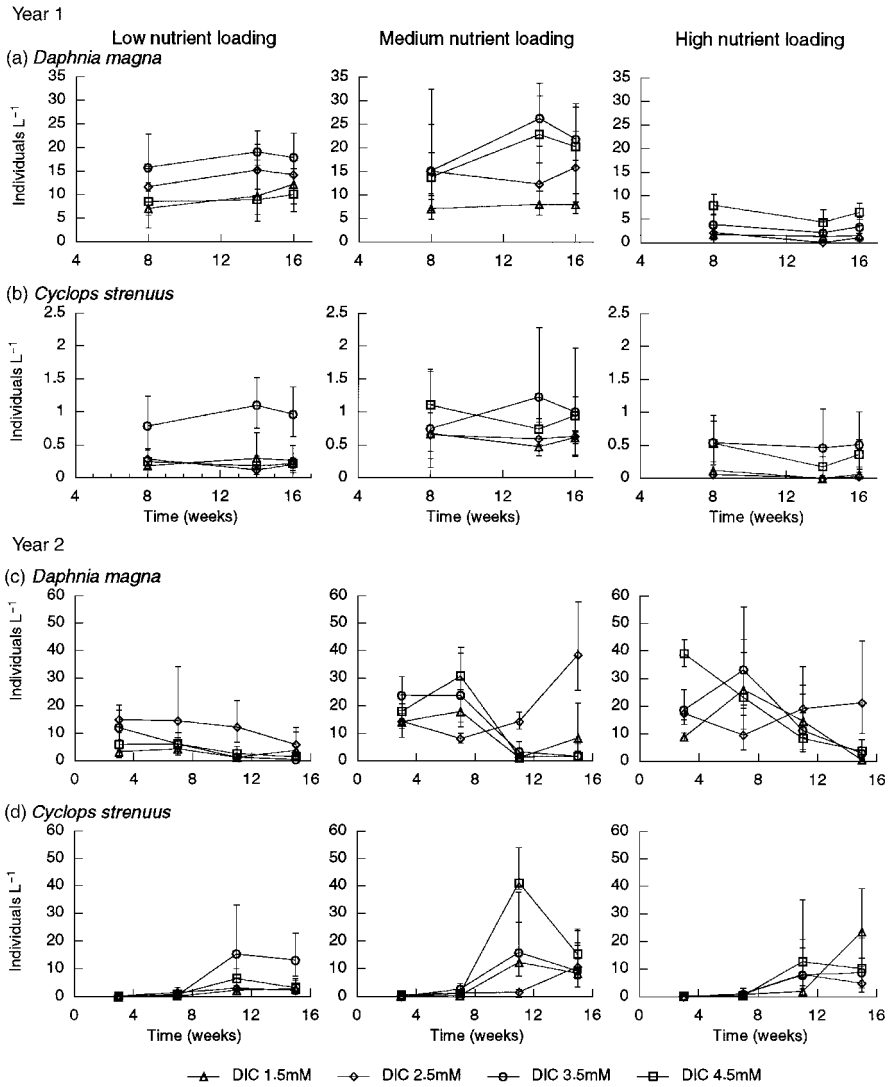

Fig. 3 Effect of DIC and nutrient loading on the mean number of zooplankton in the experimental ponds. Note differences in scales.

previous year (Fig. 2). This is in part a consequence of covering the ponds with black plastic during the set-up period, which produced clear water conditions at the start of the experiment, not achieved the year before, so that phytoplankton no longer had a head-start.

As well as more species of zooplankton (additional species were probably introduced with the fish) and more individuals being present, their response to the treatments differed from the previous year. Time had the strongest influence on all species of zooplankton (Table 1). Both *D. magna* (Fig. 3c) and *Heterocypris incongruens* (Ramdohr) decreased in number with time, whilst *Cyclops strenuus* (Fig. 3d), *Chydorus sphaericus* (Müll.) and *Cypridopsis vidua* (Müll.) increased. *D. magna* numbers were also higher with increased nutrients and DIC concentration (Table 1), whereas *Chydorus sphaericus* numbers were lowest at high nutrient loading.

Again the abundance of periphyton growing on the plants, measured as chlorophyll or dry weight ($P > 0.25$) per unit plant surface area, was not affected by any variable (Fig. 4, Table 1). Overall, however, the abundance of periphytic algae was greater than the previous year (Fig. 4): mean = 2.33 cf. 0.99 μg chl cm^{-2} plant surface.

The abundance of periphyton growing on the walls of the ponds (only assessed in year 2) showed a significant increase with increases in both nutrients and DIC (Fig. 5), and with time, particularly at high nutrients (time × nutrients, Table 1).

Again many species of invertebrates colonized the ponds, but the number of individuals was lower in the second year (Table 2). Ordination (CCA) of gut contents showed that the fish ate a wide variety of invertebrates (Table 2) as well as all zooplankton species present, but that their diet was not significantly affected by any of the treatments ($P > 0.05$, results not shown).

BOTH YEARS

Data from the two years were then combined, with each replicate regarded as independent, and year included to test for inter-annual effects. The mean

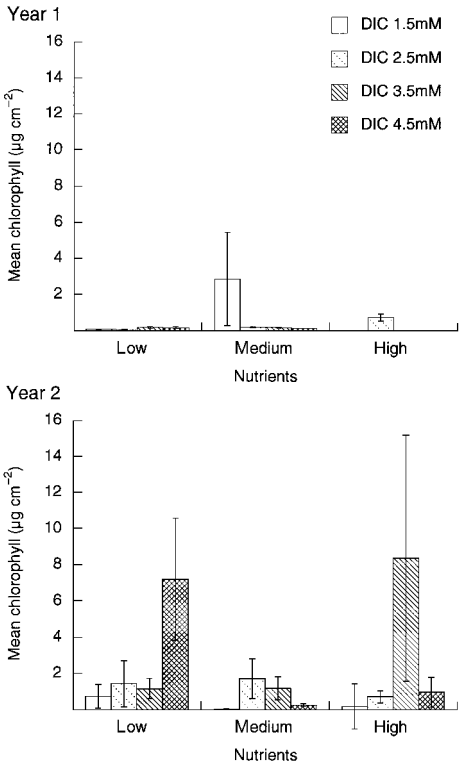

Fig. 4 Effect of DIC and nutrient loading on the mean density of periphyton (as µg chlorophyll cm^{-2} plant surface ± SE) in the experimental ponds.

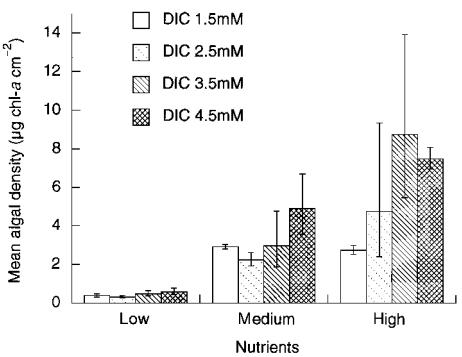

Fig. 5 Effect of DIC and nutrient loading on the mean density of periphyton (as µg chlorophyll cm^{-2}) growing on the walls of the experimental ponds in year 2.

abundance of phytoplankton was greater in the first year (127 ± 30.7 cf. 29 ± 8.6 µg chl L^{-1}, $P = 0.0001$), and its relationship with nutrients was affected by year ($P = 0.0001$), with no relationship found in the second year. Overall the number of invertebrates was lower (1702 ± 212 cf. 2297 ± 277 individuals m^{-2}; $P = 0.037$) and periphyton abundance higher ($P = 0.021$) in the second year, when fish were present. There was an interaction between nutrients × year on invertebrate numbers ($P = 0.0001$), which were reduced at high nutrient loading only in the first year. Over both years there was an increase in the number of invertebrates per unit plant surface area with increasing nutrients ($P = 0.006$).

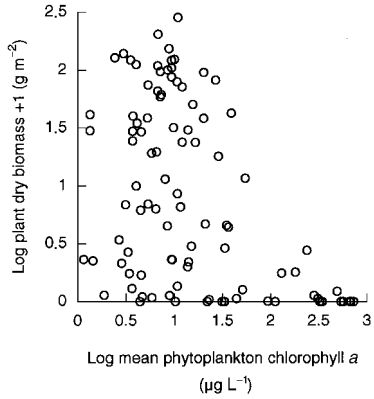

Fig. 6 Relationship between the mean density of phytoplankton and the final biomass of *Elodea nuttallii* in the experimental ponds.

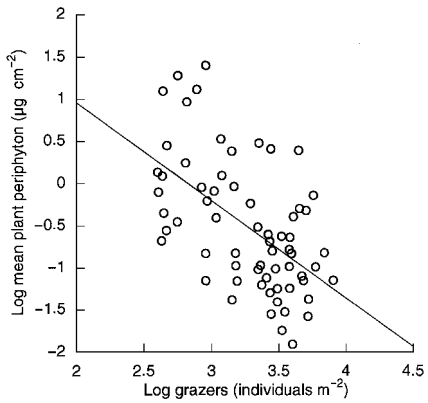

Fig. 7 Relationship between the density of grazing invertebrates and the abundance of periphyton growing on *Elodea nuttallii*. $y = 3.851 - 1.159x$, $R^2 = 0.309$, $P = 0.0001$.

When mean phytoplankton abundance over the growing season was plotted against plant biomass, it was apparent that the plants were virtually absent when phytoplankton abundance was above 100 µg chl L^{-1} (Fig. 6). These replicates all came from the first year where the phytoplankton had a head-start during the set-up period.

A negative relationship was found between the abundance of periphyton on the plants and the number of grazing invertebrates in each pond (Fig. 7). The number of grazing invertebrates was the only variable tested that was significant ($P = 0.0001$, $R^2 = 0.309$). Stepwise multiple regression indicated that the inclusion of DIC ($P = 0.011$) and nutrients ($P = 0.033$) into the model slightly (but significantly) improved the relationship ($R^2 = 0.386$ cf. 0.309), but that no other variable was significant. No significant effect of year was found.

Where plants were present, a negative relationship was found between periphyton density and the standing crop of *E. nuttallii* in the ponds, which became less severe with increasing DIC (Fig. 8). This relationship was not significantly improved by the inclusion of phytoplankton abundance or any other variable. Again, no significant effect of year was found.

Table 2 Invertebrate species found in the ponds, showing the mean densities (individuals m^{-2} benthos ± SE) in the two years of experiments. Zooplankton species have been excluded, data shown in Fig. 3 (all species found in guts of fish)

Species	Grazers	Density year 1	Density year 2	Fish guts
Platyhelminthes				
Dugesia tigrina (Girard)			1.1 ± 0.6	
Annelida				
Chaetogaster diaphanus (Gruithuisen)	*		0.7 ± 0.7	
Stylaria lacustris (L.)	*		34.5 ± 24.5	
Limnodrilus hoffmeisteri Claparede			3.2 ± 2.0	
Helobdella stagnalis (L.)			9.8 ± 2.8	
Erpobdella octoculata (L.)		0.1 ± 0.1		
Mollusca				
Bithynia tentaculata (L.)	*		0.2 ± 0.2	
Lymnaea peregra (Mull.)	*	187.7 ± 48.0	12.1 ± 6.0	†
Physa fontinalis (L.)	*		508.6 ± 97.5	†
Planorbis albus Mull.	*	34.4 ± 20.2	1.3 ± 0.5	†
Arthropoda				
Arachnida				
Hydracarina sp.				†
Crustacea				
Asellus aquaticus (L.)	*	3.8 ± 2.0	25.1 ± 9.3	
Insecta				
Agraylea multipunctata Curtis	*		228.3 ± 66.1	†
Cyrnus trimaculatus (Curtis)		10.9 ± 5.1		
Notonecta glauca L.		0.9 ± 0.7	0.1 ± 0.1	
Callocorixa praeusta (Fieber)	*	0.5 ± 0.3		
Corixa punctata (Illiger)	*	4.5 ± 0.9	0.2 ± 0.2	†
Sigara dorsalis (Leach)	*	6.6 ± 1.1	0.3 ± 0.2	†
Sigara falleni (Fieber)	*	5.5 ± 1.2	0.3 ± 0.2	†
Sigara lateralis (Leach)	*	3.8 ± 1.1	0.1 ± 0.1	†
Sigara stagnalis (Leach)	*	0.4 ± 0.3		
Cloëon dipterum (L.)	*	1816.4 ± 277.7	762.6 ± 139.9	†
Sialis lutaria (L.)			0.2 ± 0.2	
Dytiscus marginalis L.		.	0.04 ± 0.04	
Acilius sulcatus (L.)		0.2 ± 0.1		
Agabus bipustulatus (L.)		0.4 ± 0.2		
Agabus nebulosus (Forester)		0.3 ± 0.1	0.04 ± 0.04	†
Colymbetes fuscus (L.)		0.6 ± 0.3		
Hydroporus negrita (Fab.)		0.1 ± 0.1		
Hygrotus inaequalis (Fab.)		0.1 ± 0.1		
Ilybius ater (Degeer)		0.7 ± 0.4		
Laccophilus minutus (L.)		0.4 ± 0.3	0.5 ± 0.3	
Dytiscidae sp. (larvae)		0.7 ± 0.3		†
Haliplus lineatocollis (Marsham)	*	0.0 ± 0.0		
Haliplus sp. (larvae)	*	0.5 ± 0.3	0.2 ± 0.2	
Gyrinus caspius Menetries		0.1 ± 0.1		
Chaoborus crystallinus (De Geer)		18.2 ± 5.9	31.2 ± 12.5	
Chironomus riparius Miegen		665.6 ± 185.7	245.2 ± 53.5	†
Microtendipes sp.		0.6 ± 0.2	0.3 ± 0.1	
Microspectra sp.		0.5 ± 0.2	0.4 ± 0.2	
Cricotopus sylvestris (Fabricius)	*	233.0 ± 95.7	127.2 ± 27.7	†
Psectrotanypus varius (Fabricius)		2.7 ± 0.7	6.7 ± 2.3	
Culex pipens (L.)		3.0 ± 2.0	0.2 ± 0.2	
Ceratopogon sp.			0.7 ± 0.4	

*Species with the potential to graze attached periphyton.
†Species found in the guts of fish in year 2.

Discussion

There is strong evidence that positive feedback mechanisms structure the community composition of shallow lakes (Scheffer *et al.* 1993). Although the alternative equilibria of plant and phytoplankton dominance are maintained under changing conditions by such positive feedbacks, these mechanisms may not necessarily be involved in switches between these communities. Theoretical models utilize stochastic events to produce an imbalance in the relative abundance of species, from which positive feedbacks enforce attraction to an equilibrium (Petraitis & Latham 1999). In terrestrial and coastal systems, given a constant high input of recruits, this stochasticity takes the form of physical disturbance. Such events are relatively rare

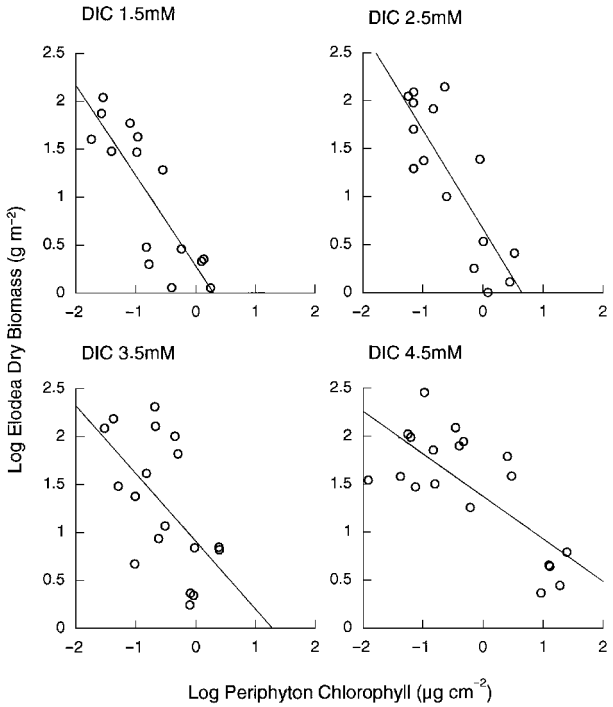

Fig. 8 The influence of DIC concentration on the relationship between the mean density of periphyton and the final biomass of *Elodea nuttallii* in the experimental ponds. (From ANCOVA, periphyton $P = 0.0001$; DIC $P = 0.023$; year $P > 0.5$).

in shallow lakes (although they do have the expected impact, e.g. Mitchell 1989; Blindow *et al.* 1993), and are insufficient to explain the community changes characteristic of eutrophication. As such a deterministic process has been suggested as being important in the switch from plant to phytoplankton dominance, namely suppression of plant growth through a nutrient-mediated increase in periphyton (Phillips *et al.* 1978).

Laboratory experiments have shown an increase in periphyton with nutrients (Jones *et al.* 2000b,c) and strong negative interactions between periphyton and plants (Jones *et al.* 1999). Although such experiments are capable of elucidating detailed interactions, they tend to focus on only one process at a time and exclude other components which may influence the outcome of the interaction in question. This limitation is of particular importance when testing interactions in a biological community structured by a suite of positive feedback mechanisms, such as in shallow lakes. We wanted to investigate these interactions at a scale that would enable all the components to be present at natural densities and concentrations, while maintaining a high degree of control not possible in the field. Whilst our experimental ponds represented a major improvement on laboratory scale experimentation, they were more spatially and temporally confined than natural shallow lakes, and conclusions from their use must be cautious.

We worked from the presumption that nutrient availability is the prime determinant of community composition in shallow lakes, but that variation in their DIC concentration is likely to have consequences for the interactions between phytoplankton, periphyton and plants (Jones *et al.* 2000b). The experiment was designed to determine whether a nutrient-mediated increase in periphyton causes suppression of plant growth, through a reduction in the supply of light and/or carbon to the host plant, thus potentially forcing the switch from plant to phytoplankton dominance. Although anecdotal evidence suggests that the loss of plants occurs rapidly (Mason & Bryant 1975; Bales *et al.* 1993; Blindow *et al.* 1993) and the outcome of one growing season may be sufficient to determine community structure for a prolonged period thereafter, the experiment was not intended to test if alternative community equilibria are stable, merely whether they are possible and what factors determine their inception.

In the first year of the experiment the different DIC treatments had no effect on plant growth, but there was a marked effect of nutrients (Fig. 1). Massive phytoplankton populations built up very rapidly under high nutrient loading, attaining chlorophyll concentrations in excess of 100 µg L^{-1} (Fig. 2). This was in part a consequence of the prolonged period involved in setting up an experiment of this size; phytoplankton populations were already established by the time the plants and zooplankton were added. Even though the phytoplankton were predominantly edible, small green algae (mainly *Chlorella* sp. and *Schroederia setigera* (Schroed.) Lemmermann), and toxic species were absent, the densities attained at high nutrient loading were too high for the added zooplankton to control them. Zooplankton numbers were reduced in the high-nutrient treatments (Fig. 3), possibly as a result of phytoplankton-induced pH and/or oxygen stresses (Hansen *et al.* 1991; Bekliogl u & Moss 1995). Although this effect may have been an artifact due to experimental design, such a

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positive feedback of high phytoplankton abundance on zooplankton has been suggested before (Gliwicz 1990). Large populations of phytoplankton at the highest nutrient loading created highly turbid conditions, making light reduction the most likely cause of the observed failure of submerged plants to grow (Fig. 1).

Contrary to expectations based on laboratory results, increased nutrient loading had no effect on the abundance of periphyton growing on the surface of the plants; neither did DIC concentration (Fig. 4). The density of periphyton in all treatments was low compared to that found in laboratory experiments (1.2–3.9 μg chlorophyll cm^{-2} for periphyton growing on *E. nuttallii* under the same range of nutrient loadings (Jones *et al.* 1999)), being more typical of communities subject to intense grazing (Jones *et al.* 2000c). A large number of invertebrates, including many species which feed upon periphyton, colonized the ponds, either through accidental introduction with the plants or naturally from surrounding populations (Table 2).

In the second year of the experiment the ponds were covered during setting up with black polythene, so phytoplankton densities were still low at the start of the experiment when plants and zooplankton were added. Zooplankton were able to control phytoplankton and prevent it reaching the massive proportions found the year before (Fig. 2). Numbers of *Daphnia magna* were higher at increased nutrient availability, presumably as a result of greater phytoplankton production. Numbers of *D. magna*, the largest zooplankter, decreased with time (Fig. 3) as they were eaten by fish, and phytoplankton numbers increased. The numbers of smaller zooplankton increased with time, presumably as they were released from competition with *D. magna*. As phytoplankton densities were far lower than in the previous year, the pronounced effect of nutrient loading on plant growth was not seen (Fig. 1). Even at high nutrient loading, the mean phytoplankton chlorophyll *a* in all the tanks remained below 100 μg L^{-1}, the level above which plants had not grown the previous year (Fig. 6).

The numbers of grazing invertebrates in the ponds was on average lower in the second year (Table 2), probably as a result of predation by fish with a wide-ranging diet, including zooplankton and benthic invertebrates (Table 2). Predatory fish are known to influence benthic invertebrates in standing waters (Diehl & Kornijów 1997). This reduction in invertebrate grazing pressure may explain why periphyton was more abundant on the surfaces of *E. nuttallii* in the second year. Over the two years, grazing intensity (rather than the availability of nutrients) determined the abundance of periphyton on the plants (Fig. 7). A range of species was included when calculating these numbers, as there is no systematic difference between taxa in the rate of periphyton removal (Cattaneo & Mousseau 1995). It has been suggested that snails are the most important grazers in shallow lakes (Brönmark & Weisner 1992). However, the density of snails alone was insufficient to explain the variation in abundance of periphyton ($P > 0.1$), as this excluded large numbers of efficient grazers such as mayfly and chironomid larvae (Jones *et al.* 1997). Estimates calculated on biomass of grazers also produced a significant negative relationship with periphyton biomass, but the fit was less good ($R^2 = 0.183$).

The increased periphyton abundance in year 2 had a marked effect on the growth of the plants. Although there was no significant influence of nutrients on the final biomass of *E. nuttallii*, there was a significant effect of DIC concentration (Fig. 1). This cannot have been a direct effect of carbon availability since the same conditions did not affect plant growth in the previous year (Fig. 1). Over the two years there was a negative relationship between periphyton density and final plant density, which became significantly less steep with increasing DIC (Fig. 8), indicating that periphyton and plants were competing for carbon as well as light (albeit asymmetrical competition). At low DIC concentration both the availability of free CO_2 and the buffering capacity of the water are reduced, accentuating any competition for inorganic carbon between plants and algae (Jones *et al.* 2000b). The further influence of time and nutrients on pH and free CO_2 was a reflection of increasing biological activity, as photosynthesis depletes CO_2 in still water bodies, where replenishment rates from the atmosphere and benthos are insufficient to balance demand (Jones *et al.* 1996).

It may have been possible for the plants to ameliorate the effect of periphyton on CO_2 availability by adapting their physiology to exploit HCO_3^- as a carbon source where necessary (Jones *et al.* 1993). *E. nuttallii* was specifically chosen for this ability common to many aquatic plants (Maberly & Spence 1983). However such use of HCO_3^- incurs considerable physiological cost (Raven & Lucas 1985; Spence & Maberly 1985), which would be reflected in reduced growth of the plants. Marl formation on the adaxial leaf surface, a characteristic symptom of HCO_3^- use by members of the Hydrocharitaceae (Jones *et al.* 1993), was not seen in any of the treatments.

The results of these experiments appear to contradict directly the hypothesis proposed by Phillips *et al.* (1978) to account for macrophyte decline in progressively eutrophicated fresh-waters. Although periphyton did reduce plant growth, periphyton abundance was not determined by nutrient availability but by the grazing action of invertebrates. Increased nutrient loading merely resulted in an increase in the numbers of invertebrates per unit plant surface, indicating a positive feedback which increased grazing pressure on periphyton (Jones *et al.* 1999). The periphyton growing on the sides of the tanks, on the other hand, did show a marked response to increased nutrient availability. As the fish presumably had greater access to the sides of the ponds, it is probable that the predation pressure was more intense here, and that the plant beds were acting as refugia for invertebrates, in much the same way as for zooplankton (Timms & Moss 1984). Modifications

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of invertebrate behaviour in response to the presence of predatory fish may also have contributed to higher algal abundance in exposed positions, as has been described for grazing invertebrates in streams (McIntosh & Townsend 1996). Thus, grazing invertebrates appear to act as a positive feedback mechanism controlling periphyton abundance in a close parallel to the action of zooplankton on phytoplankton.

The concentration of DIC did have a significant influence on the performance of the primary producers in the ponds. As predicted (Jones *et al.* 2000b), carbon competition between periphyton and plants was more intense at low DIC concentration, with plants growing less well at the same density of periphyton. A consequence of this is that lakes of low DIC concentration will be more prone to plant loss with eutrophication than those of higher DIC. There was also an effect of DIC concentration on the growth of periphyton on the sides of the tanks. Increased DIC resulted in a greater biomass of algae (Fig. 5). Both these effects indicate that the concentration of DIC may be very important to the functioning of lakes and that this largely neglected parameter should be considered when comparing lakes, particularly when describing alternative stable states which require identical initial environments. Nevertheless, the results presented here support, rather than contradict, the theory of alternative equilibria in shallow lakes. Communities dominated by either plants or phytoplankton developed under high nutrient loading, dependent upon the initial abundance of phytoplankton. Although there was the expected negative relationship between periphyton abundance and plant performance, periphyton abundance was not determined by nutrient availability. Instead it was governed by population densities of grazing invertebrates, which in turn were greatly influenced by predatory fish, as is likely to be the case under natural conditions. Trophic cascades can and do occur in structured environments (Pace *et al.* 1999). In contrast to terrestrial and coastal systems, where there is a constant high input of recruits, the influence of stochastic events on the fish community, through the vagaries of colonization and reproduction, is likely to be important in determining the final outcome for aquatic plants (Brönmark & Weisner 1992).

Acknowledgements

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Jeff Kelble <jeff@potomacriverkeeper.org>

[FWD: RE: Cow poop dumping]

leslie.mitchell@fnfsr.org <leslie.mitchell@fnfsr.org> Mon, Jul 9, 2012 at 1:29 PM
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>

Jeff - looks like you were probably right about the algal mat. I hadn't seen that type of thing before. I have a couple of pictures if you are interested. I will pass on what Ted and Billy come up with. I will also pass your info. on to the person that called in about the issue - you can talk to her about her recreational experience.

Leslie

> -------- Original Message --------
> Subject: RE: Cow poop dumping
> From: "Kain, Donald (DEQ)" <Donald.Kain@deq.virginia.gov>
> Date: Mon, July 09, 2012 1:21 pm
> To: "leslie.mitchell@fnfsr.org" <leslie.mitchell@fnfsr.org>
> Cc: "Welcher, Jennifer (DEQ)" <Jennifer.Welcher@deq.virginia.gov>
>
> Hi Leslie,
>
> I just returned from the river. The material in your photo was indeed still there. Water was still very clear in the river, so the heavy rains must have been local and not influenced the river greatly.
>
> I brought back samples that we will examine in the lab, but based on the appearance and odor (both definitely nasty), I think what we are seeing is decaying blue-green algae mats. I took a trip down the river 2 weeks ago from Deer Rapids to Strasburg with Jeff Kelble specifically to evaluate nuisance algae problems. The material at Black Bear crossing looks the same as the mats we observed on that section of the river. Today I collected some of the material from Black Bear crossing and also from Strasburg Town Park (matted on a log). We'll compare these samples microscopically to see if they are different or the same type of material.
>
> By the way, these blue-green algae mats are quite often mistaken for sewage, due to both appearance and odor.
>
> Was there actually an incident where someone observed or heard firsthand that cow manure was dumped in the river? We would definitely be interested in following up on any credible reports.
>
> I'll let you know Billy and Ted's lab findings.
>
> Thanks.

Don

Don Kain
Water Monitoring & Assessment Mgr.
DEQ Valley Regional Office
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540-574-7878 (fax)
E-Mail donald.kain@deq.virginia.gov

From: leslie.mitchell@fnfsr.org [mailto:leslie.mitchell@fnfsr.org]
Sent: Monday, July 09, 2012 9:08 AM
To: Kain, Donald (DEQ)
Subject: RE: Cow poop dumping

Don - I doubt that there is anything to see at this point - especially after the torrential rain last night. For my future reference, is this something that you guys try to track down - the source of the material?

Leslie

-------- Original Message --------
Subject: RE: Cow poop dumping
From: "Kain, Donald (DEQ)" <Donald.Kain@deq.virginia.gov>
Date: Mon, July 09, 2012 8:04 am
To: "leslie.mitchell@fnfsr.org" <leslie.mitchell@fnfsr.org>

Thanks, Leslie. We'll look into it today.

Don Kain
Water Monitoring & Assessment Mgr.
DEQ Valley Regional Office
P.O. Box 3000
Harrisonburg, VA 22801

540-574-7815
540-574-7878 (fax)
E-Mail donald.kain@deq.virginia.gov

From: leslie.mitchell@fnfsr.org [mailto:leslie.mitchell@fnfsr.org]
Sent: Friday, July 06, 2012 4:02 PM
To: Kain, Donald (DEQ)
Subject: Cow poop dumping

Hi Don - I had a report earlier this week that some one dumped a great deal of cow dung in the river, upstream of Blackbear Crossing, east of Maurertown. They observed the dung floating in the river and today I went

> > down and a small amount appeared to have gathered at the low water bridge there.
> >
> > Is this something DEQ is concerned and will do anything about? I don't know the source or how the stuff got in the river, just had the reported observation and saw what was there today - pictures attached.
> >
> > Thanks,
> >
> > Leslie

RESEARCH ARTICLE

Taxonomic and functional composition of the algal benthos exhibits similar successional trends in response to nutrient supply and current velocity

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Abstract

In an effort to identify the causes and patterns of temporal change in periphytic communities, we examined biomass accumulation, taxonomic and functional composition, rate of species turnover, and pairwise species correlations in response to variability in current velocity and nutrient supply in artificial stream flumes. Divergent patterns in community growth and succession were observed between nutrient treatments and, to a lesser extent, between flow treatments best described by shifts in taxonomic and functional composition. Specifically, understory low profile species, tolerant to low resource supply, became dominant under low nutrients, while overstory high profile and motile species with higher nutrient demands dominated the high nutrient treatments. Increased resource supply or current velocity did not influence the species turnover rate, measured by a time-lag analysis. Interspecific interactions, especially competition, did not appear to be driving community dynamics, as the number of positive and negative pairwise species correlations ranged between low and extremely low, respectively. The overwhelming majority of correlations were not significant, indicating that species within the biofilm matrix were not perceptibly influencing one another. Thus, temporal trends in taxonomic and functional composition were largely environmentally driven, signifying that coexistence in biofilms is defined by the same mechanism along the hierarchy from species to functional groups.

Introduction

Temporal species replacements or succession has long fascinated ecologists, who have sought to answer whether community development is strongly influenced by interspecific interactions, such as competition for limiting resources or species' individualistic responses to local and regional environmental conditions (Odum, 1969; Drury & Nisbet, 1973; Tilman, 1985; Huston & Smith, 1987). Competitive outcomes depend on resource supply rates and over time, communities exposed to different resource conditions become more dissimilar (Inouye & Tilman, 1988, 1995). This divergence has been attributed to compositional differences (Inouye & Tilman, 1988, 1995), but little is known about whether it can also arise from the presence of unique species in each environment or presence/absence differences. As communities are commonly characterized by dominance of just a few species and lower abundances of many more rarer species, comparisons between communities based only upon species lists or presence/absence data may be misleading if there is a considerable overlap in the presence of rare species (Magurran, 1988). Additionally, if community succession proceeds according to the predictions of the 'initial floristic composition' hypothesis (Egler, 1954), where propagules of the entire sequence are present from the beginning, then succession is merely the development of that initial flora. As algae possess passive and generally unlimited dispersal (Finlay, 2002) and do not necessarily settle in environments matching their requirements, their succession may resemble the initial floristics model. Species that are unsuitable for the local conditions can still

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remain at very low densities despite a lack of reproduction. Therefore, presence/absence data may not reflect actual differences between communities, which may be detectable only by compositional analyses.

Biofilms are complex, three-dimensional entities, composed of species differing in resource requirements and spatial position, that is, tolerant vs. sensitive forms (Passy, 2008; Passy & Larson, 2011). Tolerant species, with a short habit and an understory location, have low resource demands and can withstand nutrient limitation that is either environmental (low ambient levels) or biotic (overgrowth and subsequent resource depletion). Sensitive forms, on the other hand, need high resource levels and for that reason must obtain a beneficial spatial position in the overstory, where resource acquisition is unimpeded. This can be achieved either through late successional colonization or extended and motile habit, which explains why larger diatoms tend to be eutrophic (Pringle, 1990; Passy, 2007; Passy & Larson, 2011). Thus, nutrient enrichment in the spatially complex biofilm promotes coexistence of tolerant and sensitive species and greater species richness (Passy, 2008; Passy & Larson, 2011). Conversely, eutrophication in spatially simple communities, such as grasslands and phytoplankton, can cause competitive exclusion and diminished biodiversity (Interlandi & Kilham, 2001; Harpole & Tilman, 2007). The rate of competitive displacement, and with this the rate of succession in the latter communities, increases with resource supply (Tilman, 1988; Prach *et al.*, 1993). As biofilm species overgrow rather than displace one another at high resource levels (Passy & Larson, 2011), it is expected that the rate of succession will remain constant across nutrient regimes. However, nutrient supply can cause biofilm succession to take divergent paths – toward tolerant low profile forms under low supply but toward the coexistence of tolerant species as well as sensitive high profile and motile forms under high supply (Passy & Larson, 2011). Consequently, functional composition changes throughout succession and displays resource-driven temporal trends, but it is not known to what extent taxonomic shifts will follow those observed at the functional level.

Current velocity is another environmental attribute in streams that affects community composition and physiognomy (Biggs & Hickey, 1994; Passy, 2001, 2007). Faster flows, favoring understory species and eliminating many overstory forms (e.g., without firm attachment or loosely aggregated), cause alterations in the biofilm structure similar to nutrient limitation. Intermediate flows, however, are expected to be beneficial for biofilm growth because they stimulate algal metabolism and nutrient uptake (Stevenson, 1996). Although nutrients and current velocity influence colonization, growth, and biomass accumulation in developing biofilms, thus impacting the physiognomy and successional trajectories of these communities (Sutherland, 2001; Rickard *et al.*, 2004), experimental research examining the effects of both factors on community dynamics is limited. Several studies have examined current velocity and nutrient abundance in combination (Horner & Welch, 1981; Horner *et al.*, 1983, 1990), but these studies focused primarily on the response of algal biomass accumulation and less on temporal trends in taxonomic composition. Here, we explored the successional trends in periphyton communities across four current-nutrient treatments and tested the following hypotheses: (1) at low to intermediate velocities, successional trajectories will diverge more strongly in response to nutrient additions than current manipulations owing to the strong nutrient dependence of sensitive forms; (2) this divergence will encompass species and functional composition much more so than species presence/absence; and (3) the rate of succession will not vary appreciably across nutrient and flow regimes because competition, implicated in driving successional rates, is expected to be weak.

Materials and methods

Artificial stream flumes

Experiments were conducted in four oval-shaped laboratory streams, each with an experimental trough measuring 80 cm in length, 12 cm in width, and 13 cm in depth. Eighty liters of modified Guillard's WC medium (see below) was recirculated in each stream channel at uniform current velocity (±1 cm s^{-1}), measured by a Marsh-McBirney model 2000 flowmeter (Marsh-McBirney Inc, Frederick, MD). Current velocity in each stream was maintained by adjusting a belt and multiple drive step pulleys attached to a 1.5-hp motor and a water pump. Drop-in chillers (1/5 hp; TradeWind Chillers, Escondido, CA) maintained stream water at room temperature (~ 20 °C) in the high velocity channels, while water temperature in the low-velocity channels was at room level. In each experimental trough, 4.9 × 4.9 cm unglazed porcelain tiles were placed equidistant from one another. A 250-W metal halide lamp, positioned above each experimental trough, provided light on a 14 : 10 daily light/dark ratio at levels sufficient to saturate photosynthesis of attached algae, that is, ~ 200 μmol m^{-2} s^{-1} (Hill, 1996).

Algal succession was examined under different current and nutrient regimes. Streams were subjected to constant flows of either 10 or 30 cm s^{-1}, shown in natural streams to cause differences in algal immigration and emigration rates, and biomass accumulation (Stevenson, 1996). Additionally, nutrient concentration was varied across current

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regimes with either high (800 μmol N-NO_3 and 50 μmol P-PO_4) or low (20 μmol N-NO_3 and 1.25 μmol P-PO_4) levels in modified Guillard's WC media (Guillard, 1975). Other than the manipulation between treatments of nitrate and phosphate, modified WC media consisted of all constituents in their normal concentrations. Nutrient analyses of water samples, collected at the time of algal sampling, were performed with AutoAnalyzer III (SEAL Analytical Inc., Mequon, WI). In our low nutrient treatments, average concentrations (μg L^{-1}) of NO_3^- and PO_4^{3-} were 343–407 and 6.44–6.53, respectively, which were within the ranges shown to limit algal communities (Earl *et al.*, 2006; Hill & Fanta, 2008). Conversely, concentrations in the high nutrient treatments, averaging 7730–9123 for NO_3^- and 840–1165 for PO_4^{3-}, greatly exceeded these values. Hence, there were four different treatments: low nutrients at 10 and 30 cm s^{-1}, referred to as 10-low and 30-low, respectively, and high nutrients at 10 and 30 cm s^{-1}, referred to as 10-high and 30-high, respectively. The limited number of channels did not allow for replication in each flow × nutrient treatment, so the experiment itself was replicated three times in November and December 2006 and February 2007, with each experimental run lasting 35 days when sloughing occurred in some channels. For the duration of each run, 24 L of water was replaced every third day with new medium.

All artificial streams were seeded once, at the beginning of the experiment, with epilithic algae from several small streams in the Dallas-Fort Worth area encompassing diverse physicochemical conditions. Seed algae were suspended in carbon-filtered water, and 2 L of this mixture was dispensed to each stream. Not unexpectedly, taxonomy varied between the three seed communities; however, the multivariate procedures we employed in PRIMER (see below) account for variability between replicate runs.

Sample preparation and analysis

After allowing an initial period of 7 days for biofilm colonization, two tiles were randomly retrieved from each stream channel. Tiles were taken from the same locations within each stream on days 7, 11, 14, 18, 21, 25, 28, and 35. Each retrieved tile was placed into an accompanying Petri dish with enough distilled water to cover the tile. Following procedures in Larson & Passy (2005), five random fields on each tile were examined with a Zeiss Axioplan 2 LSM 510 META confocal microscope (Zeiss, Jena, Germany), using a Achroplan 40 ×/0.80 numerical aperture water-immersion objective. Total algal biovolume in each field was quantified with 3-D for LSM software (Zeiss, Jena, Germany), and a mean value for the five fields was obtained and converted to total biovolume of algae per square centimeter (Larson & Passy, 2005). After biovolume quantification with confocal microscopy, the biomass on the surface of each tile was removed with a razor blade and a toothbrush until visibly clean. The tile was then returned back into the streams but never retrieved again for the duration of the experiment. Biomass from the two tiles was consolidated, suspended in carbon-filtered water, and preserved in 4% buffered formalin solution. Samples were uniformly mixed by pulse sonification, and a subsample was placed into a Palmer-Maloney counting cell and observed under a light microscope at 400× magnification. Algal community composition was assessed by counting a minimum of 500 algal units, where an algal unit was an individual cell for unicellular organisms, a 10 μm length for filaments and a 10 × 10 μm area for colonies. Soft algae were identified in this count, and diatoms lumped into a single taxonomic category. For diatom species identification, material was acid-digested, washed with carbon-filtered water, and mounted in Naphrax® (Brunel Microscopes Ltd., Chippenham, Wiltshire, UK) mounting medium. At least 300 units (one frustule or two valves) were counted and identified for each sample at 1000× magnification. Counts were converted to density of cells per surface area of tiles (number of cells cm^{-2}).

Designation of functional guilds

Soft algae, including cyanobacteria and green algae, and diatoms were grouped into three guilds based on growth morphology, that is, low profile, high profile, and motile species, following Passy (2007) and Passy & Larson (2011). Briefly, species in the low profile guild were short-statured, encompassing prostrate, adnate, erect, solitary centric forms, small colonies, cenobia, and slow moving species. The high profile guild comprised species of tall stature, including erect, filamentous, branched, chain-forming, tube-forming, and stalked species, and colonial centric diatoms. The motile guild was composed of comparatively fast moving species, for example, flagellated green algae or biraphid diatoms. The low profile forms can be classified as tolerant, as they peak in oligotrophic conditions, while the high profile and motile species as sensitive because of their preference for high nutrient concentrations (Passy, 2007).

Statistical analyses

Determination of successional composition

Differences in algal community structure between treatments were analyzed with PRIMER software application (version 6.1; Plymouth Marine Labs, Plymouth, UK). Compositional and functional group similarities between

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samples were computed with the Bray–Curtis coefficient, while presence/absence similarities with the Sørensen's coefficient. Within the PRIMER software, MDS, ANOSIM, SIMPER, and BIOENV routines were performed.

Analysis of similarities (ANOSIM) of species abundance and presence/absence data was used to determine the differences between experimental treatments. ANOSIM calculates an R-statistic, which varies between 0 and 1. A value of 1 indicates that all replicates within a treatment are more similar to each other than to any replicate from a different treatment, while a value of 0 indicates that similarities between and within treatments are the same on average (Clarke & Warwick, 2001). Therefore, the value of the R-statistic reflects the observed differences between treatments, contrasted with differences among replicates within treatments; however, the number of replicates in the groups being compared does not bias the R-statistic (Clarke & Warwick, 2001). Ordination with nonmetric multidimensional scaling, considered a robust ordination technique for ecological analyses of successive time points, which are not independent (Clarke & Warwick, 2001), was employed to provide a graphical summary of the relationship among communities. Additionally, average abundance for each taxon was calculated across the three replicates in the different treatments for days 7, 21, and 35, representing early, mid, and late successions, respectively. For each of the 3 days, a subset of species contributing a minimum of at least 90% of the total abundance in each treatment was used in the BIOENV procedure. It calculates the Spearman correlation coefficient between the rank similarity matrices of biota (measured as Bray–Curtis similarity) and environment (measured as Euclidean distance) (Clarke & Ainsworth, 1993). The environmental data for each treatment consisted of binary variables, representing the two levels of flow and nutrient regime.

The pairwise R-statistic values for each sampling time point obtained from ANOSIM were regressed against day of colonization using the curve-fitting software TABLECURVE 2D 5.01 (SYSTAT Software Inc., Richmond, CA). Total algal biovolume accumulation and rates of succession were also regressed against day of colonization with TABLECURVE 2D. For these analyses, we adopted a parsimonious approach of selecting the simplest and biologically meaningful model with a good fit and high r^2.

Determination of successional rates

Successional rates for each treatment were calculated using a time-lag analytical approach shown to be an effective technique for examining rates of change (i.e., successional rates) when there are a limited number of observations in a time series (Collins *et al.*, 2000). As the number of data points in our time series (e.g., eight time measurements) was limited, giving more weight to potentially extreme values, we chose the more robust time-lag approach than measuring distances between sequential time points. We calculated a triangular dissimilarity matrix from a species-by-time rectangular data matrix using Bray–Curtis similarity and Sørensen's coefficient (for species composition and presence/absence, respectively). Similarity values were obtained between all pairs of each of the eight sampling periods or time lags in each treatment. For each treatment, Bray–Curtis similarity and Sørensen's coefficient were regressed against time lag and the relationship examined for statistical significance and/or nonlinearity. A linear model best explained the relationship for each treatment, and model II regression with no constant was employed for the comparison of treatment slopes using the model:

$$y_{ij} = b_{1,1}x_1 + b_{1,2}x_2 + b_{1,3}x_3 + b_{1,4}x_4 + b_{0,1} + b_{0,2} + b_{0,3} + b_{0,4} + \varepsilon$$

where, y_{ij} = Bray–Curtis similarity or Sørensen's coefficient between all pairwise sample dates i within each treatment j (j = 1–4), where, x_{1-4} = lag period in 10-low (1), 30-low (2), 10-high (3), and 30-high (4); $b_{1,1-4}$ and $b_{0,1-4}$ = slope and intercept for the respective treatments; and ε = error term. Slope values (b_1) for the four treatments indicated the rate of succession.

Species interactions

True measures of interactions between pairs of species in multispecies assemblages are not possible, yet a pseudo-measure of the type of interactions can be calculated using pairwise correlations (Pearson's r) of ln-density values over the course of the experiment. Sixteen species, each comprising > 1% relative abundance over at least two consecutive time periods in an experimental run, were used in these analyses. No apparent nonlinearities were observed in the pairwise species relationships when fitting with a LOWESS smoother; therefore, we proceeded with a correlation analysis. Significant correlations ($P < 0.05$), either positive or negative, were taken as an indicator of whether pairwise interactions between species were 'positive' or 'negative', respectively, while nonsignificant correlations suggested 'neutral' interactions. Notably, the presence of a correlation between two species is ambiguous; that is, it can originate from shared responses to a common environment and/or from an interspecific interaction. However, the lack of correlation indicates that the two species do not interact perceptibly. This may

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be due to the absence of direct interactions, such as facilitation or inhibition, or to community effects, like competitive networks, which weaken the strength of individual pairwise interactions (Huisman & Weissing, 1999). The three types of pairwise correlations were tabulated and converted to a percent of all interactions per experimental run. This percent was then used as a dependent variable in a two-way ANOVA, with treatment and correlation type as factors.

Results

Total community biovolume

Total algal biovolume increased initially and subsequently leveled off in all treatments, captured by the model $y = a + b/x^2$ (Fig. 1). The highest total biovolume (as indicated by the 'ceiling' parameter a in this model) was observed in the 30-high treatment and the lowest total biovolume in the 30-low treatment. The temporal response of total biovolume in the high nutrient treatments was similar between current treatments, with higher biovolume in the 30-high treatment late in succession as evidenced by a higher 'ceiling' parameter (95% CI: 20.41–20.59 for 30-high vs. 20.00–20.28 for 10-high). In the low nutrient treatments, total biovolume was higher in the 10-low treatment compared to the 30-low treatment (95% CI: 18.98–19.49 for 10-low vs. 18.26–18.95 for 30-low).

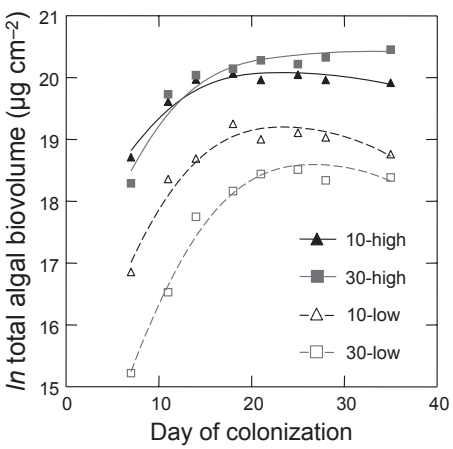

Fig. 1. Change in total community biovolume with day of colonization for current-nutrient treatments. All treatments were fit with the model $y = a + b/x^2$, with all parameters significant at $P < 0.05$, where a = maximum observed ln community biovolume. 10-high: $y = 20.14 - 67.01/x^2$, $r^2 = 0.937$, $P < 0.0001$, 30-high: $y = 20.50 - 105.91$, $r^2 = 0.989$, $P < 0.0001$, 10-low: $y = 19.24 - 112.96/x^2$, $r^2 = 0.929$, $P = 0.0001$, 30-low: $y = 18.61 - 175.46/x^2$, $r^2 = 0.945$, $P \leq 0.0001$.

Community composition, presence/absence, and functional groups

Examination of an MDS of mean species densities revealed early separation between nutrient treatments, which remained distinct throughout succession (Fig. 2a). Within nutrient treatments, differences between flow regimes were more subtle, with dissimilarity arising early in succession at low nutrients but later at high nutrients. Specifically, early in succession, the flora across experiments was largely composed of *Achnanthidium minutissimum*, *Gloeocystis ampla*, and *Gomphonema parvulum*; however, the most discriminative species were the dominant *A. minutissimum* and the subdominant *Nitzschia palea* (Supporting Information, Table S1). Mid-successional communities were dominated by *Lyngbya vandenberghenii*, *N. palea*, *Scenedesmus bernardii*, and *G. ampla* in the high nutrient treatments, while in the low nutrient treatments, *Ankistrodesmus braunii* was abundant in 10-low and *A. minutissimum* in 30-low. With the exception of *A. braunii*, all of the above species were good discriminative species on day 21. In the later stages, the low nutrient treatments became dominated by *A. braunii* in 10-low and *A. minutissimum* in 30-low, with the cyanobacterium *Chamaesiphon fuscus* abundant in both flow treatments. All these species were tolerant to low nutrient conditions (Sládeček, 1973; Van Dam *et al.*, 1994; Rott *et al.*, 2006). Late successional communities in the high nutrient treatments were dominated by several species of *Scenedesmus*, several motile diatoms (e.g., *N. palea* in both current treatments, *Luticola mutica* in 30-high, and *Fistulifera saprophila* in 10-high), and several filamentous cyanobacteria, for example *Phormidium inundatum* in 30-high and *L. vandenberghenii* in 10-high, all of which were characteristically eutrophic and pollution-tolerant (Palmer, 1969; Pringle, 1990; Vis *et al.*, 1998). On day 35, *S. bernardii*, *A. braunii*, *C. fuscus*, *L. mutica*, and *F. saprophila* were the species responsible for discriminating among treatments.

An MDS of mean presence/absence data revealed some separation between nutrient and current treatments, albeit not as pronounced as that seen for densities (Fig. 2b). Furthermore, points were more scattered and the stress for this MDS was fairly high (0.18), making the interpretation of the plot difficult. Many species in these experiments were observed in all treatments (even if at very low densities), further contributing to the lack of considerable separation between treatments. Of the species that were abundant, *Scenedesmus bijugatus* and *Ankistrodesmus fusiformis* were unique to the high nutrient treatments. The same successional patterns as seen for densities were detected using only three functional groups, indicating high congruence between compositional and functional

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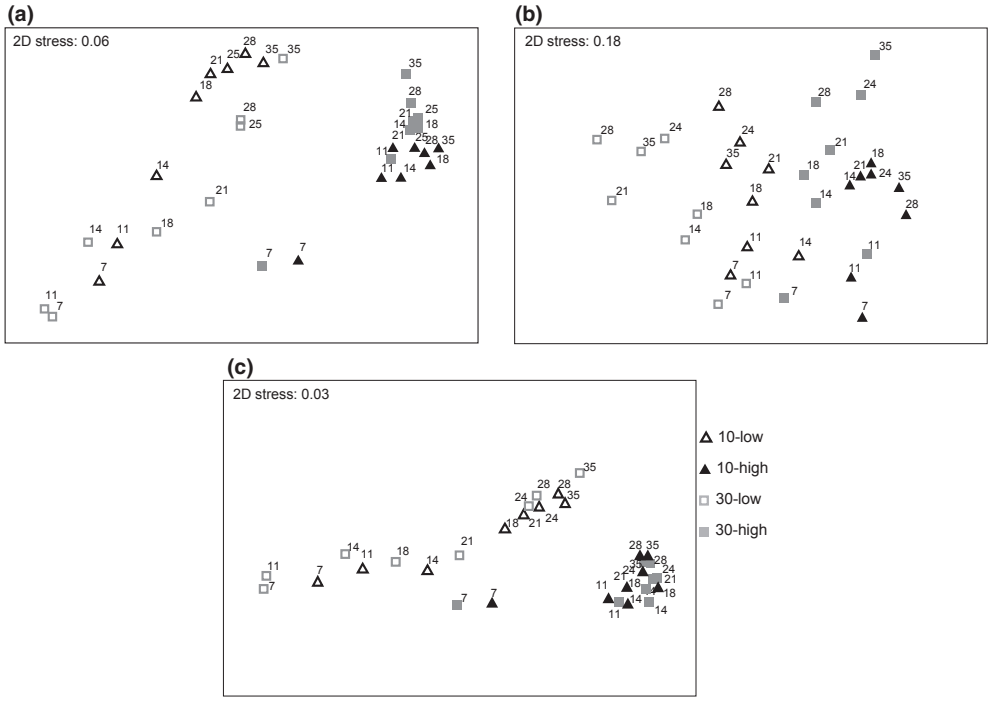

Fig. 2. MDS plots showing divergence between periphyton communities growing in artificial stream flumes exposed to various flow and nutrient supply treatments using (a) Bray–Curtis similarities of untransformed species abundance data (two-dimensional stress = 0.06), (b) presence/absence data using Sørensen's coefficient (two-dimensional stress = 0.18) and (c) untransformed functional guild densities (two-dimensional stress = 0.03). Symbols represent mean values across experiments, and numbers next to symbols represent day of experiment.

response to nutrients and flow (Fig. 2c). In both low nutrient treatments, low profile species were dominant, while all three guilds were abundant in both high nutrient treatments.

R-statistic values using densities and presence/absence were plotted against time, and the trends were similar to those seen in the MDS plots. Values based on abundance increased for all pairwise treatment combinations, revealing an increasing dissimilarity through time (Fig. 3a–f); however, divergence was the strongest between nutrient treatments irrespective of the current treatment (Fig. 3c–f vs. Fig. 3a and b). In the MDS plots, divergence between current treatments was greater and arose sooner in low than in high nutrient treatments. Examination of values obtained with presence/absence data showed that only one comparison was statistically significant, that is, 10-high vs. 10-low treatments with differences starting to emerge at mid-succession (Fig. 3c).

Rate of succession

Increasing dissimilarity in periphyton communities with time was measured with both Bray–Curtis similarity and Sørensen's coefficient (Fig. 4). Linear models best described the trends, meaning that the rate of change was consistent through time; however, the rate was greater using Bray–Curtis similarities, as evidenced by the steeper slopes (Fig. 4a). The rates of change in species composition and presence/absence were not significantly different across treatments as indicated by the considerable overlap in the 95% confidence intervals of the slopes. However, a lack of observed differences in successional rates between treatments should not be construed as a lack of treatment effects, as communities in each of the treatments, most notably in the nutrient manipulations, were progressing toward different end points, that is, tolerant low profile forms at low nutrients and sensitive high profile and motile forms at high nutrients.

Species interactions

In each treatment, the overwhelming majority of pairwise comparisons was nonsignificant, suggestive of predominance of 'neutral' interactions (Fig. 5). The comparatively few significant correlations were generally positive and only occasionally negative, implying that 'positive' but especially 'negative' interactions were uncommon, the latter comprising no more than 3% in each treatment. The three interaction types had significantly different percentages across all treatments, that is 'neutral' (mean of

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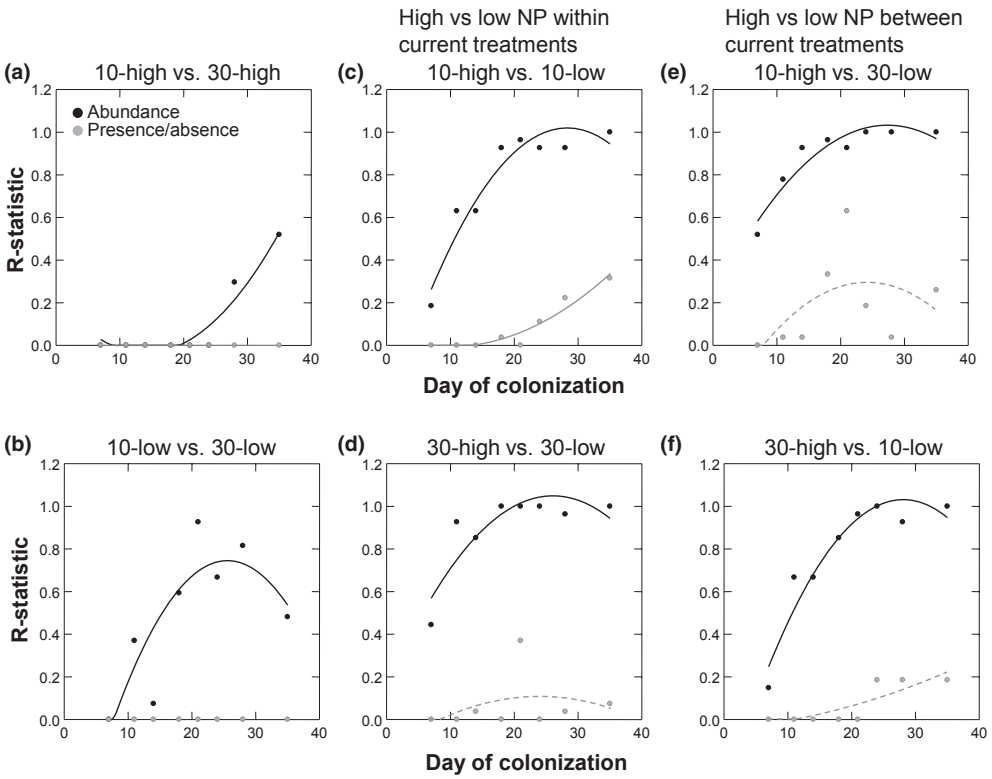

Fig. 3. Change in ANOSIM R-statistic with day of colonization for species abundance and presence/absence data for comparison between flow and nutrient supply treatments. The fits were generated by the following models with all parameters significant at $P < 0.05$. (a) 10-high vs. 30-high, abundance: stepwise regression, presence/absence: no model significant; (b) 10-low vs. 30-low, abundance: $y = -0.79 + 0.12x - 0.0023x^2$, $r^2 = 0.72$, $P = 0.042$, presence/absence: no model significant; (c) 10-high vs. 10-low, abundance: $y = -0.32 + 0.09x - 0.0017x^2$, $r^2 = 0.92$, $P = 0.0019$, presence/absence: $y = -0.085 + 0.0018x^{1.5}$, $r^2 = 0.81$, $P = 0.0009$; (d) 30-low vs. 30-high, abundance: $y = 0.15 + 0.07x - 0.0013x^2$, $r^2 = 0.76$, $P = 0.027$, presence/absence: no model significant; (e) 10-high vs. 30-low, abundance: $y = 1.02 - 25.13/x^2$, $r^2 = 0.98$, $P = 0.000001$, presence/absence: no model significant; (f) 30-high vs. 10-low, abundance: $y = 1.01 - 43.09/x^2$, $r^2 = 0.94$, $P = 0.00002$, presence/absence: no model significant. Nonsignificant trends shown as dashed lines.

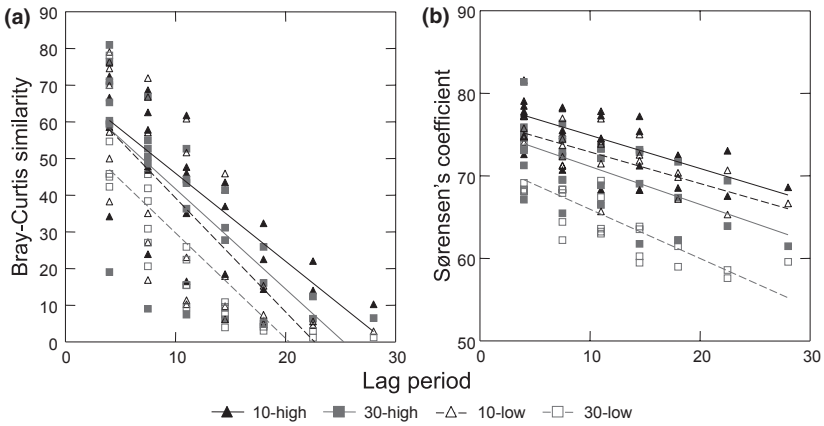

Fig. 4. Rates of temporal change for the different flow and nutrient supply treatments. Sampling days were 7, 11, 14, 18, 21, 24, 28, and 35 with lag period representing the number of days between sampling days. Linear models significant for all treatments and model II regression were performed to estimate 95% confidence intervals for slopes using (a) Bray–Curtis similarities (slope: 10-low: −3.98 to −2.24, 10-high: −3.27 to −1.53, 30-low: −3.75 to −2.00, 30-high: −3.59 to −1.86) and (b) Sørensen's coefficient (slope: 10-low: −0.56 to −0.21, 10-high: −0.58 to −0.23, 30-low: −0.77 to −0.42, 30-high: −0.63 to −0.29).

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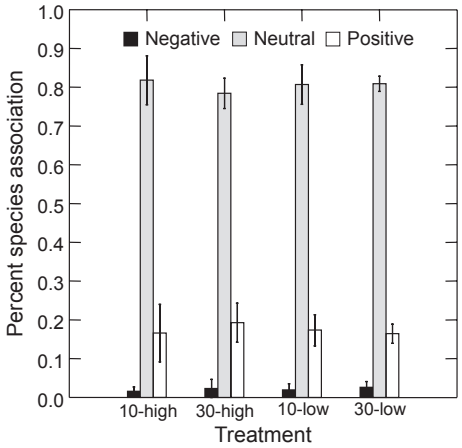

Fig. 5. Percent of pairwise species interactions for various current-nutrient treatments. Interactions classified as either 'positive', 'neutral', or 'negative' based on Pearson's correlations between ln-transformed densities.

80%), followed by 'positive', and finally 'negative' interactions (two-way ANOVA, $F_{2,24} = 726.21$, $P = 0.000001$, followed by Tukey pairwise comparisons, all significant at $P = 0.00001$). These trends did not differ between treatments ($F_{3,24} = 0.00$, $P = 1.00$), and the interaction between treatment and interaction type was not significant ($F_{6,24} = 0.21$, $P = 0.97$).

Discussion

Periphyton communities, subjected to various treatment combinations, exhibited a stronger temporal response to nutrient supply, ranging from low to high, than to current velocity, which was manipulated at low to intermediate levels. Divergence between communities resulted largely from changes in the abundance of different species populations and, to a lesser extent, disparity in species' presence/absence. Species lists or presence/absence data are commonly used to examine similarity between sets of communities, yet our results suggest that these data do not adequately capture the actual differences between communities owing to substantial overlap in species distributions. Conversely, the temporal patterns in composition repeated at the level of algal ecological guilds were distinct across treatments, indicating that succession is defined by taxonomic as much as by functional changes. Examination of both species and functional groups revealed the highest similarity among all treatments early in succession, with dissimilarity between nutrient treatments increasing with time.

Conspicuous and diverging trajectories toward the establishment of species of differential tolerance to nutrient limitation were observed across nutrient treatments, that is, tolerant species at low nutrient supply but sensitive forms in the high nutrient supply. Early in these experiments, there was considerable similarity in species composition between treatments, dominated by small tolerant forms, for example, *A. minutissimum*, which is not unexpected, given treatments were seeded the same. Later in succession, both nutrient treatments sustained their low profile tolerant flora, but high nutrient treatments also accumulated high densities of various high profile sensitive forms, as predicted by the recently proposed benthic model of coexistence (Passy, 2008). Our results support the premise that succession in algal biofilms is largely influenced by environmental or abiotic factors. It proceeds toward dominance of the functional form, both trophic and morphological, that is selected for by the particular environment. These results are in agreement with a growing body of research in both natural and experimental settings, which reveals a predictable response of the present algal ecological guilds to a broad array of environmental impacts, including eutrophication, light availability, flow disturbance, organic pollution, and pesticide exposure (Passy, 2007; Berthon *et al.*, 2011; Lange *et al.*, 2011; Passy & Larson, 2011; Rimet & Bouchez, 2011).

The separation in species composition between current regimes within nutrient treatments was not as striking. Current can have both positive and negative effects on periphyton communities (Stevenson, 1996), but limited influence on nutrient uptake when benthic algae are nutrient replete (Borchardt, 1994). In our experiments, dissimilarity between current treatments at high nutrients only emerged toward the end of our experiments and was largely because of the differences in the relative abundance of a few species at each treatment. Similarly, total biovolume accumulation in current treatments at high nutrients differed only at the end of succession. Under low nutrient conditions, differences between current treatments were apparent earlier as *A. minutissimum* became very abundant at 30-low, while *A. braunii*, with no mode of attachment, at 10-low. In the low nutrient treatment, accumulation of total algal biovolume was reduced in the high current treatment; perhaps reflecting the difficulty some species had dealing with current stress. These results indicate that the flow gradient exerted stronger control over succession in the nutrient-deplete communities than in the nutrient-sufficient communities.

Admittedly, divergence between treatments may have been partially facilitated by a limited supply of propagules emanating only from the developing communities within each stream, as high immigration rates can lead to greater homogenization in species composition (Leibold *et al.*, 2004). However, in microbial communities, the importance of local factors such as resource availability may be

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more important than immigration after initial colonization. In the periphyton, colonization ability influences early dynamics on bare substrates (Stevenson, 1983; Stevenson & Peterson, 1991), while specific differences in growth rates become important once surfaces are colonized (Peterson, 1996a, b). Despite continual immigration in natural streams, periphyton communities are highly responsive to environmental conditions, especially water chemistry, which has led to their frequent use as bioindicators of stream conditions (Hill *et al.*, 2000). In our experiments, many of the late dominant species were initially nearly undetectable, either in the seed algae or early in community development, but became very abundant later in succession, for example, *Scenedesmus* and *C. fuscus* in high and low nutrients, respectively. This is more consistent with the predictions of the 'initial floristics' than the 'relay-floristics' model of succession (Egler, 1954), which is to be expected in a community marked by species overgrowth rather than by displacement.

Using a time-lag analytical approach to examine the differences in species composition between samples at increasing time intervals, we observed significant linear trends, which confirmed that our assemblages were undergoing strong directional changes. Temporal trends were best explained by linear models, indicating the rate of succession was uniform for the duration of our experiments. This was consistent with our initial predictions but contrary to other studies which have shown a decreasing species turnover through time (Prach *et al.*, 1993; Myster & Pickett, 1994; Anderson, 2007). Transient dynamics or competitive displacement of successive species has been suggested as a mechanism for higher rates of succession in fertilized systems (Tilman, 1988). This does not seem to be the case in our experiments, where most interactions in the benthos appeared to be 'neutral'. The very low number of 'negative' interactions suggests that strong competition was not a major driving force in our periphyton communities. A diminished role of 'negative' interactions is expected under the benthic model (Passy, 2008) and in agreement with field observations of 'three-dimensional' communities, shown to be driven by tolerance to overgrowth rather than competitive exclusion (McCormick & Stevenson, 1991; Airoldi, 2000; Passy, 2008). Furthermore, the high spatial complexity of biofilms translates into greater internal resource gradients, providing more opportunities for coexistence and easing negative interspecific interactions (Passy & Legendre, 2006; Passy, 2008). 'Neutral coexistence' of functional groups, neither facilitating nor suppressing one another, was proposed as a mechanism of succession in the biofilm at a functional level (Passy & Larson, 2011). Here, we see that at a species level, coexistence throughout biofilm succession was largely neutral as well. This suggests that contrary to many terrestrial systems, in periphytic biofilms, interspecific interactions may play a marginal role in temporal community organization, while the environment and particularly nutrient supply control both the taxonomic and functional composition of this community. These findings also emphasize the effectiveness of algal functional groups in capturing as general a community pattern as primary succession.

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Authors' contribution

C.A.L. and S.I.P. conceived and designed the experiments. C.A.L. performed the experiments. C.A.L. and S.I.P. analyzed the data and wrote the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Species abundance (density cm^{-2}) averaged across replicates for each treatment on days 7, 21, and 35.

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WATER QUALITY CRITERIA

SECOND EDITION
By
McKEE & WOLF
(1963)

Publication 3-A
(Reprint, June 1, 1974)

CALIFORNIA STATE WATER RESOURCES CONTROL BOARD

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SECOND EDITION

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PREPARED WITH ASSISTANCE FROM
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REVISED 1963

THE RESOURCES AGENCY OF CALIFORNIA
STATE WATER RESOURCES CONTROL BOARD

Publication No. 3–A

PREFACE TO THE FIRST EDITION

Strictly speaking, the first edition of WATER QUALITY CRITERIA did not have a preface as such. Instead, the information normally presented in a preface was contained in the letter of transmittal. The following material has been excerpted from that letter.

In compliance with the terms of the contract by and between the State of California and the California Institute of Technology, dated 4 January 1951, an investigation has been made of the technical and legal literature pertaining to water-quality criteria for the various beneficial uses of water. The report submitted herewith embodies a condensation and critical evaluation of such literature, along with a bibliography and appendices.

A prominent feature of the new legislation for water-pollution control in California is the provision for a "case-by-case" study of each problem of pollution, with abatement requirements to be based upon the economics of waste treatment as well as upon the injury to potential beneficial uses of polluted waters. Of necessity, this "case-by-case" stipulation precludes a broad-brush treatment on a state-wide or even regional basis, and it rules out rigid stream standards and the arbitrary zoning of streams or underground basins. In effect, then, it depends on astute judgment by members of the water-pollution-control boards and such judgment must be founded on the most recent, complete, and reliable compendium of data pertaining to the limiting and threshold concentration of each potential polluting substance, for each possible beneficial use of the water.

It was the primary aim of this project to assemble, condense, and evaluate the readily available literature pertaining to water quality and its effects upon the beneficial uses of water. Such literature includes the fundamental work of original investigators, as well as standards and requirements of other state and interstate agencies. The scope of the survey included, furthermore, a review of judicial expressions to determine court rulings and decisions that have dealt with the reasonableness or unreasonableness of water-quality standards as established by public agencies. The second aim of the project was to present the material in a manner that would be most useful to the water-pollution-control boards and their staffs, i.e., to design the report to serve as a manual or handy reference for water-quality criteria.

The State Water Pollution Control Board has been designated as the state agency with which the U.S. Public Health Service deals for the purposes of the Federal Water Pollution Control Act. Because the state board has ultimate over-all power and responsibility for enforcing the state laws relating to water pollution, the scope of this report has been broadened to include all potential pollutants and contaminants, whether chemical, physical, biological, or radioactive, regardless of their origin in municipal sewage or industrial wastes. Each substance that may enter the waters of the State is deemed to be a "potential" pollutant—potential in the sense that, if concentrated sufficiently, it can adversely and unreasonably affect such waters for one or more beneficial uses; and yet, if diluted adequately, it will be harmless to all beneficial uses.

Inasmuch as the concentration of a potential pollutant in water is a function not only of the amount of polluting substance added but also of the factors of dilution and self-purification in surface waters and ground waters, a chapter entitled "General Considerations" has been included in the report to describe and explain such factors. Other chapters include an enumeration of the standards promulgated by other state and interstate agencies, a review of judicial expression pertaining to water-quality criteria, and a summary of the quality requirements for the major beneficial uses of the water.

The bulk of the report, however, consists of a survey of the potential pollutants, whether chemical, physical, biological, radiological, or mixed, arranged in alphabetical order according to the most logical designation, with appropriate cross-referencing. For each pollutant is given a brief statement of general information, such as its characteristics and typical sources, followed by cross-references and bibliography, and finally a summary of limiting and/or threshold concentrations for various beneficial uses, as demonstrated by original investigators or as promulgated by enforcement agencies. It is this chapter that should prove of most value for the California concept of water-pollution control.

The factors that influence pollution and enter into case-by-case studies include, in addition to the strength and volume of the polluting waste, the effects of pH, temperature, synergism or antagonism, dilution, mixing, chemical and biochemical changes, velocity of flow, requirements of beneficial uses, and many other variables. The combination and interplay of these many factors are so complex and they exert such an influence on each pollution problem that any attempt to establish a classification system or a tabulation of standards appears to be inadvisable. In fact, the use of the word "standard" has been avoided in the title of this report, for it signifies "any definite rule, principle, or measure established by authority". Instead "criterion" has been chosen for it designates "a means by which anything is tried in forming a correct judgment respecting it". This report, therefore, presents water-quality criteria, and any attempt to generalize or condense them into rigid standards or requirements has been avoided as contrary to the California concept of water-pollution control.

The conduct and the management of this project and a large proportion of the detailed work of abstracting, evaluating, and organizing the material was the responsibility of Mr. Richard H. Gilman, III, project engineer. In this work he was ably assisted by Mrs. Thelma Linderman, who also served as secretary and typed most of the manuscript. For the review of the law literature and the preparation of much of Chapter IV, "Judicial Expression", we are indebted to Mr. Earl C. Borgeson of

the Los Angeles County Law Library. Mrs. Pearl Horowitz served as biological consultant and abstracter during the early phases of the project and later prepared much of the manuscript for Chapters V and VI. We are also grateful for the assistance of several part-time abstractors and stenographers. To the many people throughout California, the entire U.S., and many foreign lands who responded so generously to our requests for information and data, and to the staff of the California Institute of Technology library which secured innumerable obscure references, we offer our deepest thanks and appreciation.

As Euripides wisely observed, "One man does not see everything". With this thought in mind and cognizant of the limitations of the project staff, an Advisory Committee was selected to provide assistance and guidance. The names and affiliations of the members of the Advisory Board are tabulated on the following page. Members of the Committee twice met formally with the project staff, and several individual members called at the project office, or communicated with the staff, to provide valuable suggestions and sources of information. We are deeply indebted to the members of the Advisory Committee for their helpful assistance and advice, and we trust that this report fulfills their expectations.

J. E. McKee

Pasadena, California
June, 1952

PREFACE TO THE SECOND EDITION

The overwhelming response to the published version of the original report on WATER QUALITY CRITERIA came as a surprise to the authors and to the California State Water Pollution Control Board. Written to fill a need in the case-by-case analysis of water pollution in California, this compendium was sought, accepted, and used extensively throughout the United States and indeed in many other countries. By permission of the state board, the book was translated into Japanese and published in Japan. Despite a second printing by the State of California in 1957, the steady local, national, and international demand exhausted the supply within a few years. A third printing, however, would have been ill-advised in view of the tremendous amount of literature that has accumulated in the past decade and the progress that has been made in water-quality control. Instead, the State WPC Board elected to have a thorough revision and expansion of the original report.

The second edition bears many resemblances to the first report. The objectives, scope, and limitations are generally the same as before. Format and style remain unchanged, perhaps to the chagrin of some readers. But there are many improvements in the second edition. The bulk of it has been rewritten to incorporate a wealth of new information. Indeed, data on certain aspects of water quality have accumulated to the extent that separate chapters were needed to do justice to their presentation. For this reason, biological pollutants, radioactivity, pesticides, and surface-active agents became the subjects of chapters separate from the parent chapter on potential pollutants.

A significant change in the second edition has been the attempt to summarize. It is not easy to do so. As pointed out in the first edition, the independent variables tending to cause a diversity of data (e.g., synergism, antagonism, temperature, species, prior exposure) often outweigh the factors of central tendency. As a consequence, definitive threshold concentrations and limiting values are difficult to establish. It was the policy in the first edition to present a condensation of the literature and let the reader draw his own conclusions.

It has been reasoned, however, that the abstracters and project staff are in a better position than the reader to summarize the data. For that reason, summaries are presented for many potential pollutants in Chapters V to X inclusive. They are given, however, only when the data are so definitive that they can be summarized logically. Where there is much of a question about threshold concentrations, no attempt has been made to summarize.

The chapter entitled "Judicial Expression" has been completely rewritten, this time by Mr. Paul Freeman, an attorney who specialized in water law. Having personally abstracted much of the literature in relation to pesticides, Dr. Ralph Pressman was asked to prepare the original draft of the chapter on this subject. For a similar reason, Mr. Jack Patton was the logical person to write the chapter on surface-active agents. We are grateful indeed for the assistance of these three researchers and writers.

The project was managed by Mr. Harold W. Wolf, Senior Sanitary Engineer of the U.S. Public Health Service, who proved to be a real "bird dog" in tracking down obscure references. To him goes the credit for the fact that this second edition covered the original version of many references in German, French, and Russian, and not merely their English summaries or abstracts. Mr. Wolf also wrote two of the chapters and helped to edit the others. He was ably assisted during most of the project by Mrs. Sharon McMichael, who served as secretary and also typed some of the manuscript. She was retired to motherhood in the later stages of the project and replaced by Miss Joann Kiekover to whom we are indebted for much of the final manuscript.

To cover thousands of references, it was necessary to rely on abstracters with technical training in chemistry, biology, engineering, and pharmacology. In this work we received excellent service from Dr. Ralph Pressman, Mr. Jack Patton, and Mr. Adolph Gottfurcht who among them handled German, French, and Russian literature as well as that in English. The project also utilized the services of seven other abstracters, including Mrs. John F. Kennedy. Finally, we are indebted to the staff of the library at the California Institute of Technology who secured innumerable obscure references and provided valuable advice in the literature survey. To the many authorities throughout the world who responded so generously to our requests for information and technical data we shall be eternally grateful.

During the preparation of the first edition an Advisory Committee was formed to provide assistance and guidance. The services rendered by this group made imperative the appointment of a similar committee for the second edition. The names and affiliations of the members of this second Advisory Committee are tabulated on the following page. Although the group did not meet formally, individual members provided valuable suggestions and sources of information. To the entire Committee we wish to extend our sincere thanks.

J. E. McKee

Pasadena, California
February, 1963

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FOREWORD

This is the second edition of WATER QUALITY CRITERIA (Publication No. 3A). The general format remains the same, but the second edition has been thoroughly revised to incorporate the great amount of new information available. One of the authors in the "Preface to the Second Edition" describes the revisions in more detail. His "Preface to the First Edition" gives background information, particularly the need for the publication in relation to water pollution control.

WATER QUALITY CRITERIA was first published in 1952 and proved to be a necessary and exceedingly valuable reference book in the California water pollution control program. An addendum was published in 1954 to bring WATER QUALITY CRITERIA up to date. In 1957 a second printing was made of the combined original edition and the addendum. This printing was exhausted in a few years.

In light of the demand for WATER QUALITY CRITERIA and realizing the importance of keeping this publication up to date, the former State Water Pollution (later Quality) Control Board on July 1, 1960, contracted with the California Institute of Technology for a revised and updated review and evaluation of technical and scientific literature pertaining to criteria of water quality. The project was under the direction of Dr. Jack Edward McKee, Professor of Environmental Health Engineering, and was managed by Mr. Harold W. Wolf, Senior Sanitary Engineer of the U. S. Public Health Service. The succeeding pages present the report prepared (with others) and edited by Dr. McKee and Mr. Wolf. It was submitted on February 28, 1963. Printing and distribution of the report was authorized by the State Board on May 7, 1963.

Although the investigation reported herein was conducted under the sponsorship of the State Water Pollution Control Board, and with the assistance of the U. S. Public Health Service, it should be pointed out that the conclusions and recommendations given in the report WATER QUALITY CRITERIA, Second Edition, are those of the editors and authors. Attention is also invited to the fact that this report is primarily a survey and evaluation of the literature and a compendium of data on water quality criteria. It does not represent, establish, or recommend specific values for standards, requirements or objectives of water quality for either the State of California or the Public Health Service; nor is it intended to reflect official opinions or policies.

* * *

The Legislature of the State of California by enacting Chapter 284, Statutes of 1967, consolidated the functions of the former State Water Rights Board and the State Water Quality Control Board (successor agency to the former State Water Pollution Control Board) under the State Water Resources Control Board. This change is reflected on the cover of this publication but not in the text.

April, 1971

W. M. KECK LABORATORY OF ENVIRONMENTAL HEALTH ENGINEERING
California Institute of Technology
Pasadena

28 February 1963

State Water Pollution Control Board
Mr. Paul Beermann, Chairman
State of California, Résources Agency
1227 "O" Street
Sacramento 14, California

Gentlemen:

In compliance with the terms of Standard Agreement No. 12-14 between the State of California and the California Institute of Technology, dated 1 July 1960, an investigation has been made of the technical and scientific literature pertaining to the criteria of water quality for various beneficial uses of water. The report submitted herewith embodies a condensation and critical evaluation of such literature, along with an extensive list of references.

Supplementary to the requirements of the contract relative to scientific and technical information, a thorough search was made of the legal literature by a practicing attorney hired by the California Institute of Technology for that purpose. This literature is summarized in Chapter IV entitled "Judicial Expression".

It is difficult to summarize this investigation, for the entire project represented a condensation of voluminous literature on the subject of water quality. In effect, then, the entire report is a summary. Although specific recommendations are not given, limits are delineated for many special pollutants in Chapters VI to X inclusive.

As indicated in the agreement, the U.S. Public Health Service participated in this investigation by assigning an engineer to the project and by providing valuable advice and encouragement. We are grateful for this collaboration. We are especially appreciative of the assistance and cooperation rendered by your executive officer, Mr. Paul R. Bonderson.

Respectfully submitted,
J. E. McKee
Professor of Environmental
Health Engineering

CHAPTER I

INTRODUCTION

BACKGROUND OF WATER POLLUTION CONTROL IN CALIFORNIA

The control of water pollution in the State of California turned a monumental corner in 1949 with the passage of Assembly Bill No. 2034, Chapter 1549, which added Division 7 to the Water Code. This act provided means for coordinating the programs of the various state agencies and political subdivisions of the State in the control of water pollution, through a State Water Pollution Control Board and nine regional boards. That such coordination was necessary is evident from the Report of the Assembly Interim Fact-Finding Committee on Water Pollution (1) popularly known as the Dickey Report, after its Chairman, the Honorable Randal F. Dickey. For further background information dealing with problems of water-pollution abatement in California and the legislative hearings that led to the present law, the reader is referred to the Dickey Report and its appendices.

A decade of experience and progress in water-pollution control in California revealed that certain changes in the original act were advisable in order to implement, clarify, and strengthen the statutes. At its 1959 Regular Session, therefore, the Legislature made substantive changes in the Water Pollution Control Act as set forth in Division 7 of the California Water Code (2). Provisions in the act were added, repealed, or amended by the enactment of three bills: Assembly Bill No. 1974 (Chapter 1299), Assembly Bill No. 1063 (Chapter 2053), and Assembly Bill No. 1947 (Chapter 854). A full review of the 1949 Act and the changes of 1959 is beyond the purview of this report. All further references to the California Water Pollution Control Act relate to the statutes in effect after 1959.

In Division 7 of the State Water Code (2), the Legislature wisely recognized that problems of water pollution in California are primarily regional and dependent upon factors of precipitation, topography, and population, as well as upon recreational, agricultural, and industrial development, all of which may vary greatly from region to region. Insofar as problems of water pollution are involved, the snow-capped mountains of northern California differ from the Mojave Desert as significantly as Vermont differs from Arizona; and the industrialized San Francisco Bay area is as different from the San Joaquin Valley as New York Harbor is from central Texas. Consequently, Division 7 established nine regional water-pollution-control boards that have the primary responsibility for administration, investigation, and enforcement of the program.

It is State policy in California "that the water resources of the State be put to beneficial use to the fullest extent of which they are capable and that the waste or unreasonable use or unreasonable method of use of water be prevented." Moreover, "because of the widespread demand and need for the full utilization of the water resources of the State for beneficial uses, it is the policy of the State that the disposal of wastes into the waters of the State shall be so regulated as to achieve highest water quality consistent with maximum benefit to the people of the state and shall be controlled so as to promote the peace, health, safety, and welfare of the people of the State" (2).

Although the primary responsibility for the program rests at the regional board level, there are certain powers and duties reserved unto the State Water Pollution Control Board. It is significant to note Section 13022, which reads: "The state board shall formulate and adopt a state-wide policy for control of water pollution with due regard for the authority of the regional boards. Such policy shall be in conformity with the policies set forth in Chapter 1" (largely described in the foregoing paragraph), and also Section 13025, which states: "Whenever a regional board fails to take or obtain appropriate action to correct any particular existing or threatened condition of pollution or nuisance, the state board shall direct that action to correct the condition be taken by any state agency having jurisdiction or may, itself, take such action."

In the light of these two important duties, the state board considered early in 1950 that it had the responsibility to make known its ideas on the subject of water-quality requirements, the factors that enter into their determination, and the means by which water-pollution problems may be analyzed so as to reach an equitable decision as to water-quality requirements. At that time, the board recognized that it should analyze and summarize the thoughts of its members on this subject before any issue demanding decision came before them, and it also recognized that the regional boards were entitled to be advised in advance of the procedure that the state board would follow in reviewing the actions of the regional boards.

To facilitate matters, the state board appointed from among its members a Committee on Water Quality Criteria and charged it with studying the problem, reviewing what had been done in other states, and preparing a preliminary draft of a document that would set forth the principles involved in the establishment of equitable water-quality requirements.

The first progress report of the committee (3) appeared on 2 March 1950 and was given wide circulation among technical and trade journals, trade associations, and individuals throughout California and the rest of the United States in order to stimulate criticism, suggestions, and discussion. It was also published in the Journal of the American Water Works Association (4) concurrently with the new set of standards adopted by the New York State Water Pollution Control Board. The report was divided into three parts: (a) a statement of the new concept of water-pollution control in California,

(b) an outline of a proposed method of analysis of waste-disposal problems with an example, and (c) a table of water-quality requirements, known as "Exhibit A."

As they would to any such document that was distributed so widely, criticism, suggestions, and other comments poured into the State WPC Board. Most of the replies were complimentary to the statement of philosophy and the approach to problems of pollution abatement, but some were critical of the tabulation of water-quality requirements or to certain parts thereof. Among the constructive criticisms were several suggestions that "Exhibit A" be deleted until after a thorough survey of the technical literature had been made and research had established numerical water-quality criteria that, with judgment and discretion, could be applied safely and equitably in California.

The Committee on Water Quality Criteria took cognizance of many of the favorable comments and constructive criticisms by amending its original progress report. The second report, entitled "Water Quality Evaluation", differed from the initial report primarily in that "Exhibit A" and the example of analysis of waste-disposal problems were deleted. This revised report was approved at the 3 May 1951 meeting of the state board.

CURRENT STATEWIDE POLICY

As a result of 1959 legislative amendments to the California Water Pollution Control Act, and in light of ten years of operating experience, the two documents described above were superseded by rules and regulations of the State WPC Board pertaining to statewide policy for the control of water pollution, filed in the California Administrative Code under Subchapter 4, Chapter 3, Title 23.

Article 1 of Subchapter 4, titled "Declaration of Policy", was adopted on 8 June 1960. In general terms, it sets forth the general and fundamental principles that are inherent in the pollution control legislation, by which state and regional boards will be guided in exercising their assigned duties. For the most part, these principles are described above under the heading of background information.

Article 2, adopted 11 January 1961, is the first of a series of detailed policy statements. Titled "Principles and Factors to be Considered in Formulating Waste Discharge Requirements for the Control of Water Pollution", it makes known the ground rules upon which the state board will base its decisions in the event that discharge requirements established by a regional board need to be reviewed. Although it was developed primarily for the use of the state board in fulfilling its own duties, this regulation also serves as a suggested procedure for the guidance of the regional boards. Much of the following information is quoted directly from Article 2.

"In the formulation of waste-discharge requirements, it is recognized that:

(a) Beneficial uses made of a water are the controlling factors in determining water-quality levels that are to be maintained.

(b) For every beneficial use, there are certain water-quality requirements which must be met to assure that the water will be suitable for that beneficial use.

(c) Receiving waters have varying degrees of waste assimilative capacity, and the addition of waste materials may change the chemical, physical, and biological characteristics of the water without necessarily creating significant adverse effects on the beneficial water uses.

(d) The formulation of waste-discharge requirements should be so designed as to (1) secure that degree of care in the planning and operation of works for the treatment and disposal of sewage and industrial wastes as will adequately protect the public health and the beneficial uses of waters of the State and (2) at the same time permit the legitimate planned usage of those waters for receiving suitably prepared wastes so that an orderly growth and expansion of cities and industries may be possible."

Before a regional board can prescribe requirements for specific waste discharges, it must determine the beneficial water uses that it intends to preserve and protect. The board must next define water-quality objectives which will assure the suitability of the water for the recognized beneficial uses, giving due regard to the environmental characteristics of the hydrographic unit under consideration. The enunciation of beneficial uses and the definition of water-quality objectives can be accomplished by either of the following methods:

"(a) Under the statutory authority of Water Code Section 13052(e), adopting water-quality plans and policies for the major hydrographic units within the jurisdiction of the regional board or, until such plans and policies are adopted,

"(b) Considering, on a case-by-case basis, the beneficial uses and water-quality objectives in the immediate area of the waste discharge.

"Where practical and feasible, the state board encourages use of the first method on the grounds that it provides the most sound and rational long range approach to an effective water-pollution-control program by making it possible to simplify and integrate the prescribing of waste discharge requirements on major hydrographic units. The second method, however, may be used pending the formulation and adoption of basinwide or regionwide plans and policies."

It is important for the out-of-state reader to note at this point that California does not have statewide or even regionwide water-quality standards or the planned zoning of streams or underground basins. Instead, each problem of actual or potential pollution is studied on the basis of local conditions and current knowledge. Hearings are held to establish the beneficial uses of the water to be protected and to enable all interested parties to submit relevant information. The regional board then promulgates "requirements" which govern the quality of the effluent, and/or the conditions to be maintained in the receiving waters (surface or underground). Examples of these requirements are presented in Chapter III.

It is the present policy of the state board to maintain the highest possible water quality, in contrast to follow-

ing a direction of making maximum use of receiving waters for waste assimilation. Waste-discharge requirements are designed to achieve this objective so as to give full protection to recognized beneficial water uses. If requirements developed under this philosophy appear unreasonable to the discharger, the burden of proof rests with the discharger to show why it is reasonable and proper that the requirements be made less restrictive. In evaluating reasonableness, the board will recognize the principle of cost-benefit relationship.

Of necessity, the "case-by-case" study of each problem of pollution precludes a "broad-brush" treatment on a statewide or even regional basis and it rules out rigid stream standards and the planned zoning of streams or underground basins. In effect, then, it depends on astute judgment by the members of the water-pollution-control boards and such judgment must be based on the most recent, complete, and reliable compendium of data on the limiting and threshold concentration of each potential polluting substance for each possible beneficial use of the water.

THE ORIGIN OF WATER QUALITY CRITERIA

Recognizing the need for such a compendium upon which water-quality objectives or requirements could be based, the State WPC Board entered into an agreement with the California Institute of Technology on 4 January 1951 to make a literature survey dealing with water-quality criteria for various beneficial uses of water, and to submit a final report of its survey. This contract led to the printing in 1952 of Publication No. 3 of the State WPC Board, entitled WATER QUALITY CRITERIA. By permission of the state board, the book was translated into Japanese and published in Japan. As a handy source of information on the limiting and threshold concentration of hundreds of potential water pollutants, the first edition proved to be a necessary and exceedingly valuable reference book in water-pollution control, not only in California but throughout the United States and in many foreign countries.

Realizing the importance of keeping this publication up to date, in view of the rapid accumulation of literature in this field, the state board on 1 July 1953 contracted with J. E. McKee to prepare an Addendum to WATER QUALITY CRITERIA, which was published in 1954. A steady local, national, and international demand for the original volume, which was quickly out of print, led the U. S. Public Health Service to assist the State in financing a limited second printing in 1957; but it too was soon exhausted.

The Addendum of 1954, although patterned after the original volume and thoroughly integrated with it, proved to be cumbersome and inconvenient to use. A second addendum would have compounded the difficulty. For this reason and in light of the 1959 amendments to the California Water Pollution Control Act and the new statewide policy as described hereinbefore, it was deemed advisable not to make a second reprinting of the original volume of WATER QUALITY CRITERIA and not to prepare further addenda. Instead, a thorough resumption of the investigation and critical evaluation of water-quality criteria was authorized by the State WPC Board, to be undertaken by the California Institute of Technology through an agreement dated 1 July 1960. It was expected that this project would result in a complete, up-to-date, integrated second edition of WATER QUALITY CRITERIA. The scope of this work and the aims of the project staff in preparing the following report are described later in this chapter.

OTHER ASPECTS OF THE NEED FOR WATER QUALITY CRITERIA

The foregoing paragraphs describe the origin of this report on criteria of water quality insofar as the State of California is concerned; but who else needs such criteria and why? What is the need in other states and other countries? How can the need be defined? How urgent is it? Why hasn't it been filled over the decades? Why is the term *criteria* used? Why not speak of the need for *data*, or *standards*, or *objectives?* It is germane for this report to examine these questions and especially to determine the magnitude and scope of the need for criteria of water quality. It is also important to describe some of the problems facing authorities who are responsible for the management of this nation's water resources (1742).

Criteria of water quality are needed (1) by water-pollution-control authorities on federal, state, and local levels who must restrict or regulate the discharge of sewage and industrial wastes to the nation's water courses and ground-water basins, (2) by waterworks engineers and chemists who are responsible to the consumer for the quality of treated and delivered water, despite any pollution of the source, (3) by aquatic and marine biologists who strive to protect fisheries resources and other water life, (4) by irrigation engineers who are responsible to the farmer for the quality of water delivered to crops, (5) by veterinarians and animal husbandrymen who are concerned about the tolerance of livestock to polluted water, (6) by public-health authorities and by engineers in environmental health who are concerned with all of man's contact with his environment, including bathing and boating, (7) by hydraulic engineers who design and operate impounding dams, navigational facilities, water power plants, and harbor works, and finally (8) by municipal, industrial, and consulting engineers who must design and operate systems for handling municipal and industrial wastes.

What criteria are needed? What analyses are important for the protection of our water contact? Can some tests be dispensed with or perhaps minimized? How are decisions made relative to the significance of criteria? At present, chemists and microbiologists in water-supply organizations and regulatory agencies for the most part are performing the tests that they know how to perform. More specifically, they are running the tests that are conventional. It is possible, indeed probable, that several important trace constituents are being overlooked. Conversely, some of the conventional tests are relatively meaningless for the protection of water quality in certain instances.

To establish a current picture of water quality, it is essential to collect, collate, and evaluate all available information relative to the effects of each potential pollu-

tant on each possible beneficial use of water. Such a compendium is bound to reveal that there are many gaps in current knowledge and it will indicate areas where further research and investigation are needed.

Urgency of the Need for Criteria

That the need exists for better criteria of water quality is beyond contention, but how urgent is this need? Why hasn't it been filled before? Until recently, many people believed that the nation's water resources, except in the arid West, were abundant and virtually inexhaustible. The flow of these waters has been measured for many decades by the U. S. Geological Survey, and the probability of each magnitude of flow in future years can be predicted with increasing reliability.

One thing is certain. Barring a major change in climate or geological configuration, the average volume of available water resources will not increase. Indeed, it may even decrease slightly as more reservoirs permit higher evaporative losses and as more water is used consumptively. Attempts are being made to increase the supply of fresh water by desalinization of sea water, by artificial nucleation of clouds, and by suppression of evaporation. It is unlikely, however, that these measures will provide a sizable increase in the total fresh-water resources. This nation must learn to live within its natural water budget and to manage its present water resources wisely.

In contrast with a fixed supply of water, the demand for fresh water is being increased by a growing population, expanding industry, and complex changes in technology. Concomitant with the expanding demands for fresh water of good quality are the increased discharges of waste products to the nation's water courses. Worse still are the magnitude and variety of new substances being discharged—substances about which little is known in relation to their effects on the beneficial uses of water.

In his message to Congress on 23 February 1961, President Kennedy said, "Pollution of our country's rivers and streams has—as a result of our rapid population and industrial growth and change—reached alarming proportions. To meet all needs—domestic, agricultural, industrial, recreational—we shall have to use and reuse the same water, maintaining quality as well as quantity. In many areas we need new sources of supply—but in all areas we must protect the supplies we have" (1743).

To epitomize, the supply of natural water remains constant, the demand for it increases, and its quality deteriorates. Can there be any question that the need is urgent for additional information relative to the effects of water quality on beneficial uses?

Why hasn't this need been filled more adequately in past decades? One reason has been the lack of an acute urgency. More plausible, however, is the fact that until recently analytical techniques and instruments for measuring many of the pollutants were not available. Where once the water chemist relied on simple physical and chemical tests, he now has at his disposal equipment such as the flame photometer and the infra-red spectrophotometer to measure trace elements, molecular-filter-membrane techniques for bacteriological and biological analyses, adsorptive devices such as the carbon filter for microanalysis of odoriferous compounds, and chromatographic apparatus for partition separation of organic liquids and gases.

While many of these techniques are still confined to research laboratories, their impact will be felt increasingly in waterworks and irrigation practice and in water-pollution control. In addition to improved laboratory techniques, there is a trend toward electronic instrumentation to provide continuous analyses and graphic records of water quality of a flowing stream.

Criteria, Objectives, Requirements, and Standards

The title of this report and the foregoing discussion deal with *criteria*. Why not use the more common designation of *standards* or *objectives* or *requirements?* Wherein do these words differ?

The term "standard" applies to any definite rule, principle, or measure established by authority. The key words in this definition are *definite* and *established by authority*. The fact that a standard has been established by authority makes it quite rigid, official, or quasi-legal. An authoritative origin does not necessarily mean that the standard is fair, equitable, or based on sound scientific knowledge, for it may have been established somewhat arbitrarily on the basis of inadequate technical data tempered by a cautious factory of safety. Where health is involved and where scientific data are sparse, such arbitrary standards may be justified. There is a tendency, however, for regulatory authorities to promulgate standards of questionable scientific justification to serve as a crutch that facilitates administrative action and enforcement. A further discussion of various types of standards and their use is contained in Chapter III.

A far better word to describe an administrative decision by a regulatory body is "requirement." It represents a requisite condition to fulfill a given mission. It does not necessarily have the connotation of scientific justification nor does it give an impression of immutability. Requirements are less likely to be as rigid or fixed as standards. In California, the regional water-pollution-control boards are directed to prescribe requirements for every existing or proposed discharge of sewage or industrial waste, but such requirements may be revised from time to time (Section 13054 and 13054.1 of Division 7, California Water Code). Indeed, "No regional board, by prescribing requirements, shall be precluded thereafter from revising requirements relative to the same disposal area or receiving waters. A discharge pursuant to the prescribed requirements shall not create a vested right to continue such discharge under the same requirements" (Section 13002, Division 7, California Water Code). Examples of requirements by California WPC Boards are given in Chapter III.

The word "objective" represents an aim or a goal toward which to strive, and it may represent an ideal condition that is difficult, if not impossible, of economic attainment. Most certainly, however, it does not imply strict adherence nor rigid enforcement by a regulatory agency. It is gaining favor among engineers on boards and commissions that strive to achieve water-pollution control by persuasive methods and cooperative action. It avoids the rigidity and authoritativeness of standards and it does not have the enforcement element of requirements.

A "criterion" designates a means by which anything is tried in forming a correct judgment respecting it. Unlike a standard it carries no connotation of authority other than that of fairness and equity; nor does it imply an ideal condition. When scientific data are being accumulated to serve as yardsticks of water quality, without regard for legal authority, the term "criterion" is most applicable. For this reason, this report has been entitled WATER QUALITY CRITERIA. As a compendium of criteria, it should be useful in prescribing requirements in California, and it can be used as a guide by any agency that desires to establish standards or objectives.

To be useful, a criterion should be capable of quantitative evaluation by acceptable analytical procedures. Without numerical criteria, vague descriptive qualitative terms are subject to legal interpretation or administrative decisions. A criterion should also be capable of definitive resolution, i.e., unaffected insofar as possible by synergism, antagonism, or other complicating factors.

There is a tendency, which should be avoided assiduously, to let criteria become rigid and perhaps ripen into standards. For this reason, every criterion should be regarded as flexible information to be kept constantly under surveillance.

AIMS OF THIS INVESTIGATION

Compliance with the contract between the State WPC Board and the California Institute of Technology required resumption of the previous investigations and reports involving a review and critical evaluation of the technical and scientific literature or other sources of information that have appeared since the preparation of WATER QUALITY CRITERIA and its Addendum dealing with water-quality information related to various beneficial uses of fresh and/or salt water, such as domestic water supplies, industrial water supplies, irrigation, stock and wildlife watering, the propagation of fish and other aquatic or marine life, recreational use, and navigation.

The agreement specifies that the investigation and report shall include, but not necessarily be limited to, the following items:

1. A collation and evaluation of up-to-date water-quality standards or requirements embodied in and applied through ordinances, legislation, and the rules and regulations of state and interstate agencies dealing with water pollution.

2. A review and critical evaluation of literature reporting original research to establish deleterious concentrations of potential pollutants for specific beneficial uses of water.

3. A survey of recent technical papers dealing with dilution, mixing, tidal actions, purification mechanisms in surface and ground waters, synergism, antagonism, and other factors that may influence the application of water-quality criteria to individual problems of water pollution.

4. Findings, conclusions, and recommendations from which the State WPC Board can promulgate guidelines of water quality for specific beneficial uses.

5. Recommendations for further investigations and/or research into water-quality problems.

6. A final report, including a bibliography of reference material, consisting of a manuscript ready for printing.

The original project and the first edition of WATER QUALITY CRITERIA included an investigation of court rulings and judicial expressions that have involved water-quality standards as established by public agencies. Although this phase of the project was not renewed for the Addendum and not specified in the current contract, the Executive Officer of the State WPC Board asked that it be included in this report and made current.

Shortly after the inception of the original project, it became apparent to the staff that the California philosophy of water-pollution control, involving a case-by-case study of each potential problem of pollution, called for more than a conventional literature survey. Each regional board and its staff, as well as the state board, needed a handy reference book in which could be found a synopsis of the literature relating to the effect of each potential pollutant upon each possible beneficial use of water, with proper bibliographical notations so that further reading could be pursued, when necessary.

The enthusiastic reception and international use of the first edition of WATER QUALITY CRITERIA indicated to the State WPC Board that publication of this information filled a need far beyond that of the regional boards within California. Revision and modernization of the text, therefore, represented a contribution to the science and practice of water-pollution control throughout the U.S.A. and elsewhere. For this reason, the cooperation and assistance of the U. S. Public Health Service was solicited and obtained, as described in the Preface and Acknowledgements.

It is the primary aim of this report, therefore, to serve as a compendium of current literature on the subject of water-quality criteria. Correlated with the primary aim is an attempt to present the material in a manner that will be most useful to the water-pollution-control boards and their staffs. For this purpose, the report has been cross-referenced extensively and may seem to be repetitious in places, but the repetition helps to make the volume easier to use, as explained under "Organization of the Report," which follows.

SCOPE AND LIMITATIONS

The scope of this investigation is delineated for the most part by the agreement and the aims described above. In addition, however, cognizance should be taken of certain definitions in Division 7 of the California Water Code as they relate to the scope of this report.

Section 13005 is explicit in its definitions of three terms that otherwise are frequently confused or used synonymously. "Contamination" is defined as "an impairment of the quality of the water of the State by sewage or industrial waste to a degree which creates an actual hazard to public health through poisoning or through spread of disease. Contamination should include any equivalent effect resulting from disposal of sewage or industrial waste whether or not waters of the State

are affected." "Pollution" is defined as "an impairment of the quality of the waters of the State by sewage or industrial waste to a degree which does not create an actual hazard to public health but which does adversely and unreasonably affect such waters for domestic, industrial, agricultural, navigational, recreational, or other beneficial use, or which does adversely and unreasonably affect the ocean waters and bays of the State devoted to public recreation." "Nuisance" is defined as "damage to any community by odors or unsightliness resulting from unreasonable practices in the disposal of sewage or industrial waste."

The term "degradation" is frequently used to express a deterioration in water quality as a result of natural causes. Whether a deterioration of water quality results from contamination, pollution, nuisance, or degradation has no bearing on the scope of this survey, however, inasmuch as the investigation pertains only to the concentration of potential pollutants without regard to their origin. Attention is invited particularly to what is referred to in this report as "corollary pollutants," i.e., substances that are of natural origin but grow excessively and cause an impairment of the waters of the State as a result of the discharge of sewage or industrial wastes. Such a corollary pollutant would be the heavy algal growth that may result from the discharge of a highly oxidized and nitrified sewage or industrial waste. Although they are of natural origin and seldom added directly by sewage or industrial wastes, corollary pollutants are considered to be within the scope of this report.

In defining the aims of the report it is also well to delineate the limitations of the project. No attempt has been made to do any original research work in connection with this project; in fact an effort has been made to exclude any opinions or special unpublished knowledge of members of the staff. Literature dealing with methods of treating municipal and industrial wastes or domestic and industrial water supplies (including boiler-water treatment) were deemed to be beyond the scope of this survey. Other limitations are referred to throughout the body of the report. No claim is made, moreover, that the survey is exhaustive or that very important references have not been overlooked, for the members of the staff are subject to human oversight. Every source of information that was recommended by the Advisory Committee and other interested parties, however, was investigated. It must be recognized, furthermore, that any literature survey is outdated shortly after its publication, and may become less useful as new information appears in current publications.

This second edition differs in one significant respect from the original volume. In the first edition, no attempt was made to draw conclusions or to make recommendations relative to any potential pollutant. Instead, a certain amount of background information was given for each substance, the available literature was abstracted and cited in relation to each beneficial use, and the reader was expected to draw his own conclusions. This policy was in line with the philosophy of case-by-case analysis, for it was reasoned that the proper concentration for one situation might not apply in a separate instance. Indeed, it was feared that any attempts to summarize or suggest a limiting criterion would lead to rigid interpretations that might gel by continuous use into fixed standards.

Nevertheless, many users of the first edition have commented that the volume would have been more useful if conclusions, summaries, or recapitulations had been added where feasible. These users contended that the authors of the report were generally better qualified than the readers to draw such conclusions.

Accordingly, many of the reviews of potential pollutants in this second edition include brief statements or conclusions to summarize the current status of knowledge. Summaries could not be attempted for most of the substances because the available data are too sparse. In other instances, it was unwise to specify limiting concentrations because the effects of synergism, antagonism, temperature, pH, and many other factors far outweigh the exact concentration of the specific substance in determining its effect on a beneficial use. Where summaries and recommendations are feasible, however, they have been included in this edition.

CONDUCT OF THE SURVEY

At the outset of the original project in 1951, it became evident that the scope of the survey was so broad and the variety of potential pollutants affecting the numerous beneficial uses was so great that all possible direct assistance from known authorities should be sought, not only from within the State of California, but also from the entire country and even foreign nations. For this reason, an Advisory Committee was established in 1951, as acknowledged and listed in the front of this report. Comprising representatives of diverse background and varied interests, the 1951 Advisory Committee was of tremendous assistance in recommending numerous sources of material and specific references to the project staff. Furthermore, several of the members of the Committee reviewed the manuscript prior to publication and suggested important modifications.

The service of the original Advisory Committee was so valuable to the project staff that a decision was reached early in the resumption of the investigation to form a new Advisory Committee, as shown hereinbefore. Several members of the new committee followed the progress of the survey carefully and made numerous suggestions that helped to guide the project.

A literature survey of this type involves a thorough search of all possible sources of information. Of inestimable value are the various abstracting agencies and journals such as Water Pollution Abstracts (British), Public Health Engineering Abstracts, Journal of the American Water Works Association, Journal of the Water Pollution Control Federation, Chemical Abstracts, Biological Abstracts, and the book reviews of many journals. These abstracting services provided a good starting point for the survey, but the original article for each abstract was consulted if it could possibly be obtained. To make sure that no significant references were missed, many journals that normally or even occasionally carry articles on water quality were combed for relevant material, through December 1961. Finally, extensive correspondence with numerous authorities in the U.S.A. and foreign countries produced references to obscure articles, reprints of manuscripts or unpublished

reports, and other sources of information relative to water quality. Needless to say, each technical article referred to several others, each of which in turn also contained a bibliography, so the list of potential sources of information soon pyramided.

Each reference became the subject of an 8″ x 5″ card, the margins of which were punched in accordance with a code that designated the journal, the beneficial use involved, the nature of the potential pollutants, legal or statutory considerations, and other factors. During the course of the original survey which resulted in the first edition of WATER QUALITY CRITERIA over 2340 references were investigated, of which 1369 were cited in the text and listed in the bibliography. The Addendum of 1954 utilized and cited 371 additional references. The current survey scrutinized a total of over 3560 additional references of which 2087 have been added to the bibliography.

Relatively few of this great number of references actually proved to be significant to the project, that is to say, significant to the extent of containing the results of original investigations with actual threshold or limiting concentrations. The remainder of the references were either couched in general terms, without specific values of use to this project, or else they were rewrites or abstracts of previously published material. Consequently, the bibliography at the end of this report is much smaller than the list of references investigated.

In connection with the review of standards utilized by other states and interstate agencies, a questionnaire was sent to the primary water-pollution-control organization in each state and to each interstate agency. The fact that replies were received from all states and interstate agencies is a tribute to the quality of personnel engaged in water-pollution-control work throughout the nation. These replies form the framework for Chapter III of the report. Other questionnaires dealing with court decisions that passed on the reasonableness or unreasonableness of water-quality standards were sent to the Attorneys General of each state in 1951, the replies of which assisted in the preparation of Chapter IV. Without the formality of a specific questionnaire, numerous inquiries were directed to industrial associations, schools of animal husbandry, foreign laboratories and research centers, state departments of fish and game or natural resources, and similar groups. Each recipient of a letter of inquiry was most cooperative and each agency was most willing to contribute to the future usefulness of this publication, for almost all had heard of the original volume and had used it extensively.

Finding sources of information is the least work involved in a survey of this type. Much more effort and thought must be directed toward scanning the available literature, abstracting the significant articles to obtain water-quality information, and finally collating and evaluating the profuse data. In this respect the project was fortunate in having the services of the abstractors listed under "Acknowledgements."

ORGANIZATION OF THE REPORT

Following this chapter of introduction, in which the background, scope and aims, and progress of the survey have been described, a chapter entitled "General Considerations" has been inserted. This chapter was deemed necessary to explain and describe factors and areas of surface-water and ground-water pollution that affect many, if not all, specific pollutants and beneficial uses. It deals with such matters as available dilution in streams, estuaries, oceans, and ground-water basins; natural purification; frequency and reliability of sampling; lateral and longitudinal mixing in streams and ground water; and the significance of synergism and antagonism. An understanding of all of these factors is essential to an appreciation of the entire field of water-quality criteria.

The third chapter contains an enumeration of the standards that are utilized, in some manner or other, by other state and interstate agencies, along with a brief history of the development of such standards. Although it is conceded that Chapter III will not be nearly as useful as Chapters V–X to the state and regional WPC boards of California, it is included in the report for the sake of completeness and interest, and as a possible source of data. For background information and a general appreciation of the problems involved in formulating and administering water-quality standards, Chapter III should be significant. Furthermore, it is useful for a clearer understanding of Chapter IV, "Judicial Expression."

Inasmuch as references to instances where the courts have ruled on the reasonableness or unreasonableness of water-quality standards are rare in engineering and technical publications, a thorough search through legal channels was indicated. For this purpose, the original project was fortunate in securing the services of Mr. Earl C. Borgeson of the Los Angeles County Law Library. In this resumption of the investigation, Mr. Borgeson was no longer available, inasmuch as he transferred to the Harvard Law Library in 1953. To bring the chapter on judicial expression up to date, it was deemed advisable to utilize the service of an attorney trained in methods of legal research and interested in problems of water quality. The assistance of the County Counsel of Los Angeles County, Mr. Harold Kennedy, was solicited in this problem and he kindly agreed to have one of his deputy attorneys, Mr. Paul Freeman, handle this phase of the project. The methods by which Mr. Freeman proceeded and the results of his literature search are described in Chapter IV.

The bulk of the report (Chapters V through X) consists of summaries of literature relating to threshold and limiting concentrations for each potential pollutant with respect to each beneficial use of the water. Each substance or waste that may enter the waters of the State is deemed to be a "potential" pollutant—potential in the sense that, if concentrated sufficiently, it can adversely and unreasonably affect such waters for one or more beneficial uses; and yet, if diluted adequately, it will be harmless to all beneficial uses. In order to make the report of maximum utility to the state and regional WPC boards, the same data have been presented in two ways: (1) tabulated by beneficial use in Chapter V and (2) segregated by potential pollutants in Chapters VI through X.

It is anticipated that case-by-case studies will bring up many instances when WPC boards will have to weigh

the effect of many substances upon a specific beneficial use. In such instances, the arrangement of data in Chapter V should prove most useful. Consider, for example, a large river that has been receiving raw or partially treated wastes from several municipalities and industries, without adverse or unreasonable effects upon any existing beneficial use. Suppose also that an irrigation district (or a state or federal agency) proposes to take water near the mouth of this stream and pump it to distant lands for irrigation, which in this example constitutes a new beneficial use. In determining whether or not any of the existing discharges of liquid wastes will cause an adverse or unreasonable effect upon the new beneficial use, the WPC board should consult Chapter V and note the limiting and threshold concentrations tabulated for agricultural use.

More frequently, however, the WPC boards should have reason to refer to Chapters VI through X. Here, for each pollutant, appears a brief statement of general information, such as typical sources of the substance, characteristics, formulae, and principal effects; followed by cross references to other related substances; bibliographical reference numbers that apply; limiting and threshold values for each beneficial use; and finally where feasible conclusions or summaries that might form the basis of usable criteria. Listed among the potential pollutants are mixed wastes or collective terms such as "gas-manufacturing wastes" or "sulfite waste liquors" involving many chemical substances in unknown concentrations. All potential pollutants, whether chemical, physical, biological, radiological, or mixed, are listed in alphabetical order according to the most-common designation.

The second edition of WATER QUALITY CRITERIA differs from the organization of the original volume in that four additional chapters have been added. Under the category of potential pollutants, the subjects of surface-active substances, radioactivity, pesticides, and biological agents were so important and so profuse that they were removed from Chapter VI and placed in separate chapters.

The technical and scientific literature cited in the text is tabulated in a lengthy bibliography. While most of these references have been used directly in the body of the report, several of them are not referred to directly. They are included in the bibliography for the sake of completeness and because their philosophies guided the writers of this edition.

A word of explanation is in order here to account for the literary style used in this report. Conventional literature surveys are written in an established style that credits each original investigator with his findings; but to have complied with the conventional style would have rendered Chapters V through X of this report ponderous and dull. In the interest of utility and readability, therefore, the work and publications of many investigators are summarized in a few sentences, with the several references being cited at the end of the paragraph. In a few instances, where only one or two references are involved, direct credit is given.

CHAPTER II

GENERAL CONSIDERATIONS

There are certain aspects of water-pollution control that are not strictly part of this literature survey, but they have a direct bearing on the critical evaluations and use of the data contained herein. It is the aim of this chapter to present these aspects for consideration, and to indicate how they affect case-by-case studies of pollution. To many of the readers, the contents of this chapter will bring few new ideas or thoughts, but it is hoped that the references to established principles will help in the evaluation of the detailed data of Chapters V to X inclusive. To other readers, the concepts described herein should serve as a condensed text and handy reference, inasmuch as such considerations are essential to an understanding of the California philosophy of water-pollution control and the application of water-quality criteria.

Not all of the general considerations in this edition are confined to Chapter II. Indeed, the reader will find extensive discussion of the development and use of criteria, standards, requirements, and objectives in the first part of Chapter III. General considerations in the application of criteria to specific beneficial uses, such as agricultural water supply and the propagation of aquatic life, are described in appropriate parts of Chapter V. Finally, the opening pages of each subsequent chapter are devoted to general discussion relative to the use of data presented therein. The factors described in Chapter II, however, do not fall logically into any subsequent chapter and hence they are presented here as general considerations.

The concentration of many pollutants in water depends primarily upon two variables: (a) the amount of polluting substances, and (b) the volume of diluting water. An accurate determination of the former without a similarly precise evaluation of the latter is virtually meaningless. In order that the criteria of Chapters V to X inclusive can be applied properly, it is essential that the concentration of pollutants be determined accurately, and hence that the volume of diluting water be known. For fresh surface waters, fortunately, this problem is relatively simple and straightforward, although complicating factors such as longitudinal mixing and short circuiting are involved in the translation of the pollution to downstream points. For ocean waters, tidal estuaries, and ground waters, however, the problem is much more complex, as described later in this chapter.

There is a need for further investigation and research dealing with factors such as dilution, mixing, self-purification, and synergism. Indeed, such work may be the key to increased economy in waste disposal and improved water quality in streams, tidal estuaries, and ocean shore lines. A striking example of the advantage of such studies is the research that led to the design of the new multiple diffusers for the ocean outfalls of the Los Angeles County Sanitation Districts (1911), the Hyperion plant of the City of Los Angeles, and the metropolitan sewer system at San Diego. Similar basic research is needed to determine the fundamental reactions and mechanisms that govern the decomposition of pollutants in receiving waters, so that such reactions may be accelerated, or in some instances retarded.

DILUTION IN SURFACE STREAMS

The volume of water for dilution in surface streams is seldom constant, inasmuch as the discharge varies from year to year, day to day, and even hour to hour. While most hydrometric investigations deal with the total availability of water from a stream or the magnitude and frequency of flood flows, the sanitary engineer is more interested in minimum flows and their duration. The probability of occurrence of low flows can be determined from a study of stream discharge data contained in the USGS Water Supply Papers, in records of state departments of water resources, or in reports of local flood control districts. In themselves, these tabulations are sometimes difficult to interpret; but when properly analyzed and presented in graphical form, stream-flow data become very useful.

One of the more convenient methods for the presentation of minimum discharge data is the "duration curve," i.e., a plot of stream flow against the probability of occurrence. This curve may be plotted with arithmetic scales as in Figure 2-1, but such a presentation obscures the low flows during which pollution is most severe. An improved plotting utilizes a logarithmic scale for stream flow with an arithmetic scale for the time axis, as shown in Figure 2-2. Better still, the probability scale can be substituted for the arithmetic time axis, so as to give prominence to infrequent occurrences, as demonstrated in Figure 2-3.

In northern California, water flows in the larger streams throughout the year; but in southern California many streams have no visible or measurable surface flow during several months of each year. For either region, however, the duration curve is applicable and it provides a rapid and convenient means of studying the availability of diluting water. A duration curve for the American River at Fair Oaks, California, which is typical of northern California, is shown in Figure 2-3. The solid line represents the duration curve for the period of record up to 30 September 1955. Folsom Dam was placed in operation on 25 February 1955, just upstream from this gaging station. Thereafter, flows were regulated to reduce peaks and augment periods of low discharge as shown by the dot-dash curve. The broken lines show the duration curves for the driest and wettest years on record prior to construction of Folsom Dam. The duration data corresponding to the solid and dot-dash curves are given in Table 2-1.

The initial preparation of duration tables is a relatively simple but time-consuming procedure. Once a tabulation has been made for the entire period of record, however, the tabulation is relatively easy to keep up-to-

date, and duration curves can be made from the most recent tabulation whenever such curves are needed. Duration curves are utilized extensively by the Ohio River Valley Water Sanitation Commission (1914).

Having a duration curve at his disposal, how can a sanitary engineer use it in determining the availability of dilution water and the concentration of polluting substance at each rate of flow? On what percent of the time should he base his calculations? The answer to these questions involves many factors, primary among which are the beneficial uses to which the water will be put and the damage that will be done if available dilution is too low. No hard and fast rules can be set. In a classic study of the Merrimack River, Camp (6) based his computations of dilution on the 10% flow, i.e., a discharge that would be exceeded 90% of the time but might not occur for 36 days of a year on the average. The flows of this river were highly regulated and discharge virtually stopped on Sundays during periods of low flow. Moreover, the maintenance of fish and wildlife did not constitute the primary beneficial use, and occasional periods of severe droughts could be tolerated. (See Chapter V relative to the tolerances of fish to short-time adverse conditions.) In the Clarion River investigation, however, Camp et al. (7) let the 1% flow govern, inasmuch as fish, wildlife, and the effect of taste and odor upon water supplies were important considerations.

In selecting a minimum stream flow on which to base pollution computations, therefore, a sanitary engineer should consider the following factors:

1. Beneficial uses of the water.
2. Probability that the selected dilution will not be reached, and the duration of periods during which such dilution will not be attained.
3. The economic damage that will be done if dilution is insufficient.
4. The cost of increased treatment to meet stricter dilution requirements.

TABLE 2-1

DURATION DATA FOR STREAM FLOW OF AMERICAN RIVER AT FAIR OAKS, CALIFORNIA *

(July 1909 through September 1955, and October 1955 through September 1960)

Discharge	*Time When Discharge Did Not Exceed Stated Value*			
	(A) Prior to Folsom Dam (1909-1955)		*(B) Regulated by Folsom Dam (1955-1960)*	
cfs	*Days*	*Percent*	*Days*	*Percent*
5**	16	0.095	0	0
10	31	0.185	0	0
20	55	0.328	0	0
30	72	0.429	0	0
50	108	0.643	0	0
70	266	1.58	0	0
100	405	2.41	0	0
200	1,392	8.29	0	0
300	2,755	16.4	0	0
500	4,848	28.9	2	0.109
700	6,430	38.3	186	10.2
1,000	7,521	44.8	234	12.8
1,500	8,713	51.9	517	28.3
2,000	9,583	57.1	718	39.3
3,000	10,814	64.4	1,043	57.1
5,000	12,485	74.4	1,494	81.8
7,000	13,832	82.4	1,628	89.1
10,000	15.237	90.77	1,717	93.98
15,000	16,271	96.93	1,776	97.21
20,000	16,556	98.62	1,797	98.36
30,000	16,707	99.523	1,815	99.343
50,000	16,763	99.857	1,823	99.781
70,000	16,773	99,917	1,827	100.0
100,000	16,784	99.982	1,827	100.0
150,000**	16,787	100.0	1,827	100.0

* Flow regulated by Folsom Dam, beginning 25 February 1955. There are many diversions above this station for irrigation, municipal, and domestic water supply.
**Instantaneous peak discharge of record = 180,000 cfs on 21 November 1950.
Absolute minimum flow = 3.6 cfs on 16 August 1924.
Mean discharge 1909-1955 = 3748 cfs
Mean discharge 1955-1960 = 3748 cfs (regulated)

In computing the availability and tolerances of dilution, consideration must be given to many other factors. Certain substances such as chlorides, sulfates, sodium, and potassium are relatively stable in water and their concentration beyond the point of initial dilution depends only on subsequent dilution, evaporation, and percolation. Other substances, such as cyanides, phenols, and even nitrates and phosphates, are subject to many factors that tend to change their concentration. Among these factors are biological, chemical, and biochemical action, volatilization, sedimentation, adsorption, and other factors that are discussed in more detail in the sections on natural purification in streams later in this chapter. Although many of these factors are difficult to evaluate, serious consideration should be given to them by the sanitary engineer. In addition, the hydraulic phenomena of turbulence that govern mixing and short-circuiting must not be neglected.

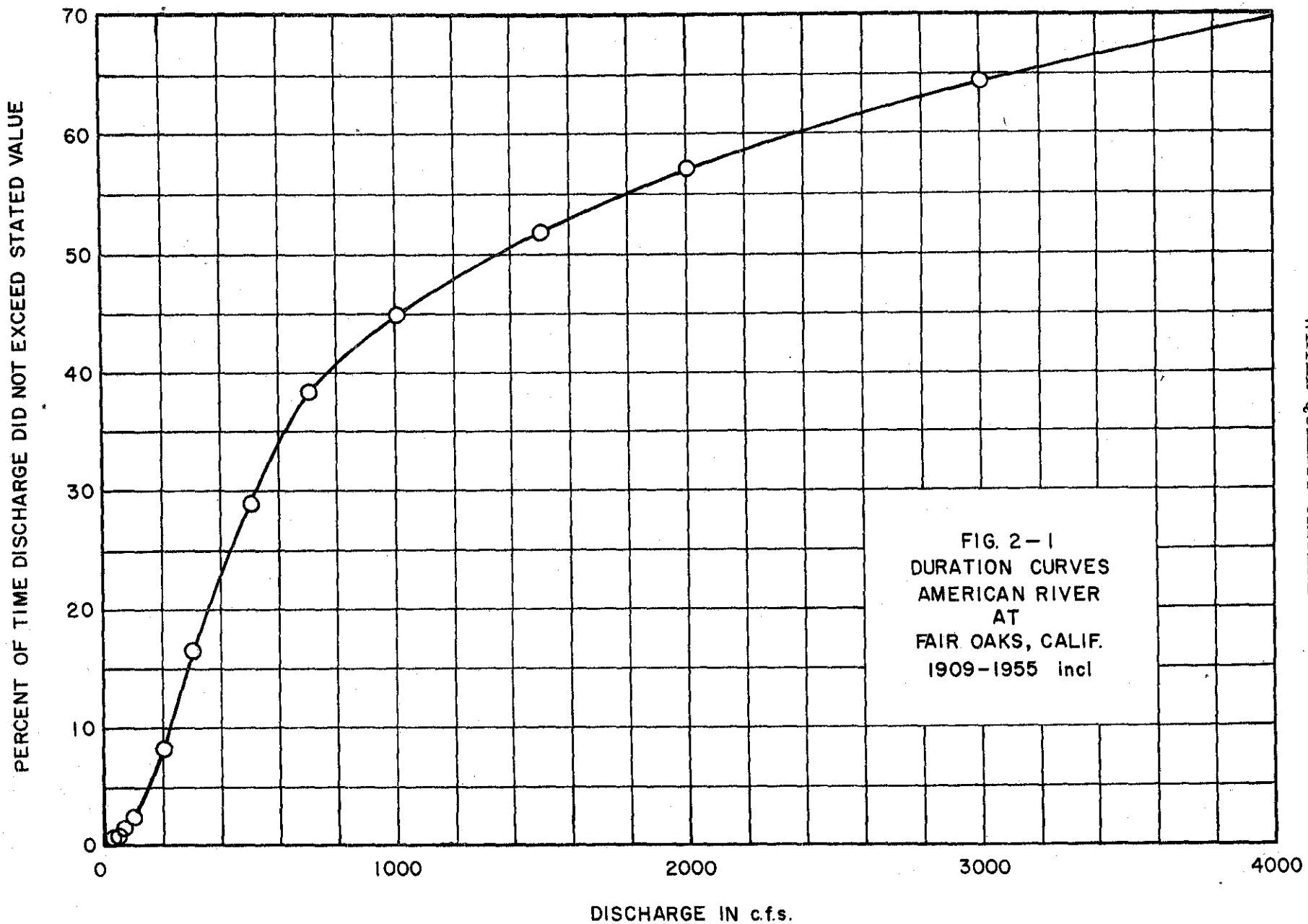
The use of duration curves in case-by-case studies can best be explained by a simple hypothetical example. Let us imagine that an industry planning to locate along the American River near Fair Oaks prior to the construction of Folsom Dam notified the regional WPC board that its wastes, after the proposed treatment, will contain 1000 lbs per day of substance X. By reference to Chapter VI of this report, the engineers of the regional board determine that the "threshold" concentration of substance X for the established beneficial uses is 1.0 mg/l while the "limiting" concentration beyond which economic damage may occur is 2.0 mg/l. The required diluting flow to achieve the threshold concentration is computed to be 186 cfs and the required flow to keep below the limiting concentration is found to be 93 cfs.

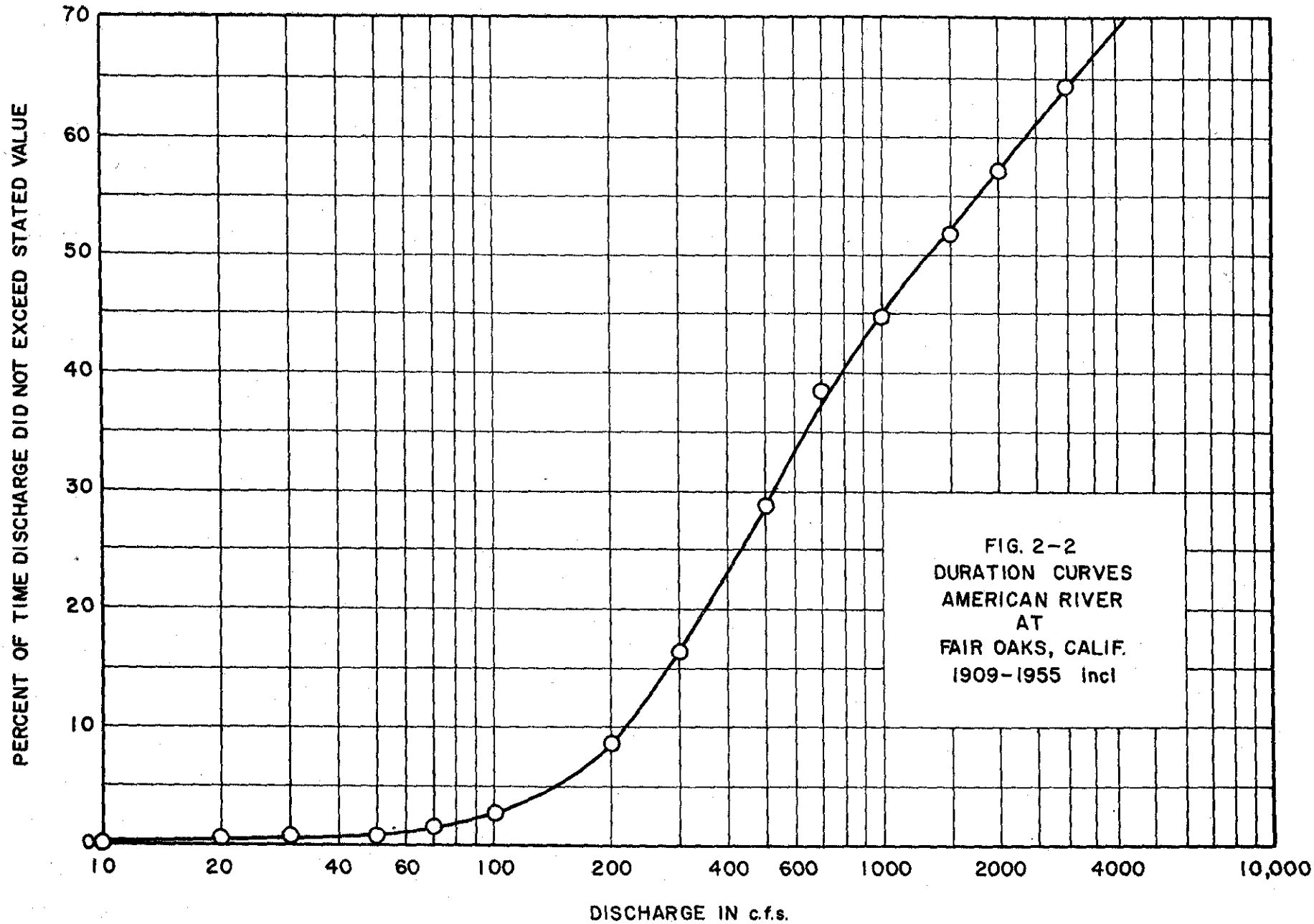
Reference to the solid line on Figure 2-3 shows that the stream flow will fall below 186 cfs about seven percent of the time and below 93 cfs about two percent of the time for all of the years of record. In the driest year, 1924, the threshold concentration would have been exceeded almost 29 percent of the time or for about 106 days, and the limiting concentration would have been exceeded about 25 percent of the time, or 92 days. During the wettest year, 1909, dilution would have been adequate at all times.

Equipped with these estimates of the probability of occurrence of excessive concentrations of substance X, the engineer can then determine the economic losses involved during periods of excessive concentration as compared with the cost of providing further treatment of the waste prior to discharge.

Duration curves have advantages for specific applications, but they do not reveal the probability of occurrence of drought flows for extended periods, such as a week or longer. For this purpose it is necessary to turn to other methods of analysis.

Phelps and Barry (1374) used an analysis of low-flow frequencies based on the publications of Gumbel (1375, 1376, 1913), who found that maximum and mini-


PERCENT OF TIME DISCHARGE DID NOT EXCEED STATED VALUE
70
60
50
40
30
20
10
0
0
1000
2000
3000
4000
DISCHARGE IN c.f.s.
FIG. 2-1
DURATION CURVES
AMERICAN RIVER
AT
FAIR OAKS, CALIF.
1909-1955 incl


PERCENT OF TIME DISCHARGE DID NOT EXCEED STATED VALUE
70
60
50
40
30
20
10
0
10
20
40
60
100
200
400
600
1000
2000
4000
10,000
DISCHARGE IN c.f.s.
FIG. 2-2
DURATION CURVES
AMERICAN RIVER
AT
FAIR OAKS, CALIF.
1909-1955 incl

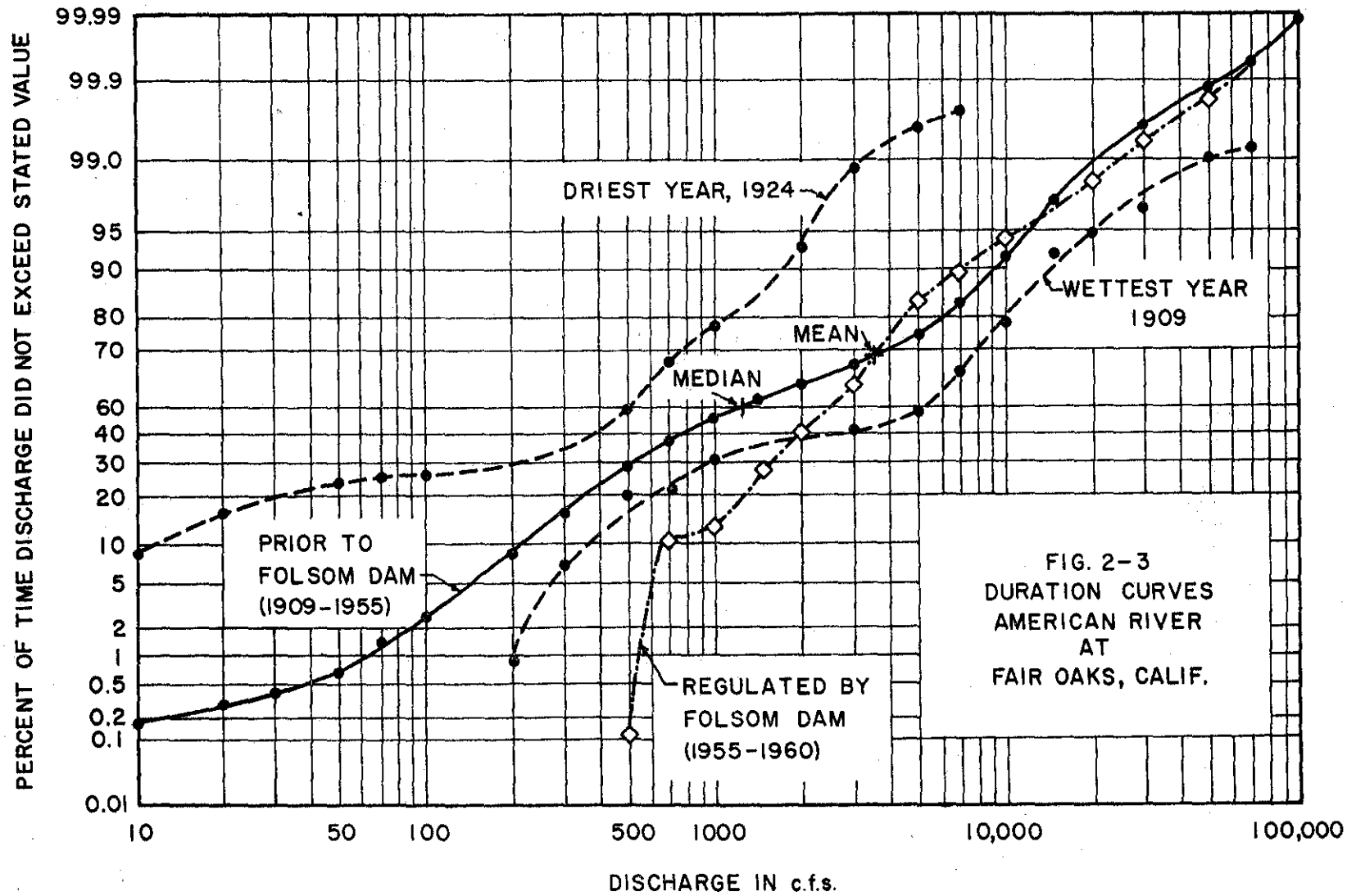


FIG. 2-3
DURATION CURVES
AMERICAN RIVER
AT
FAIR OAKS, CALIF.

mum flows in streams are distributed as to magnitude and frequency according to a statistical pattern which is a probability function. An "extreme value" is defined as a maximum or minimum runoff during any one day of a calendar or water year. Gumbel's analysis indicates that a record over N years gives a series of N extreme values which can well be described by a linear trend line in the form, for minimum flows according to Phelps and Barry, of

$$x = \bar{x} + aS - bSy$$

where x is the drought flow of any value, $\bar{x}$ is the mean drought flow of the series, S is the standard deviation of the distribution of the series, y is a dimensionless variate introduced for the purpose of linearizing the relation, and a and b are constants. This line may be plotted and extended for purposes of prediction. Gumbel also developed a skewed probability paper for straight-line plotting of the original data.

The frequency of drought flows has also been analyzed by Thomas (1377) who developed a method involving the use of the binomial coefficient to determine from past streamflow records the probability of recurrence of a given drought flow. Like Gumbel, he considered the extreme 24-hour run-off for each year, irrespective of its magnitude in comparison with other extreme values, as a variable subject to statistical analysis. Thomas's general formula is:

$$\phi_k = \frac{m\binom{t}{k}\binom{n}{m}}{(m+k)\binom{t+n}{m+k}}$$

where ϕ_k is the probability that in t future years the *mth* in magnitude of n past drought flows will be exceeded exactly k times. The notations ${}^{t}_{k}\binom{\ }{\ }^{n}_{m}$ denote the binomial coefficient, i.e.,

$$\binom{t}{k} = \frac{t!}{k!(t-k)!}$$

If $k = 0$, i.e., for the probability that the *mth* drought flow will *not* be exceeded:

$$\phi_0 = \frac{\binom{n}{m}}{\binom{t+n}{m}}$$

As an example, consider a stream with 25 years of record for which the lowest daily flow in any year was 8 cfs and the next lowest daily flow (in a different year) was 10 cfs. What is the probability that during the next five years a flow less than 10 cfs will not occur (i.e., that 10 cfs will be exceeded exactly five times)? Here $n = 25$, $m = 24$, $t = 5$, and $k = 5$, and hence

$$\phi_5 = \frac{24\binom{5}{5}\binom{25}{24}}{(24+5)\binom{30}{29}} = 0.69$$

Thus the chances are 69% that a flow equal to or less than 10 cfs will not occur during the next five years, or 31% that it will. If a minimum flow of 8 cfs had been considered for the same period (i.e., that $m = 25$) the probability that a worse drought might occur is computed to be about 17%. In their Florida studies, Phelps and Barry (1374) adopted a 25% expectancy value, that is, a mean recurrence period of four years, for the lowest annual 24-hour flood to be used in computing critical concentrations for water quality.

Some engineers prefer to use a period longer than a day to judge low flows, especially for a major stream where the discharge varies slowly. Le Bosquet and Tsivoglou (1378) advocated the use of an average 2-week flow for one large river. They called attention to a "lag period" between the time when the discharge is a minimum and the dissolved oxygen content reaches a minimum. Cleary and Robertson (1914) describe drought-flow analyses based on the frequency of occurrence (or the recurrence interval, e.g., once in 5 years or once in 10 years) of minimum daily, weekly, bi-weekly, and monthly flows. Their figures were based on the statistical theory of extreme values as developed by Gumbel (1375).

Todd (1915) reviewed the basic principles of frequency analysis of streamflow data, while Chow (1916) compared several of the better-known analytical procedures for extending frequency curves. Chow developed a general formula that he maintains is applicable to all of the other methods. Whisler and Smith (1917) found that the frequency of annual peak (or minimum) flood flows can be estimated with sufficient accuracy by an analysis of monthly peak (or minimum) flows. Having determined the probability (P_m) that a given event will occur in any one month, they compute the probability that it will occur in a year (P_y) as follows:

$$P_y = 1 - (1 - P_m)^{12}$$

Thus if the probability of occurrence in any one month is 0.01 or 1.0 percent, the probability of occurrence in a year is 0.114 or 11.4 percent.

A fundamental paper describing the frequency of natural events and utilizing much the same approach as did Gumbel, Thomas, Todd, and others, was published by Riggs (1918).

In California and other western states where stream beds may be dry for many weeks and even months of each year, the methods described above do not appear to be applicable. For western mountain areas where the streams are fed primarily by melting snows, Riggs (1379) proposed a method of forecasting low flows by means of base-flow depletion curves on the premise that stream-flow hydrographs will not fall below the depletion curves. This method is not applicable until the cessation of direct surface run-off from rainfall or snow melt, that is, until stream flow consists solely of groundwater depletion. Thereafter, low flows can be predicted for several months by Rigg's method.

Todd (1380) compared flow-duration curves with frequency-distribution curves to demonstrate that this derivative of the duration curve is more sensitive in illustrating the effects of flood and drought flows. The flow-duration curves are seldom straight lines on logarithmic probability paper, especially near the extremes, owing to varying topography, precipitation, ground cover, and other factors. The frequency distribution curves often

reveal two or more peaks that show more clearly the effects of snow melt, ground-water depletion, storm patterns, and other variables.

IMPOUNDMENTS AND LOW FLOW AUGMENTATION

To provide more efficient utilization of water resources for stream flow, according to Velz (1919), two possibilities exist, viz, regulate the waste discharge and tailor it to the varying pattern of stream flow, or regulate the stream flow to provide greater dilution during periods of naturally low flows. The first approach is sometimes employed for seasonal industrial wastes when large storage lagoons are sufficient to retain the wastes until stream flows increase. A modification of this concept occurs when spent sulfite liquors are discharged intermittently to control the growth of *Sphaerotilis natans* in receiving streams (1920). The second situation is a by-product of the construction of multi-purpose dams that impound flood flows for subsequent release for power generation or for low-flow augmentation as an aid to navigation. On the other hand, intermittent discharges from dams that are used for peak power generation may be troublesome to downstream waste dischargers (5).

The construction of dams for flood control, power, river regulation, navigation, and diversion of water for irrigation or municipal use will have a marked effect upon duration curves and the availability of water for dilution. Studies such as that by Camp et al. for the Clarion River (7) and by Le Bosquet (8) for the Kanawha River in West Virginia illustrate the benefits to pollution abatement from augmentation of low-water flows. Hazen (1921) describes the effect of storage reservoirs in increasing the minimum flows of typical eastern streams, as measured by duration curves and probability analyses. Costs of improved minimum flows are also computed.

A comprehensive study of low-flow augmentation has been conducted by Hull and his colleagues at The Johns Hopkins University (1922, 1923, 1924, 1925, 1926, 1927, 1928). This investigation included legal aspects, state and national policy, economic factors, and several technical considerations relating to water quality.

In addition to improved dilution from low-flow augmentation, impoundments may produce both beneficial and detrimental effects on water quality (5, 1928, 1929, 1930, 1931, 1932, 1933, 1934, 2007). Among the beneficial effects are decreases in coliform bacteria, turbidity, suspended matter, color, silica, and biochemical oxygen demand. Furthermore, storage tends to smooth out sharp variations in chemical quality. The potential detrimental effects include a decrease in dissolved oxygen, especially when water is withdrawn from below the thermocline, and increases in carbon dioxide, odor, ammonia, iron, manganese, alkalinity, and algae. Diminished temperatures when water is released from below the thermocline are generally beneficial, especially when downstream thermal pollution is a problem. Such changes in temperature are normally considered to be favorable for fish life; although the fauna may be altered in species and numbers as a result of cold summer water in place of the normal warm water.

MIXING IN SURFACE STREAMS

Three types of mixing influence dilution in surface streams and affect the short-circuiting of polluted waters, viz, lateral, vertical, and longitudinal mixing. Lateral mixing governs the rate at which pollution spreads or diffuses from one bank to the other, vertical mixing determines the extent of stratification, and longitudinal mixing controls the rapidity with which a portion of water moves downstream in advance of the average longitudinal velocity. Each type of mixing is a function of the turbulence of the stream, which in turn depends on discharge, slope, depth of flow, channel roughness and configuration, wind action, density currents, and temperature.

The rate of lateral mixing may be important in many ways. Where it is rapid and thorough, it enables a polluting substance that has been introduced at one bank of the stream to be diffused rapidly into the entire diluting flow, thereby dissipating high local concentrations that may be toxic or deleterious. In broad, deep, slowly moving streams, however, lateral mixing may be so slow that the polluted flow clings to one bank of the stream for many miles, with relatively little effect upon the entire flow. Such a condition may be advantageous in streams used for fish propagation, for the fish may be able to avoid the high concentrations of pollutant by remaining near the clear bank. If the pollutant is of such a nature that the fish cannot detect it and consequently swim unwittingly into toxic concentrations, then the insufficiency of lateral mixing may be deterimental. Water works that draw from the river directly, or from infiltration wells near the river, might also benefit by using water from the less-polluted side. There are many instances of one-sided pollution of streams, according to Mueller (1440). The Elbe River above and in the city of Dresden is heavily polluted especially by waste waters of factories manufacturing cellulose, all of which are on the left bank. This one-sided pollution can be traced for many kilometers downstream before an even distribution of the load across the stream is achieved.

Lateral mixing is a function of turbulence and velocity gradients in a stream. In shallow, steep, rocky channels, turbulence is high and conducive to rapid diffusion of pollution across the width of the stream. In deep, flat, smooth channels, the flow approaches a laminar condition and lateral mixing is inhibited. A precise evaluation of the rate of lateral mixing cannot be made for a general case, for each stream has unique characteristics that govern such mixing.

An understanding of lateral mixing is important in the establishment of sampling stations. Where mixing is incomplete, samples may be concentrated more or less than would be indicated for thorough mixing, depending upon where the sample is taken. In such cases, it is wise to take samples at several points across the stream and blend them into a composite sample. The State of Mississippi specifies in its standards (9) that ". . . The sampling place shall be chosen after the waste has been dispersed in the receiving waters and not more than one thousand (1000) feet below or above the waste or sewage outfall."

Even far downstream from a point of pollution it may be advisable to sample at several points along a

traverse across a stream, for lateral mixing also influences the longitudinal displacement of wastes. Haney (1370) illustrates the problem clearly with a chart showing the concentration of phenols from bank to bank in a large stream. In this particular illustration, the phenol concentrations are high near the banks and low at the center. Such a condition might occur, for example, when a discharge of phenolic wastes that had been thoroughly dispersed in the stream is then diluted by a clear stream. The unpolluted water from upstream will dilute and clear up the center of the stream much more quickly than the banks.

According to an abstract from the Russian by Straub (1381), Nesmejanow gives a formula for calculating the point of discharge of wastes in a stream to prevent the possible pollution of water near a bank which may be used for recreational purposes, as follows:

$$L = \frac{q}{hvn}$$

where L is the distance from the bank to the point of discharge, q is the volume of wastes discharged, h is the depth of the stream at the point of discharge, v is the velocity of the stream, and n is a dilution factor. In this simple relationship, quite obviously, the author assumes a complete and presumably rapidly mixing between the point of discharge and the shore, the degree of pollution at the shore depending wholely on the assumed dilution ratio. For domestic wastes, according to Nesmejanow, the required dilution factor ranges from 1:500 to 1:1000 and for industrial wastes it must be determined by laboratory experiments. With small volumes of wastes the value of L will be small and the calculation is straightforward. For lakes, ponds, and open ocean, however, the calculation is more difficult inasmuch as there is no simple velocity relationship. Similar studies have been reported by another Russian (1935).

Vertical mixing in most surface streams generally occurs rapidly in relation to lateral mixing, owing to the low ratio of depth to width. In slow deep rivers, however, thermal differences may cause a density stratification that will interfere with normal turbulent diffusion. This problem may occur when warm wastes are discharged at the surface of cold quiescent receiving waters. In most streams, however, turbulence is so great and depths so shallow that rapid vertical mixing takes place (1936).

Longitudinal mixing influences the translation of pollution downstream and affects the rate of diminution of a pollutant by factors of natural purification in streams. It is related to short circuiting of flow through lakes or deep rivers, as a result of density currents. Mathematical parameters of longitudinal mixing have been derived and evaluated by Thomas and McKee (10) and radioactive tracers have been used to measure longitudinal mixing by Thomas and Archibald (11), and by Parker (1937). An outline of the causes, effects, and parameters descriptive of longitudinal mixing is given by Thomas (1938).

An exact mathematical analysis of longitudinal mixing is exceedingly complex. By assuming that flow in the stream is steady, uniform, and two-dimensional, that no sedimentation, chemical actions, or adsorption occurs, that the vertical velocity distribution at a cross-section is parabolic, and that the diffusion of substances is not influenced by density currents or solubilities, it is possible to simplify the analysis of longitudinal mixing to the differential equation (11):

$$e\,\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} - v\,\frac{\partial c}{\partial x} - kc = 0$$

where e is the kinematic eddy viscosity, c is the concentration of pollutant, x is the distance in the direction of flow, y is the distance perpendicular to the direction of flow, v is the velocity and k is a constant to be evaluated experimentally. In view of the many factors that counteract the assumptions, this formula loses its practical significance in natural stream channels; however, the basic concepts of longitudinal mixing must not be overlooked, for they have a great influence on natural purification in streams. Fortunately, they can be taken into account in the formulations used for analysis of oxygen balance in streams.

Whether lateral, vertical, or longitudinal in dimension, the mixing or dispersion of wastes in streams (and in lakes and oceans too) is largely a function of the turbulence, as measured by eddy diffusion. Strictly speaking, diffusion is a combination of molecular and eddy action; but in open bodies of water, eddy diffusion is 10^3 to 10^6 times greater than molecular diffusion. Hence, molecular diffusion is generally ignored in quantitative evaluations of mixing.

Eddy diffusion is attributed to the large-scale eddies and small-scale eddies in which the velocity energy of a stream is dissipated. The intensity of turbulent flow is often related to a parameter of distance, such as the "mixing length," or average distance that a small mass of fluid in an eddy will move before it loses its momentum. In their dissipation, large-scale eddies generate numerous small-scale ones, of shorter mixing lengths. A full discussion of the coefficient of eddy diffusivity, e, and its relation to mixing in surface streams is beyond the purview of this report. The reader is referred, instead, to modern textbooks on fluid mechanics or to the recent articles by Orlob (1939) and Parker (1937).

DILUTION IN TIDAL ESTUARIES

The concepts of dilution of sewage or industrial waste by a river are markedly altered in regions where the river water mixes with and measurably dilutes sea water. In such tidal estuaries normal dilution is complicated by tidal action which carries a portion of the pollutant back and forth through the region for many cycles; by differences in density of fresh water, sewage, and sea water; by factors such as wind action and density currents that militate against vertical mixing and favor longitudinal mixing; by coagulating and flocculating effects of saline waters; and by configurations in the shores and bottoms of estuaries.

Early attempts at evaluation of dilution in estuaries utilized the tidal-prism concept. Taken as the difference between the volumes of water in the estuary at high and low tides, the tidal prism is contributed in part by fresh river water and in part by ocean water that enters through the seaward boundary of the estuary on the flooding tide. The proportion of fresh water and sea

water depends on the size and configuration of the estuary as well as the discharge of river water into the estuary on each tidal cycle. In the classical application of the tidal-prism concept, the volume of water entering the estuary from the ocean is presumed to be the volume available for dilution and for the removal of pollution added during each tidal period. This concept assumes, therefore, that *mixing* of the polluted fresh water and the diluting sea water is complete and uniform during each cycle and that the mixture escapes to the open ocean on each ebb tide and does not return to the estuary on the next flood tide.

That the tidal-prism concept was grossly in error became apparent after the wartime perfection of the salinity-temperature-depth indicator, as described by Ketchum (18). Extensive measures of several estuaries in the Pacific Northwest and along the East Coast revealed stratification and stability that militated against mixing within the tidal-prism. These measurements led to redevelopment of the theory of dilution in tidal estuaries, by Tyler (17) and by Ketchum (18, 19, 20).

In his modification of the tidal-prism concept, Ketchum (20) divides the estuary into segments defined by the average excursion of a particle of water on the flooding tide. He assumes, furthermore, that the water within each segment is completely mixed at high tide, with the proportion of water removed on the ebb tide being given by the ratio between the intertidal volume and the high-tide volume of the segment.

Ketchum's theory is a distinct improvement on the former tidal-prism concept, but still it does not eliminate entirely the effects of incomplete vertical mixing within a segment and the transverse or longitudinal mixing in a horizontal plane. As pointed out by Rawn in his discussion of Ketchum's paper (19), there is a tendency for fresh water, because of its lower density, to stratify and flow over the top of the salt water and for colder, heavier sea water on the incoming tide to move shoreward underneath the overlying warmer and less dense fresh waters.

Salinity studies at the mouth of the Columbia River described by O'Brien (1393) showed a marked tendency for the reversal of current from flood to ebb to occur first at the surface, while the change from ebb to flood started near the bottom. Furthermore, at maximum current strength, the ebb velocities increased almost linearly from bottom to surface, while the flood velocities showed the level of maximum velocity to be depressed markedly below the surface.

Ketchum's success in applying his theory to actual observations in the Raritan River estuary led Arons and Stommel (1390) to attempt a translation of Ketchum's fundamental idea (that the element of mixing volume is bounded by the length of the tidal excursion) into the language of physics of continua. Arons and Stommel computed salinity distribution using an eddy diffusivity based on the distribution of tidal currents and excursions. By a relatively simple mathematical analysis they arrived at the relationship:

$$\frac{S}{\sigma} = e^{F(1-1/\lambda)} = e^{F(1-L/x)}$$

where S is the actual salinity and σ is the ocean salinity; F is a "flushing number" depending on the geometry of the estuary, the fresh-water discharge, the coefficient of diffusivity, and the angular frequency of the tide; and λ is the ratio of the distance downstream (x) to the length (L) of the estuary, both measured from an origin where $S = 0$. Empirical data for several estuaries appear to fit this theoretical formulation quite well. It should be recognized, however, that the methods of analysis based on segmentation and complete mixing within each segment apply only to estuaries that are so intensively turbulent that they exhibit no vertical stratification. In such cases, the salt is carried upstream against the main river flow by turbulent eddies.

In a later paper, Stommel (1940) used the distribution of river water in an estuary as a means of discovering the magnitude of the turbulent *diffusion* coefficients at various locations. Using these coefficients, he devised a method to yield the dilution of pollution at any point in the estuary. Again, however, this analysis applies only to vertically unstratified estuaries in which the mixing is due to tides.

In a review of the theories of tidal dilution, Diachishin, Hess, and Ingram (1391) point out that there are a host of variables that are difficult to evaluate accurately, especially in view of long-range tidal changes. These authors favor the use of models to study the range of tides, the effect of density currents, changes in river flow, fluctuations in ocean level, and other variables.

Simmons (1941) discusses the application and limitations of models in the analysis of pollution in estuaries. By proper distortion of horizontal and vertical scales and by adjustment of channel roughness and other friction effects, it is possible to construct models to operate in accordance with Froudian scale relationships for time, velocity, discharge, etc. With a salinity scale of 1:1 it is possible to establish density gradients and to maintain stability during the test.

Tracers can be used to study the movement and dispersion of wastes in a model estuary, either by instantaneous release of a slug of tracer or by continuous release at a predetermined rate over a relatively long period of time. Based on the instantaneous release of a tracer, it is possible to study the movement of the peak concentration, the movement of the center of mass of the contaminant, and the physical dilution of the peak concentration, all as a function of time and tidal cycles. Continuous release of a tracer will reveal the extent and ultimate level of contamination of a given area for a given source of waste. It must be recognized, however, that models can only reproduce the physical phenomena of dispersion, dilution, and transport. They cannot simulate to scale the chemical and biochemical reactions that affect the decay of non-conservative wastes, nor other physical factors such as atmospheric reaeration. These phenomena in an estuary are not amenable to model analysis, but determination of physical dilutions and residence time by means of models will greatly assist in the theoretical evaluation of other factors. Selleck and Pearson (1942, 1943) and Pritchard and Carpenter (1955) have recently utilized conservative tracers in large estuaries for the direct observation of movement and dispersion.

Considerable research and several investigations of pollution in tidal estuaries have been undertaken in recent years (1944, 1945, 1946, 1947, 1948, 1949, 1950).

but a thorough review of this work is beyond the scope of this chapter. It is significant, however, to call attention especially to the publications of Pritchard and his colleagues at the Chesapeake Bay Institute (1392, 1951, 1952, 1953, 1954, 1956).

In his earlier work (1392, 1951), Pritchard classified estuaries as positive, inverse, or neutral. A positive estuary is one in which there is a measureable dilution of sea water by a fresh-water stream or other land drainage, e.g., where a large river discharges to the ocean. Inverse estuaries are those that receive little or no fresh water so that evaporative losses exceed the fresh-water input, i.e., the estuarine water becomes more saline than the flushing sea water. A neutral estuary is one in which neither fresh-water inflow nor evaporation dominates.

An example of a neutral tidal estuary is that of San Diego Bay, as reported by Nusbaum and Miller (1394). The bay was divided into three regions and a modification of Ketchum's analysis was applied. It was assumed that no stratification occurred, which is essentially true inasmuch as very little fresh water enters the bay, and that the tidal prism of each region was thoroughly mixed and distributed both on flood and ebb tides with the adjacent regions. Thus, it required three complete tidal cycles for water entering the mouth to pass into the furthermost region. Based on this analysis, the total dissolved-oxygen content of the bay, as provided by tidal exchange, was computed.

Positive estuaries are discussed by Pritchard in a subsequent paper (1952) and further delineated as type A (highly stratified), type B (moderately stratified), type C (vertically homogeneous, with large tidal velocities and great width), and type D (vertically homogeneous but with no upstream net advection). The type of estuary depends on river flow, tidal velocities, depth, and width.

In his later papers (1952, 1953, 1954), Pritchard groups coastal-plain tidal estuaries into four classes according to the physical structure of the water and to the net motion. Class I is a salt-wedge estuary in which the river inflow is large compared to the tidal effect. Here the fresh water flows seaward on top of a sea-water wedge. The extent to which the wedge intrudes the estuary depends on the frictional drag between the lighter river water and the denser sea water. For low flows, the sea-water wedge is only slightly diluted by fresh water and extends many miles upstream, e.g., over 100 miles up the Mississippi River. At high flows, frictional drag restricts the wedge in this river to only a mile or so. A fresh-water pollutant, introduced at the surface of a salt-wedge estuary, will be quickly flushed to sea. On the other hand, if a dense pollutant is introduced into the sea-water wedge, it will disperse very slowly and take a long time to be flushed from the estuary, for the currents in the wedge are relatively weak and exchange with the surface layer is slow.

Class II is a partially mixed estuary in which tidal movements are relatively large compared to river inflow, vertical mixing is sufficiently strong to destroy the sharp boundaries between the salt wedge and the upper waters, and the wedge is no longer identifiable. Most of the estuaries along the eastern coast of the United States fall into this class. A combination of oscillatory tidal currents and fresh-water inflow produces a circulation pattern in which there is a net seaward flow in the surface layers and a net flow towards the head of the estuary in the deeper layers. There is also a small net vertical exchange from the lower layers to the surface layers. These net motions govern the movement and dispersion of contaminants introduced into either the bottom or surface layers. In either case, however, any dissolved or suspended pollutant ultimately reaches the surface layers and is eventually flushed from the estuary.

Class III estuaries are vertically homogeneous owing to the fact that tidal movements are very large compared to the motion induced by the inflowing fresh water. The salinity decreased from the mouth to the head of the estuary. In relatively wide estuaries in the northern hemisphere, the rotation of the earth causes a net seaward flow along the right bank (looking toward the mouth) and a net inward flow along the left bank. A laterally directed flow carries water from the left side to the right, so that there is a large-scale horizontal mixing. These patterns of flow will largely govern the movement of pollutants.

Class IV involves sectionally homogeneous estuaries that are comparatively narrow and vertically homogeneous. Tidal mixing is sufficient to destroy any lateral salinity gradient, although the longitudinal gradient from the mouth to the head of the estuary remains. In such estuaries, a pollutant is rapidly mixed vertically and horizontally. It is also dispersed longitudinally by turbulent diffusion and carried slowly seaward with the net flow. Kent (1956) presents a mathematical analysis of a well-mixed sectionally homogeneous estuary from which quantitative predictions of the mean distribution of pollutant concentrations may be obtained.

According to Pritchard (1952, 1953) the generalized equation for the local time rate of change of concentration of a pollutant in an estuary is given by the following differential equation:

$$\frac{\partial c}{\partial t} = -v_1\frac{\partial c}{\partial x_1} - v_2\frac{\partial c}{\partial x_2} - v_3\frac{\partial c}{\partial x_3} + \frac{\partial}{\partial x_1}\left[e_1\frac{\partial c}{\partial x_1}\right] + \frac{\partial}{\partial x_2}\left[e_2\frac{\partial c}{\partial x_2}\right] + \frac{\partial}{\partial x_3}\left[e_3\frac{\partial c}{\partial x_3}\right]$$

where c is the concentration of pollutant at the point (x_1, x_2, x_3) at time t: v_1, v_2, and v_3 are the x_1, x_2, and x_3 components of the velocity in the longitudinal, lateral, and vertical directions respectively; and e_1, e_2, and e_3 are the corresponding coefficients of eddy diffusion. The first three terms on the right side of the equation show the effects of advection while the last three terms represent the non-advective or turbulent diffusive processes. In the three-dimensional form, the equation is too complex for analytical solution. Even when reduced to two- or one-dimensional forms, it is necessary to make simplifying assumptions regarding the velocity fields and diffusivities in order to obtain analytical solutions.

Any sanitary engineer, confronted with the problem of disposal of sewage or other wastes into a tidal estuary, must recognize that the problem of dilution is exceedingly complex and not capable of precise theoretical evaluation. Each tidal estuary presents problems of density currents and configurations that distinguish it markedly from other estuaries, and consequently each must be studied carefully. The older techniques utilizing

floats, both surface and submerged, are still valuable for studies of currents in estuaries. The salinity-temperature-density indicator and the new tracer methodology in prototype estuaries will serve as additional handy tools in such evaluations. Where the estuary is complex and the construction of waste treatment works is costly, the use of models may be indicated. For judicial expression with respect to tidal estuaries, see Chapter IV.

DILUTION IN OCEANS AND LARGE LAKES

The first edition of WATER QUALITY CRITERIA and its Addendum of 1954 described briefly the work of Rawn and Palmer (21, 22), Clifton (23, 1395), Rice and Johnson (1396), and Kersnar and Caldwell (1397) in evaluating the dilution and dispersion of sewage in open ocean waters. Owing to the fact that more than 125 coastal communities in California, including 11 of the 13 largest cities, dispose of their sewage through submarine outfalls, the State Water Pollution Control Board contracted with Dr. Erman A. Pearson of Berkeley for a comprehensive investigation of the efficacy of submarine outfall disposal of sewage and sludge. His report, Publication No. 14 of the California State Water Pollution Control Board (1957), summarized all available information on dilution in marine waters through the year 1955.

Subsequent to that time, the design and construction of several large outfall sewers on the Pacific coast and the problems associated with disposal of radioactive wastes in the ocean have stimulated research and publication related to dilution and dispersion in oceans and large lakes (e.g., 1911, 1958, 1959, 1960, 1961, 1962, 1963). To evaluate and summarize all such literature is far beyond the purview of this chapter. Indeed, Gunnerson (1964) has already published a comprehensive review of literature (256 references) related to marine waste disposal through the year 1960. In addition, Ingram and Wastler (1945) have compiled a bibliography of selected studies on estuarine and marine pollution, including physical as well as biological aspects. Finally, attention is invited to the Proceedings of the First International Conference on Waste Disposal in the Marine Environment (1965) stimulated by, and sponsored in part by, the California State Water Pollution Control Board.

The physical phenomena of dilution, dispersion, and movement of sewage or other liquid wastes in the ocean or large lakes are generally considered in two separate categories: the initial dilution caused by jet action and/or density differences, and the subsequent dispersion and transport of diluted wastes by ocean currents. Initial dilution, described thoroughly by Rawn, Bowerman, and Brooks (1911), is favored by strong density gradients, rapid jet velocity, and high ratios of depth below the surface to the diameter of the jet. Where a thermocline exists, the rising plume of diluted waste may not break through this stable barrier, but rather spread out below it. The most feasible engineering control over these factors is to provide multiple outlet ports of small diameter and to locate the multiport diffuser in deep water.

Subsequent dispersion and transport of diluted wastes by ocean currents are beyond the control of engineering design once the location of the outfall has been chosen and the multiport diffuser designed; but these factors are important in selection of the discharge site and depth.

A thorough analysis of diffusion in ocean waters is presented by Brooks (1960). Relatively little information has been published on the dilution and dispersion of liquid wastes in large lakes, but increasing attention is being given to this subject.

DILUTION AND TRAVEL OF POLLUTANTS IN GROUND WATER

In attempting to estimate the effect of sewage or industrial wastes upon the quality of ground water, a sanitary engineer must consider not only the natural dilution that may be available but also the effects of soil upon the quality of water passing through it. This section deals with dilution only, while the subject of natural purification or other changes in quality is discussed in a later section of this chapter.

Wastes from municipalities or industries may be discharged into underground waters by means of spreading grounds (percolation beds) or recharge wells, provided that such wastes are relatively free of substances that clog the interstices of the soil. From spreading grounds, the wastes may pass through a zone of aeration before reaching the ground-water table, or they may build up a ground-water mound such that the soil beneath the spreading basin is completely saturated with water. In the zone of aeration, which in southwestern states may be scores of feet deep, the only available dilution is that from intersticial water (and after a short time this water comes into equilibrium with the seeping waste) or from percolating rain water during the wet season. Above the ground-water table, then, little or no dilution will be available. If a perched ground-water table is formed by the percolating wastes, concentration of pollutants therein can be diluted only by percolating rain water, or by self-purified waste water where such purification occurs.

When percolating wastes reach a ground-water basin or enter an underground stream, they may be diluted measurably under certain circumstances. The factors of lateral and longitudinal mixing, however, are different from those in surface waters, for ground-water flow is almost always laminar whereas surface-water flow is generally turbulent. Thus, a small ribbon of polluted water injected into ground-water flow will move in a well-defined streamline with a minimum of lateral or vertical diffusion and dilution. In accordance with the same phenomenon, percolating wastes may reach a sloping ground-water table and then follow along its upper surface with a minimum of vertical diffusion. In many instances, vertical diffusion is inhibited by horizontal clay lenses or by extensive aquacludes. Consequently, the total volume of water in an underground stream or basin cannot be considered as effective for diminishing the concentration of pollutants.

The velocity of flow of ground water may be as low as 10 ft. per year, and only in coarse material or fissures

does the velocity exceed one mile per year. Coupled with the aforementioned minimum rates of lateral and vertical diffusion, these low velocities of flow cause two significant conditions to develop in ground-water basins or streams. First, pollution that is being added to the ground at one point may not affect the quality of water-supply wells at nearby points for many years, or at more distant points for decades; consequently no complaints are registered and no one may be aware of the damage being done. Second, when pollution is finally discovered or when the quality of water supplies is degraded, the damage cannot be repaired or otherwise rectified merely by stopping the pollution, for purification by leaching and dilution will require a longer time than the period of original pollution.

Excellent reviews of the geologic and hydrologic factors relating to the movement and dilution of polluted liquids in ground water are contained in the recent Symposium on Ground Water Contamination (1966, 1967), and in the American Society of Civil Engineers Manual on Ground Water Basin Management (1980).

The advent of synthetic detergents and long-lived radioisotopes has brought renewed interest to problems of ground-water flow, for these substances are excellent tracers of pollution by certain waste waters. Moreover, the need for waste-water reclamation and reutilization has focused attention on ground-water reservoirs as a means of natural purification and storage. One Task Group of the American Water Works Association reports periodically on the status of ground-water contamination (1400, 1968, 1969) while another reviews developments in artificial ground-water recharge (1970, 1971).

Recognizing the importance of underground streams and basins as receiving waters for waste disposal as well as a major source of water supply in many areas, the California State Water Pollution Control Board sponsored investigations to determine the effects of travel of pollution and waste-water reclamation in relation to ground-water quality. Those reports (1398, 1972, 1973, 1974) are largely concerned with chemical and biological factors but they also relate to problems associated with lateral, vertical, and longitudinal mixing.

Several investigators (1975, 1976, 1977, 1978, 1979) have reported on the underground travel of radioactive substances and the feasibility of using radioisotopes as ground-water tracers. The hazard of ground-water contamination from underground nuclear explosions and the possibilities of using deep aquifers for the storage of atomic wastes have also been studied. For information relative to these investigations, the reader is referred to the publications of the Atomic Energy Commission.

It should be realized that a thorough treatment of the subject of dilution in ground water, like that of ocean dispersion, is beyond the scope of this report. A few complicating factors are mentioned herein, merely as a warning to the water-pollution-control official. Each problem of ground-water dilution must be studied individually in the light of geophysical and hydrological data, the nature of the pollutants, the type of soil, the location of water-supply wells, and the beneficial uses to which the water may be put.

NATURAL PURIFICATION IN SURFACE WATERS

Pollutants in receiving waters are frequently classified as conservative or non-conservative. A conservative substance is one for which the concentration is directly related to the extent of dilution, i.e., the substance is not decomposed, altered chemically, or removed physically as a result of natural processes. Consequently, it is possible to predict from a measured concentration of a conservative pollutant at a known stream flow what concentration would occur at other stream flows (provided, of course, that physical parameters of mixing do not also change with flow) and also what would be the effect of varying degrees of treatment. The chloride ion is a good example of a conservative substance.

A non-conservative pollutant is one that may be subject to chemical, biological, or physical processes, (other than dilution) that tend to alter it or remove it from solution or suspension. Ammonia, for example, may be oxidized biochemically to nitrates, then assimilated in algal synthesis, and eventually consumed by animal life. Carbonaceous wastes may be converted largely to carbon dioxide, part of which may escape to the atmosphere. Heavy metals may be precipitated as insoluble salts and adsorbed on rocks or removed by sedimentation. The processes by which non-conservative wastes are altered or removed from water (surface or sub-surface) are grouped into the collective term of natural purification.

Water-pollution-control authorities are interested in three major aspects of natural purification; (a) the rate and extent to which pollutants are stabilized or removed, (b) the effect that such stabilization has upon other significant parameters of water quality such as pH and dissolved oxygen, and (c) corollary action such as nutrient enrichment that may lead to algal blooms and other undesirable qualities.

The rate of diminution of many unstable chemical substances in streams can be estimated and evaluated by routine procedures of physical chemistry. In many instances the oxidizing of unstable compounds follows a first-order equation, but in some cases the formulation more closely approximates a second-order reaction. In a solution containing ample dissolved oxygen, for example, sulfites are oxidized to sulfates in a true first-order reaction at a fairly rapid rate, thus

$$\frac{dc}{dt} = -Kt = -2.3kt$$

or

$$C_t = C_o \cdot e^{-Kt} = C_o \cdot 10^{-kt}$$

where $\frac{dc}{dt}$ is the rate of change of concentration with time, C_o is the initial concentration of sulfites, C_t is the concentration after time t, and K or k is a reaction-velocity coefficient. For sulfites, k has been evaluated at values ranging from 0.236 per day to 0.412 per day (7). It is possible to evaluate similar reaction-velocity coefficients for other unstable compounds such as nitrites, polythionates, and cyanides. There is a need to accumulate such data for use in predicting the rate of diminution of non-conservative substances; but one must recognize that in a natural stream the reaction-velocity coefficients may be

nfluenced by temperature, pH, concentrations of other substances, and the extent to which the stream has previously become acclimated to such pollutants (1981, 1982).

German engineers have long recognized that a stream partially polluted by municipal sewage is much better able to absorb new industrial wastes than a stream previously not exposed to mild pollution and consequently not seeded with the necessary organisms for biochemical decomposition (1983, 1984).

The second major aspect of natural purification, specifically its effect upon the dissolved oxygen in surface waters, has received considerable attention from sanitary engineers and chemists for several decades. The combined effects of many putrescible substances have been grouped into the test for biochemical oxygen demand (BOD), the rate of which is commonly considered to be a first-order reaction. Thus, for the standard laboratory test for BOD, when samples are tested every day for a week or so, the equation of the curve so established can be expressed as

$$y_t = L(1 - 10^{-K_1 t})$$

where y_t is the BOD exerted in any time t, L is the ultimate first-stage BOD, and k_1 is the reaction-velocity coefficient or "bottle k." If BOD were exerted in a stream in the same manner as it is in a laboratory bottle, then at any point B, downstream t hours from point A where the initial ultimate BOD had been L_a, the remaining ultimate BOD, L'_b, would be

$$L'_b = L_a - y_t = L_a \cdot 10^{-k_1 t}$$

or

$$\log L'_b = - k_1 t \log L_a$$

Many factors, however, tend to make the changes of BOD in a stream different from those in a laboratory bottle, so that the actual remaining BOD at the downstream station, L_b, will be

$$L_b = L_a \cdot 10^{-k_r t}$$

$$k_r = \frac{1}{t} \log \frac{L_a}{L_b}$$

where k_r is the "river k" as contrasted with k_1, the "bottle k." Inasmuch as k_r differs from k_1, the difference ($k_r - k_1$) is designated as k_3 (k_2 having been assigned previously to reaeration, as described hereinafter). The value of k_3 is positive if k_r exceeds k_1, and negative if k_1 exceeds k_r. Factors that tend to make k_3 positive include:

1. Sedimentation
2. Volatilization of organic acids
3. Adsorption, as influenced by the area/volume relationship
4. Flocculation
5. Biological activities of attached growths.

Factors tending to make k_3 negative include:

1. Previously deposited sludge banks that exude BOD to the stream
2. Channel scour
3. Longitudinal mixing and short-circuiting.

The use of the k_3 concept is convenient because k_3 can be used to group all of the unknown variables and factors that are difficult to evaluate. Longitudinal mixing, which has previously been shown to affect the downstream translation of pollution, is accounted for handily by k_3. To separate and evaluate the many factors that constitute k_3 are tasks that are difficult, if not impossible, for k_3 depends on the interplay of all of the factors. Thus, k_3 may be positive in the upper reaches of a stream during the summer, especially where preceding spring freshets have washed upstream sludge deposits into the downstream pools. In the spring, however, this same stream may show a negative k_3 in upper reaches, owing to scour, and a positive value in lower reaches where sedimentation occurs. By evaluating k_3 for any reach of a stream, an engineer can learn much about the condition of the channel and the ability of the stream to oxidize or otherwise diminish a pollutional load.

The evaluation of k_3 is relatively simple and straightforward. It depends, however, on the accurate determination of three other variables, viz, k_1 and L at each end of the reach and the time of flow, t, through the reach. Simplifications in the analysis of BOD data, as developed by Moore, Thomas, and Snow (12), lead to a rapid determination of k_1 and L from the BOD curve; consequently these parameters can be evaluated readily for each stream sample.

The remaining variable, time of flow, is just as important as k in the formulation 10^{-kt}, but more difficult to determine precisely. It represents the time required for the centroid of pollution at point A to travel to the corresponding centroid at point B. Unfortunately for the engineer who must determine it, this time does not correspond to the theoretical detention period, for the actual time of passage is influenced by short-circuiting and longitudinal mixing. In small streams, the time of passage, or flowing-through time, can be measured by the use of dye or salt, but in large streams the required quantities of such substances become unreasonable. Radioactive tracers present a possible approach to the problem, but as yet the potential hazards and controls governing the use of radioactive isotopes preclude their serious consideration. Recent innovations in analysis of a stream's capacity to assimilate pollution, as described hereinafter, have an advantage in that they do not require determination of the time of flow.

The foregoing analysis gives a reliable formulation for the change in putrescible organic matter in a flowing stream, but it does not indicate the manner in which the dissolved-oxygen content of the water will be affected. The concepts of oxygen balance in a stream have been formulated and developed by many engineers, foremost among whom have been Streeter and Phelps (13), and Fair (1985). The use of the theory has been improved somewhat by Thomas (14) on whose analysis much of the next few paragraphs has been based.

It has been shown that the rate of deoxygenation in a flowing stream is proportional to L, the ultimate first-stage BOD, or

$$\frac{dD}{dt} = K_dL = 2.3k_dL$$

where $\frac{dD}{dt}$

is the rate of change of the oxygen deficit, D, and K_d or k_d are proportionality coefficients. Note that neither k_1, the bottle value, or k_r, the river coefficient, was used in this equation. In the case of an incubated sample, $k_d = k_1$; but in a river the rate of deoxygenation is not the same as the rate of reduction of BOD, owing to such phenomena as sedimentation, volatilization, scour, and longitudinal mixing that do not involve deoxygenation. Moreover, k_d is not the same as k_r, for some of the removal of BOD in streams is not related to oxygen utilization. As yet, no way has been developed to measure directly the deoxygenation rate in a river and hence past investigators have assumed it to be equal to k_1. In certain heavily polluted, shallow, swift-flowing streams, however, the rocks in the channel resemble a trickling filter and give a tremendous k_3 value as a result of biological oxidation. The deoxygenation rate coefficient in such streams is probably closer to k_r than to k_1. For most streams, however, the value of k_d is approximated by k_1, so that

$$\frac{dD}{dt} = 2.3k_1L$$

At any downstream station, however, $L = L_a \cdot 10^{-k_r t}$, where k_r is used rather than k_1, hence:

$$\frac{dD}{dt} = 2.3k_1L_a^{-k_r t}$$

To offset deoxygenation, a polluted stream receives oxygen from the atmosphere in accordance with Henry's law. The rate at which oxygen will pass from the atmosphere into solution depends on the rate at which it crosses the air-water interface, the rate at which it diffuses through the depth of the water, and the oxygen deficit. For a given stream with fixed surface characteristics and turbulence, the rate of reaeration is proportional to the deficit, thus with no simultaneous reaeration,

$$\frac{dD}{dt} = K_2D = 2.3k_2D$$

where k_2, the reaeration-rate proportionality coefficient, is a function of the stream characteristics. The magnitude of k_2 can be determined approximately from the physical characteristics of the waterway, such as slope, depth, and velocity of flow; or it can be computed for an actual situation from field observations of temperature, dissolved oxygen, and BOD by means of the following equation for oxygen balance.

Combining the rate of deoxygenation and the rate of reaeration, the resultant differential equation expressing oxygen balance in a stream is

$$\frac{dD}{dt} = 2.3k_1L - 2.3k_2D$$

which, upon integration gives

$$D = \frac{k_1L_a}{k_2 - k_r}\left(10^{-k_r t} - 10^{-k_2 t}\right) + D_a \cdot 10^{-k_2 t}$$

The foregoing analysis of oxygen balance in a stream can be used to predict the effects of certain substances and varying degrees of treatment; provided, of course, that the characteristics of the stream that govern k_2, k_r, and t are well known. It has been used successfully in several instances, but it requires careful control and adequate experimental data. Some of its limitations are discussed in the following paragraphs.

Literature on the oxygen balance in surface waters is profuse. Hull (1926) has prepared a recent bibliography on biochemical oxygen demand, but it does not cover articles relating to other factors of the oxygen balance in surface waters. Other resumes of current knowledge have been prepared from time to time (1384, 1924, 1986, 1987, 1988, 1989).

Orford and Ingram (1385) criticize the use of the unimolecular or first-order reaction to formulate the BOD curve pointing out that the k_1 and L values do not remain constant over the entire curve. This effect had been noted by several previous investigators who proposed factors in the first-order formulation to compensate for it. Orford and Ingram propose instead that the recognized formulation be abandoned in favor of an entirely new logarithmic BOD equation. They claim that the new equation has more biological significance, that it is better adapted to the interpretation of biological oxidation phenomena, that it is easier to compute, and that the parameters of the formula are constants over a time range of 85 percent of the first-stage BOD. Woodward (1386) refutes these arguments, pointing out that the BOD curve can be formulated by a second-order reaction to give a fit as good or better than the logarithmic BOD equation. He defends the present first-order equation as an established and worthy method, within the limits of reliability of the data, which can be formulated quickly by the new technique with the method of moments. Schroepfer et al. (1990) reappraised deoxygenation rates of various wastes, noting that raw sewage, primary effluent, and chemical-treatment effluent were characterized by a rapidly decreasing reaction rate constant during the first day of incubation.

Photosynthetic action by algae may represent a major source of oxygen for streams, lakes, and estuaries; hence it should be evaluated along with other factors in the analysis of oxygen balance (1394, 1925, 1991, 1992). Similarly, organic bottom deposits cannot be neglected, for they may cause a significant portion of the oxygen demand (1993, 1994).

A great deal of work has been accomplished and published in recent years to evaluate more precisely the reaeration of streams and estuaries. To review all of this work is far beyond the scope of this chapter, but the reader may wish to investigate these cited references (1925, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2097, 2518, 2951, 2952).

Of particular significance in the study of oxygen balance has been the work of Hull at The Johns Hopkins University and Churchill of the Tennessee Valley Authority

Using the concept of a self-purification coefficient (f) as developed by Fair (1895), whereby $f = \frac{k_2}{k_1}$, Hull (1927) demonstrated that this coefficient can be determined readily at the critical point of the oxygen-sag curve by the relationship:

$$f_c = \frac{k_{2_c}}{k_{1_c}} = \frac{L_c}{D_c}$$

Where L_c and D_c are the first-stage BOD and the oxygen deficit at the critical point, where the dissolved oxygen reaches its minimum value. By this technique, it is possible to compute f by knowing only the stream temperature, first-stage BOD, and the dissolved-oxygen content at the point of minimum dissolved oxygen. There is no need to determine k_1, k_2, or the time of travel. It should be recognized, however, that the self-purification coefficient is not a constant, for it may change along the course of a stream and it will vary with the discharge; hence the f_c value may not apply for computing the oxygen balance over the length of a watercourse.

Churchill (2008, 2009, 2010) presented a method for finding the decrease in dissolved oxygen in a typical oxygen-sag curve without using the original formula by Streeter and Phelps, or any of its subsequent refinements. The conventional procedure requires determination of the first-stage BOD value (L) and the rate of deoxygenation (k_1), both of which require long-time BOD incubation. Also, the reaeration coefficient (k_2) and the time of flow (t) must be evaluated for each reach of the stream and for each rate of discharge. Instead, Churchill proposed a method of analysis based on multiple linear correlation of observed dissolved-oxygen drops (to the lowest point on the oxygen-sag curve) with 5-day BOD, stream temperature, and stream discharge at a stream sampling point nearby. For the example described (in the Tennessee River area) the coefficient of correlation between observed and computed drops in dissolved oxygen was $R = 0.869$. The formula can be used to predict the improvement in the stream that can be achieved by reducing the pollutional load.

In addition to oxygen-sag analyses, other less-sophisticated methods have been developed along empirical lines to determine the allowable load of pollution that a stream can handle (1387, 1388, 1389, 1437, 2011). They represent a compromise between thorough case-by-case studies of the deoxygenating effect on a stream of each probable or possible discharge of waste and the arbitrary zoning of streams with concurrent use of effluent standards or receiving-water standards.

The third aspect of natural purification of interest to water-pollution-control authorities is the development of corollary pollutants as a result of nutrient enrichment. The problem is especially acute in fresh-water lakes, where nutrients hasten the processes of eutrophication and result in heavy blooms of algae. Conventional processes of sewage treatment are of no help in amelioration of nutrient enrichment, for the end-products of biochemical stabilization (nitrates, phosphates, carbonates) are the offending substances. Only by advanced waste treatment methods or by complete removal of effluents can the eventual eutrophication of lakes be delayed.

In flowing water, nutrient enrichment is not generally a serious problem. Where discharged wastes are high in certain carbohydrates, however, heavy growths of *Sphaerotilis natans* may develop in streams.

For further discussion of the effects of fertilization on receiving waters, the reader is referred to an excellent review by Lackey (2012), the transactions of a 1960 Seminar on Algae and Metropolitan Wastes (2014), and a description of the eutrophication of Swiss Lakes by Jaag (2013).

NATURAL PURIFICATION IN GROUND WATER

While extensive investigations have been conducted on the physical, chemical, and biological changes that occur in polluted surface waters, relatively little attention has been directed toward similar phenomena in ground water. The need for research in ground-water pollution has been pointed out by Stanley and Eliassen (2015), by McKee (2016), and by recent seminars on this subject (2017, 2018). A Task Group of the American Water Works Association (1969) makes periodic surveys of groundwater pollution and waste-disposal practices.

In estimating the anticipated concentrations of pollutants in ground water, with due allowances for dilution as described hereinbefore, one must recognize that certain substances will pass through soil with little or no physical, chemical, or biochemical changes, whereas other substances will be removed or altered markedly. Stated otherwise, some wastes will be conservative, or unaltered by flow through soil, while others will be non-conservative. Chlorides, for example, will pass through soil with few if any changes in concentration except for the possible effects of dilution or evaporation. Indeed, the chloride ion is probably the best tracer of ground-water flow, for it is least affected by adsorption-desorption lag or by other physical or chemical phenomena.

Non-conservative substances in ground water may be altered by physical, chemical, and/or biochemical phenomena such as oxidation or reduction, adsorption or desorption, ion exchange, precipitation or dissolution, aerobic or anaerobic decomposition, and antibiosis or symbiosis. Sometimes the reaction products from decomposition of non-conservative substances may be more deleterious than the original compounds. Carbonaceous wastes, for example, will be degraded biochemically in the presence of oxygen to produce carbon dioxide, which remains in solution in ground water. The increased CO_2 tension, in turn, might cause dissolution of calcium carbonate from the soil and thereby increase the hardness of the ground water.

It is to be expected that wastes of high organic content will quickly deplete any dissolved oxygen in ground water. Moreover, no opportunity will be available for reaeration. Anaerobic ground water will have a negative redox potential that may lead to the reduction of ferric salts and an increase in soluble ferrous iron in pumped water. In some instances, if the proper bacteria are present, ground water of strongly negative redox potential will lead to dinitrification of nitrates (2019, 2020) and possibly to reduction of sulfates to sulfides. Ground water from organic formations such as peaty soil is notoriously characterized by a high content of hydrogen sulfide and possibly methane.

Some of the most thorough investigations of the behavior of pollutants in ground water have been conducted at the University of California and the University of Southern California under the sponsorship of the California State Water Pollution Control Board (1398, 1972, 1973, 1974, 2021, 2022, 2023, 2024, 2025, 2026, 2027). The reader is referred directly to these publications for details. Attention is invited also to the legal problems associated with possible pollution from cemeteries and other judicial aspects of ground-water flow as described in Chapter IV.

Prior to the widespread use of synthetic detergents, especially the ABS-type compounds, the chloride ion was the only effective tracer of ground-water pollution. Nitrates have also been used as a tracer of ground-water pollution (2028). As a conservative substance in saturated ground water, ABS has proved to be an ideal tracer, for it does not appear to be subject to biochemical decomposition and it is only slightly retarded by the chromatographic effect of adsorption and desorption. Several studies have been made to assess the rate of travel of ABS through various types of soils under known conditions of flow (2029, 2030). For more detail, see Chapter X.

One aspect of natural purification in ground water is the direct application of waste water to soil, either by ridge-and-furrow irrigation or by sprinklers. The past decade has shown an increased interest in land disposal of municipal and industrial wastes. In general, the discharge of polluting substanees into water courses is meeting with increased resistance, according to an AWWA Task Group report (1400) and consequently the attractiveness of land disposal of wastes is enhanced. A similar philosophy to show why industry is turning more frequently to land disposal by percolation beds or injection wells is presented by Henkel (1401).

In lieu of lagooning, which frequently results in strong odor problems and unstabilized effluents, the food-processing industries such as canneries and dairies have experimented successfully with spray irrigation, which represents a form of land disposal. According to Sanborn (1402) the objective of spray irrigation of cultivated areas for waste disposal is to apply the maximum amount of waste water that can be absorbed by the soil and vegetation without surface run-off or damage to cover crops. This intent is in direct contrast with the objective of agricultural irrigation, which is to grow the maximum crop with the minimum amount of water. Spray irrigation involves a possible risk of pollution of ground-water strata, especially if persistent toxic substances such as boron are contained in the waste water, inasmuch as a large proportion of the water percolates beyond the root zone of the cover crop and enters the shallow or deep ground-water reservoirs.

Problems of the land disposal of sewage and industrial wastes, too complex and too lengthy to include in this brief synopsis of general considerations, are discussed in detail by several authors (1400, 1402, 1403, 1404, 1438, 1439, 1878, 2021, 2026, 2031, 2032, 2033, 2034).

FREQUENCY OF SAMPLING

In studying and utilizing water-quality data, consideration must be given to the frequency of sampling and the methods by which such data were obtained. This consideration should take into account many of the factors described hereinbefore, such as dilution, mixing, duration of stream flow, and natural purification. In addition, the evaluation should recognize the nature of polluting substance, the frequency and rate of its discharge, and the harm to beneficial uses caused by infrequent high concentrations.

Municipal sewage is discharged at a fairly uniform rate, with daytime peaks and early-morning minima that can be established relatively well for a given community. Many industries follow a similar pattern of discharge, related to working hours and unit processes for which the character and strength of wastes can be predicted. Some industries, however, utilize batch processes from which discharge is infrequent and irregular, and some may have accidental spillages or surreptitious discharges. One such unpredicted slug of toxic substance might destroy the aquatic life of a stream or ruin the crops in an entire irrigation district.

An ideal stream-sampling program, therefore, should involve continuous sampling and analysis by means of automatic equipment analogous to the continuous stage recorders used for discharge measurements. This is a goal for which the State Water Pollution Control Board should strive, and hence it is recommended that an effort be made to encourage the development of such equipment. In this connection, attention is invited to the 1960 U. S. Public Health Service Seminar on Water Quality Measurement and Instrumentation (2035).

Without continuous water-quality recorders, samples should be taken as frequently as possible, commensurate with the cost of manual sampling and analysis, in regions where slugs of pollution are likely to occur. On other streams not subject to intermittent discharges of wastes, less-frequent sampling is indicated. For ground-water basins and underground streams, changes in water quality occur so gradually that quarterly or semi-yearly sampling may be adequate.

In evaluating water-quality data, an engineer should note whether the results are based on grab samples, daily composites, or monthly or yearly averages. Where averages such as the arithmetic mean or median are employed, the number of samples used to compute the parameters should be considered. In a similar fashion, any standards that are promulgated to govern water quality should specify the number and frequency of samples. The USPHS Drinking Water Standards (2036) recognize this effect insofar as bacteria are involved by designating the minimum number of samples to be analyzed per month. Insofar as the mandatory chemical standards of the USPHS are concerned, analyses need be made only semi-annually, and for the recommended limitations on other physical and chemical tests no mention is made of frequency of sampling. Streeter (26) points out that a noteworthy feature of the stream standards of certain state and interstate agencies is their "use of the month as a time unit in fixing limiting requirements for coliform bacteria, dissolved oxygen, and BOD, and also the separation of these requirements according to monthly averages and daily maxima or minima. The monthly average time unit, first proposed in the Ohio River Basin standards, is in effect a stricter requirement than an an-

nual average, all other things being equal, but tends to narrow very materially the wide range of variations above and below the mean which in the past has introduced difficulties in the application of standards based on the annual period. Moreover, it recognizes the fact that a monthly period affords a fair basis of indicating sanitary conditions in a stream during critical times of the year with respect to various stream uses. The added specification of a daily maximum or minimum limit differing from the monthly average is desirable because critical conditions in a stream with respect to certain water uses, notably the maintenance of aquatic life and certain aspects of water supply sanitation, may occur within periods of a few hours, and must be safeguarded by setting tolerances accordingly. Thus, for desirable stream qualities, each one of the four standards listed has specified a daily minimum dissolved oxygen content of 5 ppm., in order to protect streams from short-time lapses in this respect which might cause serious damage to aquatic life.

"It has been well said that a fish dies only once, and it is a matter of common observation that extensive fish killings often occur as the result of stream conditions prevailing for relatively short periods of time. Although such mortality may be due to the temporary presence of toxic substances rather than asphyxiation due to oxygen deficiency, the principle involved is the same in both cases, and should be applicable to both of them alike, in order to be effective. In general, the tendency appears to be to specify either the total absence of certain toxic substances in streams, or their maximum allowable concentration at any time. This question has an important bearing on the fitness of streams for water supplies as well as for the maintenance of fish life.

"A question may arise as to how the proper relationship between a monthly average stream characteristic and its daily maximum or minimum may be derived. In developing the Ohio River Basin recommendations, and also those of the Tennessee Valley streams this relationship was worked out from a study of the daily frequencies with which various observed limits were exceeded, as related to the corresponding monthly average figure for the same characteristic. For example, it was found from a study of a large number of observations in different streams of the Ohio River Basin that the daily minimum dissolved oxygen content tends to remain above 5 ppm. when the monthly average is not less than 6.5 ppm. Although the findings were slightly different in this respect from the analysis of stream data covering more limited areas, such as those in the Tennessee Valley, and thus were reflected by some degree of variation in the actual figures proposed in the different standards, the method of their derivation was substantially similar in each case."

The effects of diurnal fluctuation in dissolved oxygen, the importance of brief heavy discharges of strong industrial wastes, and the phenomena of mixing are discussed by Haney (1370), Chase (1423), Black and McDermott (1373), and Hoak (1371). These factors were among many that led Hoak to reiterate the necessity for continuous water-quality recorders, as recommended in the original volume of WATER QUALITY CRITERIA.

VARIABILITY OF WATER QUALITY DATA

In any analysis of water-quality data, one must recognize that concentrations of specific substances in natural waters change from hour to hour for many reasons. The discharge of polluting material, for example, will seldom be constant throughout the day. The quantity of diluting water, moreover, will change as a result of stream flow, tides and currents, ground-water fluctuations, and other variables of the receiving water. Sometimes water-quality criteria are specified to apply to the low-flow condition in a stream. As a consequence of these two important fluctuations, the concentration of any substance in natural waters may be expected to vary widely from hour to hour.

The question then arises: should the average (arithmetic mean) or maximum concentration be considered for threshold and limiting values? In some instances there is no question that the extreme value must govern. A low concentration of oxygen, for example, may persist in a stream for a few hours only; but it may result in the death of many fish. On the other hand, fluorides slightly in excess of 1.5 mg/l for a short period of time will cause no serious mottling of teeth, although prolonged excesses will be deleterious.

It is possible to define each analysis in terms of the prescribed frequency of sampling, a measure of central tendency (e.g., the arithmetic mean), and a parameter of variability such as the standard deviation. In lieu of these three logical yardsticks, the 80 percent or 20 percent values have frequently been selected as representative measures of central tendency and variability. Where an occasional high (or low) value will cause no serious harm, regulations often specify that the prescribed value should not be violated in more than 20 percent of any 20 consecutive samples. If sampling is infrequent, however, the water may be in violation for a long period of time before this error is evident. To avoid a long wait, with possible irreparable damage, the additional requirement is superimposed that no three consecutive samples can exceed the stipulated concentration. If a water just barely meets the 20 percent rule, the probability that three consecutive samples will be in violation is only 6 percent. Hence, this additional precaution superimposes no further severe restriction and yet it provides a warning signal for violations of the quality requirements.

Reference is made frequently to the 95 percent confidence limits, rather than to the 80 percent rule. Ninety-five percent of all observations should fall between these confidence limits. Thus, a stated value cannot be exceeded more than about 2.5 percent of the time. Expressed otherwise, any value that is outside the 95 percent confidence limits varies from the mean by more than two standard deviations.

BIOLOGICAL INDICES OF POLLUTION

No discussion of self-purification in surface streams would be adequate without reference to the role of biota and their use as indicators of stream conditions. However, the decomposition of complex organic compounds into stable substances by biological and biochemical processes and the synthesis of many end-products of decomposition into algae, crustacea, and fish are fascinating

subjects that defy attempt at condensation and synopsis. Instead of a review of current literature the reader is referred to classical standard works such as "Fresh Water Biology" by Ward and Whipple (15) and "Microscopy of Drinking Water" by Whipple, Fair, and Whipple (16). For more advanced and recent work, the references given for the specific biological pollutant in Chapter VII should be consulted.

Interest and enthusiasm concerning the relationship of macroscopic organisms to self-purification in streams enjoy periodic surges, and currently there appears to be renewed vigor along this line, especially for the use of biota as indicators of pollution and purification. Strictly speaking, the presence, absence, numbers, and diversity of all types of organisms, from viruses and bacteriophages through fishes and even aquatic mammals, could be used as criteria of water quality and consequently should be considered as part of this report. Inasmuch as the chemical, physical, and bacteriological criteria are easier to evaluate and apply, and inasmuch as the biological indices of pollution are relatively undeveloped, most of this report is confined to the conventional criteria. In Chapter VII, however, certain biological pollutants, such as pathogenic bacteria and toxic algae are covered. It should be recognized, furthermore, that biota other than those of fecal origin are seldom primary pollutants, i.e., they are seldom added directly to a stream by a polluting agency; instead, they are generally potential corollary pollutants whose numbers depend upon the concentration of primary polluting substances as well as upon natural conditions of temperature, stream flow, etc.

SYNERGISM AND ANTAGONISM

Throughout the literature relating to water-quality criteria, two terms appear frequently to describe the effects and interaction of two or more substances. Inasmuch as these terms, "synergism" and "antagonism," as used hereinafter may not be familiar to some readers, a few words of explanation are in order.

Webster's New Collegiate Dictionary (Merriam) defines synergism as "cooperative action of discrete agencies such that the total effect is greater than the sum of the two effects taken independently, as in the action of the mixtures of certain drugs." The corresponding adjective is "synergistic—of or relating to synergism." The terms "synergy" and "synergetic" are sometimes used but there appears to be a shade of difference in their meaning as compared with synergism. Thus synergy is "combined action or operation, as of muscles, nerves, etc." and the adjective synergetic is "working together, cooperating, as synergetic muscles." Insofar as water-quality criteria are involved, therefore, the terms synergism and synergistic seem to be more descriptive.

To stress the importance of synergism, let us consider a hypothetical example. Substance A may not be toxic to fish in a concentration of 2.0 mg/l, and substance B may not be toxic at 5.0 mg/l; yet when only 1.0 mg/l of A and 2.0 mg/l of B occur together in water the combined effect may be rapidly toxic. Actual examples of synergism are noted later in this report, especially in Chapter VI. Synergism should not be confused with direct chemical reactions that increase the inimical effects of separate substances, e.g., as chlorination intensifies the taste of phenols by the formation of chlorophenolic compounds. For an interpretation by the courts of synergistic action, see Jessup and Moore Paper Company v. Zeitler, as described in Chapter IV.

The opposite of synergism is antagonism, in which the total effect of discrete agencies is less than the sum of the separate effects taken independently. Thus, by itself substance C may be toxic in concentrations as low as 1.0 mg/l, but in the presence of substance D, the toxic concentration of substance C may be raised to 5.0 mg/l. Part of this antagonism may result from direct precipitation of the toxic compound, but more often the antagonistic action is not so well defined or understood.

The entire subject of synergism and antagonism is worthy of further investigation, for little is understood about the basic mechanisms or fundamentals governing these processes. Where their effects have been observed in the literature, they are noted in this report, but seldom is an attempt made to explain them.

DELINEATION OF TOXIC CONCENTRATION

The toxicity of many potential pollutants in water toward plant and animal life is a time-concentration phenomenon, i.e., for a given concentration, toxicity increases with continued exposure. For other substances, however, toxicity is relatively independent of time, i.e., if a given concentration is not toxic in one or two hours, it will not be acutely or directly toxic.

Experimental data on toxicity are sometimes reported as "minimum lethal dose" (MLD), or the minimum concentration required to kill one or more of the test species. More frequently in bioassay work the term TL_m (tolerance limit, median) is used to designate the concentration required to kill 50 percent of the tested organisms. For either of these parameters (MLD or TL_m) it is essential that the time of exposure be specified. Thus, one reads about 24-hr. TL_m values or 96-hr. MLD's. For direct feeding experiments or injections, the median toxic dosage is noted as LD_{50}, or the lethal dose for 50 percent of the animals. Many LD_{50} values are based on single feedings or injections, in which case time of exposure is not a criterion; but where ingestion continues over a period of days, the time of exposure should be specified.

Most bioassay work relates to "acute" and "direct" toxicity, i.e., the lethal action within a period of 96 hours or less. The deleterious effects of many substances, however, may not be evident for weeks, months, or longer. Such long-term effects are known as "chronic" toxicity. They may be related to changes in appetite, metabolism, disorders of the nervous system, reproduction, or other vital functions, the alteration of which does not produce early death. For this reason, many investigators prefer to measure toxicity not in terms of death of the test species but rather in relation to respiration, food consumption, reproduction, or other function. It is reasoned that changes in these functions are precursors of chronic toxicity and eventual diminution or elimination of the species. Even more remote in the time sequence is possible mutation of genes that may cause a long-term chronic effect.

Toxicity may be indirect as well as direct, i.e., a substance may have no direct toxic action toward a given species either on an acute or chronic basis; but it may result in eventual elimination of the species by toxic action against one or more of the organisms in the food chain of the species in question. Very little quantitative information is available on indirect chronic toxicity, for such determinations require a complicated study of the entire ecology.

In this report, toxicities are expressed for the most part in the language of the original investigators. Some data are given as MLD values, others as TL_m's, and others in terms of non-lethal effects. In establishing criteria of toxicity, some authorities speak of "threshold" and "limiting" concentrations. A threshold concentration is defined as one at which a given beneficial use of the water is not damaged to any measurable degree by pollution, but slight effects are noticeable. A limiting concentration is one at which the beneficial use is severely inhibited.

Throughout the following report an attempt is made to differentiate between limiting and threshold concentrations, but unfortunately such careful segregation of values is not always possible. Many criteria are reported merely as "desirable" or "optimum", while others are objectives or goals toward which to strive. This confusion of terms and blending of values is particularly noticeable in Chapter V where criteria for various industrial water supplies are given. Many industries will specify water of high purity but will accept inferior quality if other economic considerations so dictate.

The confusion in parameters of concentration is particularly evident where biological requirements are involved. Here it is virtually impossible to establish threshold concentrations inasmuch as effects of a specific substance on a particular organism will not portray the over-all changes in the complex interrelationships of ecology. For example, fish, minnows, daphnia and other organisms may be exposed to one mg/l of substance X for 100 hours or longer without apparent injury; yet the continued discharge of substance X so as to give one mg/l in a natural stream might alter materially the natural fauna of the stream and result in the absence of fish.

How can TL_m or MLD values from short-time bioassay tests be converted to safe, threshold, or limiting concentrations? Unfortunately there is no simple application factor or formula to convert 24-, 48-, or 96-hour TL_m or MLD data to concentrations that are considered safe for long-time exposure. Simple factors such as one-tenth of the 48-, or 96-hour TL_m have been proposed, while other investigators prefer formulas involving ratios of 24-, and 48-hour TL_m values. For a discussion of the merits and limitations of application factors, see the papers by Henderson (2037, 2038) and Pearson (1772).

For man, livestock, and wildlife the concentration of a specific substance in drinking water may represent only a small fraction of the total intake of that substance. As pointed out by Cass (2039) it is essential to consider the total intake from (a) atmospheric exposure through nose, eyes, lungs, and digestive tract, (b) foodstuffs, (c) water and beverages, and (d) skin contact. Man's total intake of sodium, calcium, chloride, and sulfates, for example, is generally far more dependent on his diet than on the concentrations of these substances in his drinking water.

CHAPTER III

WATER QUALITY CRITERIA PROMULGATED BY STATE AND INTERSTATE AGENCIES

The aims of this chapter are restricted to: (1) a brief commentary on the historical development of water-quality standards and water-pollution-control practices; (2) a discussion of the various types of standards or criteria with an evaluation of their significance or utility; and (3) a summary of the standards and/or provisions for standards of the states, interstate agencies, and international groups of which the United States is a member.

No attempt is made to consider the legislation by which water-pollution-control boards or commissions are established, nor the powers and duties delegated to such bodies other than for the establishment of standards and/or systems of stream classification. Furthermore, this investigation does not deal with the action of boards in requiring permits, approving plans, or operation of programs; nor with enforcement, penalties, or appeals from decisions of the boards, except insofar as such actions are relevant to water-quality considerations.

HISTORICAL BACKGROUND

The earliest water-quality criteria were those that employed physical tests such as temperature, taste, odor, color, and turbidity. Although old and familiar to the layman, these criteria scarcely deserve the faith placed in them by a Mississippi jurist in 1904 when he declared, in part "It is not necessary to weigh with tenderness and care the testimony of experts. Any ordinary mortal knows whether water is fit to drink and use" (27).

Many of the physical criteria have been refined and may now be evaluated quantitatively with precision and understanding. Temperature falls in this category. Others, however, are still expressed in contemporary standards by vague terms such as "No discharge shall cause objectionable discoloration or turbidity," thereby leaving to the administrative agency or to the courts the final interpretation.

Chemical criteria of water quality were proposed almost two centuries ago. As early as 1784 waters were said to be "drinkable" if they would "dissolve soap without forming lumps . . . and deposit nothing or very little by tests" (28). Organic matter in water was viewed with suspicion by early observers and attempts were made to determine it quantitatively and relate it to the incidence of disease. That human and animal wastes could contaminate water and render it dangerous for drinking and other domestic uses has been known for centuries (29, 30). Such tragedies as the cholera epidemic resulting from sewage contamination of the Broad Street well in London in 1854 served to increase interest in developing criteria to detect impure and dangerous water.

Such criteria were proposed by De Chaumont (31) who held that a water was "pure and wholesome" if it would yield less than 17 mg/l of residue upon evaporation and the ". . . solids on incineration should scarcely blacken"; a water was "usable" if it had less than 51 mg/l of residue and the ". . . solids may blacken a little, but no fumes should be given off"; a "suspicious water" would have 54 to 85 mg/l of residue, with ". . . much blackening on incineration or nitrous fumes given off"; and an "impure water" would have more than 85 mg/l of residue with ". . . much blackening and nitrous fumes given off, or smell of burnt horn." These criteria were tempered, however, by the remark that "In peat waters the incinerated solids may blacken considerably" (31). Other early tests were patterned on the rate and quality of color changes of potassium permanganate added to a small sample of the water.

About 1870, criteria based on the amount of ammonia derived from proteinaceous substances in the water came into prominence, and Leeds (27) proposed that, in streams used for water supply, the free ammonia should not exceed 0.12 mg/l and the albuminoid nitrogen 0.28 mg/l.

An early method of measuring the microbiological quality of water depended on its "keeping power." Thus, a relatively pure water could be stored for a much longer time than could an impure water without changes or growths that were visible to the naked eye. With the development of the microscope, criteria were formulated on the number and type of organisms and organic debris observed in the water.

Thresh (32) makes an interesting comparison of two early bacterial criteria relating the total number of bacteria to the quality of the water. His tabulation of the recommendations of Miquel and Mace is shown below:

Designation of Quality	*Number of bacteria per ml, after*	
	Miquel	*Mace*
Very pure water	0-10	0-10
Very good water	10-100	20-100
Good or pure water	100-1,000	100-200
Passable water	1,000-10,000	200-500
Impure water	10,000-100,000	500-1,000
Very impure water	Over 100,000	Over 1,000

Hence, a water containing 750 bacteria per ml would be "impure" by Mace's standards and "good" by Miquel's.

As might be expected, almost all criteria for determining water quality have been subject to criticism of some sort. Many standards, just by posing as a target for criticism, have served a useful purpose by instigating and inspiring further research, thereby increasing the total knowledge. Bean, in a symposium (1744), discusses the need for high-quality professional standards within the water industry. In a later paper (1745), he presents some finite values for an ideal water. Lubratovich and

Ruble (1746) point out that the U. S. Public Health Service Drinking Water Standards define a safe water, but not necessarily a good quality water, and then proceed to suggest finite high-quality standards. Both sets of standards were published with the same intent, namely, to create interest and discussion. Other standards, however, have been meekly and blindly accepted and somehow did not serve to spark the scientific curiosity that leads to progress.

That some criteria have thus served their exciting or antagonizing purpose, then slid out of sight or been improved upon, is an encouraging fact, for many criteria of today are undoubtedly in error and will be improved upon in time or cast away. Criteria are merely imperfect human-made tools and will be supplanted by other criteria as scientific knowledge advances. Among water-quality criteria there are few if any that can be considered absolute and final, for absolute truth is a rare thing.

DEVELOPMENT OF WATER POLLUTION CONTROL PRACTICES

In the last three decades, there has been an extremely rapid expansion of governmental functions and responsibilities in the field of water-pollution control, closely following the development of the greatly widened governmental activities in other areas of our highly integrated and complex society. Inasmuch as water-pollution control has remained to a large extent a state responsibility, the principles, laws, and methods adopted by each state for such control differ markedly, depending upon the particular needs and experiences of the individual state. In reviewing each state's program of water-pollution control, however, certain patterns, similarities, trends, and styles appear.

The authority to control water pollution began with the common law. Several articles, papers, and reports have been published to describe the water-pollution aspects of the body of law dealing with public nuisances, riparian rights, private damages, equity, and similar facets of law (33).

The basic doctrine threading through the numerous court decisions affirms the principle that no right can be acquired for an unreasonable abuse of a water to the extent that a downstream user suffers loss or hardship. The earliest and most apparent results of uncontrolled or indiscriminate pollution of water were the threats to public health largely from the disposal of untreated sewage, and the mass killing of fish largely from the disposal of strong industrial wastes. It was natural, therefore, that the first efforts to control water pollution were directed in the fields of public health and wildlife conservation.

The tools for controlling water pollution furnished by the common law were largely punitive, applicable after the pollution was accomplished and the damage was done. However, there appears to be no fundamental reason why an efficient and adequate system of control could not develop, over a period of years and with the accumulation of custom and precedents, largely in such a shadow of potential litigation. Water-pollution controls based upon the common law have been used in part, and still are, by various states. In many states it is common to find two different sets of laws applied to the same substance—water. These states may use the riparian doctrine or the doctrine of prior appropriation to govern the use of surface waters while the common law prevails for the use of ground waters (1747). Although older, the common-law criterion can be quite severe (1748).

As the need for some sort of restraint to the pollution of water grew, health departments of many states were given statutory powers and responsibilities for pollution control. Corollary powers and responsibilities were usually given to other agencies for the protection of fish and game. The legislation of each state differed greatly in scope and powers, but usually the boards of health were given the power to protect the supplies of domestic water from contamination, to abate nuisances, and to pass rules and regulations to insure this protection. The departments of fish and game usually were given analogous powers for the protection of fish and wildlife. Many states adopted a scheme of licensing or issuing permits for the disposal of various wastes in natural waters to facilitate the control of water pollution.

In general, the primary responsibility for water-pollution control fell upon the divisions of sanitary engineering or environmental sanitation in the state health departments. These agencies drew their power from three sources: the common law, specific statutes, and quasi-legal rules and regulations of the boards of health. In many states such a foundation in the areas of health and wildlife was deemed to be unsatisfactory for the control of water pollution, inasmuch as there was a lack of delegation of authority and responsibility for pollution abatement to a single administrative agency. Furthermore, there were peculiarities that made such abatement unenforceable in certain conditions, and there was confusion over the very definition of pollution and the decision of what was to be abated.

To correct some of these inadequacies, the legislatures of several states enacted specific water-pollution-control legislation. Generally, these newer acts supplement the existing statutes by providing for a single administrative agency that is responsible for control and abatement of water pollution in the state. Provisions are often made (a) for representation of the several interests concerned in water pollution, either on an advisory board or on the control board itself, (b) for the definition and determination of pollution, usually by stream standards or classifications, (c) for the enforcement of abatement orders, with penalties for non-compliance, and (d) for hearings and appeals of parties affected by actions of the agency.

More recent developments include the closer alignment of state water-pollution-control agencies with other state water-resource-development activities (see New York, North Carolina, and California), the participation of the Federal Government in interstate pollution-control agencies, and the formation of state-federal basin committees on water-resource activities whereby the various state and federal agencies will have representation.

TYPES OF CRITERIA FOR WATER POLLUTION CONTROL

There are two basic types of criteria or standards that have been used for the control of water pollution. One type dealing with the quality of the receiving water, whether stream, lake, river, estuary, open ocean, or

ground water, is commonly designated as "stream standards" or "receiving-water standards." The other type, referring to the quality of the wastes to be discharged from a given plant, is called "effluent standards." Each type has its advantages and disadvantages, each type has its advocates and opponents, and each type is in common use today (1749, 1750).

Stream standards may be divided into two distinct categories; (a) dilution requirements and (b) standards of receiving-water quality. Largely outmoded now (1751), dilution requirements were favored at the turn of the century as handy yardsticks and rules-of-thumb. They were proposed first in 1887 for the Chicago drainage canal, when Rudolf Hering recommended a dilution rate of 3.3 cfs per 1,000 persons sewered (35). Later Hazen, Goodnough, Stearns and others in New England proposed requirements varying from 2 to 10 cfs per 1,000 persons, depending on the characteristics of the receiving waters (26).

Considerations of dilution entered into the recommendations in the Eighth Report of the Royal Commission on Sewage Disposal, 1913. Although these recommendations were never legalized by Parliament, they are generally quoted in bills before parliamentary committees and in courts of law in Great Britain (36). The Royal Commission recommended that a general standard and several special standards be established, the choice of which in each instance would depend on local circumstances. In order to comply with the general standard, an effluent had to contain not more than 30 mg/l of suspended matter and not more than 20 mg/l of 5-day, 18.3°C BOD, without reference to dilution. In specifying special standards, however, the Royal Commission considered dilution as the chief factor. Where dilution exceeded 150 to 1, the limitation on BOD was omitted and the suspended solids content was allowed to be as high as 60 mg/l. Where dilution exceeded 300 to 1, the standard for suspended solids was relaxed further to 150 mg/l; and with dilution in excess of 500 to 1, all tests could be waived, provided of course that esthetic requirements with regard to screenings and floating solids were considered.

Standards of quality of the receiving water are based on threshold and limiting values for specific substances in the water, and depend on the beneficial uses to which the water may be put. Widely used now and gaining in favor among those who feel that some sort of formalized criteria are necessary, this category of stream standards is frequently correlated with a system of stream classification or zoning whereby separate standards are set for each stream or zone. The principal advantage of standards of stream quality over effluent standards lies in the fact that they take into account dilution and the assimilative capacity of the receiving water and consequently lead generally to an economy of treatment works for pollution abatement. On the other hand, such standards are difficult to formulate and define, and more difficult to administer. Where stream classification is mandatory and complicated, the program may become extremely cumbersome. For an example of stream standards without classification, see the brief description of the Indiana program and for an example of standards coupled with classification see the New York plan. An example of stream standards that have not been legislated but which have been generally accepted and used as guides (in England) are: a maximum limit of 4 mg/l of BOD; pH in the range of 6.0 to 9.0; suspended solids that will not cause silting of streams; and virtually complete removal of highly toxic substances such as cyanides and certain metal ions (1752).

The second general type, effluent standards, may also be divided into two general categories; those that restrict the strength and/or amount of substance that can be discharged, and those that specify the degree of treatment or percentage removal of a specific pollutant that must be accomplished by treatment or by changes in industrial processes. The first report of the Rivers Pollution Commission in England, 1868, recommended "standards of purity" for effluents, proposing limits for suspended organic and mineral matter, organic carbon, and nitrogen, sulfides, metals (other than the common alkaline earth metals), acidity, alkalinity, arsenic, free chlorine, oil, and color (1753). As an example of restrictions on the strength of wastes, the Interstate Commission on the Delaware River (Incodel) specifies that effluents discharged into Zone 1 shall have a BOD not greater than 50 mg/l, and into Zone 2 not greater than 100 mg/l.

Pennsylvania has pioneered in effluent standards that specify the amount of pollutant that may be discharged by special industries and each process within such industries. This program is based on a percentage removal of pollutants from "normal" for each process, as established by representatives of industry. Consequently the effluent requirements favor good housekeeping and penalize the inefficient operator.

Standards that specify the degree of treatment are also typified by those of Incodel, which require in addition to the limitations on BOD as specified above that the BOD reduction of wastes discharged into waters of Zones 1 and 2 shall be at least 85 percent and that wastes entering Zone 3 shall have a BOD reduction of at least 35 percent.

Effluent standards have the advantages of simplicity and ease of administration, for they are well defined and equitable among industries. Their primary disadvantage lies in their uneconomical use of the assimilative powers of receiving waters.

SIGNIFICANCE OF CRITERIA

A marked difference of opinion exists among water-pollution-control authorities, in industry, in private practice, and in public agencies, regarding the advantages and disadvantages of various types of standards and systems of classification. Dappert (1421) presents the case for the use of classes and standards, whereas McKee and Bacon (1422) outline the merits of case-by-case studies in lieu of fixed standards. In a panel discussion, Wieters, Knox, Healy, and Chase (1423) present many facets of the arguments for and against classification of surface waters, while Hopkins (1424), Hess (1413, 1754), Gloyna, Wolff, Geyer, and Wolman (1755), and Frederick (1425) discuss the background of several philosophies.

In a comparison with England, Klein notes that in the United States "stream standards rather than effluent

standards are preferred for legal purposes and (that) this is understandable in a country where, in general, rivers are large and pollutions occur at wide intervals" (1756). However, Pennsylvania has long favored effluent standards because they are easy to enforce (1757).

The difference of opinion is just as prevalent abroad as it is in the United States. In England, for example, "experience has led to the conclusion that to devise by-laws (standards) which would be satisfactory, effective, and generally applicable would be so difficult as to be almost impossible. Recognizing this, the trade effluents sub-committee of the Central Advisory Water Committee recently (1960) recommended a change in the law in England and Wales whereby the current procedure, suitably modified, would also apply to old discharges. By this, the discharges would be dealt with one by one instead of by reference to a general standard, and this would enable all the circumstances to be taken fully into account" (1758).

The Water Research Association in England discussed the philosophy of establishing limits or standards of water quality. It is their conclusion (1759) that:

"1. Limitation is clearly advantageous only if those substances that are injurious to human health in concentrations that are lower than those in which the substance may be otherwise deleterious, e.g. lead.

"2. Limitation may be advisable where the substance is not detrimental to human health, provided the ground for the limitation is generally applicable and clearly stated, e.g. iron on account of coloration imparted.

"3. Limitation is not desirable where the presence of the substance at concentrations less than that at which it is injurious to health could be objectionable on more than one ground. In such cases a list of the grounds on which the presence is harmful, together with the quantities below which no damage could result, would be advantageous.

"4. Such tables might be of great complexity as the activity of one substance can depend on the presence of another. Although present knowledge does not permit of their construction a greater understanding should be sought.

"5. Accurate information and good judgment have no substitute."

King (1760, 1761) also discusses the difficulties in formulating standards and their disadvantages and impracticability. Nevertheless, he states, in order to protect the quality of tidal waters and streams it is necessary for the authorities controlling the waters to adopt conditions of discharge.

For Australia, the impracticability of any universal standard of purification (treatment) is pointed out, but still the need to draw up standards for particular rivers or for sections of rivers is stressed (1762).

In Germany, the fixing of standards of concentration is not recommended (by Bucksteeg), as local conditions and capacity of streams vary (1763). Another writer concluded that general effluent standards are impractical, and that equitable standards for an individual effluent can be established only after careful consideration of both the effluent and the receiving stream, and as the latter change so would the standards have to change (1764).

The need for effluent standards in South Africa is cited, but separate standards will be necessary for each region of the country as a result of wide climactic and geographical differences (1765).

On 23 and 24 February 1960, the U.S.S.R. Chief State Sanitary Inspector and the boards of the All-Union and All-Russian Hygiene Societies called a meeting to discuss the problem of establishing maximum permissible concentrations. A discussion of the meeting was published by T. E. Nagibina in a Soviet journal and translated into English by B. S. Levine. According to this account, a Soviet scientist (Ya. M. Grushko) had questioned the basic principle of levying maximum permissible concentrations on harmful substances in water since this practice made it possible for industry to pollute the water "according to law." Such regulation resulted in a hindrance of the work against water pollution. These comments, published in newspapers and magazines, had precipitated a storm of controversy that resulted in the meeting.

Over 60 leading Soviet scientists participated in the meeting. The only person who spoke on behalf of Grushko was Grushko himself. He reaffirmed his position, stating that the policy of relying on standards is wrong. He added, however, that he is not against maximum permissible concentrations per se. He only wanted to see these standards lowered, i.e., a "drastic reduction in the maximum permissible concentrations."

All other participants soundly scolded Grushko's views. V. M. Zhdanov, the U.S.S.R. Chief State Sanitary Inspector, summarized the favorable (almost militant) attitude of these participants for standards with, "We in the State Sanitary Inspection Commission consider the discussion closed . . ."

The results of the meeting were discussed on 3 March 1960 by the staff of the U.S.S.R. Ministry of Public Health who reaffirmed and endorsed the principles and practice of maximum permissible concentrations (1766).

In summary, it appears that sanitary engineers have an almost universal dislike for broadly applied water-quality standards. While all of the partisans of the various views will agree that effective and economic abatement of pollution is the logical goal it remains difficult to assess the merits of each philosophy. Often the engineering and economic aspects are obscured by political and personal factors.

FEDERAL ATTITUDE TOWARD CRITERIA OF POLLUTION CONTROL

Five Federal laws containing major provisions concerned with the control of water pollution have been enacted by the Congress. Two of these, the Rivers and Harbors Act of 1899 (Section 13) and the Oil Pollution Act of 1924, are primarily concerned with preventing damage to shipping, and are administered by the Department of Defense. The 1899 Act prohibits the depositing of waste materials, other than that flowing from streets and sewers in a liquid state, in or on the banks of navigable waters and their tributaries (see Chapter IV, United States v. Republic Steel Corp.). The 1924

Act prohibits the discharge of oil into the coastal navigable waters of the United States.

The third major Federal enactment regarding water pollution was a provision in the Public Health Service Act of 1912, which gave specific authority to the Public Health Service to conduct investigations of the pollution of streams and lakes by sewage and other causes. It was under this legislation that the Service carried out early research and investigations that provided the basis for information and consultative services to other agencies.

The first comprehensive-type legislation in the pollution control field was the Water Pollution Control Act of 1948 (P. L. 845, 80th Congress) which authorized expanded activities by the Public Health Service. This law, passed after a half-century of consideration of national water pollution problems and evaluation of the responsibility of the Federal government, added the principles of State-Federal cooperative program development, limited Federal enforcement authority, and financial aid. Finally, the particular concern of the Federal government in the growing national pollution problem resulted in the new Federal Water Pollution Control Act (P. L. 660, 84th Congress), which was approved by the President on July 9, 1956. Experience had shown that many of the principles and provisions embodied in the Act of 1948 were sound, and many were carried over into the new legislation (1767).

The Federal Water Pollution Control Act Amendments of 1961 considerably elevated the significance of the entire federal water-pollution-control program. Instead of being a Public Health Service program, it is now administered directly by the Secretary of the Department of Health, Education, and Welfare. Also, the amendments greatly increased the area of federal enforcement since the previous restriction to interstate waters has been lifted to include interstate *or navigable* waters.

Section 4 of the act states, in part, that the Secretary shall, "in cooperation with other Federal, State, and local agencies having related responsibilities, collect and disseminate basic data on chemical, physical, and biological water quality insofar as such data or other information relate to water pollution and the prevention and control thereof" (1768). The 1961 amendment adds ". . . the Secretary shall develop and demonstrate under varied conditions . . . practicable means of treating municipal sewage and other waterborne wastes to remove the maximum possible amounts of physical, chemical, and biological pollutants in order to restore and maintain the maximum amount of the Nation's water at a quality suitable for repeated reuse . . ." (1769).

Following discussions among federal, interstate, state, and local agencies having allied responsibilities, it was agreed by them that the over-all objectives of the program should include:

"a. Long-term information on changes in water quality at key points in river systems, as such quality may be affected by change in water use and development.

"b. Continuous information on the nature and extent of pollutants affecting water quality" (1768).

Clearly, the federal position in regards to quality is one of extreme concern. However, no national standards or regulations have been developed for the control of discharge of wastes into surface waters (1755).

The question of the introduction of national standards was lightly touched upon at the 1960 National Conference on Water Pollution. Recommendation 21 of the Conference states, "Provision should be made within the Public Health Service for developing the water quality criteria which are suited to application on a national basis. However, many water quality criteria are not uniformly applicable because of the effects of area usage differences, stream characteristics and other factors. State and local determinations of some criteria also will have to be made. It is recognized that periodic revision of these criteria not only will be in order, but should be sought as new data are made available" (1772).

Congress recognizes that primary responsibility in the field of water-pollution control rests with the states (1773). The federal role in water pollution is to provide technical services and financial aid to states, interstate agencies, and municipalities. A "Suggested State Water Pollution Control Act" has been issued by the Public Health Service, and as of July 1961 its principles had been used by 40 states (1743). An explanatory statement with respect to classification and standards is as follows:

"Some agencies in the administration of water pollution control programs have classified the waters of the State according to their use and have established standards of quality for such waters in accordance with their respective uses. Proponents of this method have urged that classification and setting of standards is an essential element of any comprehensive program and also that no enforcement action can be undertaken without determination of the use to which a particular body of water should be put and the degree of quality which the water must have in order to be suitable for such use. This approach, however, has been severely criticized by others who maintain that the process is administratively difficult and time consuming, that classifications once made are hard to change and tend to create vested interests, and that the tendency will be to reduce waters to the level of mere carriers of wastes because of the pressure of special interests.

"The Suggested State Water Pollution Control Act authorizes the agency to classify waters and set up standards of quality for water falling within particular classifications but does not make it mandatory to do so. Classifications and standards once promulgated have a definite legal effect and their violation is made unlawful; conversely, discharges which comply with such classifications and standards are not pollution within the meaning of this Act. In view of the number of persons affected by classifications and standards, it is required that their adoption be preceded by a public hearing open to all residents of the areas affected, and that adequate notice thereof be given. The Act provides that in classifying waters and setting standards the agency will be guided by the principle of constantly seeking to improve water quality and upgrading streams for progressively higher uses to the maximum extent practicable" (1774).

The Suggested Act itself deals with the above subject matter in Section 6 as follows (also see 1793):

"Section 6. Classification of Waters; Standards of Water Quality.

(a) In order to effectuate a comprehensive program for the prevention, control and abatement of pollution of the waters of the State, the Board is authorized to group such waters into classes according to their present and future best uses for the purpose of progressively improving the quality of such waters and upgrading them from time to time by reclassifying them, to the maximum extent that is practical and in the public interest. Standards of quality for each such classification consistent with best present and future use of such waters may be adopted by the Board and from time to time modified or changed. (States which adhere to the "prior acquisition doctrine" should take cognizance of the effect that classification will have on water rights established under other laws.)

(b) Prior to classifying waters or setting standards or modifying or repealing such classifications or standards the Board shall conduct public hearings in connection therewith. Notice of public hearing for the consideration, adoption or amendment of the classification of waters and standards of purity and quality thereof shall specify the waters concerning which a classification is sought to be made or for which standards are sought to be adopted and the time, date, and place of such hearing. Such notice shall be published at least twice in a newspaper of general circulation in the area affected and shall be mailed at least twenty days before such public hearing to the chief executive of each political subdivision of the area affected and may be mailed to such other persons as the Board has reason to believe may be affected by such classification and the setting of such standards.

(c) The adoption of standards of quality of the waters of the State and classification of such waters or any modification or change thereof shall be effectuated by an order of the Board which shall be published in a newspaper of general circulation in the area affected. In classifying waters and setting standards of water quality or making any modification or change thereof, the Board shall announce a reasonable time for persons discharging wastes into the waters of the State to comply with such classification or standards, unless such discharges create an actual or potential hazard to public health.

Any discharge in accord with such classification or standards shall not be deemed to be pollution for the purposes of this Act" (1774).

Whether or not this new and suggested legislation indicates an eventual application of federal standards to interstate and to navigable waters remains to be seen.

The statutes in effect in each state with respect to standards, objectives, requirements, or criteria of water quality are described in the following sections of this chapter.

ALABAMA

The Water Improvement Commission has the primary responsibility for water-pollution control in Alabama. The Commission was created in 1947 as an investigation and advisory group (37), and was called the Water Improvement Advisory Commission. In 1949 it was given fairly comprehensive powers (38), and in 1953 it was further strengthened, renamed as indicated, and made more flexible by the creation of an Executive Committee of five designated members of the Water Improvement Commission who act for the full Commission under such powers as are delegated to it by the Commission. Among the duties of the Commission is that of establishing criteria or standards for recognized limits of pollution (1770).

The standards of quality are to be related to the reasonable and necessary use of the waters in the public interest, in the consideration that no single standard of treatment and no single standard of quality are practical, and that future as well as present uses must be considered (1770, 1771).

The Commission has not yet established any standards of water quality, relying instead upon a review of each situation on the basis of its merits and upon the permissible conditions as governed by downstream water uses. "Permits are required for the discharge of sewage, industrial waste and other wastes subject to the authority and control of the Commission. Each permit shall stipulate the conditions under which such discharge may be permitted. Preservation of water quality for all reasonable downstream uses is a primary factor in the issuance of permits" (39).

ALASKA

In 1949 Alaska enacted a comprehensive water-pollution-control law known as the Alaska Water Pollution Control Act, administered by the Water Pollution Control Board, and created within the Department of Health. The Board was responsible for the setting of standards of pollution and of water purity, and in so doing, recognized that owing to variable factors no single standard of quality and purity is applicable to all of the waters of the state (1776). Then in 1959, the State Organization Act abolished the old Board of Health and the Alaska Water Pollution Control Board. It reconstituted the Water Pollution Control Authority within a new Department of Health and Welfare. The Commissioner of the new Authority is charged with the functions and duties of the former Commissioner of Health and the Board of Health, as well as those of the former Water Pollution Control Board and the Executive Secretary of the Water Pollution Control Board (1777).

Industrial wastes from "gravel-washing plants and all phases of Placer Mining Operations shall not be considered pollution within the meaning of this Act" (1776). However, it is the public policy of the State of Alaska to maintain standards of purity of its waters consistent with public health and public enjoyment, the propagation and protection of fish and wildlife, and the industrial development of the state, and to that end require the use of all known available and reasonable methods to prevent and control pollution (1778).

ARIZONA

Water-pollution control in Arizona rests largely with the State Department of Health and with the Game and Fish Commission. The State Board of Health establishes the general policies of the Department of Health, and prescribes regulations for minimum standards of the quality of water sold or distributed to the public, for

inspection and certification of water supplies, and for correction of those systems which do not comply with minimum standards. Its regulations also provide for sewage treatment, industrial-waste treatment, and the setting of minimum standards therefore. In general, the Commissioner of the Department of Health is given the power to issue and enforce necessary special orders to protect the public health and welfare in cases of pollution of bodies of water (41).

The Arizona Game and Fish Commission is authorized to restrain the discharge of any poisonous, deleterious, or polluting substance or waste into a stream or body of water that may be injurious to fish, fishlife, or wildlife (1779).

The statutes do not provide specifically for establishing stream standards or classifications (40) and none have been established.

ARKANSAS

The Arkansas Water Pollution Control Commission was created and established within the State Board of Health by the 1949 Legislature. Among the powers and duties given to the commission was the responsibility "to make such classification of the waters of the State as it may deem advisable. . . " (42).

Amendments to the act were passed in 1953 and 1959 which were primarily concerned with administrative details. But the amendments of Act 120 of 1961 considerably broadened the definitions of the earlier act and, further, charged the commission with the power of "setting standards of water quality, classifying waters or evidencing any other determination" under the Act (1781).

The Commission is authorized to control the disposal of wastes into the waters of the state by municipalities, industries, and public and private persons; to establish pollution standards for such waters; to restrain the discharge of waste material or polluting substances into state waters; and to issue rules, regulations, and orders in carrying out these functions (1782).

As yet, no standards or objectives have been established. Any disposal of sewage or industrial wastes is subject to a permit issued by the Water Pollution Control Commission.

The Arkansas Game and Fish Commission has authority in the field of water pollution parallel to that of the Water Pollution Control Commission.

CALIFORNIA

The background and philosophy of water-pollution control in California are described in Chapter I. Primary responsibility for protection of the quality of fresh and saline waters rests with the State Water Pollution Control Board, which is intended by law to coordinate the interests of other state agencies, such as the Department of Public Health, Department of Fish and Game, Department of Water Resources, and Department of Agriculture. While the State Water Pollution Control Board establishes overall policy, budgets and distributes funds (including federal allotments under P.L. 660), sponsors research and investigations, publishes reports, and generally administers the program, the specific control measures are carried out by nine Regional WPC Boards.

With considerable freedom to conduct water-pollution abatement in accordance with local conditions and needs, each regional board has developed its own pattern of general "objectives" for the maintenance and protection of the major receiving waters in the region and has promulgated specific "requirements" for each existing or proposed discharge of municipal or industrial wastes. The general objectives relate to the quality to be maintained in the receiving waters (stream, lake, groundwater basin, estuary, or open ocean) but the specific requirements may govern the amount or concentration of pollutant in the effluent as well as the quality of receiving water. To reproduce, tabulate, collate, or otherwise describe the hundreds of objectives and requirements issued by the nine regional boards would be tedious and perhaps unwise. Instead, one example will be cited for objectives and one for requirements. These examples are not necessarily typical, nor should they be considered as models. Indeed, a major feature of the California program of water-pollution control is the individual case-by-case analysis of each specific problem, a feature that militates against typical or model solutions.

Objectives have been promulgated by the Santa Ana Regional Water Pollution Control Board (No. 8) for the quality of exported and sub-surface outflow of ground water from the Bunker Hill Basin, a fairly well-defined ground-water body underlying the City of San Bernardino and contiguous areas. These objectives, reproduced as Appendix A-1 of this report, are intended to prevent the degradation by sewage or industrial wastes of this extensive water resource and the downstream groundwater basins into which it discharges. Although they do not relate to any specific dicharge, the objectives are used as guidelines in establishing requirements for present and future waste effluents in this area.

An example of requirements for a specific disposal situation is given by Resolution No. 60-76 of the Los Angeles Regional WPC Board (No. 4), reproduced herein as Appendix A-2. This resolution prescribes requirements for the disposal of effluent from an activated-sludge plant to be used for reclamation of water at Whittier Narrows, in the County of Los Angeles. After recitation of background information and findings, the resolution specifies the maximum limit of 14 constituents in the effluent, the location of sampling stations, the type, frequency and technique of sampling and analysis, the records and reports to be maintained and submitted, and the establishment of monitoring wells to determine the effect, if any, that the spreading of discharged wastes will have on underground receiving waters.

Objectives and requirements issued by the nine regional WPC boards in California are subject to frequent review and revision in the light of new findings and improved techniques of analysis. For this reason, any interested reader should check with the responsible regional board for the most recent version relating to any specific situation.

COLORADO

The agency that has primary responsibility concerning water-pollution control is the State Department of Public

Health. However, the City and County of Denver have authority to control pollution on the South Platte River and tributaries above Clear Creek, such control to keep the waters safe for domestic purposes (1783).

Changes in the State Health Department Laws include a 1955 amendment:

"The state department of public health shall establish and enforce minimum general sanitary standards as to the quality of water supplied to the public and as to the quality of the effluent of sewerage systems and trade wastes discharged upon the land or into the surface or ground waters.

"The phrase 'minimum general sanitary standards' as used in this section shall mean the minimum standards reasonably consistent with protection of the public health, and in the case of minimum general sanitary standards as to the quality of water supplied to the public, the same shall in no event be less than the drinking water standards of the United States Public Health Service. The word 'standards' as used in this section shall mean standards reasonably designed to promote and protect the public health. . . .

"To enforce the public policy of the state of Colorado with reference to the pollution of waters of the state by human excreta as herein expressed, it is hereby declared that the health, safety, and welfare of the inhabitants of the state of Colorado require that the streams, lakes, and other waters of the state be kept free of pollution by human wastes, and it is therefore declared to be the public policy of this state that no discharge which contains human excreta shall be permitted to flow in the streams, lakes, or other waters of this state, unless such discharge shall comply with all standards of the state board of health adopted pursuant to law; and in addition thereto the following standard shall apply:

The coliform count shall not average more than 1000/ml based upon not less than four samples taken at the rate of at least one sample per day over a period of four consecutive days" (1784).

In 1959, the Health Department Law was amended to include:

"Minimum standards shall be not more than 0.5 ml/l for settleable organic matter, not more than 75 mg/l for suspended organic matter, and not more than 50 mg/l for the combined suspended and dissolved organic matter when measured in terms of 5-day, 20°C BOD" (1784).

In addition, State Board of Health Regulations specify that:

"1. The effluent of sewerage systems or trade wastes shall not contain substances in quantities toxic to man.

"2. No floating matter shall be discharged in the effluent of sewerage systems or trade wastes.

"3. If the effluent of sewerage systems or trade wastes are discharged into a watercourse used downstream in Colorado as a surface source of public domestic water supply, the dissolved or suspended matter shall not exceed 30 mg/l when measured in terms of 5-day 20°C BOD" (1785).

CONNECTICUT

The control of water pollution in Connecticut rests largely with the State Water Resources Commission, but the State Department of Health has specific powers relating to water-pollution control as it affects public health (1786). Since Connecticut is a member of both the Interstate Sanitation Commission and the New England Interstate Water Pollution Control Commission, some of her waters are subject to certain standards and classifications of those commissions (46), as described hereinafter. The Commission adopts some standards on a river-valley basis and others on individual plants and specific discharges.

In September 1952 the Commission adopted a pollution-abatement policy in the Quinnipiac River Valley which established the following effluent standards (1787):

"1. All sewage or sanitary wastes before discharge to the Quinnipiac River or its tributaries shall be treated in an adequately designed and operated plant which will produce an effluent of a quality equal to that usually produced in a treatment process including high rate trickling filters and chlorination.

"2. All water borne industrial wastes, before being discharged to the Quinnipiac River and its tributaries shall be treated in an adequately designed and operated plant which will produce an effluent which, upon laboratory tests, will meet the following standards:

a. pH between 6.5 and 8.5
b. Color and turbidity not over 50 ppm.
c. Suspended solids not over 30 ppm.
d. B.O.D. not over 25 ppm.
e. Metals (dissolved) each not over 5 ppm.
f. Oils and greases not over 20 ppm.
g. Any water borne wastes of unusual character or volume not covered by the preceding standards will be considered as individual problems."

For the Hockanum River Valley the following effluent standards were established by a policy statement of the State Water Commission in March 1953 (1788):

"1. All sewage or sanitary wastes, before they are discharged into the Hockanum River and its tributaries, shall receive a degree of treatment equal to that ordinarily expected from a well designed and well operated plant including high rate trickling filters and chlorination.

"2. All industrial wastes, before they are discharged into the Hockanum River and its tributaries, shall receive a degree of treatment which will produce an effluent having a quality falling within the following standards:

a. pH between 6.5 and 8.5
b. Suspended solids not over 30 ppm.
c. Biochemical Oxygen Demand not over 30 ppm.
d. Color and turbidity shall not increase by more than 5 ppm. the amount present in the receiv-

ing stream sampled above any industrial waste outlet.

e. Any water borne wastes containing substances of a toxic, unusual, or peculiar character and volume not specifically covered by the above items shall be subject to specific standards."

In 1961, the definition of "water" was considerably expanded (45).

The Commission and the oil industry in the state endeavor, cooperatively, to control pollution of surface waters by gasoline, oils, and other petroleum products (1431).

DELAWARE

In 1949 the State of Delaware created a Water Pollution Commission with general power to supervise all laws relating to pollution of the waters of the state. The State Board of Health also exercises some functions relating to the protection of public water supplies and the disposal of wastes by public authorities and individuals (1790). The Commission considers each instance individually, specifying the improvements necessary in terms of (1) maximum acceptable amounts of important constituents, (2) percentage reduction of significant pollutants, or (3) the type or degree of treatment required (1791).

Although Delaware is a member of the Interstate Commission on the Delaware River Basin and of the new Delaware River Basin Compact Commission, and the former Commission has promulgated minimum standards for the Delaware River within the state, no statewide water-quality standards have been formulated and announced (47).

FLORIDA

The State Board of Health has the primary responsibility for water-pollution control in Florida. The Board has general control and supervision over all waters insofar as their pollution may affect the public health or impair the interest of the public or persons lawfully using them (1794). No specific water-quality standards or classifications have been adopted. Florida has never attempted stream classification because, with few exceptions, every stream would be classified for recreational use regardless of its industrial load (1795).

In the State Sanitary Code, the following general conditions are declared to menace the health of persons, fish, or livestock and therefore are unlawful (1754):

"(a) When raw sewage or incompletely treated sewage, or wastes deleterious to a drinking water or to a water treatment plant, are discharged into a stream or body of water from which a public water supply is derived.

"(b) When raw or incompletely treated sewage is discharged into a water so as to cause an unsafe concentration of bacteria from human excreta in waters where shellfish are taken, or where there are bathing beaches established and operating.

"(c) When sewage or wastes are discharged in such concentrations as to reduce the dissolved oxygen below the natural oxygen balance.

"(d) When acids, alkalies, or other chemicals, or deleterious substances are discharged into waters so as to interfere with the biochemical functioning of the stream or waters, or where such wastes kill or interfere with the normal development of fish or other foods derived from waters" (51).

In 1957, an amendment was made to the Sanitary Code to provide for both temporary and permanent injunctive relief in the abatement of pollution (1775).

GEORGIA

On July 1, 1957, the "Georgia Water Quality Control Act" went into effect. This Act gave the State Board of Health extensive responsibility for controlling the pollution of the state's waters. It also created a "Water Quality Council" for the purpose of "making recommendations to the Board regarding rules, regulations, procedures, policies, standards, waste disposal certificates, and for the conduct of hearings in review of orders or actions of the Board" (1780).

The Board is authorized "to establish such standards of quality for any waters in relation to the reasonable and proper use thereof as it deems necessary, and to establish such general policies and standards relating to any existing or future pollution of such waters as it deems necessary, and to modify, amend, or revoke any such standards or policies so established."

No specific standards have been promulgated (1789). However, Section 6 of the Act states:

"(a) It shall be unlawful for any person to discharge or permit to be discharged into any of the waters of this State any sewage, industrial wastes, or other wastes which would adversely affect the health of the people of the State or any segment of such people through:

(1) The spread of contagious or infectious diseases;

(2) The dissemination of toxic or radioactive substances;

without having first provided treatment facilities or works for the treatment of such wastes approved as to methods of treatment, plans and specifications for the construction of facilities or works, and/or operation thereof, by the Board" (1792).

"(b) It shall also be unlawful to use any waters of the State for the disposal of sewage, industrial wastes, or other wastes so as to render such waters unsuitable for their then current uses except in such a manner as to conform to and comply with all rules, regulations, certificates and orders established under the provisions of this Act and applicable to the waters involved.

"(c) It shall also be unlawful for any person to erect, construct, enlarge, extend, open, reopen, or operate any public or community water supply system, including storage, distribution, purification treatment facilities or works, without having first secured from the Board approval of: the source of water supply; the means and methods of treating, purifying and storing water; plans and specifications for the construction of facilities or works; and the operation thereof.

"(d) Anything to the contrary notwithstanding, nothing shall prohibit a person, firm or corporation from discharging its sewage, industrial wastes or other wastes into any non-navigable stream or other non-navigable waters of this State, if

(1) Such person, firm or corporation owns all the lands immediately adjacent to such streams or waters lying between the point of entry of such sewage, industrial wastes or other wastes into such streams or waters and the point of its junction with other waters lying adjacent to lands belonging to others if at such point of junction the quality of water does not adversely affect the health of the people of the State as defined in the preceding paragraph of this Section, or

(2) Such person, firm or corporation has acquired an easement, either by express grant or prescription, to discharge such sewage, industrial wastes, or other wastes into such waters of this State provided that the water of such stream at a point not more than 3 miles from the point of entry of such sewage, industrial wastes, or other wastes, and within limits of said easement, does not adversely affect the health of the people of the State as defined by the preceding paragraph of Section 6" (1789).

HAWAII

The State Department of Health exercises primary control in the area of water pollution. Its activities in this area are administered by the Division of Environmental Health (1796). The Department of Land and Natural Resources through the Director of the Division of Fish and Game is authorized to enforce all laws relating to the protection of fish and fishing within the state. Also, the Harbors Division of the Department of Transportation is authorized to make rules and regulations to prevent the throwing into the waters of the state of garbage or other substances liable to make such waters unsightly, unhealthful, or unclean and to prevent the escape of fuel or other oils into such waters either from vessels, pipes, or storage tanks upon the land (1797).

Water-quality standards are under consideration but have not yet been adopted. The Department of Health is authorized to adopt and establish rules and regulations and will adopt water-quality standards when deemed necessary. Each case at present is handled on its merits (1798).

IDAHO

The State Board of Health, created in 1955, is the agency that has the primary function of controlling water pollution caused by sewage or sewage effluents and any other pollution that may affect the public health. It has authority to establish and enforce minimum sanitary standards (52, 1799).

In addition to the regulations of the State Board of Health, there are certain types of pollution control that may be enforced by the State Fish and Game Department. Also, wastes from dredge mining are controlled by the State Land Department (52).

The State Board of Health has the power to administer and enforce all state health laws and regulations and to establish and enforce minimum sanitary standards for "the quality of water supplied to the public and as to the quality of the effluent of sewerage systems, sewage treatment plants, and discharges upon the land or into the surface or ground waters" (1800).

On May 11, 1959, the State Board of Health adopted regulations which state, in part:

"1. All wastes discharged to waters of the state shall be subjected to such treatment that they shall not create a health hazard or nuisance and such wastes shall not impair the quality or interfere, either directly or indirectly, with the treatment processes of any public water supply. Waters of the state shall include surface waters and underground waters.

Minimum acceptable treatment for any waste shall be equivalent to the removal of readily settleable and floatable solids. Minimum treatment for waste containing domestic sewage shall include removal of readily settleable and floatable solids and effective disinfection.

"2. The Department of Health shall adopt sewage works design standards, water quality objectives, and subsurface sewage disposal standards to be used as a guide in determining adequacy of proposed treatment and to be used as a guide in the review of plans for proposed treatment facilities. Plans for waste treatment and sewage facilities shall be submitted to the Department of Health for review and approval before construction is begun" (1801).

In promulgating the above regulations, the Department of Health adopted the standards previously developed by the Northwest Pollution Control Council as described hereinafter (52).

ILLINOIS

The Sanitary Water Board has the authority to develop a comprehensive plan for controlling water pollution in the state. Its authority, however, does not extend over any sanitary district having a population of one million or more.

The Department of Public Health exercises supervision over adequacy and quality of public water supplies. The Department of Mines and Minerals has authority to make rules and regulations for the prevention of pollution of fresh-water supplies by oil, gas, or salt water (1802).

The common-law water rights doctrine has been interpreted in Illinois by the theories of natural flow and reasonable use (1803). As a signator of the Ohio River Valley Water Sanitation Commission, the state subscribes to the following requirements for effluents discharged to the Illinois, Ohio, Mississippi, and Wabash Rivers:

"Industrial wastes shall be treated or otherwise modified prior to discharge so as to maintain the following conditions in the receiving waters:

1. Freedom from anything that will settle to form putrescent or otherwise objectionable sludge deposits which interfere with reasonable water uses.
2. Freedom from floating debris, scum and other floating materials in amounts sufficient to be unsightly or deleterious.
3. Freedom from materials producing color or odor in such degree as to create a nuisance.

4. Freedom from substances in concentrations or combinations which are toxic or harmful to human, animal or aquatic life.

"These conditions to be maintained in the receiving waters following the discharge of industrial-waste effluents, are basic or minimum requirements" (1804).

The questionnaire from Illinois indicated that the above requirements apply equally to municipal wastes and also that "these are not standards nor rules and regulations but upon decision can be and would be embodied in rules and regulations" (54).

Illinois is also a member of the Bi-State Development Agency and the Great Lakes Commission.

INDIANA

Functions relating to the control of water pollution in Indiana are primarily delegated to the Stream Pollution Control Board. The functions of the State Board of Health relate to public water supplies and sewage-treatment works (1825).

Indiana law states that:

"The Stream Pollution Control Board shall have jurisdiction to control and prevent pollution in the waters of this State with any substance which is deleterious to the public health or to the prosecution of any industry or lawful occupation, or whereby any fish life or any beneficial animal or vegetable life may be destroyed, or the growth or propagation thereof prevented or injuriously affected.

"The Stream Pollution Control Board shall have the power to determine what qualities and properties of water shall indicate a polluted condition of such water, in any of the streams or waters of this State, that shall be deleterious to the public health or to the prosecution of any industry or lawful occupation for which or in which any such waters may be lawfully used or employed, or whereby the carrying on of any agricultural, floricultural or horticultural pursuit may be or shall be injuriously affected, or whereby the lawful conduct of any livestock industry, or the use of any such waters for domestic animals may be prevented, injuriously affected or impaired, or whereby any lawful use of any such waters by the State of Indiana, or by any political subdivision, corporation, municipal corporation, association, partnership, person, or any other legal entity, may be lessened or impaired or materially interfered with, or whereby any fish life or any beneficial animal or vegetable life in said waters may be destroyed, the growth or propagation thereof prevented or injuriously affected" (1805).

The above law was broadened in 1957 to include ground waters (1806).

Regulation SPC 1, promulgated by the Stream Pollution Control Board in accordance with the above laws in 1945, is still very much in effect:

"WHEREAS, the Stream Pollution Control Board of the State of Indiana has the power under Section 7, Chapter 214, Acts of 1943, to determine what qualities and properties of water shall indicate a polluted condition of such water in any of the streams or waters of this State, and

"WHEREAS, the Board recognizes the fact that the character of all surface water is affected by the mode of life of the people and the activities of industry, and that both the people and industry are dependent on said surface water to a greater or lesser extent, and

"WHEREAS, it is recognized that concentrations of population may exist on small streams where diluting water is insufficient to maintain suitable concentrations of oxygen by the use of known and reasonable methods of waste treatment, and

"WHEREAS, there is a fair economic balance between cost of treatment of waste and benefits received beyond which it is not reasonable to expend money for treatment, and the cost of treatment and the benefits to be derived must be considered in determining the extent of corrective treatment to be applied, and

"WHEREAS, natural purifying agencies in the stream should be reasonably utilized, these agencies consisting primarily of the biology of the stream which is affected by the depth of the water, the velocity of the current, etc., and

"WHEREAS, the necessary degree of purity of surface waters depends on the subsequent use which varies on different watersheds and at different points on the same watershed, and

"WHEREAS, for the above-named reasons, each stream presents a separate problem and standards may need to be modified to fit specific cases,

"BE IT RESOLVED, that in general the following regulations and standards shall be applicable to all receiving waters and any water which does not meet such standards and properties shall be deemed and considered as in a polluted condition.

1. Floating material including grease and oil shall not be discharged into any surface water in deleterious amounts, or in amounts sufficient to affect injuriously fish life, fur bearing or domestic animals, or the general biology of the water, or plant life in or in the vicinity of such water.
2. Waste which is discharged into any water shall contain nothing which will deposit in a stream or a lake to form putrescent or otherwise objectionable sludge banks.
3. Waste which is discharged into any water shall contain no materials in concentrations sufficiently high to affect adversely public health, fish life, fur bearing or domestic animals, or plant life in or in the vicinity of such water.
4. Generally the oxygen content of the receiving water, after being mixed with and affected by the waste, shall be no less than 50 per cent saturation. A lower concentration will be tolerated temporarily, but only so long as it is not injurious to aquatic life, and in no case shall it fall below 25 per cent saturation.
5. Receiving waters shall be considered unsuitable for bathing if the coliform concentration exceeds 1000 per 100 ml. (MPN.) If the receiving water is used as a source of water supply, a coliform density greater than 5000 per 100 ml. (MPN.) shall not exist at or in the vicinity of the intake. Also in the

case of wastes, bearing or producing substances objectionable from a taste or odor standpoint, which are discharged into waters which are used as a source of water supply such wastes shall be so treated as to render them unobjectionable before discharge into the stream or lake" (1807).

Indiana is a member of the Ohio River Valley Water Sanitation Commission and the Great Lakes Commission.

IOWA

The State Department of Health is the agency that has the primary responsibility for controlling water pollution. The State Board of Health acts in an advisory capacity to the State Department of Health.

The Iowa Natural Resources Council is authorized to make plans and recommendations for the development, protection, and preservation of the water resources of the state. A permit must be obtained from the Iowa Natural Resources Council for the diversion of water or any material from the surface directly into any underground watercourse or basin (1808).

Water use in Iowa was formerly controlled by riparian doctrine, but since the enactment of Chapter 455A of the Code of Iowa, as amended in 1957, the principle of reasonable use has been adopted (1809).

The Iowa Stream and Lake Pollution Law states:

"The department is empowered to adopt and enforce rules and regulations consistent with and not different from the provisions of this chapter restricting the polluting content of any waste material and polluting substances discharged or sought to be discharged into any of the waters of the state" (1810).

No rules and regulations have been published but the polluting content discharged or sought to be discharged is controlled by orders requiring abatement of existing pollution and the issuing of permits for new sewage and waste outlets. The polluting content is restricted in accordance with downstream water uses and the public health aspects.

Iowa has nonofficial affiliations with:

1. Great Lakes - Upper Mississippi River Board of State Sanitary Engineers
2. Joint Resolution of Illinois, Wisconsin, and Iowa Water Pollution Control Agencies for Control of Pollution of Mississippi River
3. Missouri River Basin Engineering Health Council
4. Five State Agreement of South Dakota, Nebraska, Iowa, Missouri, and Kansas Water Pollution Control Agencies for Control of Pollution of Missouri River" (56).

KANSAS

The State Board of Health has the responsibility of controlling water pollution. The Board is authorized to make rules and regulations necessary for the protection of the surface and subsurface water from pollution by oil, gas, salt water injection wells, or underground storage reservoirs, and to govern the disposal of domestic, industrial, and sewage wastes by municipalities, corporations, companies, or individuals (1775).

"The water-pollution control program of the Kansas State Board of Health stems primarily from a system of continuous state-wide surveillance of stream, oil field waste disposal methods, and waste treatment plant conditions by field personnel; and by the use of a permit system for new waste sources. Since permits are required for the discharge of wastes into the waters of the state, new sources of water pollution are largely controlled prior to their origination. These policies pertain to both municipal and industrial waste sources" (1811).

In certain river basins where pollution problems have been widespread, overall basin studies have been conducted which have resulted in the adoption of basin-wide pollution abatement policies. The State Board of Health has adopted the following policy, applicable to both sewage and industrial wastes:

"Throughout the Arkansas River basin below Great Bend—including the major tributaries, Cow Creek and the Little Arkansas River, but not including the Ninnescah and Walnut rivers and Rattlesnake, Slate and Walnut creeks or the streams which join the Arkansas River in Oklahoma.

A. At minimum stream flows the biochemical oxygen demand exerted on any section of the stream shall not reduce the dissolved oxygen content below 4 ppm.
B. Treatment practice will be aimed at reducing the coliform concentration in the streams to less than 20,000 organisms per 100 milliliters.
C. In addition to item A, industrial wastes should be substantially free of toxic substances, including metallic ions, phenolic compounds, chemical or immediate oxygen demand, oils, alkalies, and acids, before they are discharged to a watercourse.
D. Primary treatment of sewage and industrial wastes is the minimum degree of treatment which will be acceptable. Additional treatment will be necessary in many instances to maintain water quality in the streams as indicated under items A, B and C.
E. Oil storage facilities so located that a tank failure or surface runoff from the area could quickly contaminate a watercourse shall be surrounded by an earthen dike providing a capacity equal to the storage tank contents plus a freeboard of 1.5 feet.
F. All brine shall be disposed of in such a way that fresh water is not polluted.
G. All industrial wastes discharged to municipal sewerage systems shall be pretreated to an extent which will assure that the wastes will not adversely affect the sewage treatment process.

"The pollution abatement policy is uniform in its intent that all polluters begin planning immediately and that they start construction of waste treatment works or disposal facilities quickly. Because the Little Arkansas River is a potential source of public water supply and is used for recreational purposes the early elimination of bacterial pollution is essential. Chemical and other types of pollution of the Little Arkansas River are equally important but because of their amount and complexity more time must be allowed for planning and constructing the necessary facilities" (1811).

The criteria established for the Kansas River Basin in 1950 continues in effect:

"The Kansas State Board of Health, after giving careful consideration to the data collected and its responsibilities is adopting the following policy which is believed to be in the best public interest. This policy will apply to sewage and industrial, organic or inorganic pollution throughout the Kansas River Basin.

1. That at minimum stream flows the biochemical oxygen demand exerted on any one section of a stream shall not reduce the dissolved oxygen content below 5 parts per million.

2. That at minimum stream flows the goal for coliform bacteria is 20,000 organisms or less per 100 milliliters (this quality should not be confused with the number of coliform organisms present during heavy runoff, since at such a time they may be primarily soil organisms not indicative of sewage pollution).

3. That the primary treatment of sewage is the minimum amount which will be acceptable.

4. Where oil pollution is concerned, all above-ground oil storage tanks shall be surrounded by an earth dike with a capacity equal to the contents of the tank plus a free board of 1.5 feet.

5. All brine ponds and waste oil ponds shall be operated in such a manner that 2.5 feet is provided above the maximum brine level.

6. That no brines from any field in the river basin shall continue to be stored in ponds, or discharged directly or through seepage to a fresh water course, either surface or subsurface, after July 1, 1951, which date has previously been set by the Kansas State Board of Health. However, in no case will there be any deviations from the policy in such matters, as outlined in the Board's letter dated February 15, 1950" (62, 1811).

Quality standards have also been adopted for radionuclides in water. These Basic Standards utilize the Recommendations of the National Committee on Radiation Protection and Measurements as published in Handbooks of the National Bureau of Standards as guides or as bases for calculations. Section 28-35-11, "Maximum Permissable Dose and Concentrations" as well as the specific isotopes, are quoted in Appendix B (1812).

KENTUCKY

The Water Pollution Control Commission is charged with the responsibility for developing a comprehensive program for the prevention, control, and abatement of water pollution throughout the Commonwealth. The Commission is contained within the Department of Health. The Commission is authorized to establish or modify, after public hearing, water-quality standards for the waters of the Commonwealth according to their uses (1813).

Water-quality standards have not yet been adopted. Kentucky is a member of the Ohio River Valley Water Sanitation Commission.

LOUISIANA

The Stream Control Commission has the primary function of controlling water pollution. It also establishes pollution standards for the waters of the state, and controls all waste disposal into such waters. The State Board of Health exercises jurisdiction over the water supplies of the state. The Department of Conservation is responsible for the protection of fresh-water sands from pollution by oil-well drilling and production wastes (1814), and the Commissioner of Wildlife and Fisheries supervises the drainage of all salt water and other noxious substances into natural streams.

No one may discharge into state waters any pollution of any kind that will tend to destroy fish, other aquatic life, wild or domestic animals or fowls, or be injurious to the public health or against the public welfare in violation of any rule, order, or regulation of the Commission (1815).

The Commission promulgated the following criteria on 1 September 1955 with respect to the discharge of sugar-mill wastes:

"I. No acid, acid water, alkali water, mill washdown water, effects condensate, filter press mud or liquid from filter press mud shall be discharged into state waters or drains or pipes leading to state waters during the grinding season.

"II. No wastes impounded during the grinding season will be discharged until stabilization has taken place, until the biochemical oxygen demand has been satisfied, and then only into water volumes capable of assimilating such waste waters.

"III. That all cane wash water shall be impounded for a period of not less than thirty days prior to discharge.

"IV. That all cane wash water impoundments be compartmented so that the first compartment shall act as a settling basin. The design of the impoundments shall be approved in writing by the Stream Control Commission.

"V. That, except in cases where specific orders applicable to individual mills have been issued, that condensor water, that water used in barometric condensors, can be discharged into state waters provided first that not more than fifty parts per million of 5 day, twenty degree centigrade biochemical oxygen demand is added to the waters while being pumped through the condensors, and second that the condensor water not cause a serious oxygen depletion to take place in the receiving stream" (1817).

Correspondence with Louisiana indicates no change in the exercise of water-pollution-control administration within the state since 1953 (65). In 1955, O'Neill stated that the 1954 revision of the Louisiana State Sanitary Code contains no quantitative requirements, thereby giving the State Board of Health some degree of flexibility in the application of requirements (1818). In 1959, a court ruled against the state because the statute (in question) only generally characterized or denounced the offense for which the defendant was being prosecuted. And in 1960, the Commission attemped to have new legislation passed, but it failed (65).

Louisiana has a Waterworks Warning Network on the Lower Mississippi River to notify waterworks operators of spills of industrial waste.

MAINE

The Water Improvement Commission became, in 1955, the agency exercising primary control of water pollution (67). The Revised Statutes state:

"It shall be the duty of the Commission to study, investigate, and from time to time recommend to the persons responsible for the conditions, ways and means, so far as practicable and consistent with the public interest, of controlling the pollution of the rivers, waters and coastal flats of the state by the deposit therein or thereon of municipal sewage, industrial waste and other substances and materials insofar as the same are detrimental to the public health or to animal, fish or aquatic life, or to the practicable and beneficial use of said rivers, waters and coastal flats. The Commission shall make recommendations to each subsequent legislature with respect to the classification of the rivers, waters and coastal flats and sections thereof within the state, based upon reasonable standards of quality and use.

"The Commission shall make recommendations to each legislature with respect to abatement of pollution of the rivers, waters and coastal flats and sections thereof within the State for the purpose of raising the classifications thereof to the highest possible classification so far as economically feasible; such recommendations to relate to methods, costs and the setting of time limits for compliance.

"The Commission shall have 4 standards for the classification of surface waters and tidal flats.

"Class A shall be the highest classification and shall be of such quality that it can be used for bathing and for public water supplies after disinfection, and the dissolved oxygen content of such waters shall not be less than 75% saturation and contain not more than 100 coliform bacteria per 100 milliliters.

"There shall be no discharge of sewage or other wastes into water of this classification and no deposits of such material on the banks of such waters in such a manner that transfer of the material into the waters is likely. Such waters may be used for log driving or other commercial purposes which will not lower its classification.

"Class B, the second highest classification, shall be divided into two designated groups as B-1 and B-2.

"B-1. Waters of this class shall be considered the higher quality of the Class B group and shall be acceptable for recreational purposes and after adequate treatment for use as a potable water supply. The dissolved oxygen of such waters shall be not less than 75% of saturation and contain no more than 300 coliform bacteria per 100 milliliters.

"There shall be no disposal of sewage or industrial wastes in such waters except those which have received adequate treatment to prevent lowering of the standards for this classification, nor shall such disposal of sewage or waste be injurious to aquatic life or render such dangerous for human consumption.

"B-2. Waters of this class shall be acceptable for recreational boating, fishing, industrial and potable water supplies after adequate treatment. The dissolved oxygen of such waters shall not be less than 60% of saturation and contain no more than 1,000 coliform bacteria per 100 milliliters.

"There shall be no disposal of sewage or industrial waste in such waters to lower its classification nor shall such disposal of sewage or waste be injurious to aquatic life or dangerous for human consumption.

"Class C, the third highest classification, shall be of such a quality as to be satisfactory for recreational boating, fishing and other uses except potable water supplies and swimming, unless adequately treated to meet standards.

"Waters of this classification shall be free from scums, slicks, odors and objectionable floating solids, and shall be free from chemicals and other conditions inimical to aquatic life. The dissolved oxygen content of such waters shall not be less than 5 parts per million for trout and salmon waters and not less than 4 parts per million for non-trout and non-salmon waters.

"The Commission may take such action as may be appropriate for the best interests of the public when it finds that a "C" classification is temporarily lowered due to abnormal conditions of temperature and stream flow for that season involved.

"Class D waters, the lowest classification, shall be considered as primarily devoted to the transportation of sewage and industrial wastes without causing a public nuisance as defined in Chapter 141, Section 6, by the creation of odor producing sludge banks and deposits or other nuisance condition and such waters shall contain dissolved oxygen at all times. During a period of temporary reduction in the dissolved oxygen content in this class water, due to abnormal conditions of temperature of stream flow for the particular season involved, the Commission, provided a nuisance condition has not been created in such water and in the opinion of the Commission is not likely to be created during such season, shall take no action to reduce the amount of pollution from any source which is allowed in such class water under normal conditions.

"With respect to "C" and "D" classifications, the number of coliform bacteria, or amounts of toxic wastes or chemicals discharged into said waters shall be only those amounts which will not, in the determination of the Commission, be harmful to the public health" (1819).

The Department of Health and Welfare and the Public Utilities Commission also have functions in water-pollution control relating primarily to water supply and to sewage disposal affecting water supplies (1820).

Maine is a member of the New England Interstate Water Pollution Control Commission and has informal working arrangements with the Province of New Brunswick.

MARYLAND

Both the Maryland Water Pollution Control Commission and the State Board of Health have functions relating to the abatement of water pollution. The Commission is authorized to recommend standards for sewage or waste effluents discharged into waters of the state and to establish such reasonable water-quality standards or criteria, keeping in mind the public use to which they

are or may be put. The Board has general supervision and control over waters insofar as their sanitary and physical condition affect the public health or comfort (1821, 1822).

The Commission requires that its Regulation IV apply to all industries:

"No industrial wastes shall be placed or permitted to be placed or discharged or permitted to flow into any of the waters of the State in any manner by any person unless the industrial wastes after treatment or untreated shall meet with the nine industrial waste requirements established by the Water Pollution Control Commission before being discharged into any waters of the State. These nine industrial waste requirements are as follows:

1. Solids
   A. Solids in the effluent—Must not exceed particle size than can pass Tyler designation 20 mesh screen. Grinding, maceration or any other waste treatment or handling operation intended to reduce the size of oversize solids in the effluent to pass Tyler designation 20 mesh screen, will not be permitted or approved.
   B. Total suspended solids—Must not exceed 400 ppm.
   C. Dissolved solids—Must not exceed 1500 ppm.
   D. Total solids—Must not exceed 1900 ppm.
2. Turbidity—Must transmit 10% of light through 12 inches of sample in a 3 inch column or not to exceed 300 ppm, as determined by the Jackson candle turbidimeter.
3. Biochemical Oxygen Demand:
   A. The Biochemical Oxygen Demand—The 5 day, 20° C. Biochemical Oxygen Demand in the effluent must not exceed 100 ppm.

   or

   B. The Dissolved Oxygen in the waste receiving waters must not be depleted beyond 50% of normal saturation.
4. Toxicity or toxic compounds—Eliminate, or reduce to limits of tolerance, substances toxic to humans, livestock, fish, aquatic and wildlife.
5. Color—Color intensity regardless of light frequency must not exceed 400 ppm. on the chloroplatinate scale.
6. pH—Must not range below 5.5 or above 8.5.
7. Temperature—Must be below 100°F. in the stream within 50 feet from waste outlet.
8. Oils and grease in the effluent—Must not exceed 30 ppm.
9. Taste and odor—Effluent must not exceed threshold odor number of 1000. Mixture of the waste and receiving waters shall have a threshold odor number not in excess of 80.

"These nine industrial waste requirements are generally applicable values but are not absolutely fixed values. They can be made more stringent if a survey of the waste receiving waters indicates they are still polluted or are continuing to be degraded, or in any instance where the Water Pollution Control Commission, after due study and deliberation, deems that more stringent requirements are necessary. They can be made more liberal only by formal action of the Commission on the basis of satisfactory evidence and proof that waste receiving waters are sufficient in quantity and quality to not be affected adversely by a particular industrial waste effluent having values in excess of those stated above" (1822).

In the Baltimore Harbor Area, the Commission has adopted the following Regulation (IV-A) and standard of quality:

"A—Receiving Water Quality Standards

Item:	*Specification:*
1. Floating solids, settleable solids, sludge deposits	None which are readily visible and attributable to industrial wastes or other wastes or which deleteriously increase the amounts of these constituents in the receiving waters.
2. pH	Must not range below 5.5 or above 8.5.
3. Toxic wastes, oil, deleterious substances, colored wastes and other wastes	None alone or in combination with other substances or wastes in sufficient amounts to prevent survival of fish or aquatic life, to damage marine structures, create a nuisance and/or impair the use of these waters for industrial water supply and navigation.
4. Water quality in waters bordering the Baltimore Harbor Area	The quality of any waters receiving industrial wastes or other wastes shall be such that no impairment of the best usage of waters in any area bordering but outside the designated Baltimore Harbor Area shall occur by reason of such discharge of industrial wastes or other wastes.

"B—Desirable Effluent Characteristics

1. Solids not to exceed particle size that can pass Tyler designation 20 mesh screen. Grinding, maceration or any other waste treatment or handling operation intended to reduce the size of oversize solids to pass Tyler designation 20 mesh screen not to be used.
2. Total suspended solids not to exceed 400 ppm.
3. Toxicity or compounds toxic to fish, aquatic and wildlife to be eliminated or reduced to limits of tolerance.
4. pH not to range below 5.0 or above 9.0.
5. Oils and greases not to exceed 30 ppm" (1822).

On July 1, 1961, the Maryland Department of Health (concurred in by the Water Pollution Control Commission) established restrictions upon the disposal of commercial laundry wastes containing detergents. The requirements for treatment of these waste waters:

"1. No wastes shall be discharged or permitted to flow into the waters of the state in any manner unless the effluent characteristics meet the requirements of the Water Pollution Control Commission's Regulation IV.

"2. The treated wastes must be sufficiently free of foam-producing materials or chemicals, and the physical appearance must be such, so as not to produce a nuisance in the receiving waters.

"3. Permission will not be granted for the discharge of treated or untreated wastes into subsurface drainage or disposal systems. No new subsurface disposal system shall be permitted, and no existing subsurface systems shall be extended, or connected thereto, for this purpose.

"4. Permission will not be granted for the discharge of untreated wastes into a municipal sanitary sewerage system where there is no sanitary sewage treatment or where the degree of treatment is insufficient to properly treat such wastes" (1823).

Maryland is a member of the Interstate Commission on the Potomac River Basin and the Washington Metropolitan Regional Sanitary Board.

MASSACHUSETTS

The Water Pollution Control Agency for the Commonwealth of Massachusetts is the Department of Public Health (1824). Rules and regulations have been adopted by the Department which prohibit the discharge of sewage and wastes, except under conditions and with treatment for which approval is required. As a member of the New England Interstate Water Pollution Control Commission, Massachusetts has adopted a program for the classification of its interstate streams using the classifications and standards of quality for interstate waters as adopted by the New England Interstate Water Pollution Control Commission.

The Department of Public Health has adopted rules and regulations with respect to the protection of surface water supplies. However, such rules and regulations do not apply to the Connecticut River, the Merrimack River, the Concord River, the Ipswich River, or the Saugus River (73).

MICHIGAN

The Water Resources Commission and the State Departments of Health, Conservation, Agriculture, and Highways, whose heads constitute four of the seven members of the Commission, exercise their separately authorized controls of water pollution coordinated where pertinent with activity of the Commission. None of this control takes place, however, under established statewide water-quality standards.

Section 6 of the Commission Act establishes, in effect, water-quality criteria for the state in declaring unlawful the discharge into its waters of "any substance which is injurious to the public health or to the conducting of any industrial enterprise or other lawful occupation; or whereby any fish or migratory bird life or any wild animal or aquatic life may be destroyed or the growth or propagation thereof be prevented or injuriously affected or the value of lawfully taken fish or game be destroyed or impaired as the consequence of said pollution."

Boundary waters between the U.S. and Canada (St. Marys, St. Clair and Detroit Rivers, and Lake St. Clair) are subject to objectives for quality control adopted by the International Joint Commission in October 1950.

Many Commission orders have been adopted setting effluent restrictions and conditions controlling individual uses so designed as to prevent unlawful pollution as above defined.

Michigan is a member of the Great Lakes Commission (75).

MINNESOTA

The primary function for controlling water pollution rests with the Water Pollution Control Commission. The State Board of Health exercises functions relating to water-pollution control insofar as public health and water supply used for domestic purposes are concerned (1826).

The Commission is empowered to make such classification of waters as it deems advisable, and to establish and alter such reasonable pollution standards for any waters of the state in relation to the public use to which they are or may be put (1827).

The Water Pollution Control Commission has not established any general pollution standards or made any broad classifications of the waters of the state. Recent legislation has been provided for the creation of sanitary districts (77). Minnesota is a member of the Great Lakes Commission and the Tri-State Water Commission.

MISSISSIPPI

The control of water pollution created by municipal and industrial wastes is the responsibility of the Game and Fish Commission, but control of the contamination caused by domestic sewage is the responsibility of the State Board of Health (79). The State Board of Health has been designated as the official Water Pollution Control Agency of the state.

The State Oil and Gas Board is authorized to make rules and regulations for the prevention of pollution of fresh water supplies by oil, gas, or salt water. The State Game and Fish Commisson is empowered to set effluent standards, but does not have jurisdiction over lakes or other waters that are wholly land-locked and privately owned or the Mississippi River or its tributaries (1828).

The Commission has adopted the following standards (9):

"4. The degree of treatment of industrial wastes or sewage required shall be based upon the chemical and biological condition of the receiving waters. The minimum standards required in the receiving waters one thousand feet (1000′) below the industrial waste outfall shall be:

a. There shall be no noticeable floating solids, scum, oil, grease or sleek.
b. The degree of acidity and the degree of alkalinity as expressed by pH values shall average in the range of 6.5 to 8.4, and any single sample shall not be below pH 5.0 or above pH 9.5, during the prescribed sampling period.
c. The increased salt concentration shall not exceed one thousand (1000) parts per million of sodium chloride.
d. The industrial waste after dispersion in the receiving waters, or not more than one thousand (1000)

feet below or above the point of discharge, shall not reduce the dissolved oxygen in the receiving waters below an average of 4.5 parts per million, and no sample taken from the receiving water at the point designated shall contain less than 2.5 parts per million dissolved oxygen during the prescribed sampling period.

e. No poisons or deleterious substances shall be present in sufficient quantities to cause injury or death to fish or wildlife.

f. No substances shall be present which may cause distinct foreign tastes in fish. Sampling place and sampling—The sampling place shall be chosen after the waste has been dispersed in the receiving waters and not more than one thousand (1000) feet below or above the waste or sewage outfall. Samples shall be collected over a minimum period of four (4) hours; the samples shall be taken approximately every thirty (30) minutes, or a minimum total of eight (8) samples. The samples collected in a stream shall be taken normally in mid-channel at mid-depth, or in such a manner as to obtain samples representing the average conditions of the stream."

MISSOURI

The 1957 Legislature enacted a new water-pollution law for the state and placed its administration in a newly created Water Pollution Board within the Division of Health of the Department of Public Health and Welfare. The Board is empowered to establish and revise standards of water purity that specify the maximum degree of pollution permissible in accordance with public interest in water supply, the conservation of fish, game, and aquatic life, and agricultural, industrial and recreational uses (1829).

The Missouri Water Pollution Board has not set standards, but has adopted water quality objectives for the Missouri, Mississippi, and Blue Rivers (81). These objectives are described in Appendix C. Missouri is a member of the Bi-State Development Agency.

MONTANA

The 1955 Legislature enacted a comprehensive water-pollution law and created a Water Pollution Council with responsibility for overall policy. The responsibility for administering the Act is placed in the State Board of Health. The Board may modify the policy only when public health is involved (1830, 1831).

The Council is authorized and directed "to formulate standards of water purity and classification of water according to the most beneficial uses of such water" (1832). Accordingly, the Council has adopted the water-quality objectives shown in Table 3-1.

NEBRASKA

The 1957 Legislature passed a new Water Pollution Control Act which was amended in 1961. The Act provided a Water Pollution Control Council within the Department of Health. This Council is authorized to develop effluent standards and standards of water quality, and also to classify the waters of the state (1833, 83).

As of January 1962, a committee was in the process of preparing water-quality standards to be used in accomplishing the following water-quality objectives. These objectives were adopted by the Council to be used as a guide in all future work relating to the disposal of wastes into Nebraska waters:

"1. Toxic substances including metallic ions, phenolic compounds, oils, alkalies, and acids should be virtually eliminated from sewage effluents.

"2. Removal of settleable and floating solids to prevent sludge banks, floating materials, and nuisance conditions should be required as the minimum acceptable treatment.

"3. For industrial wastes similar in nature to municipal wastes, treatment should be based on the same premise as recommended for municipalities. For industrial wastes not comparable to municipal wastes, treatment should be provided as necessary to prevent deterioration of water quality for beneficial uses in the receiving stream.

"4. Additional treatment over that specified in item 2 above should be provided as necessary to protect downstream water uses. Quality objectives for certain water uses are:

(a) For water serving as a source of domestic supply, raw water bacteriological quality should conform to that recommended in 'Manual of Recommended Water Sanitation Practices, 1946, Public Health Service Bulletin No. 296,' pages 11-13.

(b) For bathing or swimming waters, monthly arithmetical average 'most probable number' of coliform organisms should not exceed 1,000 per 100 ml during any month of the recreation season; nor exceed this number in more than 20 percent of the samples examined during any such month; nor exceed 2,400 per 100 ml on any day. For non-bathing or non-swimming waters, the monthly arithmetical average 'most probable number' of coliform organisms should not exceed 5,000 per 100 ml in any month of the recreational season, nor should exceed this number in more than 20 percent of the samples examined during any such month.

(c) For fish and aquatic life a minimum oxygen concentration of 5.0 ppm. is desirable in waters otherwise offering suitable fishery habitat, with 6.0 ppm. for trout streams.

(d) For bacteriological quality of irrigation water:

(1) Sewage used for irrigation of forage crops should be given treatment insuring a consistent reduction in coliform bacteria of not less than 90 percent.

(2) Sewage used for irrigation of human food crops should be given treatment insuring a consistent reduction (a) in suspended solids of not less than 75%, (b) in 5 day 20°C. B.O.D. of not less than 75% and (c) in coliform bacteria

TABLE 3-1

STATE OF MONTANA WATER POLLUTION COUNCIL STREAM CRITERIA FOR WASTE DISCHARGES

Water Quality Objectives and Minimum Treatment Requirements for Montana Surface and Underground Waters

Water quality Water uses	Organisms of the coliform group	Floating, suspended settleable solids and sludge deposits	Taste or odor producing substances	Dissolved oxygen	pH	Toxic, colored, or other deleterious substances	Phenolic compounds	Oil	High temperature wastes	Minimum treatment requirements for domestic sewage
(A) WATER SUPPLY, DRINKING, CULINARY, AND FOOD PROCESSING. Without treatment other than simple disinfection and removal of naturally present impurities.	Most probable number (MPN) coliform bacterial content of a representative number of samples should average less than 50 per 100 ml. in any month.	None attributable to sewage, industrial wastes, or other wastes which, after reasonable dilution and mixture with receiving waters, interfere with the best use of these waters for the purpose indicated.	None attributable to sewage, industrial wastes or other wastes.	Greater than five (5) parts per million, except for underground waters.	Hydrogen ion concentration expressed as pH should be maintained between 6.5 and 8.5.	None alone or in combination with other substances or wastes in sufficient amounts or of such nature as to make receiving water unsafe or unsuitable for use indicated (U. S. P. H. S. Stds)	Less than five (5) parts per billion	None	Not in sufficient quantities alone or in combination with other wastes to interfere with the use indicated.	Primary treatment and effective disinfection except in special cases as determined by the State Board of Health. Sewage lagoon treatment will meet this requirement.
(B) WATER SUPPLY, DRINKING, CULINARY, AND FOOD PROCESSING. With treatment equal to coagulation, sedimentation, filtration, disinfection, and any additional treatment necessary for removing naturally present impurities.	MPN coliform bacterial content of a representative number of samples should average less than 2000 per 100 ml. and should not exceed this number in more than 20 percent of samples examined in any month where associated with domestic sewage.	(Same as for use "A" above).	None attributable to sewage, industrial wastes or other wastes which, after reasonable dilution and mixture, will increase the threshold odor number above eight (8).	Same as for use "A" above.	Same as for use "A" above.	Same as for use "A" above.	Same as for use "A" above.	None detectable	Same as for use "A" above.	Same as for use "A" above.
(C) BATHING, SWIMMING, AND RECREATION. Note: When waters are used for recreational purposes such as fishing and boating, exclusive of bathing and swimming, the number "1000" may be substituted for "240" in statement of coliform objectives.	MPN coliform bacterial content of a representative number of samples should average less than 240 per 100 ml. and should not exceed this number in more than 20 percent of samples examined in any month where associated with domestic sewage.	Same as for use "A" above.	None attributable to sewage, industrial wastes or other wastes which, after reasonable dilution and mixture, will interfere with the best use of these waters for the purpose indicated.	Greater than five (5) parts per million.	Same as for use "A" above.	Same as for use "A" above.	Less than twenty-five (25) parts per billion or none in sufficient amounts as to impart a residual taste to recreational or commercial fish or other aquatic forms	Same as for use "B" above.	Same as for use "A" above.	Same as for use "A" above.
(D) GROWTH AND PROPAGATION OF FISH AND OTHER AQUATIC LIFE, Including waterfowl, furbearers, and other aquatic and semi-aquatic life.	See note under "C" above.	Same as for use "A" above.	None attributable to sewage, industrial wastes or other wastes which will interfere with the palatability or propagation of recreational or commercial fish or other edible aquatic forms.	Same as for use "C" above.	Same as for use "A" above.	None alone or in combination with other substances or wastes in sufficient amount or of such character as to make receiving waters unsafe or unsuitable for use indicated.	Same as for use "C" above.	Same as for use "B" above	Not in sufficient quantity as to increase the temperature of the receiving water beyond that optimum for the normal aquatic life of the specific water.	Primary treatment and effective disinfection. Sewage lagoon treatment will meet this requirement.
(E) AGRICULTURAL AND INDUSTRIAL WATER SUPPLY Without treatment except for the removal of natural impurities to meet special quality requirements other than those classified under "A" above. Note: Permissible limits for total concentration percent sodium, boron, chlorides, and sulphates to receive further study, suggested value for percent sodium is less than 40. Percent sodium computed as the ratio of sodium to total cations expressed in equivalent weights. Calculated from the formula: Na X 100 ÷ Na + K + Ca + Mg when Na, Ca, K, and Mg are expressed in equivalents.		Same as for use "A" above.	None attributable to sewage, industrial wastes or other wastes which will adversely affect the marketability of agricultural or industrial produce.	Greater than three (3) parts per million.	Hydrogen ion concentration expressed as pH should be maintained between 6.0 and 9.5.	Same as for use "A" above.	None in sufficient quantity as to make receiving water unsuitable for use indicated.	Same as for use "B" above	Same as for use "A" above.	Same as for use "D" above.

Water Quality Objectives of a watercourse will apply at the point of discharge of a waste except in special cases where the sampling points will be determined by the Montana Water Pollution Council.

Determinations in the above table will be in accordance with "Standard Methods for the Examination of Water, Sewage and Industrial Wastes".

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Revised April, 1958
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of not less than 98 percent, unless discharged into a stream where the volume of dilution water, available during the low flow periods during which irrigation diversions may occur, is at least 10 times greater than the anticipated maximum daily volume of sewage, in which case a bacterial reduction of 90 percent may be acceptable.

Provided, however, that where the time of passage from plant discharge to irrigation diversion is such as to permit the normal death rate of bacteria as evidenced by the die-away curve to provide equivalent bacterial reduction, the requirements of (1) and (2) above may be appropriately modified" (83).

Nebraska is a member of the Missouri Basin Engineering Health Council.

NEVADA

The State Board of Health has the primary function of controlling water pollution. Legislation enacted in 1957 and amended in 1959 authorizes the Division of Public Health Engineering, under the direction and supervision of the Board, to develop a system of classification of the waters of the state (1834, 1835, 1836).

No standards or classifications have been promulgated, as yet.

NEW HAMPSHIRE

Both the Water Pollution Commission, established in 1947, and the State Board of Health have functions relating to the control of water pollution (1837). Surface waters of the state are classified by the Legislature. The Water Pollution Commission has the duty of enforcement of such classification. Control of pollution of unclassified surface waters is accomplished by a permit system administered by the Commission (87).

Waters are to be classified as follows:

"I. Class A waters shall be of the highest quality and shall contain not more than fifty coliform bacteria per one hundred milliliters. There shall be no discharge of any sewage or waste into waters of this classification. The waters of this classification shall be considered as being potentially acceptable for water supply uses after disinfection.

"II. Class B waters shall be of the second highest quality and shall be divided into two parts as follows:

(a) Class B-1 waters shall have no objectionable physical characteristics, shall be near saturation for dissolved oxygen, and shall contain not more than two hundred forty coliform bacteria per one hundred milliliters. There shall be no disposal of sewage or industrial waste into said waters except those which have received adequate treatment to prevent the lowering of the physical, chemical or bacteriological characteristics below those given above, nor shall such disposal of sewage or waste be inimical to fish life or to the maintenance of fish life in said receiving waters. The waters of this classification shall be considered as being acceptable for bathing and other recreational purposes and, after adequate treatment, for use as water supplies.

(b) Class B-2 waters shall have no objectionable physical characteristics, shall be near saturation for dissolved oxygen, and shall contain not more than one thousand coliform bacteria per one hundred milliliters. The waters of this classification shall be considered as being acceptable for recreational boating, fishing and for industrial purposes, and, after adequate treatment, for use as water supplies.

"III. Class C waters shall be of the third highest quality and shall be free from slick, odors, and surface-floating solids of unreasonable kind or quantity, shall contain not less than five parts per million of dissolved oxygen; shall have a hydrogen ion concentration within the range of pH 5.0 to 8.5; and shall be free from chemicals and other materials and conditions inimical to fish life or the maintenance of fish life. The waters of this classification shall be considered as being acceptable for recreational boating, fishing or for industrial water supply uses either with or without treatment depending upon individual requirements.

"IV. Class D waters shall be of the lowest classification and shall be free from slick, odors and surface-floating solids of unreasonable kind, quantity or duration, taking into consideration the necessities of the industries involved, and shall contain dissolved oxygen at all times. The waters of this classification shall be considered as being devoted primarily to the transportation of sewage or industrial wastes, or both, without nuisance.

"V. All tests and samplings for the purposes of examination of waters shall be performed and carried out in a reasonable manner and whenever practicable, in accordance with the current edition of the Standard Methods for the Examination of Water and Sewage as published jointly by the American Public Health Association and the American Water Works Association. Near saturation for dissolved oxygen shall mean a dissolved oxygen content of at least seventy-five percentum of saturation. The waters in each classification shall satisfy all the provisions of all lower classifications.

"VI. Nothing contained herein shall be construed to prohibit, under such terms and conditions as the commission may direct, the use of rotenone or similar compounds by the fish and game department in the conduct of its program to reclaim the public waters of the state for game fishing" (1838).

After adoption of a classification by the Legislature, the Commission may on its own motion or on petition

of not less than 100 inhabitants of the county or counties in which such water in question is situated, reinvestigate the conditions of pollution and make recommendations to the Legislature for reclassification. Persons aggrieved by the classification adopted by the Legislature may petition the Superior Court of the county in which the pollution is occurring for a variance in the classification as applied to his specific case.

The State Board of Health is authorized to establish such regulations as it deems necessary for the protection from contamination of public water or ice supplies (1837). Recent legislation (1961) has not been concerned with standards or classifications (87).

New Hampshire is a member of the New England Interstate Water Pollution Control Commission.

NEW JERSEY

The State Department of Health is the legally constituted water pollution control agency of the state. It is authorized, among other things, to issue orders to abate legal violations and to make and enforce rules and regulations concerning the design and operation of all public water supplies, and of sewerage systems and disposal plants for treatment of wastes discharged into any of the waters of the state (1796).

Effluent or stream specifications, especially the former, are used in New Jersey as guides. They are not law and not intended to be law, and are primarily administrative tools (1749). Effluent specifications are patterned to meet the needs in each valley or stream situation. A typical effluent specification on an inland stream is as follows (89):

"1. The effluent shall contain no free acidity; that is, all titratable acidity shall be neutralized.

"2. The effluent shall be free of noticeable floating solids, scum, oil, grease, or sleek.

"3. The effluent shall be sufficiently free of color or turbidity, or both, so that after dispersion in the receiving waters, or not more than one thousand (1,000) feet above or below the point of effluent discharge, it will not noticeably discolor or add to the turbidity of the receiving waters.

"4. The effluent shall be free of caustic alkalinity or other toxic or deleterious substances.

"5. The effluent shall be free of offensive odors.

"6. The effluent shall have a biochemical oxygen demand not exceeding in the average over any four (4) hour period of a day thirty (30) parts per million and not exceeding at any time forty (40) parts per million.

"7. The effluent shall contain a chlorine residual of at least one (1.0) part per million at all times."

An example of an effluent standard as applied to an industry discharging into a tributary of the Rockaway River which supplies the Boonton Reservoir is as follows: ". . . The pH . . . shall be between 5.0 and 8.5, . . . free of phenol and phenol-like substances, and must be free of toxic or deleterious substances and in particular cyanides, cyanates, trivalent chromium and hexavalent chromium. Chemical substances must not exceed the following concentration in parts per million: sodium, 285; chloride, 210; fluoride, trace; sulfate, 85; and nitrate, 45" (1839).

New Jersey is a member of the Interstate Commission on the Delaware River Basin, the Interstate Sanitation Commission, and the new Delaware River Basin Commission.

NEW MEXICO

The State Board of Public Health has the primary function of controlling water pollution. It establishes policies, rules, and regulations for the New Mexico Department of Public Health. The Oil Conservation Commission is authorized to make rules and regulations for the prevention of pollution by wastes from oil and gas wells. The Department of Game and Fish is authorized to protect game, fish, and spawning waters (1840). In addition, the Plumbing Administrative Board, Office of the State Engineer, and the Interstate Stream Commission have functions that relate to water pollution (90). No water-quality criteria are contained in the legislation and none have been promulgated by the various boards and commissions.

NEW YORK

Effective 1 January 1962, the Water Pollution Control Board was abolished. The functions of the Board were transferred in part to the State Commissioner of Health and in part to the Water Resources Commission. The Water Resources Commission has the authority to classify waters and establish standards. The State Commissioner of Health has the responsibility of enforcing the law.

The extensive system of classification of fresh, tidal, and ground water that appeared as Appendix B in the first edition of WATER QUALITY CRITERIA (1952) has been maintained (91). It is again reproduced in this report as Appendix D and includes several new, special classes, namely, Class A-Special (International Boundary Waters), Class AA-Special (Lake Champlain Drainage Basin), and Special Class (Lower Genessee River).

New York is a member of the Interstate Sanitation Commission, the Interstate Commission on the Delaware River Basin, the Ohio River Valley Water Sanitation Commission, the New England Interstate Water Pollution Control Commission, the Great Lakes Commission, and the new Delaware River Basin Commission.

NORTH CAROLINA

The State Stream Sanitation Committee, created by legislation enacted in 1951, is responsible for the control and abatement of water pollution in North Carolina. The original act established the Committee within the State Board of Health; however, amendments by the General Assembly of 1959 transferred the Committee to the new Department of Water Resources and established therein a Division of Stream Sanitation and Hydrology to serve as administrative agent for the Committee.

The Committee acting under authority of the Statute, adopted a comprehensive series of Water-Quality Standards on 19 November 1953. These standards, are reproduced in full and included as Appendix E (1841).

Pertinent sections of the enabling act (94) are quoted below:

"(a) Duties of Committee Under This Section: The committee is hereby directed and empowered, as rapidly as possible with the limits of funds and facilities available to it, and subject to the procedural requirements of this Article:

(1) To develop and adopt, after proper study, a series of classifications and the standards applicable to each such classification, which will be appropriate for the purpose of classifying each of the waters of the State in such a way as to promote the policy and purposes of this Article most effectively;

(2) To survey all the waters of the State and to separately identify all such waters as the committee believes ought to be classified separately in order to promote the policy and purposes of this Article, omitting only such waters as, in the opinion of the committee, are insufficiently important to justify classification or control under this Article; and

(3) To assign to each identified water of the State such classification, from the series adopted as specified above, as the committee deems proper in order to promote the policy and purposes of this Article most effectively.

"(b) Criteria for Classification: In developing and adopting classifications, and the standards applicable to each, the committee shall recognize that a number of different classifications should be provided for (with different standards applicable to each) so as to give effect to the need for balancing conflicting consideration as to usage and other variable factors; that different classifications with different standards applicable thereto may frequently be appropriate for different segments of the same water; and that each classification and the standards applicable thereto should be adopted with primary reference to an existing or a contemplated best usage to be made of the waters to which such classification will be assigned.

"(c) Criteria for Standards: In establishing the standards applicable to each classification, the committee shall consider, and the standards when finally adopted and published shall state: The extent to which any physical, chemical, or biological properties should be prescribed as essential to the contemplated best usage.

"(d) Criteria for Assignment of Classifications: In assigning to each identified water the appropriate classification (with its accompanying standards), the committee shall consider, and the decision of the committee when finally adopted and published shall contain its conclusions with respect to the following factors as related to such identified waters:

(1) The size, depth, surface area covered, volume, direction and rate of flow, stream gradient and temperature of the water;

(2) The character of the district bordering said water, including any peculiar suitability such district may have or any dominant economic interest or development which has become established in relation to or by reason of any particular use of such water;

(3) The uses which have been made, are being made, or may in the future be made, of such water for transportation, domestic consumption, industrial consumption, bathing, fishing, and fish culture, fire prevention, the disposal of sewage, industrial wastes and other wastes, or any other uses;

(4) The extent to which such water is already receiving sewage, industrial waste, or other waste as a result of present or past usage of the water, and the relative economic values involved in improving or attempting to improve the condition of such water."

NORTH DAKOTA

The State Water Conservation Commission and the State Department of Health act jointly in controlling water pollution in the state. The Industrial Commission, through the State Geologist, is authorized to prevent the pollution of fresh-water supplies by oil, gas, or salt water.

The State Water Conservation Commission is authorized to establish rules and regulations for the control of pollution in watercourses. Commission approval is required for municipal or industrial-waste disposal facility or municipal waterworks construction. Before the Commission grants approval, however, plans must first be approved by the State Department of Health. The Commission cannot declare waters to be polluted without a finding to that effect by the State Department of Health (1842).

No statewide standards of water quality have been promulgated by the Department of Health or the Water Conservation Commission (95). North Dakota is a member of the Tri-State Water Commission.

OHIO

The Water Pollution Control Board and the Division of Sanitary Engineering, both of which are established within Ohio Department of Health, have functions relating to the control of water pollution. The Water Pollution Control Act of Ohio passed in 1951 created the Board with administrative, regulatory, and quasi-judicial powers. Membership of the Board includes the Directors of the State Departments of Health, Commerce, and Natural Resources as well as two appointive members that represent municipalities and industry.

The Board has the authority to develop programs for the prevention, control, and abatement of water pollution. Studies and recommendations with respect to such programs are carried out by the Division of Sanitary Engineering. A major portion of the programs of the Board is carried out through the granting of permits for the discharge of wastes to waters of the state. Such permits are issued for a specific period of time and their renewal is granted upon compliance with certain conditions that are affixed to the permit. Failure to comply with the conditions results in formal hearing and possible Board action.

General supervision of public water supplies and waterworks, as well as of the disposal of wastes and waste treatment works is carried out by the Division of Sanitary Engineering.

Previous printings of WATER QUALITY CRITERIA (1952 and 1954) have included water-quality objectives

for the Miami and Maumee River Basins, and also of the objectives of the Lake Erie Pollution Survey Report. Since then, objectives have been promulgated for the Muskingum, Cuyahoga, Mahoning, and Scioto River Basins. All of these objectives are reproduced in Appendix F.

Ohio is a member of the Ohio River Valley Water Sanitation Commission (98).

OKLAHOMA

The Oklahoma State Department of Health has primary responsibility for protecting the municipal and domestic water supplies from pollution. In addition, the Department has the authority to control pollution resulting from municipal or domestic sewage or any pollution affecting municipal, domestic and/or recreational waters.

The Oklahoma Water Resources Board, created in 1957, coordinates the activities of the other pollution control agencies in the state and is responsible for industrial waste discharges (1843). It is also responsible for adopting and promulgating standards of quality of the waters of the state (1844).

The Oklahoma State Corporation Commission has responsibility for controlling pollution resulting from oil and gas production and/or processing. The Oklahoma State Department of Wildlife Conservation is responsible for maintaining water quality at levels suitable for sustaining and propagating fish and wildlife (1843).

The Bureau of Water Resources Research, University of Oklahoma, has prepared an extensive listing of water-quality criteria for the various cooperating agencies to be used as guidelines for acceptable quality of state waters. These criteria are not intended to be utilized as inflexible standards. They are summarized in Table 3-2 (1845).

TABLE 3-2

SUGGESTED CRITERIA OF RAW WATER QUALITY FOR THE STATE OF OKLAHOMA (1845)

Ion, organism or substance	Water use			
	Municipal	Industrial	Agricultural	Recreation—fish, wildlife
Algae—Std. Units	1000 Std. Units	1000	Absence of toxic algae	
Vol.	Vol.			
Alkalinity—ppm	120	50–150	--	--
Ammonia—ppm	0.1	--	--	0.3
Biochemical Oxygen Demand—ppm	Max. 1.0–3.0 Ave. 0.75–1.5	--	--	See D. O.
Bicarbonates—ppm	150	3–100	--	--
Boron—ppm	1	--	0.2–0.5	--
Cadmium—ppm	0	0	0	0
Calcium—ppm	--	--	40	--
Carbon Dioxide—ppm	--	--	20–40	--
Carbonates—ppm	--	200–400	10	--
Chloride—ppm	50	20–250	100	--
Chromium—ppm	0	0.05	0	10.0
Coliform-MPN/100 ml	50	--	--	500–1000
Color	20–70	100–1000	--	--
Copper—ppm	3.0	--	0.2	1.0
Cyanides—ppm	0	0	0	0
D.O.—ppm	over 4.0	0.2–2.0	0.2	over 4
Floating solids	0	0	0	0
Fluoride—ppm	1.5	1.0	--	5.0
Hydrogen Ion Concentration-pH	6.5–8.5	6.0–9.6	--	5.0–9.5
Iron—ppm	0.3	0.5	--	--
Magnesium—ppm	--	--	20*	--
Nitrate—ppm	10	--	--	44
Oil—ppm	0	0	0	0.3
Phenolic compounds—ppm	0	.001–.010	.005–.020	0.2–1.0
Radioactivity—uuc/l	Background	Background	Background	Background
Sodium—ppm	0–10	50	10	--
Sulfates—ppm	250	100–250	190	--
Suspended Solids—ppm	--	--	Absence of sludge deposits	
Total Dissolved Solids—ppm	1000	100–1000		
Temperature—°F. (desirable)	50	60	60	32–95
Total Nitrogen—ppm	10	--	--	10
Turbidity—ppm	0–40	10–50	--	2000

*SAR values show the inter-relationship.

OREGON

The State Sanitary Authority, created within the State Board of Health in 1939, has the primary responsibility for the control of water pollution in Oregon (101, 1846). Among the Sanitary Authority's powers and duties are the following: to encourage " . . . restoring and preserving the quality and purity of waters of the State in accordance with standards established by the Sanitary Authority . . . ", to " . . . amend or cancel rules and regulations pertaining to minimum requirements for disposal of sewage and industrial wastes . . . ", and to " . . . establish, modify or amend standards of quality and purity of the various waters of the State . . . " The Statutes defines standards as " . . . such measure of quality or purity for any waters in relation to their reasonable and necessary use . . . " The Statutes also identify certain pollutants in association with standards:

"Section 11. The Sanitary Authority is authorized and empowered to establish standards of quality and purity of the waters of this state in accordance with the public policy of the state of Oregon and in establishing such standards, consideration shall be given the following factors:

(a) The extent, if any, to which floating solids may be permitted in the water;

(b) The extent to which suspended solids, settleable solids, colloids or a combination of solids with other substances suspended in water may be permitted;

(c) The extent to which organisms of the coliform group, and other bacteriological organisms or viruses may be permitted in the waters;

(d) The extent of the oxygen demand which may be permitted in the receiving waters;

(e) The minimum dissolved oxygen content that shall be maintained;

(f) The limits of other physical, chemical, biological or radiological properties that may be necessary for preserving the purity of the waters of the state;

(g) The extent to which any substance must be excluded for the protection and preservation of public health; and

(h) The value of stability and the public rights to rely upon standards as adopted for a reasonable period of time to permit institutions, municipalities, commerce, industries and others to plan, schedule, finance and operate improvements in an orderly and practical manner" (1847).

Specific standards and classifications are included in Appendix G unchanged from that which appeared in the 1952 edition (101, 1848).

Oregon is a member of the Klamath River Compact Commission and the Pollution Control Council, Pacific Northwest Area.

PENNSYLVANIA

The Sanitary Water Board in the Department of Health has the primary function of controlling water pollution in the Commonwealth. The Department of Health acts as the enforcement agent for the Board, and exercises control over water supplied to the public (1849).

The control of water pollution in Pennsylvania is unique in several respects. The Sanitary Water Board established one of the first comprehensive plans for the rehabilitation of polluted streams. In an attempt to deal fairly with all municipalities, the Board has classified all streams of the Commonwealth as: (a) those into which all wastes that are discharged shall be given complete treatment or its equivalent, e.g., 85 percent BOD reduction, (b) those into which all wastes that are discharged shall be given primary treatment, e.g., settling, grease removal, and chlorination, (c) acid-impregnated streams that, for the present, require no treatment of wastes received thereby, and (d) those into which a degree of treatment somewhere between primary and complete is required. This fourth category may apply to particular reaches of a stream. The degree of treatment is specified for each particular case.

In a further attempt at an equitable handling of industrial wastes, the Sanitary Water Board has encouraged industry-wide committees to formulate specific regulations and effluent standards for each industry and for each type of waste normally discharged by such industry. Rules and regulations, including standards, have already been promulgated for five types of industries, viz., wastes from pulp and paper mills (104), wastes from milk plants (105), acid pickling wastes from metallurgical plants (106), wastes from oil and natural-gas wells (107), and wastes from wells other than oil and natural gas (108). By setting up normal values for the strength of such wastes and then requiring a certain percentage removal of polluting materials therefrom, the Board recognized that the wastes frequently can be reduced in quantity and pollutional strength by improved operation and equipment, and by good "housekeeping" methods, as well as by waste-treatment works.

The Sanitary Water Board has also adopted rules and regulations with respect to the disposal of wastes into underground strata and also disposal of heated wastes. These rules and regulations are reproduced in Appendix H (103).

Pennsylvania has also adopted policies and standards with respect to radioactive discharges. Radioactive wastes have been classified as industrial wastes. The permit granted for the Shippingport reactor is subject to certain Special Conditions, some of which are noted in Appendix H (1850, 1851).

Where the Sanitary Water Board requires the removal of BOD it specifies percentage removals equivalent to that obtained by sewage treatment. This requirement amounts to 35 percent for primary treatment and 85 percent for complete treatment (1852). Pennsylvania is a member of the new Delaware River Basin Commission, the Great Lakes Commission, the Interstate Commission on the Delaware River Basin, the Interstate Commission on the Potomac River Basin, and the Ohio River Valley Water Sanitation Commission.

RHODE ISLAND

The Department of Health has primary responsibility for the control of water pollution in the State of Rhode Island. The Division of Sanitary Engineering in the Department of Health enforces the water pollution laws of the state (1853). Although Rhode Island has promulgated no standards of its own, it subscribes to those of the New England Interstate Water Pollution Control Commission of which is it a member (109).

SOUTH CAROLINA

In 1950, the Water Pollution Control Authority was created within the State Health Department. The Authority was assigned the responsibility of preparing a general comprehensive program for the abatement and prevention of water pollution (110, 1854).

The Authority is empowered to classify the designated waters. Standards of quality and purity for each such classification are related to public use or benefit and may be later modified.

The resulting system of classifications and standards were listed in the appendices of previous printings of WATER QUALITY CRITERIA. They are again reproduced unchanged in this edition as Appendix I (110).

SOUTH DAKOTA

The Committee on Water Pollution, working through the Division of Sanitary Engineering of the State Department of Health, has the primary responsibility for water-pollution control in South Dakota (1855). No specific effluent standards are in effect, but each installation is evaluated individually. Streams have been classified as indicated by the pertinent sections of the enabling statute (113) quoted below (from Laws pertaining to Water Pollution 1960 Supplement to South Dakota Code—1939):

"61.0145 Classification of Waters. It shall be the duty of the Committee to classify the public waters of this State, or parts thereof, into classes which shall be known as 'Class A' waters and 'Class B' waters. 'Class A' waters shall be those waters, or parts thereof, in which the pollution or corruption entering such waters can be so controlled that the waters receiving such pollution and corruption shall not be unwholesome or unfit for domestic use, or unsafe as a source of public water supply, or deleterious to fish or plant life, or shall not cause a public nuisance. 'Class B' waters shall be those waters, or parts thereof, which are more important to the welfare of the people of the state as carriers of waste, providing such wastes are not detrimental to the public health. (No person, firm or corporation shall discharge or cause to be discharged in 'Class A' waters of this state any new pollution or corruption in the form of sewage, industrial or other wastes, liquid or solid, without first having applied for and received a permit from the Water Pollution Committee for such discharge. In issuing the permit, the Committee shall prescribe the standards of water quality and purity of such sewage, industrial or other wastes necessary to maintain the receiving water as 'Class A' water of this state as de-

fined in Section 61.0145, 1960 Supplement to South Dakota Code of 1939. The Committee shall have authority to require the applicant for the permit to furnish such technical information and data as may be necessary to judge the character of the waste and the degree of treatment required to maintain the necessary standards of purity.)"

South Dakota is a member of the Tri-State Water Commission.

TENNESSEE

The Stream Pollution Control Board is charged with the primary responsibility of controlling water pollution. The Department of Public Health also exercises some functions relating to water pollution, primarily in the construction of public water supply and sewerage systems (1856).

The Tennessee Stream Pollution Act of 1945, as amended in 1951 (115), gives the Board the duty and authority ". . . To establish such standards of quality for any waters in relation to their reasonable and necessary use as it shall deem to be in the public interest." The original standards or any changes therein can be promulgated by the Board only after public hearings.

Acting in accordance with the statute, the Board issued the following criteria to be used as guides (1857):

"Criteria of Water Conditions

1. Solids, Floating Materials, and Deposits

There shall be no distinctly visible solids, scum, foam, oily sleek, or the formation of slimes, bottom deposits or sludge banks of such size or character as may be detrimental to public health, fish and other aquatic life, recreation, industry, or other reasonable and necessary uses of the waters.

2. Temperature

The temperature of the receiving waters shall not be raised or lowered to such an extent as to interfere with the reasonable and necessary uses of the waters.

3. Turbidity or Color

There shall be no turbidity or color added in amounts or of such character as interferes with the reasonable and necessary uses of the waters, or that cannot be reduced to acceptable concentrations by modern water treatment processes as commonly employed, or that result in an objectionable appearance of the waters.

4. Taste or Odor

There shall be no substances added which will result in taste or odor that prevent the production of potable water by modern water treatment processes as commonly employed, or imparts unpalatable flavor to food fish, or result in noticeable offensive odors in the vicinity of the waters, or otherwise interfere with the reasonable and necessary uses of the waters.

5. Acidity or Alkalinity

There shall be no substances added which will make the waters acid or alkaline to such an extent as to interfere with the reasonable and necessary uses of the waters.

6. Hardness or Mineral Compounds

There shall be no substances added to the waters which will increase the hardness or the mineral content of the waters to such an extent as to appreciably impair the usefulness of the waters as a source of water supply or interfere with other reasonable and necessary uses of the waters.

7. Dissolved Oxygen

The dissolved oxygen shall not be depleted to an extent that will interfere unduly with the reasonable and necessary uses of the waters.

8. Bacteria

No disease-producing bacteria or other objectionable organisms shall be added to surface waters which will result in the contamination of said waters to such an extent as to render the water unsuitable as sources of domestic water supply after treatment with currently existing methods or otherwise increase the bacterial content to such an extent as to render said waters unsuitable for other reasonable and necessary uses.

9. Toxic Substances

There shall be no substances added to the waters that may produce toxic conditions that materially affect man, animals, or aquatic life, or impair the safety of a treated water supply, or adversely affect other reasonable and necessary uses.

10. Other Pollutants

Other pollutants shall not be added to the waters in quantities which may be detrimental to public health, fish or aquatic life, recreation, or other reasonable and necessary uses of the waters" (114, 1857).

Tennessee is a member of the Tennessee River Basin Water Pollution Control Compact, but as of 1 January 1962 this Compact had not been activated.

TEXAS

The Texas Water Pollution Control Law, passed by the 57th Legislature in 1961, was the first change in water pollution control in Texas since 1953. This act created the State Water Pollution Control Board, which has the authority to abate and prevent pollution of the waters of the state.

Streams are not classified formally nor have standards of water quality been promulgated, although streams or other bodies of water used as the raw water supply to a public water treatment plant must conform to standards established by the Department of Health which has adopted the U. S. Public Health Service standards (116). Although the act does not mention standards per se, Section 4 states, in part:

> The Board "after notice to the parties affected and after a public hearing if . . . (deemed) . . . to be in the public interest, may issue permits for the discharge of waste into or adjacent to the waters of this State. Each such permit shall set forth the conditions upon which it is issued by the Board, including, but without limiting such conditions, to the duration of such permit, the maximum quantity of waste which may be discharged thereunder at any time and from time to time,

and the quality, purity and character of waste which may be discharged thereunder... The Board shall adopt, prescribe, promulgate and enforce rules and regulations reasonably required to effectuate the provisions of this Act" (1858).

UTAH

In 1953 the Utah State Water Pollution Control Board was established with strong powers to control, prevent, and abate the pollution of surface and underground waters. The Water Pollution Control Act did not alter the powers of other state agencies also functioning in water-pollution control, namely, the State Health Department, the Fish and Game Department, and the State Engineer (118, 1859).

Among the powers and duties that the Board shall have and may exercise under Section 4 of the act is the following: "To adopt, modify or repeal and promulgate standards of quality of the waters of the state and classify such waters according to their reasonable uses in the interest of the public under such conditions as the board may prescribe for the preservation, control and abatement of pollution." Section 6 of the same act states:

"In order to effectuate a program for the prevention, control and abatement of pollution of the waters of the state, the board is authorized to group the waters of the state into classes according to their present most reasonable uses. Subject to the approval of the Legislature the board is authorized to upgrade and reclassify from time to time the waters of the state to the extent that is practical and in the public interest.

"The board is authorized to establish standards of quality for each classification consistent with most reasonable present and future use of such waters, and such standards may be modified or changed from time to time. Prior to classifying waters, setting standards or modifying or repealing them the board shall conduct public hearings in connection therewith. Notice of public hearing for the consideration, adoption, or amendment of the classifications of waters and standards of purity and quality thereof shall specify the waters concerning which a classification is sought to be made or for which standards are sought to be adopted and the time, date, and place of such hearing. Such notice shall be published at least twice in a newspaper of general circulation in the area affected and shall be mailed at least twenty days before such public hearing to the chief executive of each political subdivision of the area affected and to such other persons as the board has reason to believe will be affected by such classification and the setting of such standards.

"The adoption of standards of quality of the waters of the state and classification of such waters or any modification or change thereof shall be effectuated by an order of the board which shall be published in a newspaper of general circulation in the area affected. In classifying waters and setting standards of water quality or making any modification or change thereof, the board shall allow and announce a reasonable time for persons discharging wastes into the waters of the state to comply with such classification or standards.

"Any discharge in accord with such classification or standards shall not be deemed to be pollution for the purpose of this act" (1860).

In accordance with these powers, the Utah Water Pollution Control Board has officially adopted the system of classification with water quality standards as shown in Appendix J (118).

VERMONT

The Vermont Water Resources Board, formerly known as the State Water Conservation Board, had its name changed by an act of the General Assembly of the State of Vermont in 1961 (119). The Water Resources Board has the primary responsibility of controlling water pollution in all surface waters. The State Health Commission also exercises some functions dealing with water pollution. These functions relate primarily to public water and ice supplies and advice concerning the disposal of drainage or sewage (1861). The Vermont Water Resources Board administers the state grant-in-aid program whereby 20 percent of the total eligible costs for necessary pollution-control facilities is paid by the state. In administering this grant-in-aid program, the Board is responsible for establishing standards of treatment and reviewing and approving plans for these facilities. Its duties also include review of sewer extension plans, issuing of permits for these extensions for new sources, and the initiation of enforcement activities when needed.

The Water Resources Board is authorized to classify waters not already classified by the Legislature, and to determine the degree of purity that should be obtained and maintained in such waters in the future. The 1961 General Assembly also amended Section 902 of the water-pollution-control laws on classification. It now reads (1862):

"S902. Classification of waters.

The State of Vermont hereby adopts, for purposes of classifying its waters, the following classes and definitions thereof:

Class A. Suitable for public water supply. Character uniformly excellent.

Class B. Suitable for bathing and recreation, irrigation and agricultural uses; good fish habitat; good aesthetic value, acceptable for public water supply with filtration and disinfection.

Class C. Suitable for recreational boating, irrigation of crops not used for consumption without cooking; habitat for wild life and for common food and game fishes indigenous to the region; and such industrial uses as are consistent with other class "C" uses.

Class D. Suitable for transportation of sewage and industrial wastes without nuisance, and for power, navigation and other industrial uses."

The above classes correspond essentially to those of the New England Interstate Water Pollution Control Commission of which Vermont is a member. The Water Resources Board utilizes the standards of this Commission (119).

VIRGINIA

The State Water Control Board has the primary function of controlling water pollution in the state. The State Board of Health has general supervision and control over all water supplies and waterworks in the state insofar as the sanitary and physical quality of water furnished may affect the public health (1863).

The State Water Control Board is charged "to establish such standards of quality for any waters in relation to the reasonable and necessary use thereof as it deems to be in public interest, and such general policies relating to existing or proposed future pollution as it deems necessary to accomplish the purposes of this chapter, to modify, amend or cancel any such standards or policies established and to take all appropriate steps to prevent pollution contrary to the public interest or to standards and policies thus established" (1864).

The water-quality aspects of each case involving a waste discharge to state waters is considered on its own merits, taking into consideration the downstream water uses and the assimilative capacity of the receiving stream. In this consideration the Board utilizes a guide of general objectives for the setting of water-quality standards. In effect, therefore, stream quality standards, either expressed or implied, exist for each stretch of stream below a point of waste discharge. Moreover, each certificate (permit) granted contains a section making at least a general reference to stream quality.

The general criteria used by the Board as a guide in recommending standards of quality are:

"1. Solids which may settle to form detrimental sludge deposits in the quieter sections of streams should not be permitted.
2. Oil, grease, scum or floating solids which are unsightly, may be deposited on the shores, interfere with re-oxygenation of the water, or are otherwise detrimental should not be permitted.
3. pH in the stream should be maintained within specified limits.
4. Inorganic dissolved solids in the stream should be kept within concentration limits so that water is not rendered unfit for an industrial or domestic water supply, as an environment for aquatic life, or for other uses.
5. Materials in the stream toxic to fish and other aquatic life, or to man or animals using the water, should be kept at or below specified maximum concentrations.
6. Dissolved oxygen should be kept at specified levels in the stream below the point of any waste discharge, so indigenous aquatic life may be maintained.
7. Discoloration and turbidity of the stream should be kept within specified limits.
8. The most probable numbers of coliform bacteria in the stream should be kept within specified limits.
9. Radioactivity levels should be kept within specified limits.
10. Temperature should be kept within specified limits.
11. Specifications for other constituents in the stream will be set in individual cases" (120).

An example of a certificate (permit) stipulation relating to water quality is the following:

"After adequate mixing of the industrial wastes referred to in this certificate with receiving State waters, the following general conditions shall be maintained:

(a) No appreciable change in existing color.
(b) No appreciable settleable or floatable solids.
(c) No grease or oil films.
(d) Natural pH value of stream not changed more than one pH unit.
(e) Biochemical oxygen demand of waste sufficiently low to prevent depressing the dissolved oxygen content at any time or at any location below _____ parts per million.
(f) Potentially toxic substances substantially below toxicity thresholds for aquatic or other life" (120).

Virginia is a member of the Interstate Commission on the Potomic River Basin, the Ohio River Valley Water Sanitation Commission, and the Washington Metropolitan Regional Sanitary Board.

WASHINGTON

The Pollution Control Commission has been responsible for administering and enforcing the State Water Pollution Control Act since 1945. The Commission has broad authority under the Act to control and prevent pollution of state water, including the setting of standards. Other state agencies that have roles concerning certain aspects of water pollution include the State Department of Health, the State Department of Conservation and Development (Division of Water Resources), the Director of Fisheries and the Director of Game, and the State Oil and Gas Conservation Committee (1865).

The Pollution Control Commission has adopted the following standards (1866):

"1. No sewage or industrial waste shall be discharged into any of the waters of the state that will cause:

(a) The reduction of the dissolved oxygen content to less than five parts per million (5 p.p.m.)
(b) The hydrogen-ion concentration (pH) to be outside of the range of 6.5 to 8.5.
(c) The liberation of dissolved gases, such as carbon dioxide, hydrogen sulphide or any other gases in sufficient quantities to be deleterious to fishes or related forms.
(d) The development of fungi or other growth having a deleterious effect on stream bottoms, fishes and related forms, or be injurious to health, recreation or industry.
(e) Toxic conditions that are deleterious to fishes and related forms or affect the potability of drinking water.
(f) The formation of organic or inorganic deposits detrimental to fishes and related forms, or be injurious to health, recreation or industry.
(g) Discoloration, turbidity, scum, oily sleek, floating solids, or coat the aquatic life with oily films, or be injurious to health, recreation or industry.

(h) The temperature to be raised above the limit of tolerance of fishes and related forms.

"2. In those waters which are used or reasonably suitable for use as drinking water supplies, shellfish culture, bathing, or in other instances where its use presents a definite public health hazard by presence or potential presence of disease-producing organisms, the median bacteriological content of a representative number of samples shall not show the presence of coliform organisms in excess of 50 per 100 ml. expressed in terms of the most probable number, provided that the sanitary survey reveals that bacterial content is of human origin. In other instances where the use of water presents a public health hazard by the presence or potential presence of disease-producing organisms, the median bacteriological content of a representative number of samples shall not show the presence of coliform organisms in excess of 240 (M. P. N.) per 100 ml provided that the sanitary survey reveals that the bacteriological content is of human origin."

As a member of the Pollution Control Council of the Pacific Northwest Area, the Washington Commission utilizes the water-quality objectives of this interstate agency.

WEST VIRGINIA

The State Water Resources Commission, named in 1959, was incorporated in the newly formed Department of Natural Resources in 1961. This Commission has the primary responsibility of controlling water pollution in the State of West Virginia (1867).

Minimum Water Quality Objectives adopted by the Commission, as amended in 1959, conform with objectives of the Ohio River Valley Water Sanitation Commission of which West Virginia is a member. This document states (126):

"That the State Water Commission adopt as a general guide the following minimum water quality objectives, applicable to all waters of the state, that is to say:

"No sewage, industrial wastes or other substances allowed to enter any of the waters of the state shall cause therein or materially contribute to any of the following conditions thereof:

(1) Distinctly visible floating or settleable solids, scum, foam or oily sleeks of unreasonable kind or quantity;
(2) Objectionable bottom deposits or sludge banks;
(3) Objectionable odors in the vicinity of the waters;
(4) Objectionable taste or odor in municipal water supplies;
(5) Poisonous to man, animal or fish life;
(6) Dissolved oxygen concentration to be less than 3.0 parts per million at the point of maximum oxygen depletion;
(7) Objectionable color;
(8) Requiring an unreasonable degree of treatment for the protection of potable water by modern water treatment processes as commonly employed; and, be it

Further Resolved, that with respect to sewage under the control of any municipal or private corporation, any county court, government institution, agency or political subdivision, these objectives be interpreted to require at least sufficient treatment to provide substantially complete removal of settleable solids and removal of not less than 45 percent of the total suspended solids, and, be it

Further Resolved, that chlorination of sewage treatment plant effluents will be required where deemed necessary, and, be it

Further Resolved, that nothing contained herein shall be construed to be antagonistic to or interfere with the obligations incurred by the State of West Virginia as a signator to the Ohio River Valley Water Sanitation Compact; and, be it

Further Resolved, that higher or additional water quality objectives may be established by the Commission at its discretion, or exceptions may be made to these objectives in individual cases where justified."

Although the above guides are to be followed in all state streams, more stringent measures can be applied to certain streams.

West Virginia is also a member of the Interstate Commission on the Potomac River Basin.

WISCONSIN

In Wisconsin, the Committee on Water Pollution, the State Board of Health, and the Conservation Department exercise functions relating to water pollution. The Committee exercises general supervision over the administration and enforcement of all laws relating to the pollution of the surface waters of the state (1868). Changes in the statutes since 1953 have not involved water-quality criteria.

No water-quality standards have been established; each pollution problem is considered individually. The Committee has, however, adopted the policy that at least primary treatment with chlorination should be provided for all sewage discharged into surface waters, and that industrial-waste-treatment plants should provide equivalent reduction in BOD to that attained in the treatment of sewage for any given location (127).

Wisconsin is a member of the Great Lakes Commission.

WYOMING

The State Department of Health has general supervision over water-pollution control in the state. The Stream Pollution Advisory Counsel assists the Department in the development of a comprehensive program for the prevention, abatement, and control of water pollution. The State Engineer is responsible for the control of pollution of underground waters. The Oil and Gas Conservation Commission also has responsibilities concerned with prevention of pollution of fresh-water supplies by oil, gas, or salt water (1869).

The Wyoming State Board of Health has adopted criteria set forth by the Missouri Basin Engineering Health Council, of which the Wyoming Department of Public Health is a member. These criteria are as described hereinafter (128).

BI-STATE DEVELOPMENT AGENCY

The States of Illinois and Missouri created by compact in 1949 the Bi-State Development Agency which has

general planning authority only with respect to water supply (St. Louis metropolitan area). Under this authority, the Agency, in 1954 and in conjunction with other agencies, sponsored a water-pollution investigation of the Mississippi River (1870).

Among the recommendations made were that all wastes discharged into the river shall be in a condition which shall not cause:

a) excessive bacterial, physical, or chemical contamination;
b) unnatural deposits in the stream;
c) objectionable colors, tastes, or odors to water used for domestic or industrial purposes; and
d) shall be free from floating materials, such as oils, grease, sewage solids, or other refuse.

For sanitary sewage and storm water, it is recommended that treatment be sufficient for adequate removal or reduction of solids, bacteria, and chemical constituents that may interfere with the use of the water, such as substantially complete removal of floating and settleable solids, and not less than 45 percent of the suspended solids. Phenol wastes shall not produce a concentration exceeding a monthly average of 2 micrograms per liter or a maximum of 5 micrograms per liter at any point. The pH of the waters shall not be less than 6.7 nor more than 8.5. Odor-producing substances in the effluent shall be reduced so that after the final dilution, the mixture does not have a monthly threshold odor number in excess of 10 and a maximum daily odor number in excess of 30. Oils and floating solids shall be reduced to a point such that the stream is free from noticeable floating solids, oil, grease, and sleek. Highly toxic wastes should be eliminated or reduced to safe limits. Deoxygenating wastes should be suitably treated (1871).

DELAWARE RIVER BASIN COMMISSION

This new agency came into existence on 27 October 1961 as an interstate-federal compact. The Federal Government will participate with the States of New York, New Jersey, Pennsylvania, and Delaware in the planning, regulation, and execution of water-resources projects and programs in the Delaware River Basin. The new agency will probably supplant the Interstate Commission on the Delaware River Basin and absorb much of the latter's progress in and activities concerning the setting of standards (133).

Section 3.6 of the Compact authorizes the Commission to "Establish standards of planning, design and operation of all projects and facilities in the basin which affect its water resources. . ." Section 4.2 allows the Commission power "to acquire, operate and control projects and facilities for the protection of public health, stream quality control, economic development, improvement of fisheries, recreation, dilution and abatement of pollution, the prevention of undue salinity and other purposes. . ." Also, Section 5.2 states "The commission may assume jurisdiction to control future pollution and abate existing pollution in the waters of the basin, whenever it determines after investigation and public hearing upon due notice that the effectuation of the comprehensive plan so requires. The standard of such control shall be that pollution by sewage or industrial or other waste originating within a signatory state shall not injuriously affect waters of the basin as contemplated by the comprehensive plan. The commission, after such public hearing may classify the waters of the basin and establish standards of treatment of sewage, industrial or other waste, according to such classes including allowance for the variable factors of surface and ground water such as size of the stream, flow, movement, location, character, self-purification, and usage of the waters affected. After such investigation, notice and hearing the commission may adopt and from time to time amend and repeal rules, regulations and standards to control such future pollution and abate existing pollution, and to require such treatment of sewage, industrial or other waste within a time reasonable for the construction of the necessary works as may be required to protect the public health or to preserve the waters of the basin for uses in accordance with the comprehensive plan" (1872).

GREAT LAKES COMMISSION

The Great Lakes Commission is an agency created by a 1955 interstate compact among seven Great Lakes States, and serves as their joint advisory agency on Great Lakes water-resource programs. The State of Ohio is the only state eligible for membership that is not now participating in the Commission's program. Also, Congress has not yet given its consent to the Compact. Nevertheless, the member States of Illinois, Indiana, Michigan, Minnesota, New York, Pennsylvania, and Wisconsin continue to recognize the Commission as their joint agency.

Article VII of the Great Lakes Basin Compact lists "measures for combating pollution" among the subjects to be considered by the Commission. In the exercise of its powers under the Compact, which limits the Commission's authority to advisory powers, the Commission to date has not recommended any water-quality standards for the Great Lakes (1873).

INTERNATIONAL BOUNDARY AND WATER COMMISSION

The 1944 Water Treaty between the United States and Mexico set forth, among other things, the jurisdiction and functions of this Commission. Article 3 of the Treaty states that "all of the (water) uses shall be subject to any sanitary measures or works which may be mutually agreed upon by the two Governments, which hereby agree to give preferential attention to the solution of all border sanitation problems." Consequently, two international sewage treatment works have been jointly constructed, the operation and maintenance of which are under Commission supervision. The Commission does not promulgate its own standards of water quality. Instead, it is guided by the regulations of the border states involved in consultation with the federal public health agency of each country (1874).

INTERNATIONAL JOINT COMMISSION

The International Joint Commission was created by the Boundary Waters Treaty, signed in 1909, between the United States and Great Britain "Relating to Boundary Waters and Questions arising Between the United States and Canada." It was agreed in the Treaty

that boundary waters and waters flowing across the boundary ". . . shall not be polluted on either side to the injury of health or property on the other . . ." (130). The Treaty did not designate specific procedures to ensure compliance with this agreement. Hence, water-pollution problems have been handled under the general provisions of Article IX, under which considerations of any kind can be referred to the Commission for investigation, report, and recommendation (1875).

In 1946, the two governments directed the Commission to investigate conditions of Lake St. Clair and of the St. Clair and Detroit Rivers (130). Eventually, the Commission's investigation was broadened to include sections of Lakes Superior, Huron, Erie, and Ontario. A Board of Technical Advisors, assisting the Commission in the investigation, developed a series of objectives for quality control of the boundary waters (131). These objectives appear in Appendix L.

A feature of these objectives (1876) is their combination of recommendations for stream quality with suggestions for effluent concentrations, e.g., an objective of 0.3 mg/l of iron in the receiving waters "will probably be attained if plant effluents are limited to 17 ppm of iron in terms of Fe." Similar expressions are given for phenols, pH, odors, and oils. Ontario, Canada, makes use of a similar system of objectives plus effluent concentration recommendations (1877).

The International Joint Commission has established an international warning system for notifying downstream water users following industrial waste spills (1875). Louisiana utilizes a similar warning system along the Mississippi River.

INTERNATIONAL WATER SUPPLY ASSOCIATION

In June 1947, representatives from Great Britain, France, and the Netherlands met in England to discuss the formation of an International Water Supply Association. The general purposes of the new group would be to enable men engaged in water supply in all parts of the world to exchange information, to meet to discuss problems of common interest, and eventually to pool ideas and to carry out research on an international basis. The decision was made to convene an International Water Supply Congress in Amsterdam in 1949 and there to set up a new Association and adopt a constitution (1878).

Each third year commencing with 1949 the Association holds an International Water Supply Congress. The United States became a member at the time of the 1961 Congress held in Berlin. Current membership now includes 32 corporate members (nations).

Also at the 1961 Congress, the Association's Technical Commission on Pollution of Surface Waters presented recommended limits of pollution of surface waters (1879) as follows:

"1. The increasing population of the world, the rising standard of life and the increase in the demand for wholesome water supplies will make it necessary to resort more and more to surface waters for drinking water. The Commission is therefore alarmed at the extent to which pollution is allowed in rivers which are or will be used for drinking water.

"2. The Commission therefore recommends that the following principles be adopted:

(a) Rivers which are at present clean shall be preserved free from pollution.

(b) Rivers which are already polluted shall not be made worse by further pollution but shall be improved wherever possible.

(c) Rivers polluted beyond the following limits shall be improved in accordance with a definite programme aimed at reducing the pollution to the following limits measured at the point of intake.

(1) Physical Characteristics

Temperature: Permissible increase to be fixed by each country pending international agreement.

Taste and odour: No disagreeable taste and odour.

Suspended matter: Permissible increase to be fixed by each country pending international agreement.

pH range: 6.5 to 8.7

(2) Chemical Characteristics

Biochemical oxygen demand: Five days at 20°C; not greater than 4 mg/l

Dissolved oxygen content: Not less than 70% saturation

Free ammonia as N: Not greater than 0.5 mg/l

Chlorides as Cl: Desirable limit 200 mg/l

Phenols: Not greater than 0.001 mg/l

Oils and fats: No oils or fats to be allowed

(3) Toxic Substances

Not greater than the concentrations set out in the "International Standards of Drinking Water Quality" published by the World Health Organization in Geneva in 1958, namely:

Substance	Maximum allowable concentrations
Lead (as Pb)	0.1 mg/l
Selenium (as Se)	0.05 mg/l
Arsenic (as As)	0.2 mg/l
Chromate (as Cr)	0.05 mg/l
Cyanide (as CN)	0.01 mg/l

(4) Radioactivity

The limits fixed by the World Health Organization from time to time. The present figures are:

Alpha emitters—not greater than 1 micro-microcurie per litre.

Beta emitters —not greater than 10 micro-microcuries per litre over and above the naturally occurring radioisotope Potassium 40.

"3. The principles set out in paragraph 2 above apply with equal force to lakes. The Commission is to consider whether any amendment is required in the detailed application of these principles to lakes.

"4. The detailed figures set out in paragraph 2 above may require to be altered, added to, and completed in the light of further knowledge and investigation. The Commission will continue its studies and will publish a new report or reports as and when necessary.

"5. The Commission also intends to study and to make recommendations on practical measures to be taken to assist in achieving the objects set out in paragraph 2 above."

INTERSTATE COMMISSION ON THE DELAWARE RIVER BASIN (INCODEL)

Correspondence with the Interstate Commission on the Delaware River Basin (133) indicated that the information presented in the earlier printings of WATER QUALITY CRITERIA is still applicable. Therefore, most of this information is repeated.

Recognizing that the growing problem of pollution along the Delaware River was of interstate origin and concern, Delaware, New Jersey, New York, and Pennsylvania joined in a reciprocal agreement for the correction and control of such pollution. In the original agreement (132) the areas of the basin were classified into zones according to the principal uses of the water and minimum quality requirements for effluents discharged into each zone were promulgated. In this manner, Incodel combined effluent standards with stream classification. Pertinent sections of the reciprocal agreement, dealing with zones and standards, have been excerpted and included herewith as Appendix N.

However, Incodel will soon be supplanted by a new agency. On 27 September 1961, President Kennedy signed an interstate-federal compact under which the Federal Government will participate with the States of New York, New Jersey, Pennsylvania, and Delaware in the planning, regulation, and execution of water-resources projects and programs in the Delaware River Basin. This co-operation will be accomplished under the aegis of a new agency endowed with broad and strong powers over all aspects of water development, including water-pollution control. The new agency officially came into existence on 27 October 1961, a month after the authorizing compact was signed by the President.

While an effective job has thus far been done in coping with pollution in the Delaware River Basin, it has become apparent that the water-quality standards must soon be up-graded in order to keep pace with the rapid growth in industry and population. In the past few years, Incodel has been conducting studies to establish bases upon which the new standards should be founded. It is sure the new Commission will continue these tasks at an accelerated rate (133). This new Commission is to be known as the Delaware River Basin Commission and is described separately.

INTERSTATE COMMISSION ON THE POTOMAC RIVER BASIN

A compact entered into by Maryland, Virginia, Pennsylvania, West Virginia, and the District of Columbia created the Potomac Valley Conservancy District, comprising the drainage basin of the Potomac River and its main and tributary streams. The compact also established the Interstate Commission on the Potomac River Basin as a co-ordinating body for the purpose of "regulating, controlling, preventing, or otherwise rendering unobjectionable and harmless the pollution of the waters of said Potomac drainage area by sewage and industrial and other wastes. . . " (134).

Among its duties and powers, the commission is authorized "To make and, if needful from time to time, revise and to recommend to the signatory bodies, reasonable, minimum standards for the treatment of sewage and industrial or other wastes now discharged or to be discharged in the future to the streams of the Conservancy District, and also, for cleanliness of the various streams in the Conservancy District" (134).

Water-quality criteria promulgated by the Commission in 1946 and recommended to the states of the basin for their use are included in Appendix O. Also, in January 1958, the Commission adopted criteria for the Potomac River in the Washington Metropolitan area. These latter criteria are included in Appendix K.

An amended compact is currently being negotiated. Since an important aspect of the new compact concerns standards and classifications, the part concerning such in Article II, Section F2 is quoted:

"To establish reasonable physical, chemical and bacteriological standards of water quality satisfactory for various classifications of use. It is agreed that each of the signatory bodies through appropriate agencies will prepare a classification of its interstate waters in the District in entirety or by portions according to present and proposed highest use, and for this purpose technical experts employed by appropriate state water pollution control agencies are authorized to confer on questions relating to classification of interstate waters affecting two or more states. Each signatory body agrees to submit its classification of its interstate waters to the Commission with its recommendations thereon.

"The Commission shall review such classification and recommendations and accept or return the same with its comments. In the event of return, the signatory body will consider the comments of the Commission and resubmit the classification proposal, with or without amendment, with any additional comments for further action by the Commission.

"It is agreed that after acceptance of such classification, the signatory body through its appropriate state water pollution control agencies will work to establish programs of treatment of sewage and industrial wastes which will meet or exceed standards established by the Commission for classified waters. The Commission may from time to time make such changes in definitions of classifications and in standards as may be required by changed conditions or as may be necessary for uniformity and in a manner similar to that in which these standards and classifications were originally established.

"It is recognized, owing to such variable factors as location, size, character and flow and the many varied uses of the waters subject to the terms of this Compact, that no single standard of sewage and waste treatment and no single standard of quality of receiving waters is practical and that the degree of treatment of sewage and industrial wastes should take into account the classification of the receiving waters according to present and proposed highest use, such as for drinking water supply, bathing and other recreational purposes, maintenance and propagation of fish life, industrial and agricultural uses, navigation and disposal of wastes" (1880).

INTERSTATE SANITATION COMMISSION

The information concerning the Interstate Sanitation Commission that appeared in earlier printings of WATER QUALITY CRITERIA is still unchanged (1881).

The Tri-State Treaty Commission was created in 1931 jointly by New York, New Jersey, and Connecticut to study the existing conditions of pollution of the harbor and coastal waters of the New York metropolitan area and to recommend methods for the abatement of the pollution. The Treaty Commission recommended the adoption of the Tri-State Compact which established the Interstate Sanitation District and the Interstate Sanitation Commission (135).

The compact, signed by New York and New Jersey in 1936 and by Connecticut in 1941, delimited the areas and waters to be included within the district, defined two classes of water, and enumerated the quality requirements of effluents to be discharged into waters of each classification. The two pertinent articles of this compact are reproduced below (1882):

"Article VI

1. It is recognized by the signatory States that, where tidal waters are used for such varied purposes as bathing, navigation, shellfish culture, and the development of fish life and the disposal of wastes, no single standard of purity is practicable in all parts of the district. In order to attain the objects of this compact, the commission, after proper study and after conducting public hearings upon due notice, shall group the designated waters of the district into classes. Where local conditions shall have changed in the future to such an extent that changes in classification become necessary, the commission may, after conducting public hearings upon due notice, adopt such changes.

Two general classifications shall be used:

(1) Class "A", in which the designated water areas are expected to be used primarily for recreational purposes, shellfish culture or the development of fish life;

(2) Class "B", in which the designated water areas are not expected to be used primarily for recreational purposes, shellfish culture or the development of fish life."

"Article VII

1. It is agreed between the signatory States that no sewage or other polluting matters shall be discharged or permitted to flow into, or placed in, or permitted to fall or move into tidal waters of the district, except under the following conditions and restrictions:

(1) All sewage discharged or permitted to flow into Class "A" waters of the district shall first have been treated so as—

(a) to remove all floating solids and at least sixty per centum (60%) of the suspended solids; and

(b) to effect a reduction of organisms of the B. coli group (intestinal bacilli) so that the probable number of such organisms shall not exceed one per cubic centimeter in more than fifty per centum (50%) of the samples of sewage effluent tested by the partially confirmed test; provided, however, that in the case of discharge into waters used primarily for bathing this bacterial standard need not be required except during the bathing season; and

(c) to effect a reduction in the oxygen demand of the sewage effluent sufficient to maintain an average dissolved oxygen content in the tidal waters of the district and in the general vicinity of the point of discharge of the sewage into those waters, at a depth of about five feet below the surface, of not less than fifty per centum (50%) saturation during any week of the year.

(2) All sewage discharged or permitted to flow into Class "B" waters of the district shall first have been so treated as—

(a) to remove all floating solids and at least ten per centum (10%) of the suspended solids, or such additional percentage as may by reason of local conditions be necessary to avoid the formation of sludge deposits in the Class "B" waters of the district; and

(b) to effect a reduction in the oxygen demand of the sewage effluent sufficient to maintain an average dissolved oxygen content in the tidal waters of the district and in the general vicinity of the point of discharge of the sewage into those waters, at a depth of about five feet below the surface, of not less than thirty per centum (30%) saturation during any week of the year."

Correspondence with the Commission indicated that in recent months the Commission has placed more emphasis on the abatement of industrial wastes. In order to clarify the industrial-waste standards required by the Tri-State Compact, the following requirements have been established (1881):

"Industrial Waste Standards Required by Tri-Sate Compact

1. Remove all floating matter such as oil, grease or solids.
2. Remove settleable solids so as to prevent the formation of sludge deposits along the shores or in the waterways.
3. Remove toxic materials, color, taste or odors that would interfere with the maintenance of fish life,

shellfish and marine life in waters designated Class 'A' or creates a condition in any District waters which is obnoxious or causes a nuisance.

4. pH—No corrections or neutralization required unless it causes a condition which violates one of the preceding standards or creates a condition which is obnoxious or causes a nuisance.
5. Reduction in oxygen demand sufficient to maintain an average dissolved oxygen content in the waters of not less than 50 percent saturation during any week of the year in Class 'A' waters and 30 percent saturation during any week of the year in Class 'B' waters."

It is to be noted that these are not new standards or regulations, and have been issued only to re-emphasize the requirements that have always been covered by the original Compact (1881).

It should be pointed out that Standard No. 3 is basically for Class "A" waters where certain materials might interfere with maintenance of fish life, shellfish, and marine life, but also would apply to "B" waters if they flow into and affect Class "A" waters. There is some flexibility to these standards as may be found under No. 5. This standard allows a determination to be made of an industrial plant on the basis of its location. For example, if a plant discharges waste with a soluble BOD and the water area into which it discharges has sufficient DO, no reduction in the BOD would be necessary. However, in an area with deficent DO, the industry would be required to reduce the oxygen demand of its wastes (1883).

KLAMATH RIVER COMPACT COMMISSION

In 1957, the Klamath River Basin Compact between the States of Oregon and California created the Klamath River Compact Commission. Article VII of the Compact states that, in order to aid in pollution abatement and control, the Commission shall have the duty and power "to cooperate with the states or agencies thereof or other entities and with the United States for the purpose of promoting effective laws and the adoption of effective regulations for abatement and control of pollution of the waters of the Klamath River Basin, and from time to time to recommend to the governments reasonable minimum standards for the quality of such waters.

The basic data to support minimum water-quality standards has been collected. However, the character of development within the area and the existing water quality has not required action by the Commission to establish minimum requirements (1884).

MISSOURI BASIN ENGINEERING HEALTH COUNCIL

The Missouri Basin Engineering Health Council consists of the chief sanitary engineers of the ten Basin States (Colorado, Iowa, Kansas, Minnesota, Missouri, Montana, Nebraska, North Dakota, South Dakota, and Wyoming) who meet yearly to discuss common engineering health programs. As an organization, it is not an interstate agency for adoption or administration of water-pollution-control laws (1885).

In 1952 this group adopted tentative objectives for water-pollution-control programs in the Basin. The state engineers recognize that the objectives are guides only and that changes in conditions, particularly as they affect water uses, may require modifications thereof. In 1954, the Council reviewed and reaffirmed these objectives, eliminating the qualification "tentative" (1886). The objectives are quoted in Appendix P.

NEW ENGLAND INTERSTATE WATER POLLUTION CONTROL COMMISSION

Following a recommendation of the New England Conference of State Sanitary Engineers in 1946, the several New England States negotiated a compact for the control of pollution of interstate streams. The New England Interstate Water Pollution Control Compact was ratified by the States of Connecticut, Massachusetts, and Rhode Island and approved by Congress. Since the creation of the Commission, New York, Vermont, New Hampshire, and Maine have also become members. All eligible states are now members (1887).

"The New England Interstate Water Pollution Control Compact is built around a classification of interstate waters according to their highest use based on physical, chemical and bacterial standards adopted by the Commission. Under the Compact, each signatory State agrees to submit classifications of its interstate waters to the Commission for approval, and then to establish programs of treatment of sewage and industrial wastes to bring about the improvements required to meet the approved classifications" (137).

The establishment of standards for water classification has involved extensive studies. The former "Tentative Plan for Classification of Water" has been revised as "Classification and Standards of Quality for Interstate Waters" and appears in Table 3-3 (1887).

OHIO RIVER VALLEY WATER SANITATION COMMISSION (ORSANCO)

The following material on the Ohio River Valley Water Sanitation Commission has been taken from a publication of the Commission (1888), which is rather inclusive with respect to water-quality criteria.

"The Ohio River Valley Water Sanitation Commission, created by the Ohio River Valley Water Sanitation Compact which became effective on June 30, 1948, is an agency representing the states of Illinois, Indiana, Kentucky, New York, Ohio, Pennsylvania, Virginia and West Virginia. The Commission is charged with the responsibility of administering the provisions of the Compact . . ."

This "Compact establishes basic standards for the treatment of wastes and empowers the Commission to supplement these standards. In addition, the Commission is empowered to prescribe and promulgate rules and regulations for the enforcement of treatment standards and for the administration of the provisions of the Compact . . . Primary enforcement of regulatory controls is sought through the appropriate state regulatory agencies . . .

"Compact provisions—The express objective of the Compact is to place and maintain the waters of the Ohio River basin in a satisfactory sanitary condition, available for safe and satisfactory use as public and industrial water supplies after reasonable treatment, suitable for recreational usage, capable of maintaining fish and

other aquatic life, free from unsightly or malodorous nuisances due to floating solids or sludge deposits, and adaptable to such other uses as may be legitimate.

"The Compact contains the following guides and specific-performance requirements regarding control of waste discharges:

1. All sewage discharged to those portions of the Ohio River and its tributaries which form boundaries between, or are contiguous to two or more signatory states, or which flow from one signatory state into another signatory state shall be so treated as to provide for substantially complete removal of settleable solids and the removal of not less than forty-five percent of the total suspended solids, and to such higher degree as may be determined to be necessary by the Commission after investigation, due notice and hearing.
2. All industrial wastes discharged to waters under Compact jurisdiction shall be modified or treated, in order to protect the public health or to preserve the waters for other legitimate purposes, to such degree as may be determined to be necessary by the Commission after investigation, due notice and hearing.
3. All sewage and industrial wastes discharged to waters situated wholly within one state shall be treated to that extent necessary to maintain such waters in a sanitary and satisfactory condition at least equal to the condition of the waters of the interstate stream immediately above the confluence.

"Water-Quality objectives—As a guide in establishing sewage-treatment requirements and in evaluating water-quality conditions, the Commission on April 4, 1951 adopted objectives regarding the concentration of coliform bacteria." These objectives are presented in Appendix M.

"Sewage-treatment standards—The Commission, in the exercise of the authority granted it by the Compact, has promulgated seven sewage-treatment standards, each applying to a particular stretch of the Ohio River." These standards are also shown in Appendix M.

"Recommendations regarding pollution-abatement needs for interstate tributaries—In cooperation with state agencies, the Commission has investigated pollution-abatement needs for the Wabash River between Terre Haute, Indiana and Vincennes, Indiana and for the West Fork and Monongahela Rivers between Weston, West Virginia and Pittsburgh, Pennsylvania. The results of these investigations have not been transposed into formal treatment standards, . . . but conclusions have been reached and recommendations made regarding degrees of treatment needed in these areas to maintain quality conditions that would be acceptable from an interstate point of view." These conclusions and recommendations are presented in Appendix M.

"Basic industrial-waste requirements—Minimum or basic requirements for the treatment and control of industrial wastes in the Compact District were promulgated on April 6, 1955. These requirements, designated as IW-1, were derived from an interpretation of Compact provisions requiring that all waters are to be free from unsightly or malodorous nuisances due to floating solids or sludge deposits. On September 13, 1958, the basic requirements were amended by the addition of restrictions on the discharge of toxic substances.

"The basic requirements were enunciated as part of a statement of policy and procedure regarding industrial-waste control, which statement represents an agreement on basic principles among the eight states signatory to the Compact and establishes the frame-work within which additional waste-control measures are to be developed. Waste-control measures adopted in accordance with this statement of policy and procedure may be transposed into formal treatment standards when furtherance of the objectives of the compact warrants or requires." The industrial-waste requirements (IW-1) and also Orsanco's chloride-control resolutions appear in Appendix M.

Publications by the Commission have been extensive. They include the Annual Summaries, the Progress Reports of the Aquatic Life Advisory Committee, the Physiological (and other) Studies by the Kettering Laboratory on specific pollutants, including fluoride, lead, chloride, phenol, common inorganic salts, copper, cadmium, iron, cyanide and thiocyanates, cresol, cobalt, and a comprehensive evaluation of river-quality conditions during a 16-week shutdown of upper Ohio River Valley steel mills, as well as numerous articles dealing with aspects beyond the scope of WATER QUALITY CRITERIA.

Many of the above reports terminate with recommendations to the Commission regarding the specific pollutant involved. Such recommendations are included hereinafter under the write-up of each pollutant.

POLLUTION CONTROL COUNCIL, PACIFIC NORTHWEST AREA

This council is an informal organization of the sanitary engineers in direct charge of water-pollution-control programs in Alaska, British Columbia, Idaho, Montana, Oregon, and Washington, with representation of the Canadian Department of National Health and Welfare and the U. S. Public Health Service. The group was organized in May 1949 for the purpose of standardizing pollution-control activities in the Pacific Northwest area. A number of committees were appointed, including a Committee on Water Quality Objectives, which submitted a report on 10 September 1952. The table of water-quality objectives and minimum treatment requirements contained in this report appears in Table 3-4.

The members of the Pollution Control Council were reported to have used these water-quality objectives effectively since their adoption in 1952, in carrying out their individual pollution-control programs. In recent years, Montana and Idaho have formally adopted comparable objectives to guide their state programs. Oregon and Washington, together with the U. S. Public Health Service, have adopted an action program on the interstate boundary waters of the Lower Columbia River in keeping with these objectives. Similarly, Oregon and Washington have consistently aimed their programs at the same goals.

TABLE 3-3

NEW ENGLAND INTERSTATE WATER POLLUTION CONTROL COMMISSION
CLASSIFICATION AND STANDARDS OF QUALITY FOR INTERSTATE WATERS
(As Revised and Adopted October 1, 1959)

	CLASS A	CLASS B	CLASS C	CLASS D
SUITABILITY FOR USE				
	Suitable for any water use. Character uniformly excellent.	Suitable for bathing and recreation, irrigation and agricultural uses; good fish habitat; good aesthetic value. Acceptable for public water supply with filtration and disinfection.	Suitable for recreational boating, irrigation of crops not used for consumption without cooking; habitat for wildlife and common food and game fishes indigenous to the region; industrial cooling and most industrial process uses.	Suitable for transportation of sewage and industrial wastes without nuisance, and for power, navigation and certain industrial uses.
STANDARDS OF QUALITY				
Dissolved oxygen	Not less than 75% sat.	Not less than 75% sat.	Not less than 5 p.p.m.	Present at all times
Oil and grease	None	No appreciable amount	Not objectionable	Not objectionable
Odor, scum, floating solids, or debris	None	None	None	Not objectionable
Sludge deposits	None	None	None	Not objectionable
Color and turbidity	None	Not objectionable	Not objectionable	Not objectionable
Phenols or other taste producing substances	None	None	None	
Substances potentially toxic	None	None	Not in toxic concentrations or combinations	Not in toxic concentrations or combinations
Free acids or alkalies	None	None	None	Not in objectionable amounts
Radioactivity	Within limits approved by the appropriate State agency with consideration of possible adverse effects in downstream waters from discharge of radioactive wastes; limits in a particular watershed to be resolved when necessary after consultation between States involved.			
Coliform bacteria	*Within limits approved by State Department of Health for uses involved.	Bacterial content of bathing waters shall meet limits approved by State Department of Health and acceptability will depend on sanitary survey.		

* Sea waters used for the taking of market shellfish shall not have a median coliform content in excess of 70 per 100 ml.

NOTE: Waters falling below these descriptions are considered as unsatisfactory and as Class E.
These standards do not apply to conditions brought about by natural causes.
For purpose of distinction as to use, waters used or proposed for public water supply shall be so designated.

The Pollution Control Council, during 1959, reviewed these water-quality objectives and found them sound, important, and of continuing value in conserving the quality of the water resources of the Pacific Northwest. The Council, at its October 1959 meeting in Portland, Oregon, re-adopted the objectives for the purpose of bringing them up to date and insuring their continued use (1889).

TRI-STATE WATER COMMISSION

A compact among the States of Minnesota, North Dakota and South Dakota created the Tri-State Water Commission. Although Congress approved the Compact in 1938, the Commission has been inactive since 1953 and it is doubtful that it will function again as an organized group (1890).

WASHINGTON METROPOLITAN REGIONAL SANITARY BOARD

The Washington Metropolitan Regional Sanitary Board was organized on 16 July 1959, through efforts initiated by the Water Supply and Pollution Abatement Committee of the Washington Metropolitan Regional Conference, a voluntary organization of elected representatives of jurisdictions in the region. These jurisdictions include, in addition to Washington, D. C., the Counties of Loudoun, Fairfax, Arlington, and Prince William in Virginia, and Montgomery, Charles, and Prince Georges in Maryland (1891).

This voluntary Conference, to which the Board is subordinated, has accepted water-quality objectives (with some minor differences) based on those promulgated by the Interstate Commission on the Potomac River Basin (1892). These objectives appear in Appendix K.

TABLE 3-4

WATER QUALITY OBJECTIVES AND MINIMUM TREATMENT REQUIREMENTS

Water Quality Objectives, Applicable to Receiving Waters, for Salt and Fresh Surface Waters and Underground Waters

Water quality→ water uses ↓	Organisms of the coliform group	Floating, suspended & settleable solids & sludge deposits	Taste- or odor-producing substances	Dissolved oxygen	pH	Toxic, colored, or other deleterious substances	Phenolic compounds	Oil	High temperature wastes	Minimum treatment requirements for domestic sewage
A. WATER SUPPLY, DRINKING, CULINARY & FOOD PROCESSING Without treatment other than simple disinfection and removal of naturally present impurities	Most probable number coliform bacterial content of a representative number of samples should average less than 50 per 100 ml. in any month	None attributable to sewage, industrial wastes or other wastes or which, after reasonable dilution & mixture with receiving waters, interfere with the best use of these waters for the purpose indicated	None attributable to sewage, industrial wastes, or other wastes	Greater than five (5) parts per million except for underground waters	Hydrogen ion concentration expressed as pH should be maintained between 6.5 and 8.5	None alone or in combination with other substances or wastes in sufficient amounts or of such nature as to make receiving water unsafe or unsuitable for use indicated (U.S.P.H.S. Stds.)	Less than five (5) parts per billion	None	Not in sufficient quantities alone or in combination with other wastes to interfere with the use indicated	Sedimentation and effective disinfection
B. WATER SUPPLY, DRINKING, CULINARY & FOOD PROCESSING With treatment equal to coagulation, sedimentation, filtration, disinfection and any additional treatment necessary for removing naturally present impurities	M.P.N. coliform bacterial content when associated with domestic sewage of a representative number of samples should average less than 2000 per 100 ml. and should not exceed this number in more than 20 per cent of samples examined in any month	Same as for use "A" above	None attributable to sewage, industrial wastes, or other wastes which, after reasonable dilution & mixture, will increase the threshold odor number above eight (8)	Greater than five (5) parts per million except for underground waters	Same as for use "A" above	Same as for use "A" above	Less than five (5) parts per billion	None alone or in combination with other substances or wastes as to make receiving water unfit or unsafe for the use indicated	Same as for use "A" above	Sedimentation and effective disinfection
C. BATHING, SWIMMING AND RECREATION Note: When waters are used for recreational purposes such as fishing & boating, exclusive of bathing & swimming, the number "1000" may be substituted for "240" in statement of coliform objective	Coliform bacterial content of a representative number of samples should average less than 240 per 100 ml. and should not exceed this number in more than 20 per cent of samples examined when associated with domestic sewage (see note under "C" at left)	Same as for use "A" above	None attributable to sewage, industrial wastes, or other wastes which, after reasonable dilution & mixture, will interfere with the best use of these waters for the purpose indicated	Greater than five (5) parts per million	Same as for Use "A" above	Same as for Use "A" above	Less than 25 parts per billion or none in sufficient amounts such as to impart a residual taste to recreational or commercial fish, shellfish, or other aquatic forms	Same as for use "B" above	Same as for use "A" above	Sedimentation and effective disinfection
D. GROWTH & PROPAGATION OF FISH, SHELLFISH & OTHER AQUATIC LIFE	Coliform bacterial content of a representative number of samples should not have a median concentration greater than 70 per 100 ml. in waters used for the growth & propagation of shellfish	Same as for use "A" above	None attributable to sewage, industrial wastes, or other wastes which will interfere with the marketability or propagation of recreational or commercial fish, shellfish, or other edible aquatic forms	Greater than six (6) parts per million	Same as for use "A" above	None alone or in combination with other substances or wastes to sufficient amount or of such character as to make receiving waters unsafe or unsuitable for use indicated	Same as for use "C" above	Same as for use "B" above	None in sufficient quantity as to be injurious to or interfere with the normal propagation of fish, shellfish, or other aquatic life	Sedimentation for all uses under this group but disinfection required in addition only if discharged into waters used for the growth & propagation of shellfish, either commercial or recreational
E. AGRICULTURAL AND INDUSTRIAL WATER SUPPLY Without treatment except for the removal of natural impurities to meet special quality requirements, other than those classified under "A" above. Note: For agricultural water supply, salinity and sodium hazards are determined by electrical conductivity (EC × 10⁶) and sodium adsorption ratio (SAR). Waters high in both salinity and sodium are generally unsuitable for irrigation purposes. (See "classification and use of irrigation waters", circular No. 969, U.S. Department of Agriculture, November, 1955)		Same as for use "A" above	None attributable to sewage, industrial wastes, or other wastes which will adversely affect the marketability of agricultural or industrial produce	Greater than three (3) parts per million	Hydrogen ion concentration expressed as pH should be maintained between 6.0 & 9.5	Same as for use "A" above	None in sufficient quantity as to make receiving water unsuitable for use indicated	Same as for use "B" above	Same as for use "A" above	Sedimentation and effective disinfection

CHAPTER IV

JUDICIAL EXPRESSION

By PAUL R. FREEMAN, B.A., LI.B.*

The object of this chapter is to marshal the judicial decisions that illustrate the principles adopted by the courts in the application and evaluation of water-quality criteria to the solution of legal problems in the field of water pollution. A brief resume of the research techniques followed may be of some value to the lay reader in understanding how legal research is accomplished.

Broadly speaking, there are two general avenues of examination employed in searching for the law on any particular subject. One is to search in indices, digests, treatises, etc., for the subject matter under its topical arrangement. In some instances, it is the only approach that can be used. Every lawyer has a working familiarity with the main topics, classifications, or titles of law that are found in legal encyclopedias, digests, compilations, and texts. He needs no assistance to help him find general information on Contracts, Criminal Law, Vendor and Purchaser, Corporations, Evidence, Code Pleading, Fish and Game, etc.; but very often he is in a field that may be related to or discussed in many categories of the same legal topic. Under such circumstances, the second method, that of examining the cases in the subject field, may furnish a better means for beginning a search. Such a method may not be available in all situations. Yet, it has the peculiar advantage that one need not stop to consider what classification or topic to examine. Its use is limited to instances where one can start with some particular decision bearing on the question he wishes to investigate. Then, by citation reference, he can go directly to the volumes and pages of all the reports where that case has been cited and find everything that the courts have said about it. The later cases will tell whether the case in question has been approved, disapproved, limited, distinguished, or in any way weakened or overruled. Very often by this process one can quickly gather all the legal authorities needed. Then, by following these authorities through the application of the principle of the case, he can find the very decision sought. The fountainhead of the law is the decision of the courts as contained in the reported cases. Even the effects of the statutory rules of law is dependent on judicial decisions. Judicial decisions do two things: (a) they declare the law on points not covered by the statute, and (b) they determine by judicial construction of statutes, rules, regulations, and administrative findings their ultimate effect.

In this study the textbooks, digests, and legal encyclopedias were used not so much as a source of information in themselves, but rather as a means of finding the decisions in which water pollution was a facet of the case. It is impossible in a review of this general character to go into detail as to the precise method employed in searching for the cases in which there was judicial expression relative to criteria for determining water quality. Suffice it to say, however, that an exhaustive search was made not only of the most likely topics or subdivision of topics where cases dealing with water pollution were cited, but in every place where there was a possibility of a case in point having been digested or referenced, and where there was a possibility that a cross reference might open a new avenue of research. Thus, for example, many cases that were examined came to the attention of the writer under such diverse headings and classifications as Actions, Appeals, Courts, Costs, Compromise, Criminal Law, Damages, Drains, Eminent Domain, Equity, Evidence, Fish, Parties, Navigable Waters, Trial, Towns, Barters, Pleadings, Pollution, Negligence and Nuisance, Health, Injunction, Limitation of Action, Master and Servant, Mines, Landlord and Tenant, Judgments, Municipal, Corporation, Waters, Watercourse, and Witness.

While this review of legal decisions is concerned primarily with those cases in which the courts have considered water-quality criteria, keeping in mind the ultimate objective of determining the validity of such criteria through judicial acceptance, it is nevertheless necessary and appropriate to include briefly a summarization of the legal principles that have been developed in water-pollution cases even though the quality of the water per se was not the issue in the case.

This summarization becomes apparent in view of the fact that there has been no significant case discovered to date in which specific water-quality criteria have been discussed from the purely scientific approach. While the criteria and standards promulgated have been discussed, as will be seen, their reasonableness or scientific accuracy as such has never alone been determinative of the legal issues involved in the particular case. It is indeed a rare situation when the litigation concerns itself only with the quality of the water, since so many times the polluted condition of the water is presumed to exist from the extrinsic facts and the question for decision is solely whether the condition imposes a remediable situation in one party and liability in the other.

It is recognized, moreover, that the acuteness of the water-pollution problem for any given area may be measured in terms of the availability of water, and that the attitudes of courts and juries must certainly be influenced by and reflect the abundance or paucity of the supply. Thus, subconsciously, standards for decisions that serve as guides for the judge or jury are formulated, based on the existence or non-existence of certain rudimentary factors, and these influences are translated into decisions in the tribunal. A brief review of the cases in which such extrinsic factors have indirectly affected the outcome of a water-pollution case may aid

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in a clearer understanding of the rules being sought and will help possibly in reconciling the opposing positions that courts in different jurisdictions have sometimes taken.

Despite the examination of well over 1,200 cases dealing with some aspect of water pollution, this writer is not presumptuous enough to imply he has found every case that contained some expression of the court relative to water-quality criteria or that all such cases have been reported or cited herein. The principles developed by the cases are comparatively few but their applications are very numerous. The manner in which these principles are applied can only be learned through an examination and comparison of judicial decisions. It is the aim of this chapter to bring these principles into focus, thereby aiding the reader in understanding some of the principles that will be applied by the courts. Also, thereby, he will be provided with some foreknowledge of the questions with which he may have to deal and methods needed to assist the courts in finding the true rule of the case.

INDIRECT NON-TECHNICAL CRITERIA

Sections 1831 and 1832, California Code of Civil Procedure, state that direct evidence proves a fact without inference, while indirect evidence, usually termed circumstantial evidence, tends to establish one fact by proving another. People v. Goldstein (1956) 139 C.A. (2d) 146, 293 Pac. (2d) 495 contains an excellent delineation of the difference between the two.

> "The terms 'indirect evidence' and 'circumstantial evidence' are interchangeable and synonymous (18 Cal. Jur. 2d 434, Sec. 12). Direct evidence is that which is applied to the fact to be proved, immediately and directly, and without the aid of any intervening fact or process: as where, on a trial for murder, a witness positively testifies he saw the accused inflict the mortal wound, or administer the poison. Circumstantial evidence is that which is applied to the principal fact, indirectly, or through the medium of other facts, from which the principal fact is inferred. The characteristics of circumstantial evidence, as distinguished from that which is direct, are, first, the existence and presentation of one or more evidentiary facts; and, second, a process of inference, by which these facts are so connected with the fact sought, as to tend to produce a persuasion of its truth (Burrill on Circumstantial Evidence, 4, 5). An inference is a conclusion as to the existence of a material fact that a trier of fact may properly draw from the existence of certain primary facts (Blank v. Coffin, 20 Cal. (2d) 457, 126 Pac. (2d) 868). Inferences drawn from physical facts amount to circumstantial evidence (McCready v. Atlantic Coast Line R. Co., 212 S.C. 449, 48 S.E. (2d) 193). It has been said that circumstantial evidence, as distinguished from direct evidence, is testimony not based on actual personal knowledge or observation of the facts in controversy, but of other facts from which deductions are drawn, showing indirectly the facts sought to be proved (Aday v. State, 28 Okla. Crim. 201, 230 Pac. 280)."

In the preponderance of cases dealing with water pollution, the existence of a polluted body of water is established not by the introduction of direct evidence as to its quality as determined by actual testing of the chemical quality of the water but rather by the introduction of circumstantial evidence from which the inference is made that the water must be polluted. In several cases, this circumstantial evidence relates to the effect of the water upon fish, livestock, or even humans. It might be reasoned, therefore, that such evidence represents a form of bio-assay, albeit not standardized.

In Albough v. Mt. Shasta Power Corporation (1937) 9 Cal. (2d) 751, 73 Pac. (2d) 217, the circumstantial evidence of the growth of weeds, the foul odor that emanated from a pool, and the preference of cattle and horses for other bodies of water were sufficient to cause the jury to conclude that the water was in fact polluted. This decision was reached despite the contrary evidence of chemical analyses:

> "It is true that appellant introduced more evidence and new theories on the present trials on the issue of pollution than had been presented by it on the prior trials. Two chemists were produced who testified that from a chemical analysis the water in the pool was fit to drink. Scores of photographs showing horses and cattle apparently drinking from the pool after the diversion were introduced. This, and other evidence introduced by appellant on this issue, did nothing more than create a conflict in the evidence, which was resolved by the jury contrary to appellant's contentions. There can be no doubt, in fact, appellant admits, that the evidence thoroughly establishes that since the diversion there has been a marked increase in the weed growth (and that) in the summer months the pool has been covered with weeds, slime, and moss.
>
> "Appellant contends, however, that these conditions were due not to the diversion but to many years of subnormal run-off of Pit River. This argument is necessarily predicted on appellant's contention, already refuted, that before the diversion the Fall River water did not mingle with the Pit River water in the pool. The argument totally ignores the permissible inference that the Fall River water freshened the pool. Considerable credible evidence was introduced that since the diversion foul odor has emanated from the pool. Various witnesses for respondents testified as to the preference of cattle and horses for other fresh and nonstagnant water. Several witnesses living on the pool testified that in the years since the diversion they have never seen cattle drink from the pool. As already held on the prior appeals, the juries were amply justified in inferring that these conditions were caused by the diversion and the construction of the dam, and that such changed conditions caused a material depreciation in the market value of respondents' lands . . ."

In Knopp v. State (1936) 59 Okla. Cr. 143, 56 Pac. (2d) 1193, the defendant was convicted of contaminating a stream by placing, allowing, and causing to be run into its waters, oil, cement cuttings, slush, and waste from an oil well, and was sentenced to pay a fine of $200. In upholding the lower court, the Appellate Court stated:

> "There is evidence that the slush pits of this well were permitted to overflow and run into the stream,

and that it became murky and muddy from such contamination; that sediment settled on the stones in the creek; that numerous fish could be seen in the creek before this, and none after. This was ample proof of pollution of the stream and the judgment is fully sustained by the evidence . . . "

In Conley et al. v. Amalgamated Sugar Co. (1953) 74 Idaho 416, 263 Pac. (2d) 705, an action by grocers against a sugar-beet factory to recover damages to a grocery business and building allegedly caused by the offensive odor of a creek polluted by the factory, the circumstantial evidence was sufficient to sustain the trial court's finding that the grocers' loss was due to the odor of beet pulp. This finding was sustained despite the introduction of expert testimony to the contrary.

" . . . Apellant countered respondents' evidence with expert and lay testimony and documentary evidence that no odor of beet pulp persisted or could persist down the stream from where the waste products of appellant's plant entered into Indian Creek, as far as respondents' store, and if odors emanated from the Creek, they were from other sources entirely disconnected from appellant's business. Also, that the amount of offensive effluent from appellant's plant was so small in proportion to the regular flow of the Creek, sometimes one to 200 cubic feet per second, it was so diluted that all offending odors therefrom were dissipated before reaching respondents' place of business. All this evidence merely created a conflict, resolved by the trial court against appellant . . . "

Eckart v. City of Belleville (1938) 294 Ill. App. 144, 13 N.E. (2d) 641 involved an action brought by a farmer to enjoin the city from polluting a creek adjacent to his farm by discharging raw and contaminated sewage therein. The finding of a polluted condition was predicated on the existence of tubifex worms in the water and on the foul odor that emanated from the creek. The court stated:

" . . . In November of the same year he (referring to a sanitary engineer who had testified at the trial) made another inspection and found that a quarter of a mile south of septic tank No. 1, the creek was full of suspended matter, heavy sludge deposits, and foul odors, and that for a distance of approximately twenty miles south of the tank, the water in the creek was dark and the stream was lined with tubifex worms. Tubifex worms live only in polluted streams. Samples of the water taken from the creek on this inspection were present and exhibited at the trial of this case.

"Much contradictory testimony appears in this record, and plaintiffs and their witnesses testifying to the above foregoing conditions and the terrible effects thereof on their homes and their comforts, and also to the effect upon their livestock; the defendant's witnesses denying that such conditions exist.

"The defendant city has brought the record here on appeal, assigning many errors, three only of which are argued. The first is that the finding and judgment of the court is against the manifest weight of the evidence. A reference to this record at once meets this objection. The evidence shows not only that the court was warranted in the action that it took, but that the conditions existing in the premises described are most shocking. The least that can be said is that the testimony raises a question of fact for the court who heard the witnesses and saw them testify. A striking incident appeared in this trial as it is described by the trial court; that is, that one of the witnesses for the defendant smelled and drank some of this water in court. The court in its opinion says that this is true, but that this witness did not impress the court either with his intelligence or his integrity. This but illustrates the wisdom of the rule that the tribunal which first hears the facts and hears them from the witnesses themselves is in a better position to judge of the credit due such witnesses than is an appellate tribunal which is only concerned with the record. . ."

A rather humorous instance of testing by taste is related in the decision in Town of Smithfield v. City of Raleigh (1935) 207 No. Car. 597, 178 S.E. 114, in which the court stated:

". . . Indeed, it seems that the trial judge subjected the question to 'trial by water,' because the record discloses that his Honor 'had drunk of the water, bathed in it, and suffered no ill effects.'' The ancient mode of trial by water was aforetime deemed efficacious in determining the guilt or innocence of witches and by applying the practice of the ancient law the distinguished jurist has found the waters of the Leuse River not guilty. . ."

In Bumbarger v. Walker (1958) 393 Pa. 143, 142 A. (2d) 171, (Supreme Court of Pennsylvania—May 26, 1958, Rehearing denied June 20, 1958), an action was brought by a lower landowner against persons purportedly in charge of an open-pit mining operation on upper land for damages for contamination of plaintiff's spring. The Court of Common Pleas entered judgment for plaintiff against one of the defendants and he appealed. The Supreme Court held that the evidence presented a question for the jury as to whether contamination of plaintiff's spring was due to defendant's action in discharging drainage waters accompanying his mining operations onto plaintiff's land, or to some other cause for which defendant was not responsible. The judgment was affirmed for the plaintiff, the court stating:

". . . The plaintiff, Harvey Bumbarger, owns a farm in Graham Township, Clearfield County, which, until 1955, was enriched with a spring which supplied all his domestic needs with pure potable water but which, by October of that year became wholly useless because of certain chemical infiltrations which rendered it unfit for drinking, cooking, or cleansing purposes. The plaintiff's wife testified that, after the chemical invasion, the water made a precarious bathing agent because, 'it would burn the skin off you almost.' Allowing her a little latitude for over-zealous exaggeration, there would still seem to be no doubt from the record that the water underwent a drastic transformation. Whereas originally it had a 'good taste,' it now became sour; whereas, before it was odorless, it was now offensive in smell; whereas theretofore it flowed easily and innocuously through pipes

and fixtures, it now corroded and otherwise damaged its copper, brass and lead containers.

"As no change in nature's manifestations occurs without the intervention of some force, put into effect either by man or cosmic energy, there had to be a reason for the deterioration in Bumbarger's spring. Bumbarger contended that this deterioration, which made it necessary for him to obtain water elsewhere at considerable expense and inconvenience, was due to the discharging into his spring of drainage from an open pit mining operation on an adjoining property which was owned by one Albert Smith. He brought suit against Ray S. Walker and Robert Bailey, purportedly in charge of the strip mining in question. The jury returned a verdict in favor of Bumbarger in the sum of $10,000 against Ray S. Walker, but exonerated Robert Bailey from any liability in the premises.

"The defendant maintains that even if, arguendo, Bumbarger's spring became unusable, it was not proved conclusively that he was responsible for the resulting damage since there were seven other mining operations in the area: four deep mines and three open pit mines. Walker maintains in this connection that the jury was allowed to guess as to which mine was responsible for the befouling muck which penetrated Bumbarger's spring. But this argument is refuted by the size and content of the record which covers 450 printed pages. Landowners, chemists, civil engineers, sanitary engineers, coal operators, pathologists, coal analysts and farmers testified to every possible phrase of the controversy and it was for the jury to decide whether the plaintiff met the burden of proof of showing that the worm of corruption and contamination in the spring, which at one time contributed to life, health, and cleanliness on the Bumbarger farm but which now generated only the germs of decomposition and decay, got into the spring through the conduit originating in the mine workings on the Smith lands.

"The jury's verdict here established that the damage was not necessary and was avoidable, and that it was sufficiently obvious to have been foreseen and preventable by reasonable care and expenditure. Hence, any further discussion of the case would be like digging a channel after the river has reached the sea. Judgment affirmed."

In Klassen v. Central Kansas Coop. Creamery Association of Hillsboro (1946) 160 Kan. 697, 165 Pac. (2d) 601, an action was brought against a creamery association that permitted waste products to escape from its plants and injure a farm tenant by polluting a stream and underground water supply. In disposing of the defense that no evidence of pollution had been introduced, the court stated:

"Hogs and chickens do not die from drinking water unless there is something the matter with it. It is not necessary that there be a chemical analysis as to whether the water was polluted to make a case go to the jury. There was ample evidence that the water in the stream was polluted. . . What better proof could there be than the fact that the stock had not died before the pollution came even though they drank water from these wells, that they died after the pollution came, and that after the rest of the stock began to use water from a different source the losses stopped."

The case of Cartwright v. Stanolind Oil & Gas Company (1948) 200 Okla. 633, 198 Pac. (2d) 737 aptly states the rule of circumstantial evidence:

"The plaintiffs, suing for damages to their land and personalty allegedly resulting from oil and other deleterious substances which defendant permitted to escape from defendant's adjoining land and flow over plaintiffs land, had the burden of proving that the oil and other substances were of such nature and character as to cause damage, and also had the burden of proving, in absence of proof that oil which escaped was crude, that the oil was deleterious, and such burden could be sustained by proving effect of oil, and the plaintiffs were not required to produce direct evidence of identity or qualities of the oil and other substances. . .

"In support of the assignment of the insufficiency of the evidence to sustain the judgment, it is urged that it was incumbent upon the plaintiffs to prove that the oil as well as any other substances included in the allegation were of such nature and character as to cause the resulting damage. Also, that there is no evidence of any substance escaping other than the oil floating upon the water and that no chemical analysis was made to show that the oil so floating was poisonous. There is cited 29 O.S. 1941 273, inhibiting the escape of 'crude oil or other deleterious substance.' It is urged that the statutory recognition of the deleterious character of oil applies only to crude oil and that, since there is no proof that the oil which escaped was crude oil, it was incumbent upon plaintiffs to prove that the oil was deleterious and that, independently thereof, there is no foundation for the jury's verdict other than conjecture.

"It is true that such burden rests upon the plaintiffs. But it does not necessarily follow it can be sustained only by direct evidence of the identity or qualities of the substance. Such is true because a cause may be proven by the effect thereof. Such additional method of proof is recognized in Prest-O-Lite Co. v. Howery, 169 Okla. 408, 37 Pac. (2d) 303, as follows: 'If it had been proved that at the time the injuries were incurred there were poisonous or deleterious substances in the water, harmful to animal life, or if it had been proved that the animals and fowls died as a result of drinking the water, a different situation would prevail, but the failure to prove one of these circumstances is fatal to plaintiff's right of recovery.'"

In Rusch v. Phillips Petroleum Co. (1947) 163 Kan. 11, 180 Pac. (2d) 270, an action was brought against certain oil companies and others for damages caused by pollution of water. Proof of chemical analysis was not necessary where other evidence showed that water was injurious to livestock. The court stated:

"Appellants contend there was no evidence of a chemical examination of the water in 1942. That is

true. It was not imperative that there should be proof of pollution by chemical analysis (Klassen v. Creamery Co., 160 Kan. 697, 165 Pac. (2d) 601). There was evidence the water was tasted and found to be salty in 1942 and that the stock would not drink it; that the stock drank some of the polluted water in 1943 and lost stated amounts of weight in 1942 and 1943."

The case quoted Donley v. Amerada Petroleum Corp. (1945) 152 Kan. 518, 106 Pac. (2d) 652:

"It may, also, be noted, that appellees were not obliged to exclude every possible source of pollution after establishing facts from which it reasonably could be inferred that appellants had polluted the stream. The fact that appellants polluted the stream could, of course, be shown by circumstantial evidence."

In Divelbiss v. Phillips Petroleum Company (1954) 272 S.W. (2d) 839, a Missouri case arising out of the poisoning of plaintiff's cows by drinking water allegedly polluted by the defendant oil company, the existence of pollution was determined in an unusual manner:

"On November 18, 1950, Mr. Divelbiss, Sr., and five of his neighbors, who were also farmers, went to plaintiffs' pasture. They saw dead frogs and minnows in the branch. They dipped water from the branch at several locations. Part of the sample taken was poured out on the ground and ignited. Mr. Divelbiss and these neighbors testified that they traced the oil and gasoline to a pipe coming out from defendant's pumping station and emptying into an open ditch, which connected with the branch running across plaintiffs' pasture."

In Hillhouse v. City of Aurora (1958) 316 S.W. Pac. (2d) 883, an action by a landowner against a Missouri city for pollution of a stream because of the discharge of effluent from the city's sewerage system, the quality of the water was determined from the following testimony of plaintiff's witness:

". . . that about 1946-47 the water in the creek began to darken, a foul odor first became apparent and fish disappeared from the stream; and that thereafter the water gradually darkened in color until it became black, grayish, slimy looking, and the odor gradually became more pungent and offensive until it was similar to a toilet . . ."

Stekoll v. Wilson (1952) 207 Okla. 456, 250 Pac. (2d) 454 was an action for injuries to cattle allegedly caused by drinking salt water produced by the defendants' oil well and permitted to run into the plaintiff's creek. The court stated:

"Defendants urge two propositions for reversal. The first is that the court permitted incompetent and irrelevant evidence to be admitted; and second, that the trial court erred in overruling the defendant's demurrer to the evidence and motion for judgment. As to defendants' first proposition, plaintiff's Exhibits 1 and 2, two three-ounce bottles of water, were admitted; these bottles had labels on them showing their salt content. Plaintiff's Exhibit 3 was a letter from the Oklahoma State Health Department Bureau of Laboratories to A. S. Booten, a veterinarian in Okemah, signed by H. E. Maxey, Chemist, but unauthenticated in any way, which gave the chemical analysis of the contents of the two three-ounce bottles. In our opinion it was error for the trial court to admit these exhibits without testimony by the chemist, but we think that this error was harmless in view of the other evidence offered by the plaintiff, which in our opinion was sufficient to authorize the trial court to overrule a demurrer to the evidence. It was unquestioned that the well of the defendant Stekoll produced a little more than ten gallons of salt water every second. The plaintiff's evidence showed that this salt water seeped into the only stream of water on plaintiff's land from which the cattle could drink, and also that in times of high water large quantities of salt water flowed into this stream. There was also testimony by a veterinarian that the cattle of the plaintiff had sustained injuries and that the injuries were the result of drinking salt water, and other witnesses testified that the only salt water available came from the well of defendant Stekoll and that defendant Stekoll had not used proper methods to prevent the salt water from escaping."

In two Oklahoma cases, the court was unwilling to accept the circumstantial evidence as decisive and ruled in favor of the defendants:

"Defendants contend that there is no evidence whatever tending to show pollution of the 'north well.' In this connection plaintiff testified that his hogs and chickens drank the water from that well and a number of them were killed and others were injured. There was other evidence to the effect that the water from the well had a 'queer' taste. It also appeared that a bottle of the water was introduced in evidence and examined by the jury but no evidence was introduced by the plaintiff showing an analysis of the water; therefore, neither the jury nor this court was informed as to the nature of the claimed pollution of the water. An examination of all the evidence on this point, together with all the conclusions reasonably deducible therefrom, fails to disclose any evidence to the effect that the north well was in fact polluted. What we have said in discussing the question of causal connection in relation to the south well is likewise applicable here. In this connection the plaintiff points out the proximity of a pond in the vicinity of the north well but it does not appear that a pond contained polluted water or that any pollutive substance escaped therefrom into the fresh water strata which supplied plaintiff's well." Shell Petroleum Corporation v. Blubaugh (1940) 187 Okla. 198, 102 Pac. (2d) 163.

"The . . . conclusion of the witnesses that the salt water destroyed the fish and timber was based entirely on the assumption that the salt content was sufficient to bring about that result. That assumption was wholly without foundation in actual experience of the witnesses, or knowledge of the salt content of the water, and without the aid of visible effects peculiarly associated with salt water damage that would in some acceptable degree distinguish the asserted cause of destruction from any number of other possible causes.

"The mere fact that water tasted salty will not support an inference of evidential verity that dead fish found in such water, and dead trees near by were destroyed as a result of the salt content of the water. Nor will the courts take judicial notice that such result will follow. Shell Petroleum Corp. v. Worley 185 Okla. 265, 91 Pac. (2d) 679, 680."

In Tyrrell-Combest Realty Co. v. Adams (1927) Tex. Civ. 291 S.W. 252, the Texas court rejected the theory of circumstantial evidence as determinative of the condition of the water. In its decision the court stated:

"Whether water is contaminated with impurities, or contains ingredients rendering it hurtful, and maybe dangerous to the human system, and unfit for domestic use, if used continuously, is a matter for scientific investigation and analysis, and cannot be correctly determined other than by such scientific investigations as will disclose its elements and their effect when used for the purposes in question or by such continued use as will demonstrate its safe and suitable adaptability to same, or the contrary."

SCIENTIFIC EVIDENCE

Scientific evidence is a non-technical term sometimes used to refer to evidence produced by the use of expert scientific techniques and experimental methods. A considerable body of case law has been built up in which there has been expressed approval of certain techniques, criticism of others, and expression of a need for a more extensive reliance on such evidence.

Evidence of experiments and scientific tests is usually produced and offered in court by an expert witness. The following cases are illustrative of the inclination of the courts to accept scientific testimony as controlling the outcome of the litigation:

Commonwealth of Kentucky v. G. & H. Cattle Co. (1950) 312 Ky. 314, 227 S.W. (2d) 420:

"The testimony of Drs. Gibbs and Lyen that in their opinion the effluent entering the river from the drain would pollute the water three miles down stream and after flowing over two dams was not incompetent, but it had no probative value in view of the three chemical analyses of the water just mentioned, all of which show there was no pollution of the water. This case is much like City of Durham v. Eno Cotton Mills, 141 N.C. 615, 54 S.E. 453, 7 L.R.A., N.S., 321, where the court said, on page 328, that it would have been an easy matter for the city to have shown by a chemical or bacteriological analysis of the water at the intake whether or not it had been polluted by sewage and waste material deposited in the stream at defendant's mill. Instead of so doing, the city contented itself with introducing several physicians and two laymen who gave their opinions that the water was polluted at the intake, just as the Commonwealth did in the case at bar, and the North Carolina Court said such testimony was of little value where the fact in dispute could have been ascertained accurately by a scientific analysis of the water."

Hoke v. Sun Oil Co. (1946) 197 Okla. 261, 169 Pac. (2d) 753, an action for injury to livestock caused by drinking polluted water in which the scientific evidence of the pollution was damaging to the defendant:

"A sample of the water which drained into plaintiff's fresh water pond was taken at a point on the drain several feet from its entrance into the lake. This sample was submitted to Dr. V. G. Heller, chemist at the A & M College at Stillwater, and his report of its analysis was made by letter to the plaintiff in the following language:

" 'A partial analysis of the sample of water submitted for you by Judge Simcoe reveals that the water is slightly alkaline having a pH of 7.4. The total amount of soluble solids present amounts to 59,858 p.p.m. The chloride content of the soluble material calculated as sodium chloride amounts to 55,600 p.p.m. A considerable part of this chloride is present as calcium chloride.'

"On cross examination with reference to this report of his analysis the following questions and answers appear:

" 'Q. Well, you testified on direct examination did you not that some of this water was harmful to cattle? A. Yes, sir.

" 'Q. Why did you conclude it was harmful to cattle? A. Our experiments show if a cow would drink a water such as this containing 58,000 p.p.m., it would kill her, undoubtedly.' "

Nash & Windfohr v. Edens (1937) Tex. Civ. App. 109 S.W. (2d) 496:

"An action for damages caused by alleged seepage of salt water from stream used by plaintiff for irrigation purposes and allegedly polluted by defendants, evidence respecting alleged seepage of salt water through the soil, destroying plaintiff's pecan trees, without any showing as to salt content of water at any time, was insufficient to authorize recovery.

". . . Furthermore, we desire to say that we do not believe the evidence introduced for the purpose of establishing the fact that salt water in this creek seeped through the soil and soaked into appellee's lands and killed his pecan trees is sufficient to raise such an issue. No effort was made to show that the salt water overflowed the banks of the stream and covered the soil over the roots of these trees. And no effort was made to show the salt content in the water at any time, and we are of opinion that under such circumstances a mere layman, such as is appellee, cannot give, as his opinion, a statement that salty water flowing along or standing in a stream actually soaks into the soil for a distance of from 10 to 40 feet in sufficient quantities to kill a pecan tree, and thereby raise an issue of fact as to the death of such pecan trees from the salt water . . ."

In a recent Oklahoma case, Burge v. Sunray Oil Corporation (1954) 269 Pac. (2d) 782, livestock raisers brought an action against a manufacturer of petroleum products to recover for damages to land and livestock because of the pollution of a stream with allegedly poisonous chemicals. The District Court entered judgment adverse to the manufacturer, and the manufacturer ap-

pealed. The Supreme Court held that the evidence was insufficient to sustain a verdict for the livestock raisers. The Court relied heavily and based its judgment primarily on the scientific evidence.

"Although plaintiffs allege that defendant in the operation of its refinery used chemicals and substances that were harmful to animals, which substances defendant permitted to drain into Cow Creek, plaintiffs offered no evidence to support this allegation. On the contrary, the proof is without contradiction that the refinery maintained pits for impounding its effluent water so that by a process of oxidation the sulfides and sulfates would become free sulphur, which in the latter state, when commingled with the flowing water in Cow and Beaver Creeks, would not be injurious to livestock. Furthermore, the evidence discloses that the bad odor of the streams was attributable to organic matter in the raw sewage, and not from any chemical content in the water. The evidence is further without contradiction that enormous amounts of oil field brine from producing wells within the watershed below defendant's refinery contaminated the waters in these two streams, as has been indicated by reference to the evidence, supra.

"Although the witnesses Crawford and Sparks, the Game Rangers, had taken samples of water coming from the defendant's refinery as it entered Cow Creek, plaintiffs made no proof of the chemical contents thereof.

"The Chief Chemist of the defendant, as well as Dr. Heller, of A & M College, discloses that various samples of water taken from Cow Creek below the refinery, had substantial quantities of sodium chloride (common salt) but did not contain any other chemicals in sufficient quantities to be harmful or injurious or toxic to livestock.

"If the evidence thus produced by plaintiffs may be said to raise an inference or presumption that the defendant's effluent waters resulted in harmful pollution of the streams, then with like force it may be said the evidence that raw sewage in the streams also raises an inference or presumption that organic matter was the cause of plaintiffs' complaint. Likewise, the evidence with equal force presents an inference or presumption that the pollution of the streams was occasioned by oil producing wells making salt brine which drained into the streams.

"The fact that defendant's effluent water drained into Cow Creek, standing alone, does not create an inference that the water contained poisonous elements injurious to livestock. In view of the further evidence that defendant impounded its effluent water in pits until the sulfides and sulfates therein by a process of oxidation became free sulphur, wholly fails to sustain plaintiffs' contention that the refinery water resulted in the injury to plaintiffs' livestock when it passed through the stream on plaintiffs' farm twenty-five miles below the refinery. Furthermore, the evidence of the chemist stands uncontradicted that the water coming from the defendant's refinery did not contain any chemical properties injurious to livestock. Furthermore, plaintiffs' own evidence is to the effect that they lost one cow in 1949 from Bangs disease, and that cattle abort from many causes other than the drinking of polluted water.

"Where evidence fails to establish with reasonable certainty that damage resulted from some act of commission or omission by a defendant so charged, or that such acts resulted in the damage or injury complained of, a verdict and judgment thereon is not sustained by sufficient evidence and is therefore contrary to law."

In Magnolia Petroleum Company v. Glen A. Stinson (1957) 230 Miss. 533, 93 So. (2d) 815, an action against an upstream oil-well operator for damage to sheep, cattle, and pasture from the alleged pollution of a creek, the court relied completely on expert testimony as to the quality of the water.

"O. U. Walling, chemist at State College, testified that he received a bottle containing a liquid content from Doctor Peters on April 28, 1954. The breakdown of his analysis was as follows:

" 'Well, I found it to be essentially a solution of sodium dihydrogen phosphate. I found 24.20% sodium dihydrogen phosphate in the water. That was based on the determination of the phosphate. The pH of the water was 5.2, which is indicative of a slightly acidic condition, and it had a specific gravity of approximately 1.223 which, of course, would indicate quite a bit of dissolved substances in it. I found phosphorus present and when expressing the (phosphorus) as P_2O_5 it analyzes 14.32% and (as) sodium dihydrogen phosphate it becomes 24.20%.'

"When asked if the chemicals, shown by his analysis, were the chemicals used in drilling muds, he said that he could not be certain, but 'I know that a compound of salt is used to bring up the gravity of the mud. Whether this is always used, or commonly used, I could not say.' He said that the specific gravity of the water was 1.223 and was indicative of a high concentration in the water. He said that most any compound of salt in excessive amounts is toxic. When asked if the contents, which he found, would be poisonous to sheep, he did not wish to pose as an expert on that question. However, when counsel for the defendant asked if he would say that this was in excessive amounts, he replied, 'In my opinion, it is.'

" . . . 'Q. Now doctor, if the drinking water in a stream contained a pH of 5.2, indicative of a slightly acid condition, and had a specific gravity of approximately 1.223, which was indicative that there was quite a bit of dissolved substance in the water, and the phosphorus as P_2O_5, which analyzed, is 14.32, of (sodium) dihydrogen phosphate, it becomes 24.20% if consumed by sheep, in your opinion, would it be fatal in the event that the sheep drank this and later died? A. That, of course, would depend on the amount that they drank. Ruminants ordinarily drink great volumes of water. I should think yes, it's perfectly capable of causing death. Q. Assume that there were approximately one hundred sheep in the herd, heavy burdened with lambs, which were almost ready to be born, and they consumed the same contents and the same water, and later all aborted, now do you have an opinion as to whether the water they drank containing the sub-

stances that I have set out, would be the cause of their aborting? A. In my opinion, it certainly would be, because one of the things that we learned early in medicine, is that acid is abortive. That would be true, it would seem.'

". . . For the defense, William G. Spence, a chemist with the Game and Fish Commission, in charge of water pollution, testified that he checked the water for its acid and alkaline condition on February 22, 1952. At that time it was normal. He said that he made an examination of material in a sack at Stinson's milk barn and found it on the acid side. He examined the location at the McShane No. 1 well and found it sloppy. He saw a couple of sacks containing material that appeared to be caustic. There was a number of empty sacks around the place in the mud. In the pits he found a conglomeration of mud and water. He did not test the pH of the pits. While he did not go around the pits on all sides, he saw that the little pit had sloshed over at the end at the time of his visit. He ran a pH on the sacks that looked to be caustic, and said that whether this substance, washing down stream for half a mile, would have a pH of more than 7 would depend on the amount of the water with which it was mixed and the amount of the substance. He, of course, could not say that the contents of the other sacks had not washed down the stream before his visit."

T. H. McCasland v. Debert Burton (1956) 292 Pac. (2d) 396, an Oklahoma case, involved an action by a land-surface owner against the owners of an oil and gas lease on a portion of land for damages allegedly suffered by the escape of salt water from an oil well on the lease, which water was allegedly drunk by plaintiff's cattle and caused death and abortions of such cattle. The judgment was for the plaintiff.

"The gist of defendants' arguments is that the evidence is insufficient to show by a preponderance thereof, that the salt water which concededly escaped from the salt water pits on their lease was the proximate cause of the death of four of plaintiff's cattle that died and the abortions that seven others suffered. Their counsel say that, in order to sustain the verdict, it must be assumed: First, that the water escaping from the pits contained sufficient amount of salt to be injurious to cattle; and, Second, that if such water was so injurious, the livestock drank sufficient of it to cause the deaths and abortions.

"As to the question involved in the first of the claimed assumptions, the uncontradicted evidence shows that plaintiff, in the company of Dr. Ryan, a veterinarian, took samples of water from the salt water pit on the premises and delivered one of them to Jack Womack, Sanitarian of the Stephens County Health Department, who in turn, mailed it to Mr. A. G. Maxey, Assistant State Chemist of the State Health Department at Oklahoma City, to be analyzed. Mr. Maxey testified that this sample contained 7.09% of 'calculated' salt, or 7.09 pounds of salt to every twelve gallons of water, and that such salt content was excessive for drinking purposes. Dr. Ryan, who was called by plaintiff to examine his cattle, stated that they were definitely sick and emaciated and had diarrhea. He further testified that he believed it had been proven that '3.3 to about 6½ pounds of salt (to 12 gallons of water) is a fatal dose to a cow. . . .' He further testified that a cow drinks 10 to 15 gallons of water per day, and that in his opinion, if a cow should drink as much as 7.09 pounds of salt in 12 gallons of water 'she would die.' He further testified that he was familiar with the symptoms of salt water poisoning in cattle, described what they are, and then answered, in the affirmative, the question: 'Did you see any of Mr. Burton's cows that had any of those symptoms?' "

The necessity for scientific analysis of the suspect water was discussed in Pure Oil Company v. Renton (1952) Sup. Ct. of Okla. 248 Pac. (2d) 580. This was an action by Johnston Renton, and wife, against the Pure Oil Company, for the pollution of a stream. The Superior Court, Seminole County, entered judgment for plaintiffs and defendant appealed. The Supreme Court held that evidence was insufficient to support award of damages that could properly be measured only by depreciation in usable value of realty. The decisive question urged by the defendant is that the evidence was insufficient to support the judgment of the court. This contention was sustained by the Appellate Court.

"While plaintiffs and other witnesses testified that the water in the creek was salt(y) and the evidence showed that a considerable amount of salt water per day was dumped into the creek by defendant, there was no analysis of the water and the only evidence produced by plaintiffs as to its effect on livestock was the testimony of plaintiff, Johnston Renton, that two years before, and while the salt water easement was in effect, two of his cattle had died from drinking salt water, and his further testimony that it would kill his hogs if he . . . (let them have) access to it."

The need for expert testimony and evidence based on scientific data was again expressed in Reynolds Metal Co. v. Ball (1950) Sup. Ct. of Ark. #4-9108, 232 S. W. (2d) 441. Fred H. Ball brought an action against the Reynolds Metal Company to recover damages for injury to his land alleged to have been caused by deposits of sediment containing poisonous substance from defendant's plant. The Circuit Court, Saline County, entered judgment for the plaintiff, and the defendant appealed. The Supreme Court held that the evidence was insufficient to sustain the verdict, but that the circumstances justified remanding the case for a new trial.

". . . Defendant Reynolds denied that any substantial amount of the sediment came from its plant, and also denied that any poisonous or harmful substances whatever were allowed to flow from its plant into the creek at any time. Reynolds introduced the testimony of expert witnesses, chemists who stated that they had analyzed the chemical content of the sediment and found nothing in it that would be poisonous or otherwise harmful to vegetation, apart from the obvious effect of choking the vegetation out by covering it up. Plaintiff introduced no expert evidence as to the chemical content of the sediment. The plaintiff and some of his witnesses gave their opinions that the sediment was poisonous, but these were unscientific

statements of lay opinion, based on observations from which it might also have been concluded that the vegetation was merely choked out. Substantial proof that the sediment included chemical constituents that were poisonous to vegetation was lacking.

"We are forced to conclude that the record does not contain substantial evidence that Reynolds discharged from its plant into the creek deleterious or poisonous substances which caused the damage of which plaintiff complains. The judgment based upon the jury's verdict in the Circuit Court must therefore be reversed."

Dohany v. City of Birmingham et al. (1942) 301 Mich. 30, 2 N.W. (2d) 907, was a suit to enjoin the city from discharging raw sewage into a watercourse crossing the plaintiff's land outside the city. The evidence was held to establish the plaintiff's right to a permanent injunction against the continuance of the nuisance by the city.

"Some six months after the dam and pumping station were put into operation plaintiff obtained samples of the sewage discharged across his land. These samples were taken each week for a period of 12 consecutive weeks during the months of April, May and June. The samples were analyzed and brought into court as evidence. Three different chemists testified that these samples contained all of the usual elements of sewage,—abundant chlorides, hydrogen sulphide, active bacilli coli, ammonia and insoluble solids. At that time human excrement was still being deposited on plaintiff's land. The stench continued and was such that a nearby resident was at times, compelled to keep the windows of his house closed."

REJECTION OF STANDARDS ON EXPERT TESTIMONY

While there have been no significant cases discovered in which the courts have seen fit to reject standards that have been promulgated through appropriate legislative actions, there have been some cases in which the courts have at least indicated that they are not bound by the testimony of the experts. They may, if they see fit, draw their own conclusions where there is a conflict in the testimony, and, influentially, accept non-expert testimony as the rule in the case.

Illustrative of such a situation is the Texas case of Barakis v. American Cyanamid Co. (1958) 161 F. Supp. 25. It was brought by a tenant of land abutting on a river and leased from the county water-control district against an upstream owner discharging industrial wastes into the river. The evidence failed to establish any damages to crops attributed to the effluent from the defendant's plant despite expert testimony. The court stated:

"The preponderance of the evidence does not show that the water flowing by the plaintiff's tract was essentially useless for irrigation. The analysis of the content of the Trinity River at the plaintiff's pump site as introduced in evidence by the plaintiff was approximately 1,500 parts per million of sodium. The testimony of Dr. Gray, plaintiff's expert witness from Midwestern University, was to the effect that this content was not so high as to cause him to condemn the water for irrigation purposes on that basis. He condemned the water because its Soluble Sodium Percentage (S.S.P.) was in excess of 60% and he stated that the standard of 60% was obtained by him from the Handbook published by the Riverside California Experimental Station. However, his testimony did not establish that this was an essential standard by which irrigation waters generally might be judged. The testimony of Dr. Edward Stiber established that water of a much higher sodium content and with a much higher S.S.P. was being used successfully, commercially, in the Rio Grande Valley, and also both the Brazos and Bosque Rivers. Mr. Valentine of the Dalworth Soil Conservation District, a witness for plaintiff, testified that he, in his official capacity, started getting complaints about the irrigation waters of the Trinity River in 1956. For over 12 years the defendant had been discharging its effluent into the River and Mr. Valentine had heard no complaints. His testimony that it was not customary to even test the waters of the Trinity River would indicate that the effluent discharge had not made the waters of the Trinity unsuitable for irrigation.

"The testimony of plaintiff's witness, Leonard Lamp, of the Texas Game, Fish and Oyster Commission, made it evident that the Commission was aware of the situation that existed in and along the Trinity River during the period in question. His testimony further showed that the Commission had been diligent in the prosecution of salt water pollution cases. However, it is to be noted that the Commission had taken no action against the defendant for the pollution of the Trinity River. Witness Lamp did not purport to know anything about agricultural pollution, and his testimony cannot be interpreted as authority for the proposition that the waters of the Trinity River were then, or are now, unfit for irrigation.

"The uncontroverted fact that plant growth along the banks of the River and down to the edge of the water was dense and luxuriant is evidence that the water of the River had no serious deleterious effect upon plant life generally. Plaintiff Barakis' testimony that he pumped water from the River and onto his land many times without suspecting any detrimental qualities is convincing that the water was no more polluted or contaminated or less suited to the ordinary domestic needs, including irrigation, than is the water of most any stream similarly situated in Texas."

Thompson v. Globe Aircraft Corporation (1947) Tex. Civ. 203 S.W. (2d) 865 related an action by B. G. Thompson against the Globe Aircraft Corporation for death and injury to the plaintiff's cows allegedly caused by chromic-acid poisoning because of defendant's alleged negligence in causing the creek on plaintiff's land to become polluted with chromic acid. From an adverse judgment, the defendant appealed. The court was unwilling to base its reversal on the failure to show that there was insufficient evidence of quantity of chromic acid in the water but preferred to find other grounds to reverse and remand the cause for a new trial.

"First, it is argued that the court should have instructed a verdict for defendant because there was no testimony showing that there was sufficient chromic

acid in the waters of the creek to cause injury to plaintiff's cattle. Plaintiff undoubtedly tried the case in the court below, and still contends here, that his cattle began to suffer injury from the pollution of the creek within a short time after he moved onto the leased land in April of 1944. A sample of water was taken from the creek in April of 1945 and other samples were taken in June and later in the summer of that year. Those samples were analyzed by chemists, and found to contain chromic acid. The least chromic acid content shown by the analyses was 7.8 parts of acid to one million parts of water, the greatest content was 31 parts of acid to one million parts of water. Some of the expert witnesses testified that chromic acid was poisonous, but none of them testified as to the amount of acid which would be required to render the water injurious to the cattle. In other words, there is no expert testimony that water with chromic acid content of the amount shown by the chemical analyses would be injurious to cattle. Opposed to defendant's argument is the theory advanced by plaintiff to the effect that the poisonous nature of the polluted water may reasonably be inferred from the known fact that chromic acid was in the water, from the fact that the cattle suffered injury, and from the fact that the evidence excludes the likelihood of injury from any other cause. Since we have concluded that the judgment of the trial court must be reversed for other reasons, and since the proof may not be the same on another trial, we shall forego further discussion of this question except to say that we are unwilling, in view of the entire record, to render judgment for defendant on this ground."

Where the injurious effect upon soil and plant life of excess salt in water overflowing land is known, where the evidence shows that salt water actually overflowed the land, where the plaintiff's analysis of the soil and water showed the presence of excess salt, where lay experts testified to the observable effect of excess salt and described that effect as present, and where no other cause or reason for the loss of fertility of the soil was advanced, it was held that plaintiff had introduced sufficient evidence to justify submitting the issue to the jury (Magnolia Petroleum Co. v. Norton (1942) 189 Okla. 166, 116 Pac. (2d) 893):

"Given the admitted fact that excess salt produces such conditions, and barring the fact that a 'lay-expert' made an examination from which he was qualified to express an opinion, the jury was in as good position as the 'lay-expert' witness to draw a conclusion whether the excess salt (if they believed it present) caused the injuries. We do not believe this is an instance where a so-called technical expert, scientific expert, Wigmore, supra, vol. 2, page 635, sec. 556, was required. It is well known, and the parties all assume, that the presence of salt in water and soil above tested levels is injurious to plant life. Each party had an analysis of the soil and water made, and the report of plaintiff's analyst showed far more than enough salt to injure plant life. The lay-experts did not qualify themselves to give more than their observation of the effect of salt on soil and plant life. Having proved the presence of salt in injurious proportions, plus the admitted fact of its injurious effect in such proportions, plus the description of the soil and plant life by competent observers, there was no necessity for the use of a technical expert. This is especially so where the defendant did not resort to the evidence of technical experts to ascribe the loss of the fertility of the soil to any other cause. This is not applicable to the trees. Defendant did introduce an expert witness who ascribed the death of the trees to borers."

The rule that a conflict in the evidence presents a factual problem for determination is succinctly stated in Burr v. Adam Eidemiller, Inc. (1956) 386 Pa. 416, 126 A. (2d) 403. It involved an action in trespass against a road construction company for damages arising out of subterranean contamination of plaintiffs' water supply. The court stated:

"The plaintiffs introduced evidence by a chemist to the effect that the acid condition of the water was the cause of the destruction of the heating and plumbing system owned by the plaintiffs as well as the loss of the use of their sewage system. While the defendant offered evidence to the contrary this was an issue of fact for the jury to determine which was resolved in the plaintiffs' favor."

Hutchinson v. Rock Island Oil and Refining Co. (1953) 208 Okla. 259, 255 Pac. (2d) 234 was an action to recover damages for the loss of livestock alleged to have resulted from the drinking of polluted water out of Cow Creek. The plaintiffs, owners of the stock, contended that the injury and resulting damages arose out of the defendants' operation of some oil refineries located several miles south of the plaintiffs land. Judgment for the plaintiff was affirmed in spite of the defendants apparently expert evidence.

"Defendants then introduced the deposition of V. G. Heller, Professor of Biological Chemistry at the Oklahoma A & M College. He testified that H. M. Marney mailed him two samples of water; one taken from Cow Creek and the other from the well on the Mize farm on Feb. 3, 1949, and from these samples he found and so advised Mr. Marney as follows:

" 'I seriously doubt if it would be advantageous to make a quantitative determination of the exact amount of inorganic materials present so long as we know the total amount would not be injurious. I think the only chemical analysis that is going to help you is to find when there are sulfides or similar compounds present that are truly toxic. We can certify to the bad physical appearance, bad odor, and to the unsavory flavor, but we have to admit that there is none of the usual oil field brines, acids, or alkalies present in toxic quantities. In your waters we are dealing with a different type of material. The total amount of dissolved materials present would definitely not be toxic to either man or animal. The amount of salts present are in the form of sodium chloride, calcium chloride, or magnesium chloride, so often found in oil well brines and which are poisonous in large quantities either to plants or animals, is not present in your water. Neither do we find the presence of alkalies as are sometimes found

about refineries or do we find any free acid, so from the standpoint of all these normally discussed materials in waters, I think you would have no case for damages.' "

COLIFORM BACTERIA AS EVIDENCE OF FECAL POLLUTION

The decisions are numerous in which the courts have found that the presence of coliform organisms in water is evidence of its polluted condition. A few such cases are cited and quoted to illustrate the courts' line of thought.

Board of Health of the State of Maryland v. Edward J. Crew (1957) 212 Md. 229, 129 A. (2d) 115:

"The testimony showed that the order to Crew to discontinue the use of his well was issued after Dr. Gordon had determined, and the Health Department agreed, that a danger to health existed in the use of a private well in the Willoughby Beach area. Dr. Gordon explained that sewage from septic tanks and cesspools has to be purified by seeping through the ground. A soil that thus purifies the sewage gradually becomes contaminated itself, no longer acts as a purifier. The rate of absorption and the value of the soil as a purifier vary with the nature of the soil. Crew had testified the soil on his place was clay for a depth of seven or eight feet under several feet of top soil. A Health Department witness had tested the soil on a place adjoining Crew's and found it clay, with poor absorptive powers. Dr. Gordon's testimony was that since a clay soil will not absorb sewage, it does not act as a purifying agent and that wells in an area where the soil is clay are likely to become contaminated. An entire area may become contaminated and the contamination may reach any or all of the wells of the area, particularly where the wells are shallow. Dr. Gordon would fix no definite time as to when this contamination might occur but said, in essence, that it could be the next day, the next week or next year. 'A well is considered to be contaminated when bacteria called coliform organisms, derived from warm blooded animals, including man, are found in the water. Dysentery can result from such organisms as well as typhoid fever.' "

Looney v. Panther Coal Co. (1946) 185 Va. 758, 40 S. E. (2d) 298 described an action for the pollution of a stream by a lower riparian owner against an upper riparian owner engaged in coal mining. The judgment for the plaintiff was reversed, for it was shown that the plaintiff's wells, rather than the stream, were the object of pollution:

"The testimony for the defendant was in sharp conflict with that for the plaintiff. That testimony was to the effect that the trouble with plaintiff's sawmill was old age and improper care and operation; that stock would drink out of the branch; that soap would lather in the water from the well, and that the water would wash clothes clean, and would make good tea, and would not turn black when boiled, and that rice cooked in it would not turn black, as claimed. Experiments were made before the jury to show that these things were true, and the results of some of the experiments are before us as original exhibits. Water was brought from the wells during the trial, tasted by four witnesses, all of whom testified that so far as taste, look, and smell, were concerned, it was good drinking water. These witnesses included J. G. Burch, health officer of the county, who said that so far as taste was concerned this water was above the average of what he was used to drinking in the county . . .

"It is not surprising, therefore, that when the plaintiff's wife had this water from the wells analyzed by J. G. Burch, a sanitation officer for the State Health Department, and a witness for the defendant, as noted above, he found the water in both wells badly polluted with B-coli bacteria and unfit and unsafe for use."

Borough of Westville v. Whitney Home Builders, Inc. (1956) 40 N. J. Sup. Ct. 62, 122 A. (2d) 233 was a case involving an action by the borough against a house builder and town to enjoin the operation of a sewage-treatment plant discharging effluent into a stream that ran into a pond which was in the borough recreational park and which was used for fishing, skating, and wading. The Superior Court, 32 N. J. 538, 108 A. (2d) 660, entered judgment denying injunctive relief, and the borough appealed. The Superior Court, Appellate Division, held that evidence on the issue of reasonable use of waters did not make denial of injunctive relief erroneous. The judgment was affirmed:

"Water pollution is generally measured in important degree in terms of bacteria count and index of coliform micro-organisms (B-coli), the latter reflecting the degree of animal (including human) excrement. Analysis of the waters of the ditch and pond by an expert for defendants prior to the construction of the plant, on July 27, 1954, showed 'gross pollution' at points above and below the proposed site of the sewage plant and in the pond, in terms both of bacteria and B-coli. The measure of dissolved oxygen at the mouth of the pond was 1.9 parts per million in July, 1954, an indication of the beginning of putrefaction. All the witnesses testified that the polluted condition of the ditch and pond stemmed from the normal floatage of impurities on surface waters in such a semi-urban area.

"Tests made April 7, 1955, after three months of operation of the plant, showed a bacteria count for the effluent vastly lower than for the water at various locations in the stream and pond, and this was also true as to the B-coli index. Substantially similar results were indicated by analysis of samples taken May 16, 1955, and also by analyses made by one of the expert chemists, Corson, who testified for plaintiff. There were indications that the chlorine in the effluent had the beneficial effect of reducing the bacteria count of the stream for some distance below the point of discharge.

"The analysis of the effluent also showed a lower (more favorable) B.O.D. (0.4 P.P.M. as compared with the Incodel maximum of 50.0) than in the samples from the watercourse, in ditch and pond. The remainder of the chemical analyses of the effluent made by defendants' expert chemist showed what he described as good, or very good, conditions, in comparison with treated sewage effluent, generally. The

amount of residual solids was such as to lead him to describe the effluent as 'rather dilute.'

"Plaintiff's experts, Boyd, a sanitary engineer, and Corson, a chemist in the public health field, testified that some disease-bearing viruses or organisms found in human feces might survive the effect of the chlorination and treatment of the average sewage plant. Polio, hepatitis and typhoid were cited as examples. Neither of these experts was a bacteriologist. Corson conceded it would occur 'extremely infrequently.' Boyd admitted that on the basis of the amount of residual chlorine in the effluent, the possibility of escape of harmful bacteria was remote, and that he did not consider himself 'an expert on viruses.' Lanning testified he considered the survival of harmful bacteria of fecal origin in the effluent as highly improbable.

"The only other tangible, rather than psychological, objection advanced by plaintiff was to the possibilities of odor or stench. There was some evidence that when the plant operates at full capacity and there is simultaneously a period of low natural flow in the ditch, the volume of effluent may be expected to exceed that of the natural stream flow. Corson stated that at such times there would be stench in the pond, attributable to depletion of dissolved oxygen in the sewage. But this is seen to be without warrant, in view of the unchallenged results of the tests made by defendants' chemist, Beltz, which showed the effluent to have substantially lowered B.O.D. than the waters of the stream and pond and quite similar proportions of dissolved oxygen. Boyd offered a different thesis for prognosticating an 'odor of decay.' It was that the chemicals in the effluent would cause 'lushness of vegetation' in the pond and that such vegetation would 'smell' in dry periods. Both Beltz and Lanning testified there would not be increased plant growth. The evidence is that there generally is considerable plant life in the pond in summer and that no odors have ever emanated therefrom. The conclusion of the trial court that the evidence of potential odors from vegetation or putrescence is speculative seems to us a fair characterization, on the present record."

Board of Health of the State of New Jersey v. Borough of Vineland (1906) 72 N.J. Eq. 289, 65 A. 174:

"If coli are found to exist in considerable numbers the conditions are believed to be conducive to the propagation of pathogenic organisms. The coli are, in themselves, harmless, and the number that may safely exist in water are not entirely certain, one thousand coli to the cubic centimeter is believed to disclose a dangerous condition of the water."

City of New York v. Blum (1913) 208 N.Y. 337, 101 N.E. 869:

"The presence of colon bacilli in water indicates the presence of fecal matter from warm-blooded animals and renders the water unwholesome and unfit for human consumption."

Hamilton v. Madison Water Co. (1917) 116 Me. 157, 100 A. 659:

". . . showed 30 colon bacilli to the ounce, while the samples taken at the mouth of Getchell Brook showed 300 to the ounce. This high percentage did not of itself signify the presence of typhoid germs, but of the existence of a large quantity of sewage. Colon bacilli are normally present in the intestines while the typhoid germ is a parasite. The presence of a large quantity of colon bacilli in water indicates therefore sewage pollution, and naturally the greater the percentage the greater likelihood of the existence of the typhoid germ."

Pennsylvania Railroad Co. et al. v. Lincoln Trust Co. Administrator (1929) 91 Ind. App. 28, 167 N.E. 721:

". . . several samples of water were taken and found to contain gas formers and colon bacilli, the bacteria count being 2,700 colonies per cubic centimeter; the presence of colon bacilli indicated sewage pollution; the greater the bacterial count, the greater the danger from typhoid bacilli."

John Wiesner, Jr. v. City of Albany (1928) 250 N.Y. 551, 116 N.E. 320:

"The presence of colon bacilli in water indicates contamination from human sources. Many types are harmless in the sense that they do not furnish the origin of the disease. The typhoid bacilli (scientifically known as 'B-typhosus') are the well recognized cause of typhoid fever. While they are difficult of isolation they belong to the colon groups, and the presence of colon bacilli in the water indicates that there is grave danger that the typhoid bacilli are also present; and this becomes a practical certainty when there is an outbreak of typhoid fever, not directly traceable to other sources of infection."

Highly significant was the affirmed ruling for the plaintiff in People v. City of Los Angeles (1948) 83 Cal. App. (2d) 627, 189 Pac. (2d) 489, 335 U. S. 852, 93 L. E. 400, 69 S. C. 80, which restrained the city from maintaining sewers without permits from the State Board of Public Health, part of which stated:

"The quality of water along the beach was not safe and suitable for bathing purposes as a result of such discharge and the bacterial count of escherichia coli had exceeded 10 per cubic centimeter."

Mary Hayes v. Torrington Water Co. (1914) 88 Conn. 609, 92 A. 406:

". . . that laboratory examination of the Crystal Lake water, made while the epidemic was in progress, disclosed a persistent appearance of colon bacilli, indicating the possibility that the water was infected with typhoid germs also."

A significant case occurred in Mayor and Aldermen of Jersey City v. Jersey City Water Supply Co. (1918) 90 N.J. Eq. 14, 105 A. 494, in which the court held that on review consideration of new evidence was not possible, for the court cannot wait for the latest of scientific de-

velopments. In speaking of the evidence that was not accepted the court stated:

"This evidence is that since the trial, bacteriologists have discovered that a bacillus known as B. welchii, if present in water in considerable numbers, is productive of intestinal disorders; that this bacillus has recently been found in the Jersey City water . . ."

The foregoing cases illustrate judicial acceptance of the sanitary bacteriologists' use of coliform organisms as indicators of fecal contamination. One exception, however, occurred in People v. Bowen (1941) 376 Ill. 317, 33 N.E. (2d) 587. Here Bowen, the Director of the Department of Public Welfare of the State of Illinois, was indicted and found guilty by the trial court of palpable omission of duty for failing to take proper measures to render drinking water at a state hospital safe. As a result, an epidemic of typhoid fever resulted with much illness and many deaths. On appeal, the Supreme Court of Illinois, reversed the decision and stated:

". . . the most that can be said for any of the 158 exhibits, is that it showed the water to be either positive or negative as to coli aerogenes. It appears from the record that coli aerogenes or colon bacillus may be friendly or inimical, and that the mere presence of the colon bacillus in water proves exactly nothing as far as typhoid fever is concerned. The tests seem to have been made by a method of broth fermentation, and determined nothing more that the presence or absence of some kind of colon bacillus. It further appears that this type of bacillus is present in the air one breathes, in milk, on fruits and practically everywhere.

". . . It is further apparent that colon bacillus may be of the fecal or non-fecal types and that so far as typhoid is concerned, it is only the fecal type from man alone (not from animals) that can spread the disease. The typhoid bacillus could not possibly have been identified by the Laboratory means used in any of these reports and none of them is of any value to the People in an attempt to prove the guilt of the defendant . . .

". . . Even if these reports were of any probative value they would necessarily tend to disprove, rather than prove, a case against the defendant. The water from these wells was consumed by all inhabitants of Manteno for more than eight years prior to this epidemic. This test over a period of eight years, if looked upon as a laboratory experiment, would go a long way toward proving that the water actually was safe for human consumption, because there is no evidence of any abnormal condition as to typhoid occurring during that period of time . . ."

It is interesting to note that, among the advances of the past decade, certain pathogenic forms of the coliform group of organisms have been identified. See Chapter VII, Coliform Bacteria. Such late progress, however, would not constitute sufficient grounds for reopening the Illinois case cited above.

JUDICIAL NOTICE

Under the doctrine of judicial notice, certain matters are assumed to be indisputably true and the formal introduction of evidence to prove them is not required. Judicial notice is thus a substitute for formal proof (9 Wigmore on Evidence, Sec. 2565 et seq). This method of deciding questions of fact was in current use in the English Common Law Courts and today the increasing tendency of the judges to recognize the significance of various types of facts demonstrates that they are striving to keep pace with the rapidly growing complexity of problems with which they have to deal. Thus, the doctrine that matters of common knowledge, in addition to matters recognized at common law, need not be proved before the court has found application in a variety of situations.

The application of the principle was early seen in an action in which the plaintiff-landowner sought an injunction to prevent the discharge of effluent into a stream which crossed the plaintiff's land. The court stated in Hayes v. Village of Dwight (1894) 150 Ill. 273, 37 N.E. 218:

"Despite witnesses' testimony that in their opinion the proposed discharge of sewage would not have the affect of materially polluting the stream, the court held that little weight is to be given to the testimony of witnesses who attempt to swear contrary to known and established natural laws. That the sewage of a village of 1600 inhabitants, discharged into a small stream will materially pollute the waters of the stream and render it unfit for domestic use, for at least a few rods below the point of discharge, is a proposition too plain and too thoroughly verified by ordinary experience and observation to admit of reasonable doubt."

In Kent v. Shell Petroleum Corporation (1940) 187 Okla. 637, 105 Pac. (2d) 230, the plaintiff sought to recover damages for alleged pollution to his water supply by reason of the defendant's pollution of a creek which traversed the farm. From an adverse judgment the defendant appealed. In the decision affirming the judgment of the lower court, the Appellate Court stated:

"A portion of our opinion in Oklahoma City v. Page, 153 Okla. 285, 6 Pac. (2d) 1033, clearly demonstrates the fallacy of confusing the character of a stream's contamination in regard to permanency with the character of the injuries resulting therefrom. From the judicial pronouncements referred to it will also be noted that a stream may be considered 'permanently' polluted from a legal standpoint, when neither the condition itself nor the injuries flowing therefrom are everlasting in character. To deny that a stream once regarded as permanently polluted can thereafter become relatively pure is to disregard the operation of natural forces. It is only reasonable that when the proportion of fresh water to salt water in a polluted stream increases (whether from a curtailment of the flow of salt water or an increase in the flow of fresh water into it) the aggregate body of water therein becomes more suitable for drinking purposes. There is evidence in the present case which tends to show that this is what was occurring in Black Bear Creek during a period that may be variously estimated at from several months to three or four years preceding July, 1935, so that by the latter date, some of the farmers living along the stream in the vicinity

of plaintiff's farm were using it for watering their livestock. For the reasons we have mentioned, the evidence cited by defense counsel showing that the stream was polluted for several years before this transformation cannot be considered fatal to the plaintiffs' cause of action for damages they sustained when its salt water content again became so great that it was once more harmful to animals drinking therefrom."

In an Alabama case involving a proceeding to restrain the pollution of a stream resulting from a quarrying operation, the court took judicial notice of the economic factors involved.

" 'The court will take notice of the fact that in the development of the mineral interests in this state, recently made, very large sums of money have been invested. The utilization of these ores, which must be washed before using, necessitates, in some measures, the placing of sediment where it may flow into streams which constitute the natural drainage of the section where the ore banks are situated. This must cause a deposit of sediment on the lands below; and while this invasion of the rights of the lower riparian owner may produce injury, entitling him to redress, the great public interests and benefits to flow from the conversion of these ores into pig metal should not be lost sight of.'

"We approve the following principle extracted from Sanderson v. Pennsylvania Coal Co., 86 Pa. 401, 27 Am. St. Rep. 711: 'The exigencies of the great industrial interests must be kept standing in view; the property of large and useful interests should not be hampered or hindered for frivolous or trifling causes. For slight inconveniences or occasional annoyances, they ought not to be held responsible, and, in dealing with such complaints, juries should be held with a steady hand.' " Montgomery Limestone Co. v. Bearden (1951) 256 Ala. 269, 54 So. (2d) 571.

The Texas Court in Rudco Oil & Gas Co. v. Lemasters (1940) Tex. Civ. App., 146 S.W. (2d) 806, pp. 811 took judicial notice of the polluting potential of refinery wastes.

"As to the point that the court should have instructed a verdict because 'there was no evidence to show that the oil, gasoline, waste material and drip oil was of such character and content as would inflict the damage to plaintiff's property complained of' there certainly was evidence tending to show that damage was inflicted from such cause which necessarily includes evidence of the damaging characteristics of such substances.

"Besides, we think we must take judicial knowledge that oil, waste material (from a refinery), drip oil or gasoline may pollute water used for domestic purposes and damage land."

Daniels v. Bethlehem Mines Corporation (1958) 391 Pa. 195, 137 A. (2d) 304, is illustrative of the court taking judicial notice in a situation involving the quality of water flowing from mines:

"In the coal mining industry, whether in the anthracite or bituminous field, the elimination of water from the mines presents a very serious problem. Its elimination is an absolute necessity if mining is to continue and, yet, its elimination by discharging it into streams and creeks grievously affects lower riparian owners along these streams and creeks, and, in some instances, the public. Waters which have their source in a coal mine, of necessity, contain deleterious substances as a result of contact with the coal and its various chemical compounds. Any mine water—that is, water which comes from a mine—upon discharge into a stream deteriorates and destroys, in varying degrees, the purity and cleanliness of the natural waters of the stream."

The ability of a veterinarian to testify as to salt condition of water through having tasted it was a matter of judicial knowledge in Greis v. Mitchell (1939) 185 Okla. 136, 90 Pac. (2d) 894. The court stated:

"Complaint is also made of the admission of an identified bottle of salt water from the creek because the degree of salt therein was not definitely shown. The complaint goes to the probative force of the evidence which was for the jury and not to the admissibility thereof which would have presented a question of law for the court. We apprehend that a veterinarian familiar with salt water and its effect on stock can by taste determine with a reasonable degree of certainty whether the water is sufficiently saturated to cause injury or death to stock. Testimony to that effect was presented in this case."

The rapid growth of technical knowledge in so many branches of water pollution has removed many factual situations from the realm of judicial knowledge, and thus we see a reluctance on the part of the judiciary to rely on the convenient expediency of judicial knowledge where other persuasive and more technical evidence is available. Thus in Christensen v. Northern States Power Co. of Wisconsin (1946) 222 Min. 474, 25 N.W. (2d) 659, the court rejected the theory of judicial notice.

"The real question presented for decision is whether there is sufficient evidence that either the electrical current or the dynamite killed the fish. The testimony was that the ice was under such pressure that it had sheared off one of the three steel supports of the tower and that for a period of four seconds electricity may have discharged into the tower. It is the claim of plaintiff that the electricity escaped into the water and killed the fish in the lake. However, the record is silent on the effect this might have had. Any conclusion a jury would reach would be mere conjecture and could not be sustained. No connection as cause between the alleged escape of electricity and the death of the fish is established. What effect, if any, the electricity would have is a matter of which this court cannot take judicial notice, for the simple reason that it is not a matter of common knowledge."

In State v. Heller (1937) 123 Conn. 492, 196 A. 337, the court distinguished the factual situations in which it could take judicial notice.

"While the established scientific fact that water can serve as a carrier of disease germs to one drinking . . . is one within judicial notice (State v. Morse, 84

Vt. 387, 80 A. 189, 194, 34 L.R.A., N.S., 190, Ann. Cas. 1913B, 218), what the conditions essential to the destruction of such germs in water of a flowing stream may be, is not. But, aside from this, the tendency of such pollution to produce public injury, even though no actual injury occurs, affords ground sufficient to sustain the Legislature's act. State v. Wheeler, supra, 44 N.J.L. 88, 92; Dunham v. New Britain, 55 Conn. 378, 384, 11 A. 354. Upon the record before us we cannot hold that bathing in a tributary of a reservoir might not have such a tendency to endanger the health of users of the water that the Legislature might not reasonably prohibit it. It is our conclusion that the statute by its terms is not of such broad scope that it fails to bear a rational relation to the protection of the public health, thus constituting an unreasonable exercise of the police power."

In De Garza v. Magnolia Petroleum Co. (1937) Tex. Civ. App., 107 S.W. (2d) 1078, the court said:

"Furthermore, this court does not know judicially that salt water is poisonous or harmful to cattle. It occurs to us it would depend on the amount of salt in the water. The evidence shows that the tank had twenty-three times as much salt at one time, as it had previously had, but this does not establish the fact that the water was poisonous or harmful to the cattle. The fact that the cattle did not like the salt water proves nothing; from the evidence, they must have lived on the water for some six months."

To the same effect, see Shell Petroleum Corp. v. Worley (1939) 185 Okla. 265, 91 Pac. (2d) 679 cited hereinbefore under "Indirect Non-Technical Criteria."

It becomes apparent from a reading of the cases, however, that when complex scientific information is necessary or available, the courts are not reluctant to discard the vehicle of judicial notice. The increasing frequency with which courts resort to other sources for factual information rather than to their own impressions is very reassuring. In addition to pointing up the recognition by the courts themselves of the limitations of this method of proof, the use of technical data lends encouragement to the fact that, as scientific techniques for the detection and prevention of pollution are improved and additional criteria for determining water quality are established, the outcome of litigation will be more predictable with a greater degree of certainty. Such use is certainly to be preferred rather than subservience to the economic and social philosophy of the court where it may have been compelled to act from a minimal factual situation, relying on its own particular knowledge or lack of knowledge.

EXPERIMENTAL EVIDENCE

The principal use of experimental evidence is in the testing of theories of the case by the performance of controlled experiments out of court followed by an expert testifying as to the results. The experiment is thus merely the basis of the expert testimony as to the facts and opinions, and demonstrative evidence is only incidentally involved, as where the expert offers in evidence physical objects connected with the experiment. (See generally McCormick on Evidence, p. 359; 2 Wigmore, Sections 441 et seq.; 2 Belli, Sections 179 et seq.) Since experiments may be conducted under dissimilar conditions and often without notice to the other side, the courts have a broad discretion to admit or exclude experimental evidence.

In Southland Co. v. Aaron (1954) 221 Miss. 59, 72 S. (2d) 161, an action for damages sustained by the plaintiff as a result of pollution by the defendants of the waters of a creek, the testimony of the expert who had collected samples of the water was shown to have some weaknesses.

"J. B. Price, of the Chemistry Department of Millsaps College, testified that he had collected a sample of the water in McGill's Branch, at a point below the refinery, and samples of the water in Boguehoma Creek, above and below the mouth of McGill's Branch. The sample taken from McGill's Branch showed 114 parts per million of sodium chloride. The sample taken from Boguehoma Creek, a short distance above the mouth of McGill's Branch, showed 1510 parts per million of sodium chloride, and the sample taken from the creek just below the land owned by the plaintiffs showed 1368 parts per million of sodium chloride. The witness stated that he did not observe the presence of any petroleum products in McGill's Branch or in Boguehoma Creek. He saw no evidence of oil deposits on the roots of trees on Dr. Copeland's land, which adjoined the land of the plaintiffs. He admitted, however, that the samples of water that he had taken were taken below the surface, and that oil would not go down and mix with water."

It should be pointed out, however, that the requirement is not always that of identity of condition to establish the validity of the experiment but in some cases only substantial similarity is necessary (to wit see Pickens v. Harrison (1952) 151 Tex. 562, 252 S.W. (2d) 575).

CONSTITUTIONALITY OF CRITERIA ESTABLISHED THROUGH LEGISLATION

The courts have uniformly held that the abatement and prevention of water pollution is a matter of state concern, and legislation designed to regulate and control such pollution is within the scope of the state's police power. City of Utica v. Water Pollution Control Board (1959) 5 N.Y. (2d) 164, 156 N.E. (2d) 301.

The problem has been considered primarily from the standpoint of how far state legislation can go without violating the prohibitions in the Federal Constitution against arbitrary and unreasonable classification of all persons and things. From the legal standpoint there appears to be no constitutional limitation that would prevent a satisfactory solution. It has been well settled that any provision of a statute or ordinance regulating a nuisance, such as a pollution nuisance, is valid insofar as the due process clause of the 14th Amendment of the United States Constitution is concerned, if it is reasonably necessary for the accomplishment of the purpose and for the public welfare generally, and if it is not unduly oppressive nor arbitrarily interferes with private business or imposes unusual or unnecessary restrictions upon a lawful business.

It becomes largely a question of fact as to whether or not the regulations and standards are reasonable. No hard and fast rule can be established for all cases. It depends upon the facts and circumstances of each case. A law is not invalid solely because it may put a particular establishment out of business or require the expenditure of large sums of money to comply with the terms of the law. In some situations it amounts to a weighing of the advantages and disadvantages although the doctrine is not placed on that ground. A slight inconvenience to the public will not justify an unnecessary destruction of property rights.

To some extent and particularly in the case of private nuisance, the rule is based on the maxim of jurisprudence as stated in the California Civil Code, Sec. 3514: "One must so use his own rights as not to infringe upon the rights of another."

The rule of law is aptly stated in City of Huntington v. State Water Commission (1953) 137 W. Va. 786, 78 S.E. (2d) 833. In this action the City of Huntington brought proceedings against the State Water Commission to review an order of the commission requiring the city to cease and desist from polluting certain rivers. The Circuit Court entered judgment dismissing the proceeding, and the city brought error. The Supreme Court of Appeals held that the evidence sustained the finding that the waters of these rivers were polluted by untreated sewage of the city in violation of the act creating the commission. The judgment was affirmed, the court stating as follows:

"The uncontradicted evidence introduced at the hearing held by the Commission, on August 15, 1949, clearly shows that the waters of the Guyandotte River and the Ohio River are polluted by untreated sewage from the City of Huntington which is discharged and flows into those streams; and the finding to that effect by the Commission is neither arbitrary nor in excess of its statutory powers. The findings of the Commission is expressly authorized by Section 6, Article 11, Chapter 16, 1931, as amended by Chapter 6, Section 6, Acts of the Legislature, 1933, Regular Session, which provides, in part that: 'After a full hearing the commission shall make its findings of facts, and if it finds that any person is polluting any of the waters of the state, it shall make and enter an order directing such person to cease such pollution and such person shall have thirty days after notice of the entry of such final order to notify the commission that he will comply therewith or will install, use and operate some practical and reasonable system or means which will reduce, control or eliminate or reduce to a harmless minimum such pollution, having regard for the rights and interests of all persons concerned.'

"The question of the force and the effect of an order of an administrative board or tribunal, and particularly the force and the effect of an order of the Public Service Commission of this State, has been frequently considered by this Court. In Town of Harrisville v. Public Service Commission, 103 W. Va. 526, 138 S.E. 99, this Court held, in the syllabus, that: 'The findings of the Public Service Commission, based upon substantial evidence, will not be reviewed by this Court.' In the opinion in that case, with respect to the findings of the Commission, this Court said:

" 'Its findings are presumed to be reasonable, lawful and correct, and will not be set aside by this Court if based upon substantial evidence. Findings of fact by the Public Service Commission, based upon evidence to support them, generally will not be reviewed by this Court.' United Fuel Gas Co. v. Public Service Commission, 73 W. Va. 571, 80 S.E. 931; Mill Creek Coal (& Coke) Co. v. Public Service Commission, 84 W. Va. 662, 100 S.E. 557, 7 A.L.R. 108; City of Charleston v. Public Service Commission, 86 W. Va. 536, 103 S.E. 673; City of Huntington v. Public Service Commission, 89 W. Va. 703, 110 S.E. 192; Baltimore & Ohio Railroad Co. v. Public Service Commission, 90 W. Va. 1, 110 S.E. 475."

PRESUMPTION OF POLLUTION FROM VIOLATION OF STATUTE

In the case of C. L. McMahon v. Smith (1941) 189 Okla. 579, 118 Pac. (2d) 1022-23, the defendant appealed from a verdict for the plaintiff alleging that an instruction to the jury that a violation of statutory law was actionable negligence was prejudicial to his defense. The statute in question reads as follows:

" 'No inflammable product from any oil or gas well shall be permitted to run into any tank, pool or stream used for watering stock; and all waste of oil and refuse from tanks or wells shall be drained into proper receptacles at a safe distance from the tanks, wells or buildings, and be immediately burned or transported from the premises, and in no case shall it be permitted to flow over the land. Salt water shall not be allowed to flow over the surface of the land.' " 52 Okla. St. Ann. Sec. 296.

" 'A violation of this statutory law is actionable negligence.' "

The court stated:

"While the instruction is technically defective, in that by the use of the word 'actionable' in the last sentence it eliminates causal connection as an element of liability, this defect was cured by subsequent instructions in which the trial court positively and repeatedly told the jury that plaintiff's right to recover existed only if the injuries to his livestock were caused or contributed to by the negligent acts of defendant, so that the error was harmless. Oklahoma City-Ada-Atoka Ry. Co. v. Riddle, 183 Okla. 318, 82 Pac. (2d) 304; Marathon Oil Co. v. Sanders, 180 Okla. 642, 71 Pac. (2d) 956.

"Defendant does not contend that the use of the word 'actionable' in instruction No. 4 in any way prejudiced him, but asserts that it amounts to a peremptory instruction for plaintiff, as it in effect made the defendant liable for damages resulting from the escape of salt water and oil from its lease, regardless of whether the escape of such substance was due to causes beyond its control, and it was in fact guilty of no negligence.

"An instruction quoting the language of the statute, and telling the jury that a violation thereof is negli-

gence, is proper. Devonian Oil Corp. v. Hurt, 169 Okla. 114, 36 Pac. (2d) 24. And if the statute is violated, and injury results therefrom, the liability attaches. The violation of the statute is negligence per se. Comanche Drilling Co. v. Shamrock Oil & Gas Co., 122 Okla. 253, 254 p20; Owen-Osage Oil & Gas Co. v. Long, 104 Okla. 242, 231 p296. Affirmed."

In Magnolia Petroleum Co. v. Ford (1938) 183 Okla. 14, 79 Pac. (2d) 588, the court held that a violation of statute requiring an oil lessee to prevent waste oil and refuse from flowing over surface of land is negligence per se, and no other negligence need be pleaded or proved in an action for damages caused by violation of the statute. While the courts have not hesitated to rule that a violation of statutory law or officially adopted standards are prima facie evidence of negligence and indicative of pollution, they have hesitated to adopt the same attitude with respect to activities that apparently bear the seal of approval of some regulatory body or administrative agency.

Thus, the following recent cases may be considered decisive of the factual situations there presented. N. C. Ginther, H. C. Warren and W. L. Ginther, d/b/a Ginther, Warren & Ginther, and R. B. Lynn v. Arthur W. Long (1956) 227 Miss. 885, 87 So. (2d) 286.

"In an action for damages to plaintiff's land and timber thereon because of salt water and oil flowing from defendants' oil well on adjacent land into a creek and thence onto plaintiff's land, Game and Fish Commission's retroactive certificate, issued after infliction of damages, that defendants had complied with Commission's anti-pollution regulations on date prior to occurrence of damages, was properly excluded from evidence on plaintiff's objection. Assuming that the certificate had been issued on January 1, 1951, it did not constitute a perpetual license to the appellants to discharge its deleterious effluents upon the land of the appellee and thereby cause damage. Masonite Corp. v. Guy, Miss., 77 So. (2d) 720. To hold otherwise would be to grant to the State Game and Fish Commission the right to make an ex parte retroactive finding as to pollution or non-pollution, which retroactive finding would be conclusive upon private parties in an action for damages such as this. We think there was no error in the action of the trial court in sustaining the objection to the introduction in evidence of the certificate."

In C. H. Guy v. Masonite Corporation (1955) 223 Miss. 8, 77 So. (2d) 720, it was shown by appellant that pursuant to the provisions of Chapter 381, Laws of 1946, there was issued to appellant on November 26, 1946, a certificate of the State Game and Fish Commission, as follows:

" 'This is to certify that Masonite Corporation of the City of Laurel, County of Jones, is complying this date with the Rules and Regulations of The State Game and Fish Commission on Industrial Waste Effluent.' It is contended that this certificate of 1946 is conclusive evidence that appellant did not pollute these streams in 1950 and 1953. With that position we do not agree. The fact that appellant constructed its settling ponds in accordance with the regulations of the commission and agreed not to release its effluent therefrom except in times of high water and then only in such quantities as not to injure fish life does not grant a perpetual license to violate its agreement with immunity, and the evidence here is sufficient to show that it did release its effluent in such quantities as to pollute these streams, and to make a jury issue on that question."

In B. E. Massey v. Masonite Corp. (1955) 219 Fed. (2d) 586, an action was brought for alleged pollution by defendant of plaintiff's allegedly privately owned lake. The lower court issued summary judgment for defendant finding that the water could not have been polluted by defendant since it had a certificate of compliance from the Mississippi State Game and Fish Commission.

The Appellate Court ruled that this was insufficient proof:

"We are of the opinion that a certificate of compliance issued by the State Game and Fish Commission, which only has jurisdiction over the waters of the State, exclusive of lakes or other waters which are wholly landlocked and privately owned, and over industrial concerns using such waters for the discharge of waste, is not a legal bar to a private suit for damages for the pollution of a privately owned lake. There is a genuine issue of fact as to whether the appellant's lake was private or landlocked, and whether Masonite's effluents killed the fish in the lake. We think it was error for the court to enter a summary judgment based upon the certificate of compliance, and the judgment appealed from is reversed. Masonite Corporation v. Guy, Miss., 77 So. (2d) 720. Reversed."

In Ellison Bros. Oyster Co. v. Rayonier Incorporated (1957) 156 F. Supp. 214, the court enunciated the principle that adherence to the terms of a permit was an effective shield against actionable nuisance arising from a discharge of pollutants.

"Actions by tideland owners against pulp and cellulose mill for alleged wrongful pollution of state waters resulting in deterioration and death of plaintiffs' oysters. Defendants moved to dismiss. The District Court, Boldt, J., held that under doctrine of primary jurisdiction Washington Water Pollution Control Commission had primary jurisdiction to control and prevent undue pollution of all tideland waters of state and if mill had been operated pursuant to commission permit from time required, if rules, regulations and standards for such operation had been established by commission which were not arbitrary, capricious or unreasonable and if effluents had not been discharged in violation of standards prescribed, discharge of waste was not unlawful or unreasonable and recovery could not be granted on either trespass or nuisance theory."

MISCELLANEOUS

It may be helpful to enumerate briefly some instances of civil litigation in which the pollution of a water supply has been the focal issue and where a principal of law has evolved through the decisions in the cases which could serve as a precedent in future similar factual situ-

ations. This situation arises despite the compliance or failure to comply with promulgated standards.

COMPARATIVE INJURY DOCTRINE

The doctrine of comparative injury, or as it is sometimes called "balancing the equities" has been discussed in a number of cases in the field of water pollution. Based on equitable principles, it would seem to be properly applicable in cases of undue hardship. Yet, the reasoning in the case of its denial is difficult to answer since, insofar as the plaintiff is denied a decree enjoining an actual nuisance arising out of the pollution of the waters, the defendant in effect is given an easement over the plaintiff's land. This action amounts to a taking of property for private use in violation of the Constitution. Where the defendant is required to pay the plaintiff, the reasonable value of his property or the interest therein which is damaged, the effect is condemnation for the benefit of a private individual who does not possess the power of eminent domain.

In American Cyanamid Co. v. Commonwealth (1948) 187 Va. 831, 48 S.E. (2d) 279, an action to enjoin the defendants, the company operated a chemical plant and in the course of its operations in producing titanium dioxide discharged dilute sulphuric acid. The action was brought to enjoin the defendants' activities. The Appellate Court in this state sustained a ruling for the defendant stating:

> "The end desired is to keep the clean waters clean and to reduce the pollution in the unclean waters. Some of this pollution comes from industrial waste, discharged by industries invited into the State and furnishing employment to some of its people. The problem is to be dealt with so as to give fair treatment to the industries, to its employees and to the public. That requires a measure and balancing of the interests involved. Some waters should be kept pure. A measure of pollution in others is necessary. Not all pollution can be abruptly stopped. On the agreed facts here, for example, this industry would have to shut down if immediately required to cease discharging its acid waste into the river. The aesthetic and recreational features involved in the pollution problem are important, but the opportunity to make a living may be even more so."

The complaint against the defendant was dismissed.

Barber v. School District No. 51, Clay County, Missouri (1960) Kansas City Ct. of App's., 335 S.W. (2d) 527 was an action by owners of adjoining real estate for damages and an injunction restraining a school district from discharging water from its sewage-disposal system upon plaintiff's land.

The plaintiff's land was 25 feet from the sewage-disposal system. The septic tank had overflowed onto the farm and photographs of the water were received in evidence. The plaintiff had testified that the water had an offensive odor and that he had seen his cattle start to drink it and "turn up their noses."

The public-health engineers testified that the system was working adequately, that the water had only a slight odor, and that it would not destroy grass.

The court held that although plaintiff's property rights were technically violated by the sewer discharge and plaintiff received some nominal damages, in view of the fact that he did not suffer substantial damage and the granting of the injunction would result in great injury to the school, to the community and the public, without a great advantage to plaintiff, injunctive relief would not be granted. The court appealed the doctrine of comparative injury. To the same effect, see Johnson v. Independent School District No. 1 (1947) 239 Mo. App. 749, 199 S.W. (2d) 421.

People ex rel. Stream Control Commission v. City of Port Huron et al. (1943) 305 Mich. 153, 9 N.W. (2d) 41 states the rule as follows:

> "The doctrine of 'comparative injury' should be confined to those situations where the plaintiff can be substantially compensated. This principle is distinguished in City of Harrisonville v. W. S. Dickey Clay Mfg. Co., 289 U. S. 334, 53 S. Ct. 602, 603, 77 L. Ed. 1208, where Mr. Justice Brandeis said:
>
> " 'The discharge of the effluent into the creek is a tort; and the nuisance, being continuous or recurrent, is an injury for which an injunction may be granted. Thus, the question is whether, upon the facts found, an injunction is the appropriate remedy. For an injunction is not a remedy which issues as of course. Where substantial redress can be afforded by the payment of money and issuance of an injunction would subject the defendant to grossly disproportionate hardship, equitable relief may be denied although the nuisance is indisputable. This is true even if the conflict is between interests which are primarily private.'
>
> "See, also, Edwards v. Allourez Mining Co., 38 Mich. 46, 31 Am.Rep. 301; Monroe Carp Pond Co. v. River Raisin Paper Co., 240 Mich. 279, 215 N.W. 325; and authorities annotated in 77 L.Ed. 1222."

In Sanderson v. Pennsylvania Coal Co. (1878) 86 Pa. 401, 27 Am. St. Rep. 711, the court stated:

> ". . . The exigencies of the great industrial interests must be kept standing in view; the property of large and useful interests should not be hampered or hindered for frivolous or trifling causes. For slight inconveniences or occasional annoyances, they ought not to be held responsible, and, in dealing with such complaints, juries should be held with a steady hand.
>
> "It is certainly true that owing to the want, if not the necessities, of the present age,—of agriculture, of manufactures, of commerce, of invention and of the arts and sciences,—some changes must be tolerated in the channels in which water naturally flows, and in its adaptation to beneficial uses. Reasonable diminution of its quantity, in gratifying and meeting customary wants, has always been permitted. So its temporary detention for manufacturing purposes, followed by its release in increased volume, is a necessary consequence of its utilization as a propelling force. Nor must we shut our eyes to the tendency—the inevitable tendency—of these and other uses, in which water is an indispensable element, to detract somewhat from its normal purity. These modifications of individual right must be submitted to, in order that the greater good

of the public be conserved and promoted. But there is a limit to this duty to yield, to this claim and right to to expect and demand. The water course must not be diverted from its channel, or so diminished in volume, or so corrupted and polluted as practically to destroy or greatly impair its value to the lower riparian proprietor. 'Sic utere tuo' in such conditions is enjoined by social obligation and by law. It is difficult if not impossible, to declare a rule in language so clear and precise, as that it can be applied with certainty to every case that may arise. See Mississippi Mills Co. v. Smith, 69 Miss. 299, 11 So. 26, 30 Am.St.Rep. 546, 100 Ala. at pages 258-261, 14 So. at page 169, 46 Am.St.Rep. 46."

In Ravndal v. Northfork Placers (1939) 60 Idaho 305, 91 Pac. (2d) 368, an action was commenced for damages to land claimed to have been caused by mud and silt being dumped into a river by defendant's placer mining operations and washed into irrigation ditches upon the plaintiffs' lands. The defendant appealed from a judgment for the plaintiff. The court in affirming the judgment stated:

"Numerous authorities announce the doctrine that while a proper use of the water of a stream for mining purposes necessarily contaminates it to some extent, such contamination or deterioration of the quality of the water cannot be carried to such a degree as to inflict substantial injury upon another user of the waters of said stream. Montana Co. v. Gehring, 9 Cir., 75 F. 384: . . . Salstrom v. Orleans Bar Gold Min. Co., 153 Cal. 551, 96 p. 292; Luama v. Bunker Hill & Sullivan Mining & Concentrating Co., 9 Cir., 41 F. (2d) 358; . . . We believe the rule stated in Arizona Copper Co. v. Gillespie, 12 Ariz. 190, 100 P. 465, 470; Id. 230 U.S. 46, 33 S. Ct. 1004, 57 L.Ed. 1384, is controlling in this case, namely: 'We do not mean to say that the agriculturist may captiously complain of a reasonable use of water by the miner higher up the stream, although it pollutes and makes the water slightly less desirable, nor that a court of equity should interfere with mining industries because they cause slight inconveniences or occasional annoyances, or even some degree of interference, so long as such do no substantial damage.' "

In Otley v. City of Kewanee (1903) 204 Ill. 402, 37 N.E. 218, a bill to enjoin pollution of a stream alleged that defendant city had constructed drains which discharged polluted water on plaintiff's land and in the stream flowing thereon rendering it unsuitable for the purposes to which it was adapted, namely pasturage, cultivation, and building lots for residences; that said lands are arable lands and were used for farming purposes and for pasturages; and that lots laid out thereon were suitable for building purposes, and would be valuable therefore but for the nuisance created by the sewage which occasioned complainant great loss and irreparable injury. The court found:

". . . that the city had constructed drains and sewers which discharge upon plaintiff's lands certain noxious, filthy and polluted waters, in which are carried great quantities of poisonous acids and greasy and oily substances, defiling said stream and rendering it unfit and unsuitable for the purposes aforesaid, and emitting noxious, injurious and offensive odors so as to create a nuisance . . .; that the water from the said sewer injuriously affected cattle and hogs . . .

"The cases held that equity jurisdiction is properly invoked to afford relief to a lower riparian owner where an upper proprietor defiles or corrupts a stream to such a degree as to impair its purity and to prevent its use for any reasonable purpose to which running water may be applied.

"The pollution of a stream constitutes the taking of property which may not be done without compensation."

In an action to restrain the operation of a village sewage-treatment plant in such manner as to cause obnoxious odors, and for damages, the court directed that the village correct the offensive condition or, if correction could not be effected, that it cease and desist from operation of the equipment or appurtenance creating that condition. The Supreme Court, Appellate Division, held that in view of the important public purpose served by the plant, the property owner was not entitled to an injunction unless the condition could be remedied without undue hardship.

"Though obnoxious odors from village sewer plant (sic) constituted nuisance to a property owner, she was not entitled to injunction unless condition could be remedied without undue hardship on village, and if correction could not be so made, she was entitled only to permanent damages, in view of important public purpose served by the plant." Esphyr Slobodkina, Resp. v. Village of Great Neck, App. (1955) 285 App. Div. 908, 138 L.Y.S. (2d) 28.

In Slide Mines, Inc. v. Left Hand Ditch Co. et al. (1938) 102 Colo. 69, 77 Pac. (2d) 125, the court had to consider whether to deny an injunction against the pollution of a stream by a mining company on the ground that the plaintiffs did not come into court with "clean hands" because of their act in turning into the stream water from a reservoir carrying extraneous substances. The court ruled:

"In support of its position, the mining company cites a number of cases in which an injunction was denied a complainant who, himself, was guilty of the same wrongful acts against which his suit was directed. Under the record in the case at bar, these authorities have no bearing. In the Wilmore Case, supra, in the majority opinion on rehearing, the word 'pollution' is defined as meaning 'an impairment, with attendant injury, to the use of the water that plaintiffs are entitled to make. Unless the introduction of extraneous matter so unfavorably affects such use, the condition created is short of pollution.' Here the trial court factually determined that such extraneous matter as the farmers introduced into the stream, not only did not unfavorably affect the use of the water they were entitled to make but was beneficial to it, and hence caused no pollution in a legal sense. The farmers having been adjudged guiltless, no basis is presented for the application of the principle advanced by the mining company."

Where the evidence is not clear and satisfactory but is conflicting as to any obnoxious odors arising from the effluent from a disposal plant which are alleged to create a nuisance, an injunction will not be granted, but reasonable time may be given to improve the operation of a plant. The courts consider, among other things, whether an injunction would not destroy the sewer system of a city and subject the public to serious inconvenience and danger. In Hall v. City of Friend (1938) 134 Neb. 652, 279 N.W. 346, the court stated:

> ". . . The facts present a case wherein the principle of the greatest good to the greatest number must be permitted to operate and private interests must yield to public good, and if the erection has been skillfully performed and without negligence, and in a way to do the least mischief, it must be held to be a lawful exercise of power that equity will not restrain. The construction of sewers and outlets is sanctioned by law, and what the law grants will not constitute a nuisance per se, public or private, and if the law is obeyed no actionable wrong will result."

Although there are decisions apparently to the contrary, the general rule, as supported by the weight of authorities, seems to be that when the issuance of an injunction will cause serious public inconvenience or loss without a correspondingly great advantage to the complainant, no injunction will be granted. So, while there is some authority apparently to the contrary, it is generally held that if the injunction would have the effect of greatly injuring or inconveniencing the public, it may be refused even though as against a defendant the complainant would be entitled to its issuance. The general rule under consideration has been applied where the allowance of an injunction would seriously interfere with or work detriment to public works or works of public benefit, where the issuance of the injunction asked would prevent the necessary disposal of sewage. 32 C.J. 81, Sec. 66; 43 C.J.S., Injunctions, Sec. 31, p. 465; State v. Pearcy, 328 Mo. 560, loc. cit. 574, 41 S.W. (2d) 403, loc. cit. 409; Johnson v. Independent School Dist. No. 1, Buffalo (1947) 239 Mo. App. 749, 199 S.W. (2d) 421.

In Butler v. Village of White Plains (1901) 59 App. Div. 30, New York, the defendant village operated a sewage-treatment plant the effluent of which discharged into a river on which the plaintiff was a lower riparian owner. It was held that the injury was a continuing one and the plaintiff was entitled to injunctive relief. The court stated:

> "The court finds that such discharge does produce at times a foul and offensive odor over the lands of the plaintiff and that such effluent adds to the discolorment and pollution of the waters of the stream, and the courts have held that, to warrant an injunction against odors and gases from an offensive business, it is not necessary that the odors should be noxious, and if they are so offensive and disagreeable so as to render life uncomfortable, equity may interfere, and the fact that the nuisance recurs only when the wind is in a given direction, or that the nuisance complained of is surrounded by other nuisances does not deprive plaintiff of their right to relief."

Jessup & Moore Paper Co. v. Zeitler (1942) 180 Md. 395, 24 A. (2d) 788 was a suit in equity for an injunction against emptying waste from defendants' paper mills into a creek flowing through complainants' lands. The court considers the synergistic effects of individual pollutants and states:

> "The locality of the nuisance is the common point, like the point of convergence in an hourglass, upon which the previously aggregated results of the originally independent acts of the several defendants concentrate, and jointly operate, and from which the joint effects again radiate and distribute themselves upon all within the reach of their influence. This view was also followed in United States v. Luce, C. C., 141 F. 385, 411, where it was held that, even though there was no business connection between two defendant factories, and even though the odors from either of them alone would not so contaminate the air as to create a nuisance, nevertheless if the combined odors from both have the effect of producing the nuisance, the two factories cooperate in fact in the creation of the nuisance, and under these circumstances the managers of either or both of the factories may be enjoined from contributing to the maintenance of the nuisance."

FLUORIDATION

Although arguments ranging from the right of each individual to be free to treat his health as he pleases, to the right of freedom of religion have been raised against the introduction of fluorides into the water supply, the cases appear to hold uniformly that the fluoridation of public water supplies does not infringe on the constitutional liberties of the individual, and in more than ten states including California (De Aryan v. City of San Diego (1953) 119 C. A. (2d) 674, 260 Pac. (2d) 98) where the issue has been brought to the courts the decisions have been in favor of the public body (Murdoch).

This concurrence of judicial opinions would indicate that the water-quality standards promulgated for fluorides have met legal acceptance and where such fluorides are introduced in the quantity permitted they will not adversely affect the condition of the water supply.

POLLUTION OF GROUND WATER

Phillips v. Sun Oil Co. (1954) 307 N.Y. 328, 121 N.E. (2d) 249 was an action in trespass arising out of alleged underground travel of gasoline from defendant's land into plaintiff's waterwell. The chemist testified that gasoline in the plaintiff's well was the same brand as that dispensed by the defendant, and the representative of the defendant testified that they had found only a small leak in an underground tank, but there was no evidence that the defendant knew or should have known that gasoline would flow to the plaintiff's land. In finding for the defendant the court stated:

> "The application of the above-stated rule, in the few pertinent New York cases, to damage claims arising from the underground movements of noxious fluids, produces this conclusion: that, even when the polluting material has been deliberately put onto or into defendant's land, he is not liable for his neighbor's

damage therefrom, unless he (defendant) had good reason to know or expect that subterranean and other conditions were such that there would be passage from defendant's to plaintiff's land. Dillon v. Acme Oil Co., 49 Hun. 565, 2 N.Y.S. 289; Elsey v. Adirondack & St. Laurence R. Co., 97 Misc. 273, 161 N.Y.S. 391; Thompson v. Board of Education of Union Free School Dist. No. 2, Town of Newfield, 124 Misc. 840, 209 N.Y.S. 362. The leading New York decision seems to be Dillon v. Acme Oil Co., supra, where, in the absence of knowledge or notice, an oil refining company was held not liable for discharging, onto its own land, water containing chemicals which somehow soaked down into and through the soil, polluting a well on adjoining property. In the suit we have before us, plaintiff's position is weaker than was Dillon's, since Dillon's defendant intentionally poured, on the ground, the substance which seeped and percolated into Dillon's well."

EMINENT DOMAIN

Article I, Sec. 14 of the California Constitution provides that: "Private property shall not be taken or damaged for public use without just compensation having first been made to, or paid into Court for, the owner. . ." The Fifth and Fourteenth Amendments to the U.S. Constitution provide additional safeguards. These are the guarantees that are applicable to the usual situation of condemnation by the state, county, city, school district, or other instrumentality of the governing body. The courts have held that the pollution of a water supply is in effect a taking of private property under some circumstances.

In Clinard v. Town of Kernersville (1939) 215 N.C. 745, 3 S.E. (2d) 267, the court stated:

"The liability of the town is not to be determined by any negligent conduct on its part in the operation of its disposal plant. If in so doing it in fact discharges foul matter upon the lands of the plaintiffs, or it so pollutes the water of the stream which crosses plaintiffs' land that foul and noxious odors emanate therefrom it is liable for the resulting damage, even though in so doing it is exercising a governmental function. An action by a landowner against a muncipality or corporation possessing the right of condemnation for the maintenance of a continuing nuisance which adversely affects the value of plaintiffs' land is, by the demand for permanent damage either by the plaintiff or by the defendant, converted into an action in the nature of a condemnation proceeding for the assessment of damages for the value of the land or easement taken. The assessment of permanent damages for the maintenance of a continuing nuisance as here alleged and the payment of such damages vests the defendant with an easement entitling it to a continued use of the property in the same manner. No matter how urgent the demands of the public may be or how necessary to the progress of the country, no man's property may be taken without compensation. In those cases wherein the right is asserted to pollute streams or otherwise appropriate or subject lands to an additional burden the question of negligence is not involved. Courts uniformly hold that where the action is for damages by way of compensation, which when paid, secures an easement, the owner of the property is entitled to recover. The pollution of a stream is equivalent to a taking and an appropriation in part. Stanton v. R. R., 111 N.C. 278, 16 S.E. 181, 17 L.R.A. 838; Thomason v. R. R., 142 N.C. 318, 55 S.E. 205; Beach v. R. R., 120 N.C. 498, 26 S.E. 703; Lassiter v. R. R., 126 N.C. 509, 36 S.E. 48. The law permits the acquisition of the easement in such cases by the payment of permanent damages, the judgment having that effect. Brown v. Power Co., 140 N.C. 333, 52 S.E. 954, 3 L.R.A., N.S., 912; Thomason v. R. R., supra."

Cases in other jurisdictions have made the same rule of law.

Snavely et ux. v. City of Goldendale et al. (1941) 10 Wash. (2d) 453, 117 Pac. (2d) 221:

"Generally, polluting a stream is tortious, but, if a municipality pollutes a stream on such scale as to create a public nuisance, municipality's action may assume the character of a 'taking' or 'damaging' of property within the contemplation of the constitutional guaranty that no private property shall be taken or damaged for public or private use without just compensation having first been made. Const. Art. 1, Sec. 16."

Quoting Platt Bros. & Co. v. Waterbury (1900) 72 Conn. 531, 45 A. 154, the court states:

"The right to pour into the river surface drainage does not include the right to mix with that drainage noxious substances in such quantities that the river cannot dilute them, nor safely carry them off without injury to the property of others. The later act is in effect an appropriation of the bed of the river as an open sewer, and the proposition that it may become lawful by reason of necessity is inconsistent with undoubted axioms of jurisprudence. The appropriation of the river to carry such substances to the property of another is an invasion of his right of property. When done for a private purpose, it is an unjustifiable wrong. When done for a public purpose, it may become justifiable, but only upon payment of compensation for the property thus taken. Public necessity may justify the taking, but cannot justify the taking without compensation. It may be necessary for a city to thus mix with its drainage such substances, but it is not necessary to pour such mixture into the river without purification. Indeed the purification is coming to be recognzed as a necessity. But, however great the necessity may be, it can have no effect on the right to compensation for property taken. The mandate of the Constitution is intended to express a universally accepted principle of justice, and should receive a construction in accordance with that principle, broad enough to enable the court to protect every person in the rights of property thus secured by fundamental law."

A decision rendered in Barber v. State (1938) 135 Fla. 637, 185 So. 319 stated that although a city may not be compelled to erect a treatment plant, the discharge of effluent so as to ruin oyster leases is a "taking" of private property and is compensable. To the same effect

see Douglass v. City of Lakeland (1940) 143 Fla. 771, 197 So. 467.

FRESH WATER—TIDAL WATER

There is a marked and well-defined distinction between the pollution of a small nonnavigable stream and the pollution of large tidal navigable bodies of salt water, for the reason that in the first case the bed of the stream and the waters are owned by the riparian owners, while in the latter case the bed of the navigable, tidal salt water and the waters themselves are owned and controlled by the state, for the use and benefit of all the public. It is for the state to say what uses shall be made thereof and by whom, subject always to the right of the public, and for the state, through the legislative branch of the government, to say how much pollution it will permit to be emptied into and upon its waters, so long as the owners of the land between low-water and high-water marks are not injured.

In Squaw Island Freight Terminal Co., Inc. v. City of Buffalo (1937) 273 N.Y. 119, 7 N.E. (2d) 10, the rule was stated:

> "The general rule is that a riparian owner in a nonnavigable fresh water stream is entitled to have the water that passes his land free from pollution. Such riparian owners, therefore, are entitled to an injunction against a city which pollutes the water of such a stream. Sammons v. City of Gloversville, 175 N.Y. 346, 67 N.E. 622. As to tidal waters, the rule is that riparian owners are not entitled to have such waters free from pollution. Seaman v. City of New York, 176 App.Div. 608, 161 N.Y.S. 1002, affirmed, 227 N.Y. 572, 124 N.E. 901; Darling v. City of Newport News, 249 U.S. 540, 39 S.Ct. 371, 63 L.Ed. 759. The underlying basis for this exception as to tidal waters is that the state is the absolute owner of the bed of the stream and may grant rights therein of benefit to the public, subject only to the right of the public to use the waters for purposes of navigation. 9 R.C.L. 682. . ."

United States v. Republic Steel Corp. et al. (1960) 362 U. S. 482 was a suit by the United States to enjoin Republic Steel Corporation, Interlake Iron Corporation, and International Harvester Company from depositing industrial solids in the Calumet River (which derives flow from Lake Michigan and connects eventually with the Mississippi River) without first obtaining a permit from the Chief of Engineers of the Army. The District Court found that the defendants, who operate mills for the production of iron and related products, discharged through sewers into a navigable river of the United States industrial-wastes solids which, on settling out, had substantially reduced the depth of the channel. The court enjoined the companies from continuing this disposal practice and ordered them to restore the depth of the channel by removing the deposits. The U. S. Supreme Court in a close decision held that the discharge into a navigable river of industrial solids which reduces the depth of a channel creates an "obstruction" to the "navigable capacity" of the river within the meaning of Section 10 of the Rivers and Harbor Act of 1899. The Supreme Court held further that the discharge of such industrial solids suspended in water flowing into a river through sewers is a discharge forbidden by Section 13 of the above Act, and is not exempted as "refuse matter" flowing from sewers and passing therefrom in a liquid state.

MEASURE OF DAMAGES

The scope of recovery of damages in actions for the pollution of water is governed by the same legal principles that apply in most fields of the law, i.e., that the person injured by the pollution is entitled only to recover damages sufficient to compensate him for the injury done. Cases in various jurisdictions have applied this rule.

Oates v. Algodon Mfg. Co. (1940) 217 N.C. 488, 8 S.E. (2d) 605:

> "In an action to abate a nuisance and to recover damages allegedly caused by the wrongful pollution of a stream flowing through plaintiff's farm, an instruction that the measure of damages would be the difference between the reasonable market value of the land immediately before and after the injury was erroneous. Where damages to land is due to a cause that may be removed or a nuisance that may be abated, such as the flooding of land, the measure of damage is not the difference in the market value of the land before and after the injury, but is estimated by comparing its productiveness before and after the flooding."

Oklahoma City v. Tyeteniez (1935) 175 Okla. 228, 52 Pac. (2d) 849:

> "It is a general rule that where an upper riparian owner has caused the pollution of a water course to the injury of a lower riparian owner and the injury resulting from the pollution is of a temporary character and constitutes a nuisance abatable, the measure of damages is the difference between the rental or usable value of the property free from and subject to the nuisance, together with such special damages as may be established."

Newkirk v. City of Tipton (1939) 234 Mo. App. 920, 136 S.W. (2d) 147:

> "The appropriation by city of use of stream for sewer purposes does not deprive a lower riparian owner of his right to compensation for damages suffered by him as result thereof, and measure of damages to which city must respond in case either of condemnation or appropriation without condemnation is a diminution in market value of land damaged thereby."

United States v. Fixico (1940) 115 F. (2d) 389:

> "In one of the cases which was reviewed plaintiff seeks past and future damages for permanent injury to his restricted allotted land by pollution arising out of the operation of oil wells, it being alleged, among other things, that the fertility of the soil has been permanently impaired; and in the other case plaintiffs seek damage for the depositing of sewage at such place and in such manner that it ran and runs upon and across the restricted allotted land, it being alleged, among other things, that the land has become impregnated with the sewage, that a water course traversing

the land has become infected and polluted so that it will poison livestock drinking the water, that a well on the land has become polluted, that if a new well were dug it would be likewise polluted, that offensive, noxious and poisonous gases are generated, that a stench arises which renders their home unfit for habitation, and that such conditions prevent the use of the land as a farm. Ordinarily in a case where the source or cause of the pollution is not abatable the measure of recovery is the difference in value of the land immediately before and immediately after the injury occurs. Sinclair Oil & Gas Co. v. Allen, 143 Okla. 290, 288 Pac. 981; Mid-Continent Petroleum Corp. v. Fisher, 183 Okla. 638, 84 Pac. (2d) 22. And where the injury is permanent in nature, plaintiff may recover judgment for such damages as he has already suffered, and in addition such further damages as he will sustain in the future. Comar Oil Co. v. Hackney, 119 Okla. 285, 250 Pac. 93; H. F. Wilcox Oil & Gas Co. v. Murphy, 186 Okla. 188, 97 Pac. (2d) 84. The rendition and discharge of such a judgment creates or recognizes the existence of a right somewhat difficult to define with precision but which is measurably akin to that of an easement. Luama v. Bunker Hill & Sullivan Mining & Concentrating Co., 9 Cir., 41 F. (2d) 358. And in Oklahoma an easement granting certain rights over land is an interest in the land. Missouri State Life Ins. Co. v. Whisman, 181 Okla. 168, 73 Pac. (2d) 130."

Masonite Corporation v. Steede (1945) 198 Miss. 530, 21 So. (2d) 463:

"Where the number of patrons of the fishing resort of a riparian owner was reduced by the killing of the fish in the stream by wood fiber effluent from a manufacturing plant, the owner of the fishing resort can recover from the owner of the manufacturing plant profits he claims to have lost by reason thereof only, if at all, when it clearly appears that because of the killing of the fish his profits from the business thereafter were less than they would have been had the fish not been killed."

POLLUTION FROM CEMETERIES

Two Texas cases and one Washington case have had the occasion to consider the effect of the burial of the dead on the pollution of ground waters. In Clark v. Sunset Hills Memorial Park (1954) 273 Pac. (2d) 645, the Washington case, an action was brought to enjoin the operation of a cemetery located in the vicinity of residences of the plaintiffs. The evidence sustained the finding that the fear of plaintiffs that burial of the dead in the cemetery would pollute the water in wells on the realty of plaintiffs was wholly unfounded. The court stated:

"Appellants next contend that, irrespective of the statutory questions involved in this case, the maintenance of the respondents' cemetery constitutes a nuisance which should be abated. At the trial, appellants sought to show that the existence of the cemetery on high ground above the sites of their several water wells created a danger of pollution and contamination. In this connection, the trial court made the following finding of fact:

" 'That the land of each of the plaintiffs, except the plaintiffs Love and Hardgrove, is supplied with water from wells situated upon their property. That the evidence overwhelmingly established that the maintenance and use of the property described in paragraph II, above, owned or under option to the defendants, as a cemetery or for cemetery purposes, or for the burial of dead human remains therein, will not contaminate or pollute the wells of the plaintiffs, or other persons similarly situated, nor render the water of said wells unfit for human or animal consumption, and that the fears, if any, claimed by some of the plaintiffs in that regard are wholly unfounded.'

"This finding is strongly supported in its entirety by the expert testimony of a professor of sanitary engineering at the University of Washington, a public health engineer employed by the Washington State Health Department, the Supervisor of Sanitation for King County, and an eminently qualified pathologist."

This ruling was also adopted in Eternal Cemetery Corp. v. Tammen (1959) 324 S.W. (2d) 562 (Texas) in which the court held that the evidence introduced was insufficient to support plaintiffs contention that the burial of human bodies would contaminate the water wells and stock tanks. The Court also cited the earlier case of Jones v. Highland Memorial Park (1951) 242 S.W. (2d) 250 (Texas) where, although the possibility of pollution of underground waters by the presence of human bodies was not ruled out, the court held that the evidence was insufficient to establish firmly that the proposed cemetery would be operated in such a manner as to cause irreparable injury.

PROXIMATE CAUSE

To establish liability of defendants for damages resulting from the pollution of a stream, there must be evidence reasonably tending to prove that the defendants either caused the pollution or contributed to it.

". . . An examination of the record reveals that there was abundant proof of the fact that the plaintiff's crop was damaged and destroyed by flood water containing oil, salt water, and refuse deposited upon plaintiff's premises by an overflow of the North Canadian river at the time alleged in the plaintiff's petition. There was also evidence that the defendants, with the exception of Skelly Oil Company and Amerada Petroleum Corporation, were operating producing oil wells in the drainage basin of the North Canadian river and that said wells were located about ten miles upstream from the land of the plaintiff. However, the record shows that the only evidence which sought to connect the defendants with the pollution of the stream was that of one witness to the effect that some of the pollution was known to come from the Oklahoma City Oil field and of another to the effect that all producing oil and gas wells have more or less oil, salt water, and waste and refuse around them. There was no evidence to show directly or immediately that any oil, salt water, waste, or refuse had been permitted to escape from the wells and premises of the defendants or either of them. The most that can be said of the plaintiff's evidence

in this respect is that it establishes the possibility that some of the pollution might have come from the wells of the defendants. This was insufficient to charge the defendants with liability. As said in Magnolia Pet. Co. et al. v. Dexter, 177 Okla. 153, 57 Pac. (2d) 1155: 'Where there are several oil leases on a section of land that drains into a stream, and the stream is polluted, no lessee can be held liable without showing that polluting materials from its lease went into the stream.' " Indian Territory Illuminating Oil Co. v. Henning (1937) 179 Okla. 462, 66 Pac. (2d) 83.

In Magnolia Petroleum Co. v. Dexter (1936) 177 Okla. 153, 57 Pac. (2d) 1155, the rule was thusly stated:

"The principal question and one determinative of the case, relates to the sufficiency of the evidence to show that either of the companies contributed to the pollution. The law is definitely settled in this jurisdiction that all persons who participate in a pollution are jointly and severally liable, although there is no concurrence in action, but it is equally as well settled that a party cannot be held liable unless he contributes to the pollution. Tidal Oil Co. v. Pease, 153 Okla. 137, 5 Pac. (2d) 389; Comar Oil Co. v. Sipe, 133 Okla. 222, 271 p. 1010. In the instant case there was not a word of evidence to show that any polluting material from the lease of any one defendant entered into the stream. As far as the Texas Company and Skelly Oil Company were concerned, they were not even mentioned in the evidence, and yet their demurrers to the evidence and motions for directed verdicts were overruled and judgment rendered against them. As to the other companies, the plaintiff testified that they had wells on section 6, and that the drainage from the section was to the streams that were polluted. Plaintiff said he had been on section 6, but did not fix the time. There is testimony that the streams were polluted. Plaintiff said that there was drainage from this section into the stream, but there is not a word of testimony showing that polluting materials came from any particular lease. There is nothing to show that other companies did not have leases in the section; only the fact that the Magnolia, Carter, Coline, and Roxana had leases there at some unknown time in the past. There is nothing to show that either company polluted the streams."

In Cities Service Gas Co. v. Eggers (1940) 186 Okla. 466, 98 Pac. (2d) 1114, the court stated:

"In action by owner of farm for pollution of subterranean waters by seepage from creek into which deleterious substances from oil and gas wells had been discharged, petition alleging that defendants brought to surface crude oil, salt water, basic sediment, and other deleterious substances and permitted them to accumulate and escape from their premises into the creek, was sufficient to allege commingling of salt water by the various defendants, so as to authorize instruction that, where separate independent acts or negligence of several persons combined to produce directly a single injury, each is responsible for the entire result.

"It is common knowledge that salt water entering a stream from various sources will commingle therein and thus combine to cause any resulting injury."

SUMMARY AND CONCLUSIONS

Water pollution has become a matter of gravest public and private concern. As seen in the cases mentioned herein, important private and public interests in a wide variety of circumstances are in seeming conflict and require consideration of ultimate state public policy in determining the extent and character of water pollution to be permitted in general and specific cases. Such determination involves extremely technical, complicated, and scientific problems that reasonably might be thought more suitable for resolution by administrative procedures than by the trial of particular damage claims, the varying decisions of which may not always provide specific standards.

While the writer has found no cases in which the courts have accepted one set of standards of quality in preference to another, it is significant to note that there have been many cases in which the courts have stated, expressly or inferentially, that a scientific approach to the problem based on expert evaluation of carefully derived criteria is the most reliable method of arriving at a solution of the problem in the case. Where scientific data have not been available the courts have been obliged to resort to less desirable categories of proof in the form of circumstantial or indirect evidence, judicial notice, and even standards as expressed in criminal statutes.

The courts are ready to accept the constitutionality of water-quality standards or requirements established by public authority and administered through public agencies operating under well-defined procedures best suited for the geographical areas involved. The courts have recognized that the determination of specific issues of pollution is best left with the administrative agency and the courts are content to deal only with the adjudication of individual rights as they become the subjects of lawsuits.

The judiciary appears to be only too anxious to be ousted from the undesired position of having to determine arbitrarily that a condition of pollution exists or does not exist based on the testimony of laymen and influenced by nontechnical, albeit persuasive, indirect evidence. The absence of any litigation or reported cases in which duly promulgated standards or requirements were held up to the searching light of judicial inquiry to determine their reasonableness indicates that the courts are uniformly anxious to have, and have readily adopted, scientifically developed criteria to assist them in their determination.

The existence of definitive criteria, however, is not enough. It is hoped that the delineation of the cases in this chapter will have demonstrated that, while private litigation has its own role to play in the courts, the judiciary can be aided materially in a context of personal litigation through reliance on a proper legal presentation of the data established in determining whether an actionable condition has been proved and how it can best be compensated.

CHAPTER V

QUALITY CRITERIA FOR THE MAJOR BENEFICIAL USES OF WATER

As the most abundant compound on the face of the earth, water is called upon to serve many purposes ranging from internal consumption by living organisms, through agricultural and industrial uses, to the exploitation of its remarkable powers to transport and assimilate the wastes of civilization. The many purposes that water serves in promoting the economic good and well-being of mankind are known as "beneficial uses." They have been listed and cited by Camp (151), Bacon (2323), and others. By combining these references and rephrasing, the following tabulation of the major beneficial uses of water has been established. It should be recognized that these uses are not listed in the order of economic importance nor in the order of increasing or decreasing water quality.

- Domestic water supply
- Industrial water supply (including cooling water)
- Agricultural water supply (irrigation)
- Stock and wildlife watering (including refuge for water fowl)
- Propagation of fish and other aquatic and marine life
- Shellfish culture
- Swimming, bathing, and other water-contact sports
- Boating and esthetic enjoyment
- Water power and navigation
- Transport, dispersion, and assimilation of wastes.

The relative importance of these beneficial uses for any stream, lake, open ocean, estuary, or ground-water basin depends on the economy of the area and the desires of the inhabitants. Many of the uses, such as domestic water supply and industrial-process water supply, may be compatible within narrow ranges of water quality, whereas the use of a stream for unregulated waste disposal will be at complete variance with its use for domestic water supply.

It is the intent of this chapter to present the findings and recommendations contained in the principal references dealing with each major beneficial use of water. Only the more significant sources of literature pertaining to each beneficial use have been condensed and summarized. Bits of data from numerous other sources have not been blended into these principal references. Instead, such data are presented under appropriate headings in the chapters that follow. By no means, therefore, should these summaries be considered to be complete with respect to every potential pollutant. There are numerous substances listed in the succeeding chapters (VI, VII, VIII, IX, and X) that are not cross-filed in this chapter. In using and assessing Chapter V, therefore, the reader is urged to make frequent reference to the specific substances in the chapters that follow.

DOMESTIC WATER SUPPLY

The use of water by human beings for drinking and other domestic purposes is conceded generally to be the primary, highest, or most essential use of water. Such water, to be sure, may be given the primary procurement rights and may be afforded the highest degree of sanitary protection, but other beneficial uses may have water-quality requirements that are far more stringent in certain respects than those for drinking water. Many industrial processes, for example, cannot use without further treatment water that is suitable for domestic supplies; softened water from municipal systems is often detrimental for irrigation; and aquatic life in streams and lakes may be destroyed or inhibited by concentrations of copper or zinc that are permissible in domestic water.

In evaluating water-quality criteria for domestic use, two conditions of the water must be considered: (a) its quality at the source of supply, be it surface water or ground water, and (b) its quality at the tap or point of use. In former years when water of pristine purity was available from protected watersheds, the two conditions were virtually synonymous, inasmuch as no treatment was provided or deemed necessary. Ground-water supplies, for the most part, still enjoy the luxury of no treatment; but disinfection, softening, carbonate-equilibrium control, and other methods of treatment are gaining favor rapidly, even for supposedly protected supplies.

It is not the intent of this report to deal specifically and thoroughly with the quality of treated water as delivered to the consumer, inasmuch as this study is expected to cover only the quality of water found in natural water courses. Yet, the quality of the treated delivered water is partly relevant to this report, for there are many substances in such water that are not altered readily by present methods of treatment, i.e., their concentration in the treated water is essentially the same as in raw water.

The domestic water-supply industry is caught between two strong trends in water quality. On the one hand, domestic and industrial customers are demanding improved quality and greater uniformity in the water delivered at the tap. Indeed, many water purveyors are leading a crusade towards even higher goals of water quality for the consumers (1745, 1746). On the other hand, the sources of raw water have been deteriorating under the inexorable impacts of modern civilization and may be expected to get worse in future years. The waterworks industry, therefore, must produce a better and more uniform product while drawing upon a poorer and less predictable source of supply. Fortunately, there

are many recent improvements in technology that are capable of bridging the widening gap in quality between raw and finished waters. One of the greatest needs today in water-supply technology is reliable quantitative information relative to the potential harmful effects of trace substances in raw and treated water.

DRINKING WATER STANDARDS (U.S.A.)

The physical, chemical, and bacterial quality of drinking water in the United States is now judged in relation to the Public Health Service Drinking Water Standards of 1962 (2036). Originally promulgated in 1914, these standards were modified and reissued in 1925, 1942, and 1946 prior to the 1962 edition. A comparison of the chemical constituents of these standards is shown in Table 5-1.

Strictly speaking, the USPHS standards apply only to drinking water and water-supply systems used by interstate carriers and others subject to Federal Quarantine Regulations. They have been voluntarily accepted, however, by the American Water Works Association and by most of the state departments of public health as criteria for all public water supplies. The extent to which they can be enforced within any state depends on the state and local laws.

A complete statement of the 1962 standards is contained in public documents (2036) and will not be repeated herein. Instead, the principal provisions dealing with quantitative limits are condensed and summarized below:

1. Bacterial quality. The minimum number of samples to be collected from the distribution system and analyzed each month, and the numbers of these samples and portions thereof that may show the presence of coliform organisms are specified. In effect, these requirements limit the average monthly coliform content to a most probable number (MPN) or membrane filter count of one coliform per 100 ml. For a further discussion of bacterial quality in treated and raw waters, see Coliform Bacteria in Chapter VII.

2. Physical characteristics. The following limiting concentrations are recommended for water in the distribution system of a water supply:

a. Turbidity (silica scale), not to exceed 5 units

b. Color (standard cobalt scale), not to exceed 15 units

c. Threshold odor number, not to exceed 3

3. Chemical characteristics. The limits for chemical elements or compounds in water are divided into mandatory requirements for certain substances and recommended criteria for others. The mandatory limits are shown in the first part of Table 5-1. For further information dealing with the validity or reasonableness of these mandatory limits, reference is made to the specific substances listed in Chapter VI. The rationale by which such standards are established is discussed by Hopkins (2324), by Derby et al. (2062), by Stokinger and Woodward (2325), by Welsh and Thomas (2326), and by the advisory committee (2327).

The non-mandatory but recommended limits for less critical substances are shown also in Table 5-1. For three substances (arsenic, cyanide, and fluoride) recommended limits are given in addition to the maximum permissible concentrations. Thus, an arsenic limit of 0.01 mg/l is recommended, but a concentration of not more than 0.05 mg/l will be permitted if no alternative source of supply is available.

TABLE 5-1

COMPARISON OF CHEMICAL SUBSTANCES IN USPHS DRINKING WATER STANDARDS OF 1925, 1942, 1946, AND 1962

Substance	Concentrations in mg/l			
	1925	1942	1946	1962
A. Maximum permissible concentrations:				
Arsenic**	--	0.05	0.05	0.05
Barium	--	--	--	1.0
Cadmium	--	--	--	0.01
Chromium (hexavalent)	--	0.0	0.05	0.05
Copper	0.2	*	*	*
Cyanide**	--	--	--	0.2
Fluoride**	--	1.0	1.5	1.6-3.4#
Lead	0.1	0.1	0.1	0.05
Selenium	--	0.05	0.05	0.01
Silver	--	--	--	0.05
Zinc	5.0	*	*	*
B. Recommended limiting concentrations (provided that other more suitable supplies are or can be made available):				
Alkyl benzene sulfonates	--	--	--	0.5
Arsenic**	--	--	--	0.01
Carbon chloroform extract	--	--	--	0.2
Chloride	250	250	250	250
Copper	*	3.0	3.0	1.0
Cyanide**	--	--	--	0.01
Fluoride**	--	--	--	0.8-1.7#
Iron	0.3	0.3	0.3	0.3
Manganese	--			0.05
Magnesium	100	125	125	--
Nitrate, as NO_3	--	--	--	45
Phenolic compounds, as phenol	--	0.001	0.001	0.001
Sulfate	250	250	250	250
Total dissolved solids	1000	500	500	500
Zinc	*	15.0	15.0	5.0

* Maximum permissible concentrations were replaced by recommended limits after the 1925 standards.

**These substances have both recommended limits and maximum permissible concentrations.

\# Recommended limits and maximum permissible concentrations for fluoride vary with the annual average of maximum daily air temperature, from the lowest concentrations at 79.3-90.5°F to the highest at 50.0-53.7°F.

The changes in each subsequent edition of the standards have not been drastic, but there are certain noticeable trends. With each edition there are more substances that come under the recommended or maximum permissible limitations. In general, moreover, there is a recent tendency to tighten up on the limitations. Zinc, for example, started out at 5.0 mg/l mandatory, changed to 15.0 mg/l recommended, and recently reverted to 5.0 mg/l as a recommended limit. Arsenic was limited in 1942 to 0.05 mg/l maximum and was still at this level in 1962 for the permissible concentrations. It is now recommended, however, that arsenic be restricted to 0.01 mg/l unless an alternative supply is not available. Similarly, the restrictions on copper were relaxed from 0.2 mg/l in 1925 to 3.0 mg/l in 1942 and 1946; but in 1962 the recommended standard was tightened to 1.0 mg/l.

Radioactivity became an official criterion of the USPHS standards for the first time in 1962. In general, water supplies may be approved when "the radioactivity intake from such water supplies when added to that from all other sources is not likely to result in an intake

greater than the radiation protection guidance recommended by the Federal Radiation Council and approved by the President.

"Water supplies shall be approved without further consideration of other sources of radioactivity intake of Radium-226 and Strontium-90 when the water contains these substances in amounts not exceeding 3 and 10 μμc/liter, respectively. When these concentrations are exceeded, a water supply shall be approved by the certifying authority if surveillance of total intakes of radioactivity from all sources indicates that such intakes are within the limits recommended by the Federal Radiation Council for control action.

"In the known absence of Strontium-90 and alpha emitters, the water supply is acceptable when the gross beta concentrations do not exceed 1,000 μμc/liter. Gross beta concentrations in excess of 1,000 μμc/liter shall be grounds for rejection of supply except when more complete analyses indicate that concentrations of nuclides are not likely to cause exposures greater than the radiation protection guides as approved by the President on recommendation of the Federal Radiation Council" (2036). It is apparent, therefore, that any criteria of radioactivity in drinking-water standards are dependent upon the current decisions of the Federal Radiation Council.

DRINKING WATER STANDARDS (OUTSIDE OF U.S.A)

Except for the United States, only a few countries have published national guidelines for drinking water quality (153, 154, 155, 1457, 1545). Recently, however, the World Health Organization (WHO) has promulgated the 1958 International Standards for Drinking Water and the 1961 European Standards for Drinking Water. The WHO standards arose from the Seminars for European Sanitary Engineers sponsored by the World Health Organization and the Rockefeller Foundation. Corti (1648) discussed circumstances favorable and antagonistic to the development of international standards. He recommended that continuing research be directed along two separate lines; first to define requirements for good drinking water, and second to develop reliable laboratory techniques for adequate quantitation of water quality. The WHO group that met in Geneva in 1956 to consider requirements for water quality believed that the term "standards" should be applied to their suggested criteria even though such standards were tentative and subject to modification after experience in their application. This same group also studied various analytical procedures. Approved methods of analysis are described in the annexes of the WHO International Standards for Drinking Water (2328).

Concurrent with the development of the International Standards, the WHO Regional Office for Europe sponsored meetings in 1956 and 1959 that led to the publication of European Standards for Drinking Water (2329). The preface of this document points out the reasoning by which WHO has issued both international standards and European standards as follows: "International Standards for Drinking-Water proposes minimal standards which are considered to be within the reach of all countries throughout the world at the present time. In view of the different economic and technological capabilities of various countries there will be some areas in which higher standards than those proposed for the world as a whole will be attainable—and these areas should be encouraged to attain such higher standards. It is believed that Europe is such an area and that there is, therefore, nothing illogical in setting higher standards in Europe than internationally. One of the objects of having standards at all is to stimulate improvement in water quality, and it is hoped . . . that improvement in economic and technological resources throughout the world will allow higher standards to be suggested in the future than those at present proposed for the whole world" (2329).

To describe the international standards and European standards in detail is beyond the purview of this report. It is interesting, however, to compare the two WHO standards with the 1962 USPHS standards. Table 5-2 presents this comparison for chemical constituents. In using this table, one should bear in mind that all figures are not directly comparable, for different analytical methods may be involved. Before drawing any conclusions from this table, the reader is advised to consult the original texts.

With respect to bacteriological criteria, the WHO international standards are approximately equal to the USPHS standards in requiring that the arithmetic mean coliform density should not exceed one organism in 100 ml of water. The WHO European standard, however, is considerably stricter, limiting the average coliform density to one organism in two liters of water, or one-twentieth the density in the USPHS and international standards.

The radiological requirements of the WHO standards for drinking water, both international and European, are based upon the recommendations of the International Commission on Radiological Protection. They set the following upper limits in drinking water as supplied to consumers for life-time use for large populations:

alpha-emitters	1 picocurie/liter
beta-emitters	10 picocuries/liter

Note that a picocurie is a micromicrocurie; hence the WHO limit for beta-emitters corresponds to the USPHS limit for beta emission from strontium-90. If these limits are not exceeded, no further radiochemical analysis is required by the WHO standards. "Higher figures than those given above are safe for many radionuclides, and a water with a higher total radioactivity than the figures given above may be safe for use if the absence of the more dangerous radionuclides can be confirmed by radiochemistry" (2328, 2329). Neither the WHO nor USPHS standards give permissible limits for short-time use or for small populations.

RAW WATER STANDARDS

Of particular interest to the sanitary engineer engaged in water-pollution control are the standards or criteria that have been promulgated for natural waters used as sources of domestic supply. Such standards have been issued by the U. S. Public Health Service, as well as by several state and interstate agencies, notably the New England Interstate Water Pollution Control Commission, the Interstate Commission on the Potomac River Basin, the Pollution Control Council, Pacific Northwest

TABLE 5-2

COMPARISON OF CHEMICAL CONSTITUENTS IN THE DRINKING WATER STANDARDS OF THE WORLD HEALTH ORGANIZATION AND THE U.S. PUBLIC HEALTH SERVICE

	Concentrations In Milligrams Per Liter						
	WHO International (1958)			*WHO European (1961)*		*U.S.P.H.S. (1962)*	
Chemical Constituent	*Permissible Limit*	*Excessive Limit*	*Maximum Allowable*	*Recommended Limit*	*Tolerance Limit*	*Recommended Limit*	*Maximum Allowable*
Alkyl benzene sulfonate	--	--	--	--	--	0.5	--
Ammonia (NH_4)	--	--	--	0.5	--	--	--
Arsenic	--	--	0.2	--	0.2	0.01	0.05
Barium	--	--	--	--	--	--	1.0
Cadmium	--	--	--	--	0.05	--	0.01
Calcium	75	200	--	--	--	--	--
Carbon chloroform extract	--	--	--	--	--	0.2	--
Chloride	200	600	--	350	--	250	--
Chromium (hexavalent)	--	--	0.05	--	0.05	--	0.05
Copper	1.0	1.5	--	3.0*	--	1.0	--
Cyanide	--	--	0.01	--	0.01	0.01	0.2
Fluoride	--	--	--	1.5	--	0.8-1.7#	1.6-3.4#
Iron	0.3	1.0	--	0.1	--	0.3	--
Lead	--	--	0.1	--	0.1	--	0.05
Magnesium	50	150	--	125**	--	--	--
Magnesium + Sodium sulfate	500	1000	--	--	--	--	--
Manganese	0.1	0.5	--	0.1	--	0.05	--
Nitrate (as NO_3)	--	--	--	50	--	45	--
Oxygen, dissolved (minimum)	--	--	--	5.0	--	--	--
Phenolic compounds (as phenols)	0.001	0.002	--	0.001	--	0.001	--
Selenium	--	--	0.05	--	0.05	--	0.01
Silver	--	--	--	--	--	--	0.05
Sulfate	200	400	--	250	--	250	--
Total solids	500	1500	--	--	--	500	--
Zinc	5.0	15	--	5.0	--	5.0	--

* After 16 hours contact with new pipes; but water entering a distribution system should have less than 0.05 mg/l of copper.
** If there are 250 mg/l of sulfate present, magnesium should not exceed 30 mg/l.
# Recommended limits and maximum allowable concentrations vary inversely with mean annual temperature. See table 5-1.

Area, and the states of Maine, Montana, New Hampshire, New York, Oklahoma, South Carolina, and Utah. For a complete description or tabulation of the standards or criteria used by each interstate agency or state, see Chapter III and the appropriate appendices. The Public Health Service recommended sanitary requirements for water treatment systems is reproduced herein as Appendix Q.

Inasmuch as these standards tend to follow a pattern, they have been assembled and summarized in Table 5-3, "Ranges of Promulgated Standards for Raw Water Sources of Domestic Water Supply." The table is presented as a summary of current thought on the subject. No attempt is made at evaluation of the reasonableness or unreasonableness of each standard. For further background information relating to each specific pollutant, see Chapters VI and VII.

Earlier studies of the efficacy of water-treatment plants on the removal of bacteria have shown that an average plant using rapid sand filtration and supplying a low chlorine residual by post chlorination could provide a satisfactory drinking water if the average concentration of coliforms in the raw water did not exceed 5000 per 100 ml. At an average concentration of 20,000 per 100 ml, it was found that supplementary treatment, such as prechlorination and double-stage sedimentation, was required (141). Similar studies showed that for waters requiring only simple chlorination, ". . . the average *B. coli* index of the raw water, when considered over a similarly long period of time, should not exceed 400 per 100 cc. for more than 5% of the time . . ." (156, 157).

INDUSTRIAL WATER SUPPLY

The total use of water in this county by industries for all purposes was estimated in 1950 at 70 billion gallons per day, as compared with 15 billion gallons per day for municipal and rural non-irrigation use and 95 billion gallons per day for irrigation use (158). Industrial demands for water are increasing more rapidly than irrigation requirements, so before long industrial use of water will be the largest category. The great magnitude of the industrial demand demonstrates the extreme importance of water to industry. Water is used as an ingredient with other raw materials in the finished product, as a bouyant transporting medium, as a cleansing agent, as a coolant, and as a source of steam in heating and power production. The quantities of water used per unit of product have been published for many industries (161, 2330, 2331) and predicted for others (2332) but these figures represent averages only. The actual use by an industry in a specific location may be considerably greater or less than these averages. Inasmuch as quality, rather than quantity, is the prime consideration of this report, no attempt will be made to summarize data on quantity requirements.

The ideal quality of water required for industrial use varies widely for the many purposes to which water is put. Needless to say, it is impossible to organize the quality requirements of the waters used for each of the many different industrial processes into a single set of standards. Such quality requirements differ far too much to allow any broad generalization or simplification. Within any industrial plant, water may have several functions, the quality requirements for which vary markedly. A brewery, for example, needs soft water for bottle washing but can utilize hard water for brewing. Many industries require water of one quality for boiler feed, another quality for cooling towers, and a third quality for production processes.

Industries are generally willing to accept for most processes water that meets drinking-water standards. Where water of higher quality is needed, e.g., for television-picture-tube manufacture, certain food and beverage preparation, or for high-pressure boilers, industry recognizes that additional treatment is the responsibility of the water user (2333).

One characteristic, however, is of primary importance for all industries, namely, the concentrations of the various constituents of the water should remain relatively constant. That the water is originally of poor quality for a particular industrial use is probably not as important, once a process is started and the difficulties created by the presence of undesirable constituents in water are eliminated, as having the quality remain constant. Short-time variations in concentrations of substances in the process water require continued attention and added expense (2334, 2335, 2336).

Although many studies have been made of the quality requirements of water for use in certain industries, there remain innumerable other industries for which the requirements of water quality have not been specified in public documents except in a general and qualitative way. Perhaps the most thorough study of the water-quality requirements of various industries has been that conducted by the Committee on Water Quality Tolerances for Industrial Uses, of the New England Water Works Association. The committee presented an extensive report in June, 1940, containing tables of tolerance limits for waters for a number of industries, and text material describing the effects of various harmful substances in water on industrial operations (159). Much of this information is incorporated in the book "Water Quality and Treatment" of the AWWA (152) and many of the data appear under the appropriate sub-heading in this part of Chapter V. The NEWWA table has been reproduced in several other published articles.

In 1953 the American Society for Testing Materials published the first edition of its "Manual on Industrial Water," ASTM Special Technical Publication No. 148 (1654). There have been subsequent printings. In this book, one chapter, entitled "Uses of Industrial Water," refers to the chemical, steel, paper, leather, textile, and food and beverage industries. It tells how water is used by each industry without describing the quality requirements for each industry or each process therein.

Another chapter describes "Difficulties Caused by Water in Industry," noting that "industrially speaking, water is an essential, but troublesome raw material." The three major categories of difficulties, with descriptive subheadings, are presented in this manner:

A. Effect on Product
   1. Decay, resulting from biological action
   2. Staining
   3. Corrosion
   4. Chemical reaction and contamination

TABLE 5-3

RANGES OF PROMULGATED STANDARDS FOR RAW WATER SOURCES OF DOMESTIC WATER SUPPLY

Constituent	Excellent source of water supply, requiring disinfection only, as treatment	Good source of water supply, requiring usual treatment such as filtration and disinfection	Poor source of water supply, requiring special or auxiliary treatment and disinfection
BOD (5-day) mg/l			
Monthly average:	0.75-1.5	1.5-2.5	Over 2.5
Maximum day, or sample:	1.0-3.0	3.0-4.0	Over 4.0
Coliform MPN per 100 ml			
Monthly average:	50-100	50-5,000	Over 5,000
Maximum day, or sample:	Less than 5% over 100	Less than 20% over 5,000	Less than 5% over 20,000
Dissolved Oxygen			
mg/l average:	4.0-7.5	4.0-6.5	4.0
% saturation:	75% or better	60% or better	--
pH			
Average:	6.0-8.5	5.0-9.0	3.8-10.5
Chlorides, max. mg/l	50 or less	50-250	Over 250
Fluorides, mg/l	Less than 1.5	1.5-3.0	Over 3.0
Phenolic compounds, max. mg/l	None	0.005	Over 0.005
Color, units	0-20	20-150	Over 150
Turbidity, units	0-10	10-250	Over 250

B. Deterioration of Equipment
   1. Corrosion
   2. Erosion
   3. Cavitation
   4. Water hammer
   5. Scale deposition

C. Reduction of Efficiency or Capacity
   1. Tuberculation
   2. Sludge formation
   3. Scale deposition
   4. Foaming
   5. Organic growths.

There have been several excellent papers describing the general problems of industrial water quality and giving brief descriptions of the general problems for specific industries (1649, 1650, 1651, 1740, 2331, 2333, 2334, 2335, 2336, 2337, 2338).

In the discussions that follow, consideration has been given to the common and prominent industrial uses of water, where literature relating to water-quality criteria for such industries is available. These uses are listed in alphabetical order by means of sub-headings, with cross-referencing where indicated.

ALUMINUM INDUSTRY

The water requirements of the aluminum industry have been summarized in a comprehensive report by the U. S. Geological Survey (2339) from which the following material has been abstracted. No quantitative criteria of water quality are given in the USGS report.

Aluminum is made in two steps. The first process is the production from the bauxite ore of alumina, a white powder. The second step is the reduction of alumina to metallic aluminum by electrolytic action.

In alumina plants, water of high quality is required for the hydrate wash, which constitutes only about five percent of the total water demand. For other water uses, quality is not a serious consideration. Any impurities introduced in the hydrate wash tend to accumulate in the caustic soda solution and impair its function. Fortunately, modern alumina plants employ multiple-effect evaporators to concentrate the sodium aluminate solution and the vapor from these evaporators can be condensed and recovered for use in the hydrate wash.

In reduction plants, the major need for water is in the sprays used for scrubbing of stack gases to minimize air pollution. Water quality is not a consideration in these sprays. Minor quantities of water are used for boiler feed, engine cooling, and other processes for which high quality is necessary.

BAKING (see also Confectionary, Food Processing)

In addition to being bacteriologically safe, water used in baking should not cause undesirable tastes, odors, colors, or interference with the complex fermentation processes. Inasmuch as calcium is necessary for some yeast actions, hardness is not necessarily a disadvantage for all baking waters. Too much hardness, however, retards the fermentation and too little softens the gluten to produce a soggy bread. For some cake and cracker baking, water of zero hardness is used. The few threshold values that have been recommended in the surveyed literature are summarized below (152, 159, 160, 161, 162, 2337).

Constituent	*Range of Recommended Threshold Values in mg/l*
Turbidity	10
Color	10
Taste and odor	none-low
Iron	0.2
Manganese	0.2
Iron and manganese	0.2
Hydrogen sulfide	0.2

BOILER FEED WATER

The problem of make-up or feed water for boilers is common to almost all industries. Its importance and complexity has made it the subject of innumerable technical articles, special studies, and recipes. A thorough review of all such references is beyond the scope of this study inasmuch as they deal mostly with the addition of chemical compounds or with other treatment methods to render the water non-corrosive or non-scale-forming. Insofar as the raw-water supply is concerned, however, the presence of many substances will render boiler feed water more difficult to treat or otherwise to handle. The suggested limits of tolerance for boiler feed waters as recommended by the aforementioned Committee on Water Quality Tolerances for Industrial Uses of the New England Water Works Association (159), are contained in Table 5-4.

For boilers of very high pressure (1000 psi or greater), all hardness must be removed, dissolved oxygen should be less than 0.05 mg/l, and total dissolved solids should be as low as possible, preferably less than 0.5 mg/l. For such purposes, carefully distilled and deaerated water is necessary, for no natural water would meet these conditions (2336, 2338, 2340). Silica is especially troublesome, for it tends to form a hard scale in boilers and boiler tubes. Moreover some of it carries over in the steam and forms troublesome deposits on the blades of steam turbines (2341, 2342). For high-pressure boilers, dissolved silica should not exceed 0.2 mg/l, although 1 to 5 mg/l can be tolerated at low pressures (2341). Aluminum is troublesome, for it also tends to be deposited as scale on boiler tubes. Excessive sodium and/or potassium may contribute to the foaming of boiler water (2342). In sugar refineries, the presence of sugar in boiler feed water has caused foaming and the production of organic acids that contribute to corrosion (2343). Ideally, no ammonia should be present, for it is very damaging to copper parts (1740). Finally, toxic boiler compounds should not be employed where steam is used in the preparation of food.

BREWING (see also Malt Preparation, Fermentation Processes)

An ancient art, brewing has become in the last century increasingly reliant upon scientific principles. The quality of beer depends largely upon the ingredients used in its manufacture, among which water is a major factor. The ions of the water have a marked influence on the brewing, the fermentation, and the quality of the finished product (2344). Indeed, many breweries have been located on the basis of water quality and in earlier days there was a marked association of particular waters with certain characteristic beers, especially in Europe (2345).

In the brewing industry, the use of water is divided into two major categories: production water and process water. Each use has different quality requirements. The water of production is used in brewing and much of it remains as the major ingredient of the beer or ale. Process water is utilized for heating by steam or for pasteurization, for cooling, for clean-up operations, and for bottle washing (2345). The quality requirements for such process water are similar to those for other industries. In general, the process water should be soft, of low mineral content, and bacteriologically safe. (See Boiler Feed Water, Cooling Water, and Food Washing Equipment)

Production water is first used in some breweries to steep grain such as barley. This water must be microbiologically sound to diminish mold germination and it must not contain any substances that inhibit germination (2345).

TABLE 5-4

SUGGESTED LIMITS OF TOLERANCE FOR BOILER FEED WATERS

(From Progress Report of the Committee on Water Quality Tolerances for Industrial Uses, NEWWA) (159)

(units are in mg/l except as otherwise noted)

Pressure (psi)	*0-150*	*150-250*	*250-400*	*Over 400*
Turbidity	20	10	5	1
Color	80	40	5	2
Oxygen consumed	15	10	4	3
Dissolved oxygen**	2.0[a]	0.2[a]	0.0	0.0
Hydrogen sulfide *	5	3	0	0
Total hardness ($CaCO_3$)	80	40	10	2
Sulfate-carbonate ratio (ASME) (Na_2SO_4:Na_2CO_3)	1:1	2:1	3:1	3:1
Aluminum oxide	5	0.5	0.05	0.01
Silica	40	20	5	1
Bicarbonate **	50	30	5	0
Carbonate	200	100	40	20
Hydroxide	50	40	30	15
Total solids [b]	3000–500	2500–500	1500–100	50
pH value (Min.)	8.0	8.4	9.0	9.6

* Except when odor in live steam would be objectionable.
** Limits applicable only to feed water entering boiler, not to original water supply.
[a] Given as ml per liter. Multiply by 0.70 for ppm.
[b] Depends on design of boiler.

Steeped barley, rice, and/or corn are next crushed and mixed with water at a suitable temperature and for a correct time to allow conversion of starch, by diastase, to a mixture of simpler carbohydrates which can be fermented to alcohol and carbon dioxide by "brewers yeast". The clear sweet wort that drains from this mashing operation is then boiled in copper kettles with hops.

The enzymic reactions at mashing are extremely dependent upon the pH value, which in turn is governed by the buffering action of phosphates and bicarbonates, both of which are affected by the calcium and magnesium content of the water. The enzyme systems operative at mashing have pH optima that range from 4.6 to 5.7. Calcium, and to a lesser extent magnesium, helps to keep the pH low by precipitation of phosphates and carbonates, but this effect is offset by high carbonates or bicarbonates in the brewing water. For this reason, brewers prefer water of low alkalinity and high calcium content (2345). Indeed, calcium chloride and calcium sulfate are generally added to production water to assure optimum enzyme reactions during mashing. These salts are also advantageous during boiling with hops (2346).

Iron is detrimental in production water because it gives a bad flavor and discolors the beer. Nitrite interferes with the preservation of beer and may be poisonous to the yeast. Nitrates are similarly undesirable, for they are partially reduced to nitrites during fermentation. Nitrogen compounds in general lead to poor or cloudy appearance of bottled beer. In excess of 30 mg/l, magnesum ion has been known to retard saccharification and to impart a reddish tint and acridness to beer (2344). The chloride ion renders the beer more mellow and sweet, but it may be harmful in concentrations of over 100 mg/l (2344) or perhaps over 60 mg/l (2347). Even small amounts of copper appear to cause beer to "gush" from a can immediately upon opening (2333).

Production water should meet the bacteriological requirements of drinking-water standards, inasmuch as light beers have only a slight bactericidal effect (2308). Dark beers and ales are generally strong enough in alcohol to be bactericidal.

Water containing 1.0 mg/l of fluoride ion, from NaF or Na_2SiF_6 has no adverse effect on production of beer (2348) and indeed at concentrations of 1 to 5 mg/l, fluoride appears to stimulate yeast metabolism (2349). At fluoride concentrations of 10 mg/l or higher, inhibitory effects were noted (2348, 2349).

The various threshold or limiting values that have been recommended in numerous references are summarized below:

Constituent	*Range of Recommended Threshold or Limiting Values, in mg/l*
Color	0-10
Turbidity	0-10
Taste and odor	none-low
Total dissolved solids	500-1500 300 (for any one substance)
pH value	6.5-7.0
Alkalinity, as $CaCO_3$	75-80 (light beer) 80-150 (dark beer)
Iron	0.1-1.0
Manganese	0.1
Iron and manganese	0.1
Carbonate	50-68
Nitrite	0
Nitrate	10
Chloride	60-100
Silica	50
Hydrogen sulfide	0.2
Fluoride	1.0
Calcium	100-200 (light beer) 200-500 (dark beer)
Calcium sulfate	100-500
Calcium chloride	100-200
Magnesium	30
Magnesium sulfate	50-200
Magnesium chloride	50-200
Sodium chloride	275-500
Sodium sulfate	100
Sodium carbonate	100

References: 152, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 2344, 2345, 2346, 2348, 2349

CANNING (see Food Canning and Freezing)

CARBONATED BEVERAGES

In general, the quality requirements for water used in carbonated beverages are more stringent than those

for drinking water or domestic supply. Very low concentrations or complete absence of microorganisms, organic matter, turbidity, color, taste and odor, iron, and manganese are desired. Fruit-juice beverages are particularly susceptible to microorganism growth. Because of the acid nature of the product, uniformly low alkalinity is an important requirement. The various threshold values that have been recommended are summarized below:

Constituent	*Range of Recommended Threshold Values in mg/l*
Turbidity	1.0-2.0
Color	5.0-10.0
Taste and odor	None-low
Alkalinity, as $CaCO_3$	50-128
Total dissolved solids	850
Hardness, as $CaCO_3$	200-250
Iron	0.1-0.2
Manganese	0.2
Iron and manganese	0.1-0.5
Chlorides	250
Sulfates	250
Fluoride	0.2-1.0
Hydrogen sulfide	0-0.2
Organic matter	Infinitesimal
Algae and protozoa	None
Oxygen consumed	1.5

References: 152, 162, 164, 173, 179, 180, 181, 182, 184, 185, 186, 187, 188, 2350

CARBON BLACK INDUSTRY

The water requirements of the carbon-black industry have been summarized in a comprehensive report by the U. S. Geological Survey (2351) from which the following material has been abstracted.

Carbon black is used chiefly as a reinforcing agent in the rubber-tire industry and as a coloring pigment in ink and paint. It is manufactured from gaseous or liquid hydrocarbons either by partial combustion followed by contact with cold metal, or by thermal decomposition in furnaces or retorts. Prior to World War I, the contact process was dominant, but in recent years over 70 percent of production comes from the furnace process.

The only process water used in the contact method is for pelleting of the finished carbon black to facilitate packaging. At some plants, carbon black is dry pelleted; at others, water is added to form a slurry prior to pelleting. Because this water is incorporated into the product, it should contain very little dissolved mineral matter.

In the furnace processes, carbon black is produced by incomplete combustion of hydrocarbons at 2,200 to 3,000° F. The carbon black is carried by the hot gases to quenching towers and subsequently to electrostatic precipitators and cyclone collectors. Practically all of the water used for gas quenching is lost by evaporation or incorporated into the product. Hence it should have a low content of dissolved solids. The USGS report does not develop quantitative limits of tolerance of dissolved solids either for pelleting or for quenching.

CHEMICAL PROCESS INDUSTRIES

Many and varied are the chemical process industries that employ water directly in the product or as an auxiliary to the process (189). Such industries are listed under separate sub-headings in this chapter. For most of the industries using water as a raw material, there are undoubtedly limiting concentrations of impurities that cannot be exceeded without damaging the product (159), but many such concentrations are so high that they are rarely encountered in normal domestic supplies. In other instances, however, special water treatment is required and sometimes distilled water must be employed. Important as water is to some of the chemical process industries, it is surprising how little information concerning it is available in the technical literature. The few references uncovered in this survey are included under separate sub-headings.

CONCRETE MIXING AND CONCRETE CORROSION

The formation of concrete from cement, sand, gravel, and water represents a chemical reaction in which the quality as well as the quantity of each ingredient is important. It is well known to most engineers that the strength of the concrete is dependent, in part, on the ratio of the water and cement used. Not so thoroughly understood, however, is the effect of polluting substances in the water on the strength and durability of the concrete. The Portland Cement Association has been investigating the effects of water quality on concrete mixing and much of the following discussion has originated from such research.

"Water used for mixing concrete should be free of acids, alkalies, and oil unless tests or experience indicate that water being considered for use and containing any of these materials is satisfactory. Particularly to be avoided are waters containing decayed vegetable matter which may interfere with the setting of the cement. Most specifications require that the mixing water be suitable for drinking; such water is usually satisfactory with the possible exception of that from certain areas where the drinking water contains large amounts of sulfates" (191). Tests have shown that the compressive strength of concrete is only slightly affected by the use of sea water for mixing. With continuous wet curing for a month or more, concrete mixed with sea water has tested 10 to 20 percent lower than similar concrete mixed with fresh water (192).

Although there are reports that lime mortar behaves oppositely, a mortar made from portland cement and sand is considerably weakened by the presence of sugar in a concentration that is equivalent to 3400 mg/l in the mixing water (193). Small-scale tests have demonstrated that the hardness of the mixing water has no effect on the air-entraining qualities of concrete inasmuch as ". . . the hardness of the igneous portion of a concrete mixture is so great as not to be appreciably affected by the hardness of the water supply . . ." (194). Some preliminary tests have been made upon the tensile strength of portland cement made with mine waters of both high and low acidity. Apparent increases in strength were noted, but it was not known whether this change was attributable to actual long-time increase in strengths or merely to an acceleration in setting time (195).

The constituents in natural water causing or suppressing corrosion of the concrete are of great importance, inasmuch as many concrete structures are exposed to the action of such waters. Unfortunately, information of the complicated corrosion phenomena is largely of a qualitative rather than quantitative nature. The rate of

flow of corrosive water over concrete surfaces and the porosity of the concrete greatly affect the rates of corrosion. It is known that most acids, both organic and inorganic, attack or at least slowly disintegrate concrete (2352, 2353, 2354). Petroleum oils and many coal-tar distillates have little or no effect on concrete although creosote, cresol, phenol, and many vegetable and animal oils may disintegrate concrete slowly. Algae in contact with submerged concrete blocks have been reported to cause disintegration of the concrete (2233).

The sulfates attack and disintegrate concrete (2355); with sodium and magnesium sulfates the rate of attack seems to be proportional to the concentration up to a steady rate at 10,000 mg/l and 5,000 mg/l respectively. The effect of sulfurous acid solutions upon concrete have been studied (196). "If the SO_3 concentration is less than 25 ppm injury from this source will be mild and inconsequential. When the SO_3 is in the hundreds of ppm it is a possible source of great harm . . ."

In addition to sulfates in natural waters of streams, lakes, ground-water basins, and bodies of salt water, consideration must be given to the free carbon-dioxide content of the waters. According to Dr. Ruth Terzaghi (197) free carbon dioxide in concentrations exceeding 20 mg/l will attack concrete sufficiently rapidly to require protective action. Such concentrations occur frequently in ground water, in deep lakes, and possibly in marine estuaries (2356). There appears to be a synergistic action between free carbon dioxide and sulfates, and where this combination is present, the use of sulfate-resisting cement is recommended.

Distilled or "lime-hungry" waters, and those having less than 100 mg/l of carbonate hardness, have been reported as being corrosive to concrete under specific conditions of low temperature or continuously renewed water (2357). High concentrations of organic matter in water (e.g., sewage) may, under certain conditions of high temperature and low pH, favor the production and release of hydrogen sulfide. This gas, in turn, may redissolve in condensate on other concrete surfaces, be oxidized to sulfates, and attack the concrete (198, 2355, 2358).

CONFECTIONARY (see also Sugar Making)

Water used in making confections, as that used in other food-making processes, should have a quality suitable for drinking and should be free from constituents that interfere with the complex chemical and physical reactions of the process. Soft waters have been noted as being better for making hard candies; but ordinary distilled water, with a pH value below 7.0, may favor the inversion of the sucrose sugar to produce a sticky candy. Few definite limits of quality for water to be used in confectionaries have been recommended; however, the results of long study of such waters indicate that natural waters with less than 50 or 100 mg/l of the usual dissolved mineral constituents generally are satisfactory. Summarized below are the recommended threshold concentrations of various constituents in waters to be used for making confectionaries:

Constituent	*Range of Recommended Threshold Values in mg/l*
Taste and odor	Low
Iron	0.2
Manganese	0.2
Iron and manganese	0.2
Total solids	50-100
Hydrogen sulfide	0.2
pH	> 7.0

References: 152, 161, 162, 164

COOLING WATER

Cooling, or the transfer of heat, is a very common and important step in many industrial processes. A large portion of the water utilized by industry is employed for such cooling purposes, there being several systems or methods for using water for cooling (161). The water may be circulated in a closed system, gaining heat as the product or process is cooled and losing heat in a tower or spray pond prior to recirculation. Or the water can be used first as a cooling agent and then either be wasted or be used in subsequent processes.

If a closed or recirculating system is used for cooling an industrial process, the only water added to the system is that needed largely to replace the evaporation and wind-blown or drift losses that occur in cooling towers and spray ponds. Corrosion, deposition of scale, delignification of wooden cooling towers, and the growth of microorganisms are four phenomena particularly detrimental to such cooling systems. The dangers of circulating a water that may corrode the piping and equipment of a cooling system are obvious; but on the other hand use of a water that tends to deposit scale is also detrimental to a cooling system. Not only does scale affect the physical operation of a cooling system by increasing hydraulic friction; it also diminishes the thermal efficiency of the system. The scale acts as an insulator and makes the transfer of heat from the walls to the water far less efficient. The growth of microorganisms that may act as contributors to corrosion, or as formers of slimes that foul the surfaces of cooling towers and piping, is also hazardous. A raw water introduced into such a recirculating system initially should have characteristics tending towards none of these phenomena. The make-up water, too, must be treated to conform with the desirable quality. A gradual but steady increase of undesirable constituents is possible through the continual addition of improperly prepared make-up water. While this report is not concerned with the treatment of boiler feed water or cooling water, it is significant to note the substances that are desirable or undesirable in such waters.

In the case of water used as a coolant and then wasted or then used in subsequent processes, the initial temperature of the water assumes considerable importance. Generally, the lower the initial temperature of such a water, the more desirable it is as a cooling water. Interesting estimates of the value of low-temperature water have been made (199). One of the major sources of such cooling water is ground water, which generally has a lower and more even temperature than surface waters. The U. S. Geological Survey has made thorough studies of the temperatures of the ground water through the country (200). Water is taken from and returned to rivers and lakes, and in coastal areas much cooling water

is taken from the ocean and returned directly to it. In such cases, the prevention of growths of marine organisms and the fouling of conduits and heat-exchange surfaces is desirable. If water is used once for cooling and then wasted, the treatment processes to prevent corrosion, organism-growth, and scaling should be as inexpensive as possible. In case the water is to be used in subsequent processes, the treatment of the water must not add substances that will prove deleterious to its further uses. Sometimes the subsequent use is cooling at higher temperatures. Such step-cooling can give excellent thermodynamic efficiences.

Among the technical papers reviewed in this search of the literature are studies of the deterioration of the timber (delignification) in cooling towers (201). It was found that the destruction of the wood was largely caused by sodium carbonate in the cooling water and it was recommended that the pH be maintained at 6.5-7.5 to afford a more favorable bicarbonate-carbonate equilibrium. Inasmuch as carbonates commonly give trouble by forming scale, the effects of various additives, as well as softening treatments, in preventing scale deposition have been studied (202). Sewage effluents have proved more desirable as cooling waters in some instances than extremely hard ground waters (203). In another instance (2359), treated sewage was the only dependable source of water for a cooling tower. Actually the entire problem of preventing scale formation is extremely complex, common to many other uses of water, and beyond the scope of this study. For details the reader is referred to the comprehensive book by Powell (2360) and to the ASTM Manual on Industrial Water (1654).

A corrosive or aggressive water, the converse of a scale-depositing water, also presents serious problems when used for cooling purposes. The chemical mechanism of corrosion is extremely complex, especially in cooling systems that may comprise dissimilar metals and materials. Many studies have been made of the corrosion-suppressing effects of various additives to cooling water such as silicates, chromates, hexametaphosphates, phosphates, nitrates, hydroxides, quebracho tannins, and many other substances (202, 204, 205, 206, 207, 2337, 2338). As pointed out earlier, an exhaustive study of the problem of corrosion is not pertinent to this report. Many studies too have been made of the use of various chemicals, such as chlorine, in cooling waters to suppress the growth of aquatic plants, algae, diatoms, iron and sulfur bacteria, slime-forming organisms, and the like (208, 209, 210, 211).

Warren (2361) reports that austenitic stainless-steel equipment is subject to stress-corrosion cracking when it is in contact with chloride-bearing cooling water. His laboratory studies showed that chloride concentrations as low as 10 mg/l and temperatures as low as 40° C contribute to cracking of stainless steel.

Industries that utilize cooling water, and especially those that recycle it through cooling towers, are alerted to the need for treating such water to minimize corrosion, scale formation, delignification, and slime growths. Such treatment, however, is dependent upon a steady condition of the make-up water. For this reason, industries are not as concerned about the absolute quality of the water supply as they are about changes in quality from day to day.

In short, cooling waters should have appropriate initial temperatures and should not deposit scale, be corrosive, or encourage the growth of slimes. Among the constituents of natural water that may prove detrimental to its use for cooling purposes are hardness, suspended solids, dissolved gases, acids, oil and other organic compounds and slime-forming organisms (212). One of the most definitive lists of quality requirements for cooling waters (152) gives the following limiting concentrations:

Turbidity	50 mg/l
Hardness	50 mg/l
Iron	0.5 mg/l
Manganese	0.5 mg/l
Iron and manganese	0.5 mg/l

COPPER INDUSTRY

The water requirements of the copper industry have been summarized in a comprehensive report by the U. S. Geological Survey (2362), from which the following material has been abstracted.

About five-sixths of the domestic copper is mined in Arizona, Nevada, New Mexico, and Utah where most natural water supplies are highly mineralized. On the other hand, about two-thirds of the electrolytic refineries are located near New York City, where high-quality water is available.

At the source of the ore, water is used in open-pit mines to control silica dust. Such water may be of poor quality, but it must be free of petroleum products that interfere with subsequent concentration by flotation. Most of the domestic copper ore consists of low-grade copper sulfides that are first concentrated by flotation. Water for this purpose may be highly mineralized, but it should be free of acid, mud, slime, and particularly petroleum products that adhere to ore and change its specific gravity. Copper oxides cannot be separated by flotation, so the copper is usually separated from the ore by a leaching fluid consisting mostly of water to which sulfuric acid is added. The leachate then contains copper sulfate in solution which is later precipitated by passage through beds of sponge iron or iron scrap. For leaching, water of low pH and low alkalinity is desirable.

Copper smelters and electrolytic copper refineries are generally located where water of better quality is available. A by-product of copper smelting is waste heat, which is utilized in the production of steam power, for which high-quality boiler-feed water is needed. Cooling water is also needed, so the water-quality criteria applicable to these two water uses will apply. At electrolytic refineries, copper is transferred from anodes that contain about 98 percent copper and 2 percent impurities to cathode sheets of refined copper in electrolytic baths of high-quality water to which copper sulfate and sulfuric acid have been added. Cathodes are later washed with distilled water or other water of high quality. Refineries also use considerable water for cooling and boiler feed.

About 70,000 gallons of water per ton of finished copper are used in mining and concentrating the ore and about 30,000 gallons per ton for reducing the concentrate to refined copper. Thus, more water is used to produce a

ton of copper, on the average, than to produce a ton of any other major metal (2362).

COTTON PROCESSING (see Textiles)

DAIRY INDUSTRY

Water is used in the dairy industry primarily for the cleaning of pasteurizing equipment, for rinsing and cleaning tank trucks and cans, and for bottle washing. For this reason, according to Bonnet (2344), the water must be bacteriologically potable and free from chemicals that might leave harmful residues. He classifies water as "very good" when it has a total bacterial count of 25 organisms per ml or less, "good" up to 100 per ml, "passable" up to 500 per ml, and "bad" over 500 per ml.

With respect to chemical quality, the water should be free from copper, and very low in iron and manganese, inasmuch as these metals function as catalysts for the oxidation of fatty matter and for the hydrolysis of milk constituents. Hence they may cause fatty, oily, or fishy tastes in the milk products. Bonnet (2344) recommends the following limits for chemical and physical constituents:

Dry residue	less than 500 mg/l
Nitrate, as N_2O_5	less than 30 mg/l
Nitrite	none
Ammonia, as NH_3	traces only
Chloride	less than 30 mg/l
Sulfate	less than 60 mg/l
Organic matter, in terms of $KMnO_4$	less than 12 mg/l
Hardness, as $CaCO_3$	less than 180 mg/l
Iron	0.1 to 0.3 mg/l
Manganese	0.03 to 0.1 mg/l
Color	none
Odor	none

DISTILLERIES (see Fermentation Processes)

DYEING (see Textiles)

ELECTROPLATING AND METAL FINISHING

Plating-room processes that utilize water include the stripping or pickling operations, cleaning by organic solvents or alkaline solutions, rinsing, and electrochemical plating (2363). For acid stripping or for alkaline cleaning, the quality of water used in the baths is seldom critical, for the added chemicals far outweigh the natural constituents of the water. Hardness of water may be detrimental when soaps or alkaline cleaning agents are used.

For rinsing and for plating, water quality is frequently a major problem. High quality water is of primary importance to assure satisfactory finished work. For decorative plating, water spots and stains may necessitate reworking, wiping, buffing, and other laborious operations. Before the application of any organic corrosion-resistant coating, it is almost a necessity to use demineralized water in the final rinse, in order to achieve adhesion of the coating (2364). A high concentration of dissolved solids is especially detrimental in rinse waters (2365, 2366).

In plating baths, iron, aluminum, calcium, magnesium, sodium, potassium, carbonate, bicarbonate, sulfide, sulfite, sulfate, fluoride, chloride, silicate, copper, and lead have been reported to cause difficulties under certain conditions (161, 213, 214, 2364, 2365, 2367). Considerable evaporation occurs from plating baths and hence the ions present in the make-up water are concentrated to the extent that they are troublesome.

Calcium and magnesium are especially troublesome in plating baths, for they tend to precipitate to form scale on the heated surface or a sludge in the water. There is a probability of these precipitates being included in the electrodeposit, causing pits, porosity, and roughness (213, 2338, 2367). Magnesium may also reduce the "throwing power" in chromium baths (2364), but on the other hand, magnesium sulfate is sometimes added to nickel-plating baths to produce softer deposits, to minimize certain types of pitting, and to increase throwing power (213).

Sodium and potassium are generally not harmful in plating operations, although sodium may cause brittle deposits in nickel baths or reduce the throwing power in chromium solutions (2364). Iron is one of the most troublesome pollutants of many plating operations. In a nickel-sulfate bath, it may cause hazy, streaked, pitted, or brittle deposits; in acid copper solutions it produces rough deposits; in chromium baths it reduces the throwing power; in cadmium cyanide it causes hazy deposits; in silver cyanide stained deposits; and in zinc sulfate baths it lowers the plating efficiency and the protective value of the coating (2364, 2367).

Among the anions, bicarbonates are detrimental in heated alkaline baths, for they tend to be converted to carbonates and accelerate the precipitation of calcium. Moreover, they buffer the water and require higher dosages of acid or alkali to obtain the desired pH value. Chlorides have been reported to cause rough, modular, iridescent, and crystalline deposits in cadmium, copper, silver, and tin baths respectively (2364). Organic substances reduce chromium, and cause rough, hazy, streaked, colored, or pitted deposits under various conditions. Color and turbidity are similarly objectionable (2364).

The articles abstracted herewith give numerous qualitative considerations but no quantitative threshold or limiting concentrations were uncovered.

FERMENTATION PROCESSES (see also Brewing, Malt Preparation)

Closely related to the production of beer is the fermentation of ethyl alcohol from various grains. The water used in preparing the mash for later fermentation by yeast should be free of sediments, tastes, odors, and micro-organisms. Certain mineral constituents, such as calcium and magnesium bicarbonates, in the water may be of advantage in furnishing additional nutrients for the yeast. For use in blending the final product, undistilled water of satisfactory mineral quality is reported to be preferred to distilled water. No lists of quantitatively expressed criteria for quality of water for grain-alcohol fermentation were uncovered in the literature (163, 171, 176, 215, 2344).

FOOD CANNING AND FREEZING (see also Food Processing, General and Food Equipment Washing)

A primary consideration for the quality of water used in food canning and especially in freezing processes is its bacerial content. Not only must such water be free of pathogens that may be transmitted to the consumer, but it must also be sterile with respect to saprophytic organisms that may cause spoilage. Many such organisms, to be sure, are destroyed in the canning process or remain dormant in frozen food, but their absence from the process water gives an added factor of safety. The California Department of Public Health has promulgated coliform standards for the water used in fish-canning operations. (See Chapter VII, Coliform Bacteria.)

The effects of chemical pollutants in the water differ for the various foods being processed. Substances that produce tastes, odors, color, deposits, toughening, deterioration of quality or vitamin content, or other detrimental changes in the food are to be avoided. Chlorophenols in a process water, for example, have been reported to have produced tastes in the syrup of canned pears.

Hardness in the blanching water causes toughening of some vegetables, such as dry beans and peas, coatings or deposits on others, such as beets, and has no effect or may even be beneficial to some, such as cream-style canned corn. Peas and snap beans that had been blanched before freezing with waters containing various concentrations of calcium and magnesium showed a greater loss of ascorbic acid and a greater gain of calcium as the hardness was increased. Compounds of iron, manganese, copper, other minerals, and organic matter have been named as the causes of taste, odor, color, and deposit difficulties. Copper is reported to decrease the shelf life of packaged foods, especially dairy and vegetable oil products. Apparently, fluoride tends to concentrate in spaghetti, baked beans, and cereal (2333).

The various threshold or limiting values that have been recommended are summarized in the following table:

Constituent	*Range of Recommended Threshold Values in mg/l*
Total dissolved solids	850
Turbidity	1.0-10
Taste and odor	none-low
Hardness, as $CaCO_3$	50-85 (general)
	200-400 (peas)
	100-200 (fruits and vegetables)
	25-75 (legumes)
Iron	0.2
Manganese	0.2
Iron and manganese	0.2-0.3
Sodium chloride	1000-1500
pH	not lower than 7.5
Hydrogen sulfide	1.0
Fluoride	1.0
Alkalinity, as $CaCO_3$	30-250
Nitrates, as N_2O_5	15
Ammonia, as NH_3	0.5

References: 152, 162, 164, 173, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 2333, 2337, 2344

FOOD EQUIPMENT WASHING (see also Baking, Carbonated Beverages, Food Canning and Freezing, Food Processing, and Ice Manufacture)

The water used to wash and rinse equipment that comes in contact with food should be as safe bacterially as drinking water. In fact, it should also be free from saprophytic organisms that may attack particles of food lodged in hard-to-clean crevices or cracks in the equipment. Constituents in the water that carry or cause tastes and odors or leave deposits or stains on the equipment are to be avoided. Inasmuch as the primary use of these waters is for washing, low hardness is one of the more important qualities to be desired. The various threshold values that have been recommended are summarized below:

Constituent	*Range of Recommended Threshold Values in mg/l*
Turbidity	1.0
Color	5-20
Taste and odor	None
Iron and manganese	0.2
Chlorides	250
Hardness, as $CaCO_3$	10
Total dissolved solids	850
Organic matter	infinitesimal
Fluoride	1.0

References: 173, 226, 2337

FOOD PROCESSING, GENERAL (see also Baking, Carbonated Beverages, Food Canning and Freezing, Food Equipment Washing, Ice Manufacturing)

Although they occur commonly, even in the household kitchen, the chemical reactions and physical changes brought about in commercial processing and preserving foods are extremely complex. Since water is used in nearly all of the processes and is incorporated in many of the products, the various compounds dissolved in the water enter the physical and chemical reactions. The actions of such solutes are not necessarily detrimental; in fact, some are advantageous and desirable.

Owing to the complexity of the reactions and the great number of different foods and dissolved compounds to be considered, it is unlikely that generalizations of required quality or threshold concentrations of food waters, as such, can be made. The threshold concentrations will differ for the various kinds of foods being processed. That the waters used in the process must be bacteriologically safe, at least as potable as drinking water, is one requirement commonly recommended for nearly all types of food processes. In many cases, because of the danger of spoilage or after-growth, precautions must be taken against the presence of microorganisms other than those normally considered to be pathogenic to humans.

Attempts have been made, from time to time, to summarize or generalize the quality requirements of water used for all of the many food products or processes. Inasmuch as detailed threshold values are not available for water used with many foods, the few that have been recommended for general food use are summarized below, although their applicability to any specific problem is necessarily doubtful.

Constituent	*Range of Recommended Threshold Values in mg/l*
Turbidity	1-10
Color	5-10
Taste and odor	Low-barely noticeable
Iron	0.2
Manganese	0.2
Iron and manganese	0.2-0.3
Alkalinity, as $CaCO_3$	30-250
Hardness, as $CaCO_3$	10-250
Total dissolved solids	850
Fluoride	1.0

References: 152, 162, 2337, 2344, 2368

ICE MANUFACTURING (see also Food Equipment Washing and Food Processing, General)

Since manufactured ice should be clear, colorless, tasteless, ordorless, and bacteriologically safe, the water used to make it should have similar qualities. Steam was used for power in the early manufacture of ice and consequently distilled water for making ice was easily and economically available. The very clear, crystal-like appearance of ice produced from such distilled water became associated, in the consumer's mind, with purity and quality. With the advent of electrical power, however, the use of such relative pure water was no longer economical and the effects of various salts dissolved in the natural water had to be considered.

Dissolved salts produce opacity in the ice; hence various methods of treating both the raw water and the freezing water had to be devised. Calcium and magnesium, for example, are troublesome but they can be removed rather easily from the natural water. Several classifications of waters have been made which indicate from the results of a chemical analysis the probable quality of the ice and the treatment required during freezing. In these classifications, the effects of chlorides are weighted 75 percent of the effects of the sulfates; the carbonates, 125 percent. The weighted concentrations, in mg/l, are summed to give a number that will indicate the probable treatment required. The upper threshold values, beyond which it is said that merchantable ice cannot be made, range from 684 to 710 mg/l.

In freezing large blocks of ice, the dissolved minerals tend to concentrate in the central core, making it cloudy (2369). Some ice manufacturers pass the water for ice through an ion-exchange column prior to freezing, for the purpose of reducing the number of times that the core must be removed to get clear ice.

With the wide adoption of electric refrigerators and automatic ice-making equipment, much ice is now made in small units near the point of use, utilizing municipal water of drinking-water quality. Slight cloudiness in ice cubes is seldom noticed or considered to be detrimental.

Depending upon the processes used for the manufacture of ice, the maximum allowable concentrations of dissolved solids that have been recommended range from 170 to 1300 mg/l, with several around 300 mg/l. In Charlotte, North Carolina, an increase in the fluoride content from 0.1 mg/l to about 1.5 mg/l was reported to cause serious brittleness and cracking of ice blocks (152, 162, 164, 168, 173, 183, 227, 228, 229, 230, 231, 232, 233, 234, 235, 2337, 2368, 2369).

IRON AND STEEL MANUFACTURING (see Steel Manufacturing)

LAUNDERING

The most important constituent to be avoided or eliminated in water used for laundering is hardness, because of the wastage of soap and the formation of curds. Also of great detriment is the presence of iron and manganese which, even in low centrations, can cause stains on the materials being laundered. Manganese is especially objectionable in water used in laundry work and textile processing. Concentrations as low as 0.2 mg/l may cause dark-brown or black stains on fabrics and porcelain fixtures (2342). *Leptothrix, Crenothrix, Gallionella,* and other iron-depositing organisms are reliable indicators of iron and manganese and of the unsuitability of such water for laundries. High bicarbonate alkalinity, too, may cause staining difficulties.

A summary of the range of recommended threshold and limiting concentrations of various constituents in waters used for laundering processes is presented herewith.

Constituent	Range of Recommended Threshold and Limiting Concentrations in mg/l
Hardness, as $CaCO_3$	0-50
Iron and manganese	0.2-1.0
Iron	0.2-1.0
Manganese	0.2
Alkalinity, as $CaCO_3$	60
pH	6.0-6.8

References: 152, 161, 236, 237, 238, 239, 993, 2342

LEATHER PROCESSES (see Tannery Operations)

MALT PREPARATION (see also Brewing, Fermentation Processes)

In the preparation of malt, steeping is the process of soaking grain in water to provide sufficient moisture to start enzyme production and germination which precede the breakdown of cell walls and the hydrolysis of the stored foods. Inasmuch as the steeping solution gradually becomes an excellent medium for the growth of bacteria, it is important that the water used be relatively free of microorganisms.

The tannins, resins, and bitter substances in the husks of coarse barleys are most readily removed by alkaline waters. Water that is too soft may dissolve large proportions of mineral salts and soluble protein derivatives, which are valuable nutrients for yeast. On the other hand, water that is too hard may not remove certain undesirable constituents from the grains. Freedom from objectionable tastes and odors, low contents of nitrate, ammonium salts, and iron, and a uniform temperature of about 13°C are among the characteristics recommended for water used to steep grains for malt production. No lists of quantitatively expressed criteria were uncovered in the literature (172, 175, 176, 240, 2345).

METAL INDUSTRIES (see Aluminum Industry, Copper Industry, Electroplating and Metal Finishing, Steel Manufacturing)

MILK INDUSTRY (see Dairy Industry and Food Processing, General)

OIL WELL FLOODING

For oil-well-flooding operations it has been recommended that the water should not contain more than 0.1 mg/l of iron, should not have a low alkalinity or pH less than 7, and should not be corrosive. The growth of algae in water stored for such operations should be prevented with algicides (349). In many oil-well-flooding situations, it is necessary to filter the water or brine through sand or diatomite units in order to prevent the recharged well from clogging with colloidal particles or microbiological cells.

PAPER MANUFACTURE (see Pulp and Paper Mills)

PHOTOGRAPHIC PROCESSES

Although the photographic processes of developing and fixing images on paper or films involve complicated and sensitive chemical reactions, the quality requirements of the waters used for making up the various solutions and for washing and rinsing are relatively tolerant. An extreme example of this tolerance is the fact that sea water can be used both to wash films and paper if followed with a rinse in fresh water, and to prepare developing and fixing solutions, if the sludge of precipitated chemicals is carefully settled out. For the more usual circumstances, however, hardness in concentrations of more than 200 mg/l usually causes difficulties, whereas in concentrations of less than 100 mg/l difficulties are rare.

Municipal water supplies are generally adequate for most photographic processes. Indeed, they are better in some respects than distilled water, for washing with extremely soft water or distilled water may result in reticulation or softening. The dissolved salts in natural waters prevent reticulation (2370). Most photographic processors are willing to use municipal water provided that it is constant in quality from month to month, so that experience in its use can be developed.

In the mixing of developers, the presence in the water of the chlorides and bromides in excess of 25 mg/l exerts a restraining action, but sodium carbonate tends to speed up the action of a developer. Developers mixed with water containing sodium or potassium sulfides even in small concentrations can cause bad chemical fog. The presence of 100 mg/l of calcium sulfate is reported to cause fogging, as will colloidal sulfur and certain bacteria. High concentrations of calcium and/or magnesium may cause precipitation in alkaline processing solutions. Dissolved solids will crystallize on films and plates when dried unless precautions are taken during drying. Dissolved gases in wash waters can produce blisters on papers. Spots and stains can be caused by iron, manganese, suspended solids, turbidity, and color in the solution and wash waters. Iron in concentrations greater than 0.1 mg/l may cause staining, but some photographic products will tolerate as much as 10 mg/l of ferrous or ferric iron.

Excessive copper causes emulsions to fog. Moreover, 1.0 mg/l of copper results in significant loss of strength of color developer after two weeks and 10 mg/l causes appreciable loss in a few days (2370). Tin, lead, and zinc in concentrations up to 10 mg/l have not proved to be deleterious. Fluoride can also be tolerated up to at least 10 mg/l (161, 239, 241, 242, 2370).

PLASTICS

Plastics have been defined as organic minerals that can be easily molded or shaped by mechanical or chemical action to give tough, non-crystalline substances that are solid at ordinary temperatures. They are made from molding compositions prepared from various raw-material groups such as resin or cellulose derivatives (binders); cellulose, wood flour, cotton fiber, asbestos, mica, or fabrics (fillers); dyes and pigments; and various plasticizers, catalysts, and lubricants.

There are many types of plastics and many complicated processes for manufacturing them. The quantity and quality of water vary in importance, depending on the different manufacturing methods. In general, water of unusual quality is not needed. For special situations, involving only a small proportion of the total water requirement at a plastics manufacturing plant, de-ionized city water is customarily used (152, 162, 189, 2371).

PULP AND PAPER INDUSTRY

The water requirements of the pulp and paper industry have been summarized in a comprehensive report by the U. S. Geological Survey (2372). Much of the material relating to water quality in that report and in the following summary is based upon the publications and specifications of the Technical Association of the Pulp and Paper Industry (TAPPI).

The manufacture of pulp and paper is highly dependent upon an abundant supply of high-quality water. The major purposes for which water is used include the digestion or cooking of wood chips to make pulp, repeated washings of the cellulose pulp, transportation of the pulp and other constituents of paper in a thin slurry through preparatory processes and finally onto the paper machine, boiler feed, and cooling. This section deals only with the process water used for washing of pulp and for paper making. Boiler-feed water and cooling water are covered separately.

Suspended matter is undesirable in process waters because it decreases brightness, affects colors, interferes with texture and uniformity, clogs wire screens, and favors growth of slimes. Hardness gives difficulties by reacting with sizing compounds and causing the precipitation of calcium carbonate. It also tends to form scale on washing screens, the wire of paper machines, and similar equipment. Turbidity and color can cause trouble in the finer-grade products. Dissolved gases in the process waters sometimes cause corrosion of the machinery, and carbon dioxide, in concentrations above 25 mg/l, has been reported to affect adversely the formation of sheets on the paper machines. Iron, manganese, silica, and organic matter are among other undesirable constituents of the process waters (239, 2373).

The presence in the process waters of microorganisms, such as bacteria, algae, and fungi, may lead to difficulties from the growths of slimes, slick spots on the paper, and the development of odors.

In 1940, Miller (243) presented the information in Tables 5-5 and 5-6 to show the ranges of concentration of various substances in process waters used by different paper mills.

Other studies of the desirable quality characteristics of water to be used in paper manufacturing have been made from time to time. Perhaps of greater interest, however, are the specifications for the chemical compositions of several process waters that have been approved, as tentative standards, by the Water Committee of the Technical Association of the Pulp and Paper Industry. These tentative standards, or specifications, are summarized in Table 5-7 and the remarks, definitions, and notes to each are reproduced in the pages immediately following the table. It is understood that paper mills find these specifications to be satisfactory with the possible exception of the limit of 2.0 mg/l for residual chlorine in water used for fine paper. Where dyes are used, they

may be affected by chlorine concentrations in excess of 0.3 mg/l.

RAYON AND ACETATE FIBER INDUSTRIES

The water requirements of the rayon-fiber and acetate-fiber industries are described in a comprehensive report by the U. S. Geological Survey (2374) from which much of the following material has been abstracted.

These artificial fibers, resembling silk, are made from purified wood cellulose or cotton linters by one of three processes identified as viscose rayon, cuprammonium rayon, or cellulose acetate. The purified cellulose or cotton linters are prepared by methods similar to those used for wood or rag pulp in the fine paper industry. The wash waters for purifying such pulp should be purer than the requirements for fine paper (see Pulp and Paper Industry), and in general should comply with the recommendations in the following tabulation.

The rayon processes, viscose and cuprammonium, produce a filament of regenerated cellulose whereas the cellulose acetate process produces a compound of definite chemical composition. All of the processes involve solution of the cellulose in strong alkali or acid liquors to dissolve undesirable constituents, formation of the threads by filtration and extrusion, air drying, spinning, and finally finishing operations such as bleaching and washing. Process water is used primarily in the initial chemical solutions and in the finishing operations. In quality, it should meet the limits shown in the following tabulation.

In addition to process water, rayon-fiber plants use large quantities of water for power production and air conditioning, inasmuch as many of the processes involving the fine fibers are sensitive to temperature and humidity. Indeed, Mussey (2374) reports an average of 110 gallons of water used to produce a pound of rayon fiber, of which 32 gallons were process water and 78 gallons were used largely for air conditioning and power production (for quality requirements, see Boiler Feed Water and Cooling Water). For the manufacture of a pound of acetate fiber, the average total water use was reported at 170 gallons (2374).

Owing to the fact that rayon and acetate plants require abundant supplies of clean soft water, they are generally located in the eastern area of the United States. Recommended threshold and limiting concentrations of various constituents in water used in the viscose process are summarized below:

Constituent	*Range of Recommended Threshold and Limiting Concentrations in mg/l for Manufacture of Viscose Rayon*	
	Pulp Production	*Manufacture*
Turbidity	5	0.3
Color	5	--
Hardness, as $CaCO_3$	8	55
Iron and manganese	0.05	0.0
Manganese	0.03	0.0
Iron	0.05	0.0
Total solids	100	--
Alkalinity, as $CaCO_3$	50-75	--
pH	--	7.8-8.3
Aluminum oxide	<8	
Silica	<25	
Copper	<5	

References: 152, 162, 189, 2374, 2405

TABLE 5-5

RANGES OF CONCENTRATION OF VARIOUS SUBSTANCES IN WATER USED BY PULP AND PAPER MILLS

(After Miller) (243)

Constituent	*Range in mg/l (except pH)*	*Number of Mills Reporting Process Water in This Range*
Turbidity	0-10	34
	10-50	6
	50-635	2
Color	0-10	24
	10-50	13
	50-150	8
Iron	0-0.1	16
	0.1-0.5	16
	0.5-1.0	3
	1.0-1.5	3
	1.5-4.0	4
Manganese, as Mn	0-"trace"	19
	0.005-0.05	3
	0.05-4.0	6
Hardness, as $CaCO_3$	0-25	9
	25-50	14
	50-100	6
	100-200	9
	200-300	5
	300-400	6
	400-766	1
Alkalinity, total	0-25	19
	25-50	10
	50-100	1
	100-200	10
	200-400	3
Alkalinity, phenolphthalein	0-0.1	19
	0.1-10	4
	10-80	0
	80-200	2
Sulfates	0-10	15
	10-20	7
	20-50	8
	50-100	5
	100-200	4
	200-300	1
Chlorides	0-10	30
	10-20	6
	20-50	9
	50-100	1
Total dissolved solids	0-10	0
	10-50	4
	50-100	16
	100-200	6
	200-300	6
	300-400	3
	400-600	5
Total solids	0-50	0
	50-100	17
	100-200	5
	200-300	6
	300-600	8
pH value	5.8-6.0	3
	6.0-6.5	4
	6.5-7.0	5
	7.0-7.5	19
	7.5-8.0	3
	8.2-8.3	5
	8.3-9.2	1

RUBBER (see Synthetic Rubber)

STARCH INDUSTRY

In the manufacture of starch, water should be as soft and mineral-free as possible inasmuch as any precip-

itated salts will accumulate in the starch, which should not leave any ash on ignition. Iron and manganese are especially detrimental in that they give a yellowish discoloration to the starch. A limit of 1.0 mg/l has been recommended for iron (2344).

STEEL MANUFACTURING

About two decades ago, the Bethlehem Steel Company of Sparrows Point, Maryland, required additional sources of water for use in the manufacture of steel products. Large quantities of sea water were used for cooling and condenser purposes but a search was made for a source of fresh water with a minimum safe yield of 50 mgd. Various possible sources of a supply of this magnitude were investigated and the economic and physical requirements soon led to a consideration of the use of the treated effluent from the Back River Sewage Treatment Plant of Baltimore City. According to Wolman (249):

"The quality of water desirable for the various industrial processes was not easily definable, since the exact specifications of water quality for these uses were not known in detail. No unanimity of agreement existed apparently in the steel trade on the characteristics of water harmful or harmless in the individual industrial process.

"These waters for the most part are used for cooling of blast furnaces and open hearths, of rolls in all the rolling mills, of gas washers, of wire machines and for the quenching and granulating of blast furnace slag and for cleaning of gases. For this reason a number of plant scale experiments were carried out in the various processes and over extended periods, in order to shed some light upon the desirable or objectionable characteristics.

"The local studies, and field observations made elsewhere throughout the country, indicated that the following characteristics determine a desirable quality of water for steel manufacture. They are:

1. Temperature preferably below 75°F.
2. Chlorides preferably below 175 ppm.
3. pH between 6.8 and 7.0.
4. Hardness below 50 ppm.
5. Suspended matter below 25 ppm.
6. Organic content as low as possible.
7. Corrosion potential at the lowest possible level."

It is interesting to note that, according to the terms of the agreement between the city and the company, the city makes every practicable effort to maintain the following characteristics of the treated effluent:

"(a) The hydrogen ion content shall not exceed 7.8 or fall below 6.5, calculated on the basis of monthly averages.

"(b) The total suspended solids shall not at any time exceed an instantaneous maximum of fifty parts per million and the monthly average of such solids shall not exceed twenty-five parts per million. This requirement shall not apply to effluent discharged from the secondary

TABLE 5-6

PERMISSIBLE UPPER LIMITS OF IMPURITIES IN PROCESS WATER USED IN PAPER MILLS

(After Miller) (243)

Designation of Mill	*Sulfate, mg/l*	*Chlorides, mg/l*	*Dissolved Solids, mg/l*	*Total Solids, mg/l*	*pH*
3	—	—	425	425	7.0-7.2
10	70	4.5	140	150	7.3
12	—	30	—	100	6-7
15	—	—	59	—	7.4
17	—	0.75	50	250	7.3
19	7	13	64	—	7.0
27	—	—	—	—	7.2-7.6
29	50-200	4-12	153-283	160-290	not over 7.1
31	—	0	—	—	—
43	—	—	—	—	6.8-7.3
44	20	10	75	75	6.0-7.5

TABLE 5-7

SUMMARY OF SPECIFICATIONS FOR CHEMICAL COMPOSITION OF PROCESS WATERS FOR MANUFACTURE OF VARIOUS PAPERS

Substance (Amounts given in mg/l)	*Groundwood Paper (See Note A)*	*Soda and Sulphate Pulps (See Note B)*	*Kraft Paper (See Note C)*		*Fine Paper (See Note D)*
			Bleached	*Unbleached*	
Turbidity as SiO_2	50*	25*	40	100	10
Color in platinum units	30	5	25	100	5
Total hardness as $CaCO_3$	200	100	100	200	100
Calcium hardness as $CaCO_3$	—	50	—	—	50
Magnesium hardness as $CaCO_3$	—	50	—	—	—
Alkalinity to methyl orange as $CaCO_3$	150	75	75	150	75
Iron as Fe	0.3	0.1	0.2	1.0	0.1
Manganese as Mn	0.1	0.05	0.1	0.5	0.05
Silica (soluble as SiO_2)	50	20	50	100	20
Total dissolved solids	500	250	300	500	200
Free carbon dioxide as CO_2	10	10	10	10	10
Chlorides as Cl	75	75	200	200	—
Residual chlorine as Cl_2	—	—	—	—	2.0

*Materials causing turbidity shall not be gritty.

settling tanks connected with the trickling filter unit, which effluent shall not at any time contain total suspended solids in excess of an instantaneous maximum of eighty parts per million and not exceeding, in monthly average, fifty parts per million.

"(c) The five-day twenty-degree Centigrade biochemical oxygen demand shall not exceed an instantaneous maximum of forty-five parts per million, and its monthly average shall not exceed twenty-five parts per million. This requirement shall not apply to effluent discharged from the secondary settling tank connected with the trickling filter unit, the five-day twenty-degree Centigrade biochemical oxygen demand of which effluent shall not exceed an instantaneous maximum of eighty parts per million, and the monthly average of which shall not exceed forty parts per million.

"(d) The chloride content shall not exceed one hundred seventy-five parts per million calculated on the basis of monthly averages" (249).

Perhaps more significant than the use of treated municipal sewage for the manufacture of steel has been recirculation and re-use of water at the Fontana plant of the Kaiser Steel Co. With a make-up of only 2.5% per cycle, water is used about 40 times before being lost through steam or evaporation. Recirculated water is treated to remove accumulations of suspended pollutants (248). Water-quality requirements were not specified in the surveyed literature.

SUGAR MANUFACTURE

The refining of sucrose sugar from its natural sources in sugar cane or beets is an imporant industry, especially in California. The processes largely involve extracting and concentrating techniques, but care must be exercised to prevent the inversion of sucrose to other related sugars. Relatively little information dealing with the quality requirements of process water for sugar manufacturing was uncovered, although there is no dearth of information relating to the polluting characteristics of sugar-manufacturing wastes, especially from the beet-sugar process.

All process water in sugar manufacture should be as free as possible from dissolved solids and completely devoid of suspended solids. Hard water is especially deleterious for it may form precipitates that accumulate in the refined sugar. Process waters should be sterile, not only with respect to coliform bacteria and pathogens but also to saprophytic organisms that may cause destruction of saccharose.

In sugar refineries, great care must be exercised to make sure that no sugar gets into the boiler-feed water, where it is especially detrimental (see Boiler Feed Water).

NOTE A: Specification E 602 S-48 for Chemical Composition of Process Water for Manufacture of Groundwood Papers (244). "This specification should be considered only as a guide in determining the quality of water to be used in the manufacture of groundwood papers.

"DEFINITION—By groundwood papers are meant coarse papers composed primarily of groundwood fibers such as are used for news, telephone directories, cheaper grades of catalogues, pulp magazines, etc.

"LIMITATION—This specification does not apply to process water for groundwood papers made from southern pine, nor to process water to be used for groundwood pulp manufacture.

"NOTES—1. Chlorides are limited to 75 ppm because chloride concentrations in excess of this amount tend to promote corrosion of metal parts in the manufacturing system.

"2. A limit for organic material is not included, since the nature of the organic material is a controlling factor. For some types a much higher limit may be allowable than for others. The limit on color has a limiting effect on some of the more objectionable types of soluble organic matter. In general, organic materials should be kept as low as possible in process water.

NOTE B: Specification E 603 S-49 for Chemical Composition of Water for Manufacture of Soda and Sulfate Pulps (245). "This specification should be considered only as a guide in determining the quality of water to be used in the production of alkaline pulps.

"DEFINITION—By alkaline pulps are meant those pulps produced by chemical cooking processes known as the soda process and the sulfate or kraft process.

"LIMITATION—This specification for process water applies to pulp manufacture only and not to the subsequent manufacture of papers for alkaline pulps. It is prepared upon the premise that the pulp will be processed in the normal way and without a final treatment to offset the effects of undesirable impurities in the water. Process water of less purity is suitable if the pulp undergoes a final corrective treatment.

"This specification is prepared upon the further premise that the pulps may be bleached.

"NOTES—1. Chlorides are limited to 75 ppm because chloride concentrations in excess of this amount tend to promote corrosion of metal parts in the manufacturing system.

"2. Calcium and magnesium compounds are limited to 50 ppm each because of their tendency to form scales upon equipment and precipitates on the pulp where water comes in contact with the highly alkaline chemicals used in process.

"3. Color, iron, and manganese are kept low because of their tendency to be absorbed by and to lessen the brightness of bleached pulp.

"4. Silica is kept low because of its tendency to be absorbed by and to increase the ash content of the pulp, and its possible tendency to aggravate scaling in the evaporators and to interfere with the settling of the white liquor."

NOTE C: Specification E 601 S-53 for Chemical Composition of Process Water for Kraft Paper Manufacture (351). "This specification should be considered only as a guide in determining the quality of water to be used in the manufacture of kraft papers.

"DEFINITION—By kraft papers are meant coarse papers composed of unbleached wood fibers produced by the sulfate process and used primarily for wrapping and other packaging purposes. High-grade sulfate papers, bleached or unbleached, such as condenser tissue, writing, book, file, folder, electrical insulating, and metal wrapping, require water as specified in TAPPI Standard E 600 S-48.

"NOTES—1. Chlorides are limited to 200 ppm because chloride concentrations in excess of this amount are effective in promoting corrosion of metal parts in the manufacturing system.

"2. A limit for organic materials is not included, since the nature of the organic material is a controlling factor. For some types a much higher limit may be allowable than for others. The limit on color has a limiting effect on some of the more objectionable types of soluble organic matter. In general, organic materials should be kept as low as possible in process water.

"3. This specification deals with process water for kraft paper manufacture. It does not apply to the process water to be used for kraft pulp manufacture."

NOTE D: Specification E 600 S-48 for Chemical Composition of Process Water for Manufacture of Fine Paper. "This specification should be considered only as a guide in determining the quality of water to be used in the manufacture of fine papers. The figures given are believed to be the upper limits of concentration of the significant impurities which are permissible in the manufacture of the general run of fine papers.

"DEFINITION—By fine papers are meant bond, ledger, book, and writing papers.

"NOTES—1. Certain special papers such as facial, cigarette, filter papers, photosensitive papers condenser tissues, may require process water of a greater degree of purity than above specified.

"2. A limit for pH is not included since desirable limits for pH will depend to a large extent upon the particular type and quality of paper being made, the composition of the water, and the condition of operation.

"3. A limit for soluble sulphates and chlorides is not included since these will be limited by the amount of total dissolved solids.

"4. A limit for organic materials is not included since the nature of the organic material is a controlling factor. For some types a much higher limit may be permitted than for others. The limit on color has a limiting effect on the more objectionable types of soluble organic matter. In general, organic materials should be kept as low as possible in process water."

Recommended threshold concentrations of various constituents in water to be used for sugar manufacturing are shown below.

Constituent	*Recommended Threshold Values in mg/l*
Calcium	20
Magnesium	10
Sulfate	20
Chloride	20
Bicarbonate, as $CaCO_3$	100
Iron	0.1

References: 189, 250, 2343, 2344, 2375

SYNTHETIC RUBBER

The manufacture of synthetic rubber involves the polymerization of raw materials such as farm-grown carbohydrates, coal, crude petroleum, limestone, salt, water, and air. Large quantities of cooling water and smaller quantities of process water are required. Non-corrosiveness and freedom from suspended matter and algal growths are among the desirable qualities of water reported from a synthetic rubber plant in Pennsylvania. Water having a hardness of more than 50 mg/l and an oxygen demand greater than 3 mg/l, measured by the potassium permanganate test, was found to cause trouble in another plant in West Virigina (189, 251, 252).

TANNING OPERATIONS

An ancient art with a history of several thousand years, tanning involves complex chemical reactions that are not thoroughly understood. Only in the last 50 to 75 years have significant changes been made in the tanning processes, and even now much of the skill depends on "rules-of-thumb." In the last decade, the development of synthetic tanning substances and the extended use of chromium compounds have altered the old arts and processes.

Freedom from iron and manganese, and low concentrations of free carbon dioxide, bicarbonate hardness, color, and turbidity, are among the desirable quality characteristics of waters used in soaking of hides and tanning of leathers. Iron and manganese in such waters are objectionable, for they may cause stains and discolorations.

During the liming process, the presence of bicarbonates and carbon dioxide may cause the deposition of calcium carbonate precipitates which are dye-resistant. High bicarbonates are also reported to cause a swelling of hides (2368). Although hardness in the water may interfere with certain processes, such as leaching tannin extracts, dyeing, and fat liquoring, it apparently does not interfere with chrome tanning or waste dyes in acid baths (161).

A summary of the recommended threshold and limiting concentrations of various constituents in water used for tanning leather is shown below:

Constituent	*Range of Recommended Threshold and Limiting Concentrations in mg/l*
Turbidity	20
Color	10-100
Hardness, as $CaCO_3$	50-513
Alkalinity, as $CaCO_3$	128-135
pH	6.0-8.0
Iron and manganese	0.2
Iron	0.1-2.0
Manganese	0.1-0.2

References: 152, 161, 189, 239, 253, 254, 2368

TELEVISION PICTURE TUBE MANUFACTURE

Several processes in the manufacture of monochrome television picture tubes require distilled or demineralized water. Salts that might contaminate the phosphorus coating or form gas when the tube is hot must be eliminated (2376).

TEXTILE MANUFACTURE (see also Rayon and Acetate Fiber Industries)

For the waters used in the various textile processes, such as wool scouring, cotton, keiring, dyeing, and finishing, the absence of suspended matter, turbidity, color, iron, manganese, hardness, and organic matter is desirable. Sufficient concentrations of suspended matter, color, iron, or manganese can cause staining difficulties in textiles. Manganese is especially objectionable in water used for laundry work and in textile processing. Concentrations as low as 0.2 mg/l may cause dark brown or black stains on fabrics and porcelain fixtures. Hardness can interfere with the soaps used in the various washing operations and lead to the deposition of curds on the textiles. Hardness, too, is reported to increase the breakage of silk in reeling and throwing operations. Some hardness has been described as advantageous in waters used for wool scouring. Nitrates and nitrites are reputed to be very injurious in the dyeing of wool and silk. Organic matter and microorganisms in the process water can lead to the development of stains, odors, and growths on the textiles being treated.

A summary of the range of recommended threshold and limiting concentrations of constituents in waters used in the various textile-manufacturing processes is shown below.

Constituent	*Range of Recommended Threshold and Limiting Concentrations in mg/l*
Turbidity	0.3-25
Color	0-70
Iron and Manganese	0.2-1.0
Iron	0.1-1.0
Manganese	0.05-1.0
Hardness, as $CaCO_3$	0-50
Chemical oxygen demand	8
Heavy metals	none
Calcium	10
Magnesium	5
Sulfate	100
Chloride	100
Bicarbonate, as $CaCO_3$	200

References: 152, 161, 162, 250, 255, 256, 257, 258, 259, 260, 261, 2333, 2337, 2342, 2377, 2378

AGRICULTURAL WATER SUPPLY
(Irrigation)

When water from irrigation or precipitation is applied to cultivated land some of it may run off as surface flow or be lost by direct surface evaporation, while the remainder infiltrates the soil. Of the infiltrated water, a part is used consumptively, a part is held by the soil for subsequent evapotranspiration, and the remaining surplus, if any, moves downward or laterally through the soil and substrata. The water retained in the soil is known as the "soil solution." It tends to become more concentrated with dissolved constituents as relatively pure water is utilized by plants or lost by upward capil-

lary action and evaporation. The soil solution can only be rendered less saline by dilution with fresh irrigation water or rain, and by downward leaching of excess water.

Absolute limits to the permissible concentrations of salts in irrigation water cannot be fixed, for several reasons (262): (a) It is almost universally true that the soil solution is at least three to eight times as concentrated as the water that replenishes it, because of the evaporation of water from the soil surface, transpiration of plants, and the selective absorption of salts by the plants. (b) There is apparently no definite relationship between the concentration and composition of the irrigation water and those of the soil solution, which in some cases may be as much as 100 times more concentrated than the water (246). (c) Plants very widely in their tolerance of salinity, as well as of specific salt constituents. (d) Soil types, climatic conditions (such as temperature, rainfall and humidity), and irrigation practices may all influence the reactions of the crop to the salt constituents. (e) Interrelationships between and among constituents may be highly significant; the effect of one ion may be modified by the presence of another. (Such antagonistic influences operate between calcium and sodium; boron and nitrates; selenium and sulfates) (263). A comprehensive description of the relationships among irrigation water, soil, and crops is given in Agriculture Handbook No. 60 of the U.S. Department of Agriculture (1642).

Good drainage of the soil may be a more important factor for crop growth than the salts in the irrigation supply. Even when excellent waters are used, poorly drained land may sometimes go out of production; while saline waters, on the other hand, may sometimes be used on open well-drained soils (264). The "leaching requirement" (LR) is defined as the ratio of the equivalent depth of drainage water to the depth of irrigation water, $\frac{D_d}{D_i}$, that is required to maintain a given concentration of soil solution at the bottom of the root zone (2379). In effect, it is the percentage of irrigation water applied to soils that must be leached beyond the root zone to maintain soil solution concentrations low enough for good yields (2380).

The concentration of salts in natural irrigation waters is rarely so high as to cause immediate injury to crops. If leaching of the root zone does not take place, however, the concentration of the soil solution at this depth will increase with successive irrigations until it reaches the limit of solubility of each salt. The solubility of many salts (such as borates, chlorides, and sulfates of sodium and magnesium) is beyond the tolerance limit of many plants; consequently these salts can build up to toxic concentrations (265, 266, 267, 268). The slow filling of the soil with salts (resulting in the production of highly concentrated soil solutions) will eventually force the abandonment of an irrigated area (246). This action was probably the cause of failure of many ancient irrigation systems.

In any discussion of the quality of water for irrigation, it is necessary to consider the effects of its constituents on both the plant and the soil. The deleterious effects of salts on plant growth can result from: (a) direct physical effects of salts in preventing water uptake by plants (osmotic effects); (b) direct chemical effects upon metabolic reactions of plants (toxic effects); and/or (c) indirect effects through changes in soil structure, permeability, and aeration (246).

Owing to the many variable factors, no rigid limits of salinity can be set for irrigation waters. According to authorities in Western Australia (282), water containing up to 1000 mg/l of salinity (dissolved solids) is suitable for growing all types of plants, including the salt-susceptible ones, provided that drainage is good. Water containing up to 2150 mg/l is suitable for most plants except sensitive ones and water containing up to 3150 mg/l has been used for growing tomatoes, cabbages, and other salt-resistant plants. Generally, 3150 mg/l is near the maximum for the safe watering of any plant, and in such instances drainage must be excellent and each watering should permit leaching from the root zone.

The substances most commonly found in natural irrigation waters are often listed under the following three headings, which more or less correspond with the three types of injury just described; (a) total salts, (b) substances found in low or trace concentration, and (c) cations and anions (269). The total salt content, the main effect of which is osmotic, is stated in terms of specific electrical conductance, a measure of concentration of ions per unit of water, and/or in terms of total dissolved solids, in milligrams per liter of water. The substances found in low concentration include compounds of boron, silicon, fluorine, sulfur, phosphorus, iron, and trace elements; nitrite and ammonium ions; hydrogen-ion concentration; and organic matter. These substances contribute to the total osmotic effect; they are often essential, in limited amounts, for plant growth; and they are often toxic above certain concentrations. The cations, Ca^{++}, Mg^{++}, Na^{+}, and K^{+}, and the anions, CO_3^{--}, HCO_3^{-}, SO_4^{--}, Cl^{-}, and NO_3^{-}, contribute to the total osmotic effect. Moreover, for the most part, they are essential for plant growth; they may be toxic above certain concentrations; and they are additionally important because of their effect upon the character of the soil. Among the trace elements, toxic effects may be produced by chromium, cobalt, copper, lead, mercury, molybdenum, nickel, selenium, and zinc. These metals, as well as the substances in low concentration listed above, are discussed separately in Chapter VI.

Because of all the variables involved, the classification of waters for irrigation use must be somewhat arbitrary and the limits set cannot be too rigid. Many studies, particularly at the University of California and the Rubidoux and Regional Salinity Laboratories of the U.S.D.A., have resulted in the division of irrigation waters into broad categories designated as (a) "excellent to good" or "suitable under most conditions"; (b) "good to injurious" or "harmful to some plants under certain conditions"; and (c) "injurious to unsatisfactory" or "harmful to most plants under most conditions". Occasionally these classes have been further subdivided into groupings labeled "excellent", "good", "permissible", "injurious", and "unsatisfactory" (246, 263, 264, 267, 268, 269, 271, 272, 273, 274, 1642, 1733, 1912, 2135, 2381).

For the sake of uniformity, the classifications reviewed for this survey have been reorganized, where necessary, to fit into the three-class system used in Tables 5-9 and 5-10. The characteristics of water, which have been accepted thus far as sufficient to determine its suitability for irrigation, are (a) the total concentration of salts, expressed as mg/l or the specific electrical conductance (EC $\times 10^6$) in micromhos per centimeter at 25°C, (b) the percentage of sodium which is equal to $\frac{Na \times 100}{Na + Ca + Mg + K}$ when the bases are expressed as milliequivalents per liter, and (c) boron, chloride, and sulfate concentrations. These factors may all vary more or less independently, so that water adequate in several respects may be rendered unsuitable because of a high concentration of one lone constituent, e.g. boron.

According to Doneen (1912), calcium and magnesium carbonates and calcium sulfate should not be considered in establishing standards for total salinity, owing to their limited solubility. He suggests that the remaining soluble salts be termed "effective salinity" and he proposes a tentative classification for this new parameter.

In order to facilitate the determination of water quality for various crops, plants have also been classified according to their tolerance of salinity, and according to their tolerance of boron (246, 267, 274, 1642). The salinity problem, sodium relationship, bicarbonate effect, and boron concentration in relation to the quality of irrigation water are discussed herewith in Chapter V, rather than in the appropriate part of Chapter VI, because they are so important to an understanding of water-quality criteria for irrigation.

The uptake of fission products from irrigation water and from fallout is a problem of increasing concern. According to Bowen (2382), cesium, strontium, barium, and iodine are the only important fission products that are readily absorbed and translocated by plants. As might be expected, strontium and cesium closely resemble calcium and potassium respectively in their behavior in irrigation. For further details of radioactivity in relation to irrigation, see Chapter VIII.

Many other substances whose presence in irrigation water may be undesirable have been investigated, but their limiting concentrations have not yet been fitted into standard routine systems of classification. Some of these occur in natural irrigation waters, but others such as pathogenic bacteria and insecticides are found only when they are added to the water as a result of man's activities. While it might be useful to tabulate all of these substances and their effects on irrigated crops in this section of Chapter V, the resulting tabulation would be cumbersome. Instead, the reader is referred to the effects of various trace constituents of irrigation water, summarized as "potential pollutants", in Chapters VI to X inclusive. For an interesting legal opinion involving the sodium percentage in irrigation water, see Barakis v. American Cyanamid Co. in Chapter IV, Judicial Expression.

SALINITY PROBLEMS

Certain soluble salts are essential for plant growth, but excessive concentrations of dissolved salts are harmful. In evaluating the effects of salinity on plant growth, it is seldom necessary to determine concentrations of individual salt constituents. A measurement of the osmotic pressure of the soil solution generally suffices. The electrical conductivities (EC) and total dissolved solids contents of soil solutions have been found to be sufficiently well related to their osmotic pressures to permit the substitution of EC for the more involved osmotic pressures (OP) determination. Another simplification involves substitution of the saturation extract of the soil for the more difficult-to-obtain soil solution (2383). Hence,

$$\text{OP (in atmospheres)} = 0.36\ (EC_e \times 10^3)$$

Where $EC_e \times 10^3$ is the electrical conductivity of the soil extract in millimhos per cm at 25°C. Also:

$$\text{Salinity (in mg/l)} = 640\ (EC_e \times 10^3)$$

The values of EC_e refer to the saturation extract of the soil and not to electrical conductivity in the irrigation water. It must be remembered that salinity of the soil moisture is likely to be five-fold or even ten-fold that of the irrigation water.

Moderate concentrations of chloride in the root zone (700 to 1500 mg/l in the soil moisture) usually cause chloride to accumulate in the leaves to about 1 to 2 percent of dry weight. At such concentrations, marginal leaf burn develops, leading ultimately to leaf drop, twig die-back, and possibly death of the plant. Sodium accumulations in leaves of 0.2-0.3 percent of dry weight produces leaf burns and injury (2383, 2384a).

According to Doneen (2385) the effects of salinity can best be evaluated by the "potential salinity", defined as the chloride concentration plus half of the sulfate concentration, both in meq./l. Chloride salts are highly soluble and toxic to some plants. Half the sulfate is used because this ion is less toxic to plants than chloride and because less salinity will accumulate in the soil from sulfate than from chloride, owing to the precipitation of calcium sulfate. Limiting potential salinities in meq./l for various soil conditions and for the three classes of irrigation water described in Table 5-8 are shown below:

Soil Condition	*Limiting Potential Salinity, meq./l*		
	Class I	*Class II*	*Class III*
A. Little leaching, owing to low percolation rates	3	3-5	5
B. Some leaching, but restricted. Drainage is slow	5	5-10	10
C. Open soils, deep percolation easily accomplished	7	7-15	15

SODIUM RELATIONSHIPS

Calcium and magnesium in the proper proportions maintain soil in good condition of tilth and structure, while the opposite is true when sodium predominates. The effect of potassium on soil is similar to that of sodium, but since the concentration of potassium is generally quite small in water, potassium is often omitted

from calculations or included in figures stated for sodium concentration. In most normal soils in arid or semiarid regions, calcium and magnesium are the principal cations held by the soil in replaceable or exchangeable form, with sodium consisting of a small percentage, i.e., about 3.0 to 7.0 percent (278, 279, 280). Such soils, when not misused, represent a favorable physical condition for root and water penetration. An increase of the exchangeable sodium percentage (ESP) to as much as 12 or 15 percent (or, according to Doneen even to 8.5 percent in some cases) causes the granular soil structure to begin to break down when the soil is moistened. Various changes take place resulting in the sealing of soil pores and a decrease in soil permeability. With further increases in the sodium percentage, the soil continues to deteriorate and its pH increases to the level of alkali soils.

TABLE 5-8

DETAILED CLASSIFICATIONS OF IRRIGATION WATERS

References (a)	% Sodium $\frac{Na \times 100}{K + Na + Mg + Ca \text{ as meq/l}}$	EC × 10^6 at 25° C. (b) in micromhos/cm	Total salts mg/l	Boron—mg/l: Sensitive plants	Boron—mg/l: Semi-tolerant	Boron—mg/l: Tolerant	Chlorides meq/liter*	Sulfates meq/liter*
CLASS I								
263	<30	<500	<350	<0.5	<1.0	<1.5	<5.5	<5.5
264	<40	----	----	<0.4	----	<1.0	<2	<4
269	<30	<500	<350	<0.5	<1.0	<1.5	----	----
267	<60	<750	----	<0.5	<1.0	<2.0	----	----
272	<60	----	----	----	----	----	----	----
246	<60	<1000	<700	----	<0.5	----	----	----
271	<60	----	----	----	----	----	<5	----
273, 1912	<60	<1000	<700	----	<0.5	----	<5	<10
268	----	----	----	<0.5	<1.0	<1.5	----	----
2135	----	----	<500	----	----	----	----	----
1733	<40	<750	<525	<0.67	<1.33	<2.0	<7	<7
CLASS II								
263	30–70	500–2500	350–1750	0.5–1.12	1–2.25	1.5–3.35	5.5–16.0	5.5–16.0
264	40–70	----	----	0.4–1.0	----	1.0–2.0	2–6	4–12
269	30–70	500–2500	350–1750	0.5–1.12	1.0–2.25	1.5–3.35	----	----
267	60–70	750–3000	----	0.5–1.0	1.0–2.0	2.0–3.0	----	----
272	----	----	----	----	----	----	----	----
246	60–75	1000–3000	700–2000	----	0.5–2.0	----	----	----
271	60–75	----	----	----	----	----	5–10	----
273, 1912	60–75	1000–3000	700–2100	----	0.5–2.0	----	5–10	10–20
268	----	----	----	0.5–1.12	1–2.25	1.5–3.35	----	----
2135	----	----	500–1500	----	----	----	----	----
1733	40–80	750–3000	525–2100	0.67–1.25	1.33–2.50	2.0–3.75	7–20	7–20
CLASS III								
263	>70	>2500	>1750	>1.12	>2.25	>3.35	>16	>16
264	>70	----	----	>1.0	----	>2	>6	>12
269	>70	>2500	>1750	>1.12	>2.25	>3.35	----	----
267	>70	>3000	----	>1.0	>2.0	>3.0	----	----
272	----	----	----	----	----	----	----	----
246	>75	>3000	>2000	----	>2.0	----	----	----
271	>75	----	----	----	----	----	>10	----
273, 1912	>75	>3000	>2100	----	>2.0	----	>10	>20
268	----	----	----	>1.12	>2.25	>3.35	----	----
2135	----	----	1500–2500	----	----	----	----	----
1733	>80	>3000	>2100	>1.25	>2.50	>3.75	>20	>20

* meq /liter = mg/l equivalent weight, for example, meq/l of Cl^- = $\frac{\text{mg/l } Cl^-}{35.5}$, and meq/l of SO_4^{--} = $\frac{\text{mg/l } SO_4^{--}}{48}$

(a) Data from References 263, 268, 269, and 1733 have been changed to fit this table. The original papers set up 5 classes of water.

(b) EC x 10^6 at 25° C. is a measure of salinity. See text.

Class I Excellent to good, or suitable for most plants under most conditions.

Class II Good to injurious, harmful to some under certain conditions of soil, climate, practices.

Class III Injurious to unsatisfactory, unsuitable under most conditions.

TABLE 5-9

SUMMARY OF CLASSIFICATIONS OF IRRIGATION WATERS

Class	% Na $\frac{Na \times 100}{Na + Ca + Mg + K}$ as meq per liter	Boron, in mg/l	Chlorides in meq/l	Sulfates in meq/l	EC × 10^6 at 25° C. Specific conductivity (Concentration of ions)	Total salts in mg/l
I	Less than 30-60% (Most recent work favors a 60% limit)	Boron recommendation for water of this class is generally accepted as less than 0.5 mg/l; however tolerant plants will not be injured by 1-1.5 mg/l.	Less than 2-5.5	Less than 4-10	Earlier papers suggested limit of about 500, but more recently 1000 has been accepted.	Up to about 700
II	30-75%	0.5-2.0 mg/l, although for tolerant plants water with boron up to 3.35 mg/l may be satisfactory	2-16	4-20	500-3000	350-2100
III	More than 70-75%	More than 2 mg/l although water with more than 1.0 may be highly unsuitable for sensitive plants	More than 6-16	More than 12-20	More than 2500-3000	More than 1750-2100

It is easier for calcium to replace sodium in the exchange complex than for sodium to replace calcium, and unless the sodium in the soil solution is considerably in excess of the calcium, no calcium will be replaced. It must be borne in mind that the soil solution is always more concentrated than the irrigation water. If magnesium constitutes a high proportion of the total replaceable cations of the soil, more sodium will be absorbed than if calcium is the only divalent cation present (281). It has been widely recommended that the percentage of sodium $\left(\frac{Na \times 100}{Na + Ca + Mg + K}\right)$ in irrigation water should not exceed 50-60, in order to avoid the deleterious effects on soil which have been described above. Where the soil has a high cation exchange capacity and where the irrigation water is very dilute, values above 50 may be within safe limits (2386).

Aluminum, as well as calcium, in soluble form and in appreciable quantities, has been found to counteract the injurious effects of sodium on clay; and hence applications of these cations may be used to remedy such injury (283, 348).

In 1954 the staff of the U.S. Salinity Laboratory proposed that the sodium (or alkali) hazard of irrigation water can best be expressed in terms of the Sodium Adsorption Ratio, or SAR (1642). This ratio expresses the relative activity of sodium ions in the exchange reactions with soil. It is defined as follows:

$$SAR = \frac{Na}{[\frac{1}{2}(Ca + Mg)]^{1/2}}$$

where Na, Ca, and Mg are concentrations of the respective ions in milliequivalents per liter of water. If sodium percentage is defined as

$$Na\,\% = \frac{100Na}{Na + Ca + Mg}$$

then SAR can be expressed in terms of the milliequivalents per liter of sodium and the sodium percentage as follows:

$$SAR = Na^{1/2}\left[\frac{2Na\,\%}{100 - Na\,\%}\right]^{1/2}$$

A thorough description of the SAR and its use is contained in Agricultural Handbook No. 60, U.S. Department of Agriculture (1642). Chapter 5 of this handbook is an excellent treatise on the entire subject of the quality of irrigation water.

Based on a SAR scale from 0 to 30 and conductivity values of 100 to 5000 micromhos per cm at 25° C a diagram has been prepared for classifying irrigation waters with respect to sodium and salinity hazards, taking into account that a given SAR represents a greater hazard when the total concentration of ions is high than when it is low. This diagram appears as Figure 25 of U.S.D.A. Handbook No. 60 and it is reproduced herewith as Figure 5-1.

Water in the Cl-Sl area of the diagram can be used on almost all soils and for almost all crops without detrimental effects. With increasing salinity, less exchangeable sodium can be tolerated and more leaching will be required to prevent salinity damage. Waters with an SAR value greater than 10 will present an appreciable sodium hazard in fine-textured soil having high cation-exchange capacity, especially as the salinity increases. Water in the S2 range may be used on coarse-textured or organic soils with good permeability (1642, 2387). For further analysis of this diagram, the reader should consult U.S.D.A. Handbook No. 60.

Doneen (2385, 2388) uses the term "sodium index" or "permeability index" to combine the effects of the sodium and bicarbonate ions and the total concentration of cations (c) in the irrigation water, all measured in milliequivalents per liter, thus:

For a water having 5 meq/l of sodium, 4 of bicarbonate, and 8 of total cations, the index would be $\frac{5 + 2}{8} \times 100$ or 87.5. Doneen (2388) presents curves to show the relation of the permeability index and the total ionic concentration for three types of soil and three classes of irrigation water.

BICARBONATE EFFECTS

The sodium hazard is also increased if the water contains a high concentration of bicarbonate ions, for as the soil solution becomes more concentrated there is a tendency for calcium and magnesium to precipitate as carbonates and for the relative proportion of sodium to be increased as a consequence. Therefore the bicarbonate concentration of the water has been suggested as an additional criterion for irrigation water. It has been found convenient to express the bicarbonate value of the water in terms of the "residual sodium carbonate" (RSC) concentration, a concept devised by Eaton (2406) and defined as follows:

$$RSC = (CO_3^{--} + HCO_3^{-}) - (Ca^{++} + Mg^{++})$$

when the ionic constituents are expressed as milliequivalents (meq.) per liter.

Analyses of irrigation water and soil samples at the Salinity Laboratory have led to the conclusion that waters containing less than 1.25 meq. per liter of residual sodium carbonate are probably safe; those containing 1.25-2.5 meq. per liter are marginal; and those with more than 2.5 meq. per liter are not suitable. Marginal waters might be used successfully where good management practices are followed (1642, 2389).

BORON IN IRRIGATION WATERS

Boron is found in almost all waters used for irrigation in the U.S.A., in concentrations from a trace to over 100 mg/l. It occurs naturally in the form of borax, borates, boric acid, and various borosilicates, such as tourmaline, which are of magmatic origin. It can also be found in fertilizers and certain waste-waters, such as those from citrus washing. In most natural waters, boron probably occurs as almost completely undissociated boric acid (2379, 2390). Although traces of boron are essential for all plant growth, it is doubtful whether more than 0.5 mg/l can be applied continuously to soils without ultimately producing some plant injury (265, 275).

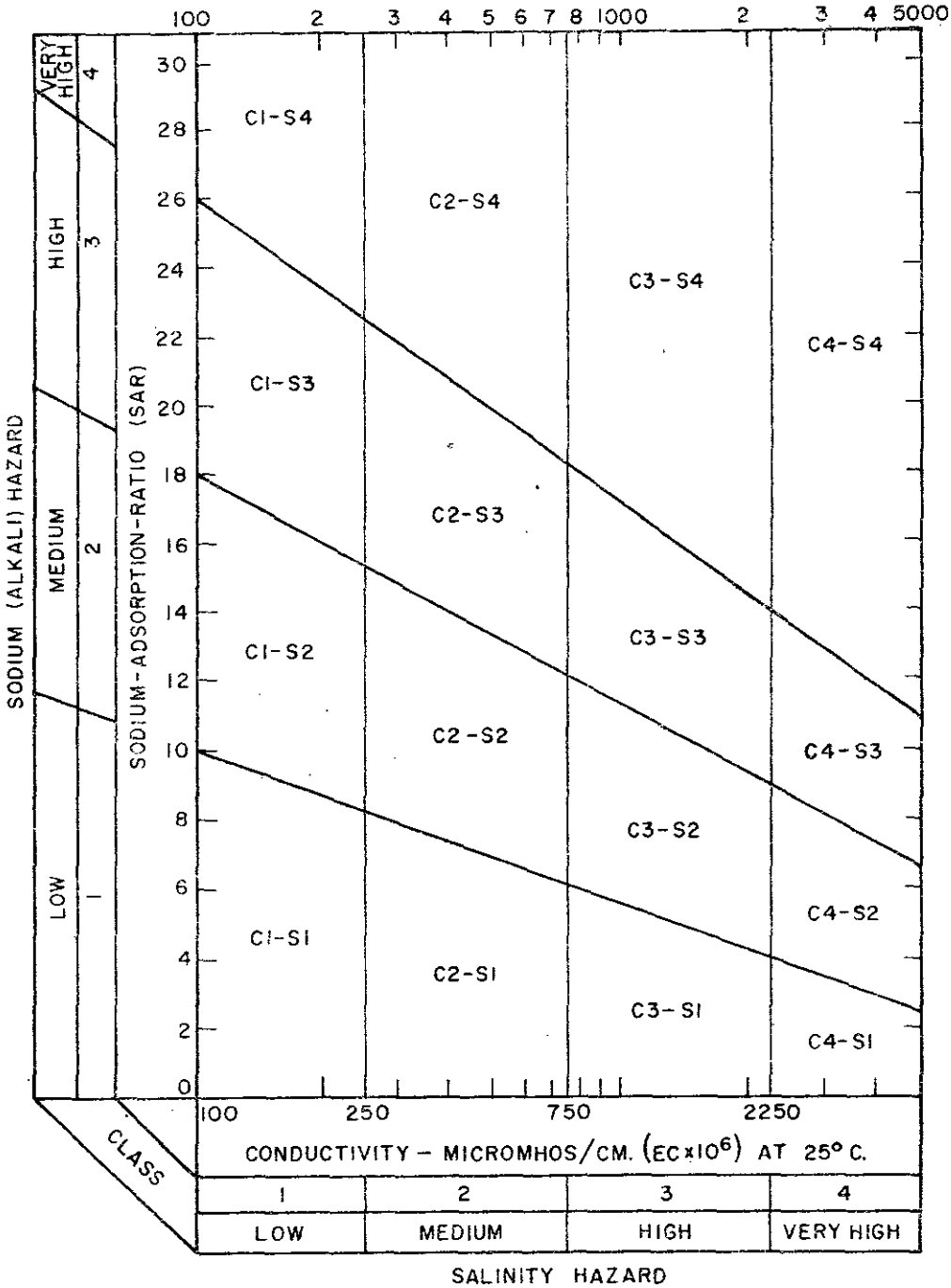

FIGURE 5-1. DIAGRAM FOR THE CLASSIFICATION OF IRRIGATION WATERS (from USDA handbook No. 60)

Agricultural authorities agree that for irrigation water the critical concentration is 0.4 to 0.5 mg/l; but because plants vary in their sensitivity to boron, waters may be classified not only according to their boron content, but also according to the tolerance of the crops to which they are applied. Tables grouping plants in the order to their sensitivity to boron will be found in several papers, including the following references (246, 263, 264, 269, 274, 1642, 2391). The most sensitive crops are citrus, nuts, and deciduous fruits; semitolerant are truck crops, cereals, and cotton; most tolerant are lettuce, alfalfa, beets, asparagus, and date palms.

While some crops such as alfalfa and date palms are stated to be uninjured by as much as 20 to 100 mg/l of

boron, it is considered that the maximum concentration safe for even the least sensitive plants is about 4.0 mg/l (276).

Symptoms of boron injury can be distinguished easily from those of most other types of injury, although occasionally they are confused with those of sulfate poisoning. Among trees, advanced damage will result in leaf-yellowing and burning, premature leaf drop, and reduced yield (276, 277). The quality of soil, drainage, and climatic and other environmental factors, such as the amount of rainfall and total amount of irrigation water applied, can modify the safe concentration limits. However, symptoms of boron injury may not become apparent for as long as several years. They develop more rapidly in light than heavy soils. Concentration of the soil solution owing to evaporation and transpiration tends to accelerate their apearance, but the absorptive capacity of the soil may delay it. Parenthetically, it is essential to remember that when boron in the irrigation water is 0.5 mg/l, its concentration in the soil solution may be more than 4 mg/l (265).

It has been suggested that where the boron concentration in irrigation water is high and cannot be reduced economically, an effort should be made to grow more-resistant crops in the area affected. A widely used classification of water according to its boron concentration is shown in Table 5-10.

TABLE 5-10

PERMISSIBLE LIMITS FOR CONCENTRATION OF BORON IN SEVERAL CLASSES OF WATER FOR IRRIGATION

(After Scofield) (263)

Class of Water	*Concentration of Boron in mg/l For Crops That Are*		
	Sensitive	*Semitolerant*	*Tolerant*
Excellent	Less than 0.33	Less than 0.67	Less than 1.0
Good	0.33-0.67	0.67-1.33	1.0-2.0
Permissible	0.67-1.0	1.33-2.0	2.0-3.0
Doubtful	1.0-1.25	2.0-2.5	3.0-3.75
Unsuitable	Over 1.25	Over 2.50	Over 3.75

STOCK AND WILDLIFE WATERING

Paradoxically, data with respect to the water-quality requirements of animals are both abundant and sparse. There is a wealth of information about the LD_{50} values of thousands of compounds fed to laboratory animals, mostly rats, mice, and guinea pigs, either in their diet or in their drinking water. Yet, there are very few quantitative data concerning the water-quality tolerances of livestock and poultry. Veterinarians and animal-husbandry personnel in this country do not appear to be particularly concerned over water quality; but in Australia and South Africa, where water for livestock is frequently highly mineralized, considerable attention has been directed to this problem.

Since the total quantities of substances ingested daily are the critical values for animal metabolism, the permissible concentrations of such substances in water will depend, to some extent, on the daily water consumption of the animals. The daily water requirements of animals vary with a number of factors, such as the temperature and humidity of the atmosphere, the water content of the diet, the degree of exertion by the individual with a resulting loss of water as sweat, and the salinity of the available supply (284, 286).

The quantity of water required for livestock and poultry has been estimated as follows (284, 286, 2392):

Animal	*Water consumption in gpd per head, except as noted*
Beef cattle	7-12
Dairy cattle	10-16
Horses	8-12
Swine	3-5
Sheep and goats	1-4
Chickens	8-10 (per 100 birds)
Turkeys	10-15 (per 100 birds)

It has been assumed that water safe for human consumption may be used safely by stock; indeed, it has been recommended that stock, for their highest production, should have such water (284, 285). On the other hand, it appears that animals can tolerate higher salinities than men, and it is conceivable also that they differ in their tolerance of specific substances.

The use of highly mineralized waters can cause among animals, as well as among men, physiological disturbances of varying degrees of severity, such as gastrointestinal symptoms, wasting disease, and death. Among the functions of animals, lactation and reproduction are generally the first to be disturbed by continuous use of waters with unfavorable mineral concentrations, so that milk and egg production are reduced, if not terminated.

It has been stated that no animal will choose to drink saline water if better water is available. Within limits, however, animals can adjust to the use of saline waters that at first were impossible to consume. On the other hand, sudden changes from slightly mineralized to highly mineralized water may cause acute salt poisoning and rapid death (282). The tolerance of animals to salts in water depends also on other independent factors, including their species, age, and physiological condition, the season of year, and the salt content of the diet, as well as the quality and quantity of salts present.

The officers of the Department of Agriculture and the government chemical laboratories of Western Australia (282, 2393) have listed the threshold concentrations of salinity tolerated by livestock in that region. The total salts include the chlorides, sulfates, and bicarbonates of sodium, calcium, and magnesium, with sodium chloride constituting as much as 75 percent of the total salinity. In general, it is stated that waters containing less than 300 grains per Imperial gallon (about 5000 mg/l can be used continuously by all livestock. Sheep are more tolerant than cattle, and cattle are more tolerant than horses or pigs. The standards in use in Western Australia as the safe upper limits for stock are reported as follows:

Animal	*Threshold Salinity Concentrations in grains per Imperial gallon*	*mg/l*
Poultry	200	2860
Pigs	300	4290
Horses	450	6435
Cattle, dairy	500	7150
Cattle, beef	700	10,000
Adult dry sheep	900	12,900

When total salts exceed the above listed concentrations, practical tests are needed to show whether or not the water is safe. When green feed is available, animals can tolerate more saline water than when "bush or scrub" is the only feed. Where feed is low in salt content, water of higher salinity is also tolerable. Sheep

have used water containing 18,600 mg/l temporarily, but lambs and ewes should be watched carefully when the water has over 10,000 mg/l. The milk production of cows is reduced by saline waters containing 7150 mg/l or even less.

It has been found that when water is heavily saturated with chlorides, sulfates, carbonates, bicarbonates, sodium, calcium, and/or magnesium, the injury caused represents an osmotic effect of the total salts present rather than a toxic effect of any constituent. The maximum concentration of salts that can be tolerated safely by domestic animals appears to lie between 15,000 and 17,000 mg/l, although it is recognized that this limit may be too high for animals in production (287, 288). Alkali salts were found to be somewhat more injurious than neutral salts, sulfates more harmful than chlorides, and magnesium chloride more harmful than calcium or sodium chloride.

Other investigators have found that concentrations as high as 15,000 mg/l, while safe for limited periods, are dangerous for continuous use (284, 289, 290, 291, 292, 293, 294, 295, 296, 1756, 2394, 2395, 2396, 2397). In areas where the salinity of the water for livestock may be a critical factor for the economic survival of the inhabitants, much lower limiting values have been recommended. For waters in the State of Colorado, a salinity as high as 2500 mg/l is considered acceptable for stock (285). For Montana waters, salt concentrations up to 2500 mg/l are considered good; 2500-3500 mg/l, fair; 3500-4500 mg/l, poor; and above 4500 mg/l, unfit (297).

A South Dakota bulletin (2398) classifies water for livestock as excellent (0-1000 mg/l), good (1000-4000 mg/l), satisfactory (4000-7000 mg/l), and unsatisfactory (over 7000 mg/l). These data were derived from feeding experiments with rats, cattle, swine, and poultry.

Apart from the total salt concentration, some salts are specifically toxic to animals, even in very low concentrations, and their presence in the water may render it dangerous for use. Studies of the effects of excesses of various chemicals on animal physiology are numerous for laboratory animals, but most results deal with the diet rather than in water. Among the compounds causing concern in water are nitrates, fluorides, and the salts of selenium and molybdenum (298). The literature dealing with specific chemical substances has been summarized in Chapter VI and will not be repeated here.

It is reasonable to assume, a priori, that bacterial diseases may be transmitted to livestock as well as to men by infected water. However, the practical significance of water as a vector of animal disease has not yet been established. Several independent investigators report that cattle and swine to which sewage, sewage effluents, and polluted water have been fed over long periods (six months to two years) have shown no signs of bacterial infection (299, 300, 301, 302, 2399, 2400, 2401) even when virulent disease organisms could be isolated from samples of the fluids administered. The risk remains, however, that the udders of cows having access to polluted water or sewage-irrigated fields may become infected by typhoid or tuberculosis bacteria.

More work should be done to determine whether cattle can be infected by bacteria in the concentrations found in sewage effluents. Until more basic data can be furnished it has been recommended that water with bacterial contamination be treated completely for use by cattle (301). On the other hand it has been suggested that water should be condemned for cattle use only when bacteria known to be pathogenic to cattle can be isolated (303). The spread of beef tapeworms through sewage must also be considered; for a discussion of this problem, see Worms, Parasitic in Chapter VII.

According to the section on animal diseases in the 1956 Yearbook of Agriculture (2402), the following diseases may possibly be transmitted through contaminated drinking water: brucellosis, bovine tuberculosis, leptospirosis, listerosis, coccidiosis, and pullorum. While water is implicated as a possible route of infection it is not a primary or major source. Waste waters from slaughter houses and dairies have also been suspect as a pathway for the transmission of animal diseases, but this method of infection has not been proved (2403, 2404).

Of far greater concern are toxic algae and/or protozoa in water consumed by livestock, poultry, and wild birds. Although no quantitative evaluation of the concentration of toxic algae is given, there are numerous citations in the literature of animal deaths attributable to the consumption of such water. The subject of toxic algae is covered in detail in Chapter VII.

Oily substances may possibly be detrimental to livestock, although the strong odor and disagreeable taste of hydrocarbons are likely to prevent animals from consuming water that is heavily polluted with oil products. Waterfowl, however, have suffered heavy losses from contact with oil that sticks to their feathers and interferes with their buoyancy in water. The entire subject of the effects of oily substances on stock and wildlife is covered in Chapter VI.

PROPAGATION OF FISH AND OTHER AQUATIC AND MARINE LIFE

The number of references dealing with the effects of various pollutants on fish and other aquatic life far exceeds those pertaining to any other beneficial use of water. A large proportion of such references has been covered by this survey, but any attempt to condense such information in this part of Chapter V was deemed to be futile; instead the effects of each potential pollutant are described in Chapters VI to X inclusive. Certain general considerations and fundamental concepts are presented at this point, however, and tentative guides for threshold concentrations in fresh and saline water are suggested.

Not only are the references dealing with fish innumerable; they are also individualistic in their approaches to the problem. The conditions under which the many investigators conducted their experiments varied widely and until recently were seldom standardized. Hence, the results by several investigators of the same pollutant may not compare closely. This wide discrepancy arises from variations in the species of fish or other organism used, its prior handling, the temperature, the season, the dissolved-oxygen content, synergistic and antagonistic substances, the hardness and other mineral content of the water, and the time of exposure (2407, 2408). These effects are described in greater detail below.

Perhaps the most variable and most confusing aspect of the numerous references is the nomenclature used to

describe terminal effects. Some authors refer to threshold and limiting concentrations in much the same manner as they are used in this report, i.e., a "threshold" concentration is a point at which a physiological effect begins to be produced or a point at which the use is not damaged to any significant degree by pollution but slight effects are noticeable; and a "limiting" concentration is one at which the quality of water for a beneficial use is markedly impaired. Some investigators measure distress as indicated by the frequency of gill action, others by feeding or the absence thereof, and others by the reflexes of the fish or their response to stimulants. Some refer to "minimum lethal concentration" or "minimum lethal dose" with or without defining the time of the test and whether one or all of the test animals succumbed. The term that is appearing in recent literature and generally approved by fish toxicologists is TL_m, the median tolerance limit, or the dosage required to kill 50 percent of the test animals within a specified period, such as 24 or 48 hours. Sometimes vague terms such as toxic concentration or lethal dose are used without further definition.

The time-concentration relationship is very important in all studies of tolerances of aquatic and marine life toward pollutants. Thus, an organism may withstand a 10-minute exposure to 200 mg/l of a certain substance, followed by a return to clear water, without any apparent deleterious effect; yet the same organism may succumb to repeated 10-minute exposures of that concentration or to a continuous exposure to only 20 mg/l of the same substance. On the other hand, by continuous exposure to gradually increasing concentrations, the organism may build up a tolerance to concentrations that would be toxic to a non-acclimated organism. The effects of long-term exposures of fish populations to very low sub-lethal concentrations are not clearly understood.

This relationship of concentration and time of exposure is extremely important in considering the effect of a slug of waste on the aquatic life of a stream. Normally a slug would be more deleterious than a steady uniform discharge with adequate mixing, but in some instances the concentrated slug may be less detrimental than the steady weak pollution. Or, perhaps the lack of lateral or longitudinal mixing in a stream or tidal estuary may be advantageous if it produces a local concentration into which fish may swim accidentally, but from which they can escape to clear water in a few minutes without permanent injury.

A unique concept relating to the effect of pollutants on fish has been presented by Hiatt, Naughton, and Matthews (1737, 1738). The objective of their studies was to find whether or not certain chemical compounds in highly dilute solution would effect a dispersal of schooling fish. Tests were conducted in 50-liter aquaria in which a small school (4 or 5) of *Kuhlia sandvicensis* was exposed to chemicals dispersed or dissolved in sea water. In the standard 2-minute span of observation, responses varied from mild to violent. Respiratory poisons induced gulping, swimming at surface, and depressed activity. Certain substances caused tetanic or flaccid paralysis, fin or operculum distension, disorientation, or convulsions. Insecticides of the irritant type were effective in dispersing schools but respiratory poisons such as cyanides were only moderately effective. The authors studied 87 compounds and presented a table describing the irritant activity of these substances. It is possible that the dispersal of schooling fish, even without other deleterious effects, might have an economic significance or modify the natural regime of the water course.

It is impossible to set up rigid quality standards or limiting concentrations for broad general application over a wide area, because the many variable factors, both physiological and environmental, can alter the responses of fish to specific constitutents of the water. Some of the most significant of these variables are considered in the paragraphs below.

a. The effects of harmful substances upon fish life vary with species, size, age, and physiological condition of the individuals. Water favorable for some species may not necessarily be adequate for others that have been adapted to somewhat different conditions.

b. The effects of deleterious substances upon fish vary with the physical and chemical composition of the water supply; for example, in soft water the damaging effects of poisons are generally greater than in hard water. In distilled water, very low concentrations of some pollutants are deleterious. Decreased oxygen concentrations and increased temperatures tend to increase the susceptibility of fish to toxicants. Interrelationships between the dissolved constituents of the water supply are also extremely important. By synergistic action, the combined influence of several substances simultaneously may result in greater damage to fish life than the sum of the individual effects taken independently. For example, a combination of sulfates of cadmium and zinc, or nickel and cobalt, are additive in effect, but combinations of sulfates of copper and zinc, copper and cadmium, or nickel and zinc can produce up to five times the reaction that would be expected if the effect were simply additive. On the other hand, certain combinations of salts act antagonistically to reduce the injurious effects of each. For example, mixtures of salts have become progressively less toxic when to sodium chloride solution has been added calcium chloride, then potassium chloride, and finally magnesium chloride (304, 305, 306, 307, 1459, 2407, 2408, 2409, 2410, 2411, 2412).

c. Hydrographical features of water courses and fluctuating water levels, particularly in impoundments, may also act to modify the effects of pollutants on fish in their natural habitats (308, 309).

d. In surveying the literature it is often difficult, if not impossible, to determine whether concentrations are reported for pure compounds, or with water of hydration.

For reasons such as these, as well as because of the complexity of many effluents, chemical and physical data alone may be insufficient to predict the results of pollution upon the aquatic life of a body of water (305, 311, 312, 343, 1459, 2410). While probable safe limits of concentrations of various materials can serve as a helpful guide for waste discharges, it is generally a good policy to conduct supplemental biological tests, or bioassays, upon the organisms involved, or upon suitable indicator organisms. Recently, attention has been given increasingly to the use of bioassays in or near the industrial plants whose effluents are potentially hazardous for fish (310, 343, 1734, 1735, 2412).

Bioassay procedures for the evaluation of acute toxicity of specific substances and mixed industrial wastes were developed in 1951 by a sub-committee of the Federation of Sewage and Industrial Waste Associations, under the chairmanship of Doudoroff (2413). These procedures, with appropriate extensions and modifications, have been incorporated into the 11th Edition of Standard Methods for the Examination of Water and Wastewater (469). The test was designed to determine whether or not a mixed industrial discharge is toxic (and if so at what concentration and in what period of time) when evaluated in water comparable to the quality of the actual receiving stream and with local fish. While reasonable uniformity of experimental procedure and the manner of presenting results is desirable, rigid standardization of the quality of water used and the test fish would defeat the purpose of the experiment.

Since the widespread adoption of this standard method, there has been a marked improvement in the bioassay results reported in the literature. These results are now expressed as the 24-, 48-, and sometimes 96-hour TL_m values, with a specified fish and recorded temperature. They indicate the concentration of waste required to kill 50 percent of the test animals in the specified time; but they do not reveal a "safe" or threshold concentration at which little or no deleterious effect will occur. Application factors for converting TL_m values to safe concentrations have been suggested, but as of 1961 no suitable factor or formula had been found (2038, 2412).

Extensive bioassay work has been performed to determine the effects on fish and other aquatic or marine animals and plants of dissolved oxygen, pH range, temperature, free carbon dioxide, ammonia, suspended and dissolved solids, turbidity, detergents, pesticides, oily substances, other biota, and hundreds of other specific organic and inorganic substances as well as mixed industrial wastes. These results have been summarized under the appropriate headings in Chapters VI-X inclusive.

Attention is invited especially to the subject of radioactivity and the fact that radionuclides can be concentrated by factors of 10^5 to 10^6 in the aquatic and marine food chains. This problem is discussed in detail in Chapter VIII and also under the next section of this chapter dealing with shellfish. It is important here to note that fish, as the end product of the aquatic or marine food chain, tend to accumulate the radioactive substances concentrated by algae, protozoa, crustacea, larvae, insects, and other links in the food chain.

CRITERIA GUIDES FOR EVALUATING THE QUALITY OF WATER USED FOR PROPAGATION OF FISH

Criteria for the quality of fresh water that will support a good mixed fish fauna were first presented by Ellis et al. (247, 313, 347) who proposed the following limits:

1. Dissolved oxygen, not less than 5 mg/l;
2. pH, approximately 6.7 to 8.6, with an extreme range of 6.3 to 9.0;
3. Specific conductance at 25° C, 150 to 500 mho $\times 10^{-6}$, with a maximum of 1000 to 2000 mho $\times 10^{-6}$ permissible for streams in western alkaline areas;
4. Free carbon dioxide, not over 3 cc per liter;
5. Ammonia, not over 1.5 mg/l;
6. Suspended solids such that the millionth intensity level for light penetration will not be less than 5 meters.

In the absence of toxic substances or pollutants, the water described above is favorable, and not merely sublethal, for a mixed warm-water fish population and its food organisms. It must not, however, be assumed that fish are not found or cannot survive in waters with concentrations beyond these limits.

A review of the general quality of water required for the maintenance of aquatic flora and fauna was presented by Stander (1736) but this publication does not differ significantly from the thorough reports by Ellis.

A most thorough analysis of water-quality criteria for fish and related organisms has been conducted by the Aquatic Life Advisory Committee of the Ohio River Valley Water Sanitation Commission, as presented in its first three progress reports (2109, 2408, 2414).

SHELLFISH CULTURE

The water-quality criteria for the growing of shellfish are sufficiently different from those for fish and other aquatic or marine life to warrant special attention as a major beneficial use of water. This section, however, is limited to the bivalve mollusks: oysters, clams, and mussels. After they have passed through the larval stage, most shellfish are non-motile benthic organisms that are incapable of changing location to avoid an unfavorable environment. The bivalve mollusks, by the very nature of their feeding mechanism, tend to concentrate and accumulate viruses and bacteria, including pathogens, from the overlying water. Moreover, unlike other marine life, these forms are frequently eaten raw. In addition to bacteria, the bivalve mollusks also serve to concentrate radionuclides and paralytic poisons, as described later in this section.

OYSTERS AND CLAMS

It is generally recognized that all good oyster beds are in the estuaries of rivers or in sounds, where they are influenced to a large extent by tidal action, topography, and bottom conditions. Tidal action controls the flushing of an estuary and combines with stream flow to govern the salinity. Along with topography, it influences the cleansing of beds, the removal or deposition of silt, and the supply of food (2415). Clams, on the other hand, live in beach sands along the open ocean as well as in mud flats of estuaries and bays. The requirements for good yields of marine shellfish include favorable bottom, adequate food, and proper water quality. The bottom must be firm enough to give support to the spat and keep the adults from sinking. Shifting sands, soft mud, slime-covered debris, and sedimentary deposits are undesirable for shellfish culture (314, 315, 316, 317, 1448, 2415, 2416). The characteristics of water that are important

for shellfish production include temperature, salinity, pH, oxygen concentration, copper content, and the presence of substances deleterious for shellfish development or hazardous to shellfish consumers.

Oysters and clams will tolerate wide variations in temperature, from freezing to 30° C, or perhaps to 35° C for hardier species. At water temperatures below 5° C, however, these shellfish are almost dormant, with little or no feeding and very slow metabolism (2416). Moreover, they will not spawn in the summer if temperatures do not rise to a critical level, e.g., 13° to 18° C for Olympia oysters *(Ostrea lurida)* and 18° C or above for Pacific oysters *(Crassostrea gigas)*. At higher temperatures, oysters metabolize their stored reserves of glycogen and consequently deteriorate in quality. Above 22° C, they are very poor (2415).

Oysters grow in brackish waters and can tolerate a broad range of salinities, which differs with species. They have been found at salinites from about 7,000 mg/l to 35,000 mg/l (314, 315, 318, 319, 320, 321) but salinities as low as 5,000 mg/l have caused death after several days (322). According to Churchill (315), the most favorable salt concentrations are between 18,500 and 34,000 mg/l and salinities below 13,000 mg/l can be tolerated for only brief periods. Salinities lower than 12,000 mg/l are definitely detrimental, for the oysters become watery and lose weight (2415). Other "most favorable" salinity ranges reported are 8,750 to 12,250 mg/l (320), 10,000 to 27,000 mg/l (318), and 30,800 mg/l (314).

The gaper clam appears to be restricted to a minimum salinity of about 27,000 mg/l while the softshell clam is found in a wide range of salinities, almost into fresh water (2417). Asiatic clams, which have become troublesome in western water supplies and irrigation systems, prefer fresh water (2418, 2419, 2420, 2421).

Oysters generally grow in neutral or slightly alkaline water. They may be injured by a pH lower than 6.5 (323) and they have been killed by a pH value as high as 9.1 (324).

Adequate dissolved-oxygen concentrations are essential. The minimum oxygen requirement of oysters at 25° C has been found to vary with the season, salinity, and pH. Minimum dissolved-oxygen utilization ranges from 1.1 to 5.8 ml per hour (325). Oysters have been found to be fairly tolerant of temporary oxygen deficiencies (326, 327).

For setting, oyster larvae require a copper concentration around 0.05 to 0.06 mg/l (328), but concentrations above 0.1 to 0.5 mg/l are toxic (329). The 96-hour TL_m value of copper to oysters was estimated at 1.9 mg/l, but such concentrations are not likely to occur in natural water. Oysters in water containing 0.13 to 0.50 mg/l of copper deposited so much copper in their tissues that they were unfit for food (2422).

Certain pesticides (DDT, toxaphene, aldrin, dieldrin) have been found to be deleterious to oysters in low concentrations (2423, 2515). For further details, see Chapter IX.

Oysters and clams feed by straining the water and extracting the plankton organisms. They are capable of pumping up to 50 liters per hour. The rate of pumping varies with the temperature, salinity, silt content of the water, and the concentration of organisms in the water. It was formerly believed that the diet of oysters consisted chiefly of diatoms, but recent findings indicate that nannoplankton, especially the small flagellates, constitute the major source of sustenance (2415). Bacteria are strained out of the water along with the flagellates, diatoms, and other higher forms of marine life, but bacteria are not believed to be a significant dietary component. Owing to the fact that oysters and claims live on plankton, any change in water quality that will alter the plankton is bound to have an effect on the shellfish (314, 315, 316, 317, 1448, 2415).

Oysters are subject to competition, diseases, and predators. Indeed, it has been estimated that only one out of every million oyster eggs survives to reach adulthood. Competition occurs in crowded oyster beds, from limpets, and from plants such as watermilfoil (2424). Among major predators are drills, flatworms, and starfish. Sponges may also be detrimental (2425). Diseases are caused by a fungus (*Dermocystidium marinum*), a flagellate protozoan (*Hexamita*), a copepod parasite (*Mytilicola orientalis*), and possibly by dinoflagellates such as *Gonyaulax scrippsae* (see also Chapter VII—Fungi). Any changes in water quality that favor the development of any of its enemies are bound to have a detrimental effect on the oyster (2415).

Considerable research has been conducted to determine the tolerance of oysters to industrial pollutants such as sulfite waste liquor and oily substances. For details of these bioassays, see the specific potential pollutant in Chapter VI.

MUSSELS

Several hundred species of fresh-water mussels are known, adapted to special environmental conditions and inhabiting lakes and rivers, shallow or deep, cold or warm. For natural occurrence they require the entry and survival of fish that serve as hosts for the young mussels during a part of their development. Mussels are often a nuisance in domestic and industrial water systems (2418, 2419, 2420, 2421, 2426).

Neither marine nor fresh-water mussels can survive on a shifting bottom; however, from clean sand or soft silt to coarse gravel and boulders, a variety of bottoms are suitable for various species. Mussels are found at various depths and under different conditions of light penetration. While some suspended organic materials may serve as food, excessive turbidity or heavy silting can smother mussels or stop their feeding movements, and contribute to oxygen depletion by blanketing decaying material having an oxygen demand. Adult mussels have become inactive when the oxygen tension of the water was reduced to 20 percent of saturation, and the young are even more sensitive to oxygen deficiency. Silt and chemical pollutants constitute a threat to the survival of fresh-water mussels (331, 332). Marine mussels are especially susceptible to the accumulation of paralytic toxic plankton, as described below.

PARALYTIC SHELLFISH POISONING

The marine armored flagellate, *Gonyaulax catenella*, contains an alkaloid toxin which is not harmful to the flagellate nor to shellfish that feed upon it and concentrate the toxin; but it is ten times as potent toward

mice as strychnine (2427), and it can cause a paralytic effect, serious illness, and even death in humans (2428). The public-health significance of paralytic shellfish poisoning is throughly described by McFarren et al. (2429) in a comprehensive monograph on this subject.

Toxicities for this substance are expressed as mouse units (m.u.). The mouse unit is defined in terms of the amount of toxin in a shellfish extract that will cause the death with paralytic symptoms of a standard 20-gram mouse in 15 minutes from the time of injection. It is a variable unit depending on the resistance to the toxin of the mice used in the tests, but one m.u. is generally equivalent to about 0.2 micrograms of purified toxin. For toxicity tests, 100 grams of shellfish meat are extracted with 100 ml of dilute hydrochloric acid. The clear extract is separated from the meat by decantation or centrifugation. If an injection of 1 ml of this extract kills a 20-gram mouse in just 15 minutes, the toxicity of the shellfish is said to be 200 m.u. Where the shellfish are highly toxic, dilutions of the extract are used (2430).

A toxicity of 200 m.u. or less is considered harmless. When toxicity exceeds 400 m.u., the collecting of shellfish is generally banned. Experience along the Pacific Coast has demonstrated that the mussel, *Mytilus californianus*, accumulates the toxin to excessive amounts during summer months; consequently the collection of mussels is generally banned during warm weather. The 1959 Manual of Recommended Practice for the Sanitary Control of the Shellfish Industry, published by the U.S. Public Health Service, specifies that "If the poison content reaches 80 micrograms per 100 grams of the edible portions of raw shellfish meat, the area shall be closed to the taking of the species of shellfish in which the poison has been found" (2431).

Oysters and clams have also been implicated in the paralytic shellfish poisoning of humans (2428, 2432, 2433). When exposed to the same water, however, the California mussel attains high toxicity levels much more quickly than oysters or clams. Oysters are least susceptible. Moreover, when the flagellate leaves the overlying water, oysters lose their toxicity most rapidly and mussels appear to retain it for the longer time (2434).

ACCUMULATION OF RADIONUCLIDES BY SHELLFISH

Radionuclides may become associated with aquatic or marine organisms in three ways: (a) through adsorption to surface areas, (b) through absorption from the surrounding medium, and (c) through ingestion as food. In some instances, especially in those tiny organisms that have a large ratio of surface to volume, adsorption is the most important mechanism (2435, 2436, 2437). Clams and oysters have been shown to accumulate and concentrate significant amounts of certain radionuclides, both in their shell and in their meat. Most of this accumulation occurs from the ingestion of nannoplankton that have adsorbed or absorbed the radio-elements, although some of the radioactivity of the shell may be attributed to direct adsorption (2438).

Mollusks, as invertebrate animals, are capable of withstanding high concentrations of radioactivity without apparent harm. A dose of 5000 to 20,000 roentgens is required to cause a 50-percent mortality in mollusks, and up to 50,000 roentgens are needed for 100-percent mortality (2439). Consequently, they can accumulate considerable amounts of radioactivity that may be harmful to human beings without being deleterious to the shellfish themselves.

It has been demonstrated by several investigators that plankton concentrate certain elements, whether radioactive or not, from the surrounding waters. These concentration factors have been evaluated for many organisms and for many elements. They range from less than 1.0 (i.e., the concentration of the element is less in the organism than in the surrounding water) to over 100,000. Moreover, the accumulation factor is different for the shell, test, or skeleton than for the organism proper, and in higher forms it varies from organ to organ. Iodine, for example, concentrates in the thyroid whereas calcium, strontium, and iron concentrate in the shell or skeleton (2435, 2436, 2440, 2441, 2442, 2443, 2444). Inasmuch as they feed on primary accumulators of radioactivity, shellfish act as secondary concentrators. For a more detailed review of this problem, see Chapter VIII.

The USPHS has recognized the potential danger associated with accumulation of radionuclides by shellfish in the 1959 Manual of Recommended Practice for the Sanitary Control of the Shellfish Industry (2431) by making low radioactivity one of the three criteria for approved shellfish areas.

Oysters appear to be especially adept at accumulating zinc from surrounding waters (2445). It has been estimated that oysters concentrate Zn-65 by a factor of about 20,000 from their environment (2446), whereas the concentration factor for Sr-89 is approximately unity (2431). The diatom *Nitzschia closterium* apparently concentrates Zn-65 very effectively and hence acts as one of the steps in accumulation by shellfish (2447).

BACTERIAL AND VIRAL CONTAMINATION OF SHELLFISH

Shellfish have long been implicated as significant vectors of typhoid fever and other enteric diseases. Recently, they have been demonstrated as the transmitting agent for infectious hepatitis. Owing to the fact that they strain bacteria as well as plankton from the surrounding water, they serve as concentrators and carriers of pathogens.

The bacterial content of the meat and shell liquor of shellfish will generally reflect the bacterial quality of the water in which the animals have grown. The relationship, however, is subject to many variable factors. For example, the soft shell clam shows a consistently higher coliform content than do other species harvested from waters of similar coliform concentration. Eastern oysters harvested from the Gulf areas have higher coliform contents than those from Middle Atlantic states. Finally, there is also a marked seasonal variation (2431). Nevertheless, it can be expected that the coliform content of shellfish, as MPN per 100 grams, will be from one to ten times as high as the MPN per 100 ml of the water from which they were taken. Furthermore, *Salmonella* organisms as well as coliforms will survive in shell liquor of harvested shellfish for 15 to 60 days, although there is little evidence that they will multiply in shellfish (2448).

The aforementioned USPHS Manual of Recommended Practice (2431) classifies shellfish growing areas into four categories: approved, conditionally approved, re-

stricted, and prohibited areas. To be fully approved, an area must meet these three criteria:

a. The area must not be so contaminated with fecal material that consumption of the shellfish might be hazardous. (The proximity of sewer discharges, even from secondary treatment plants, would be considered a hazard.)

b. The area must not be so contaminated with radionuclides or industrial wastes that consumption of the shellfish might be hazardous. (The level of radioactivity and quantitative criteria for industrial wastes are not specified.)

c. The coliform median MPN of the water must not exceed 70 per 100 ml and not more that 10 percent of the samples may exceed an MPN of 230 per 100 ml. These limits need not be applied if it can be shown by detailed study that the coliforms are not of direct fecal origin and do not constitute a public-health hazard.

A conditionally approved area is one that meets the water-quality requirements of an approved area, but the attainment is dependent upon the proper performance of a sewage treatment plant discharging effluent directly or indirectly to the area.

An area may be classified as restricted when a sanitary survey indicates a limited degree of pollution that would make it unsafe to harvest the shellfish for direct marketing. Shellfish from such areas may be marketed after self-purification in mildly chlorinated water, or after transplantation in clean water for a suitable period. For a restricted area, the coliform median MPN must not exceed 700 per 100 ml and not more than 10 percent should exceed an MPN of 2300 per 100 ml. Otherwise, the criteria are the same as for approved areas.

If an area is so contaminated with radionuclides or industrial wastes that consumption of the shellfish might be hazardous and/or if the coliform content exceeds a median value of 700 per 100 ml or a 10 percent value of 2300 per 100 ml, the area is classified as prohibited (2431).

No bacteriological criteria have been formally established in the U.S.A. for shellfish as marketed and consumed. In 1956, however, the Shellfish Sanitation Workshop (2449), sponsored by the USPHS, adopted two-year interim bacterial standards for marketed shucked oysters with three classes as follows:

Class	*Description*	*Coliform MPN per 100 ml*	*Standard Plate Count per ml*
1	Acceptable	Not over 16,000	Not over 50,000
2	Acceptable on condition*	Not over 160,000	Not over 1,000,000
3	Rejectable	Over 160,000	Over 1,000,000

* Oysters will be accepted on the condition that the shellfish sanitation authority in the originating state will make immediate investigations of the producer's plant and operations, and submit a report to the control agency in the market area.

In 1951, Canada adopted a similar system of classification except that the MPN limit on the acceptable class was set at 2400 per 100 ml.

It is certainly incongruous that the USPHS Drinking Water Standards restrict the coliform content of potable water to one per 100 ml, yet oysters containing 16,000 coliforms per 100 ml are considered to be acceptable. Most persons, to be sure, consume over 16,000 times as much water as oysters. Nevertheless, a significant number of people frequently eat a dozen raw oysters or clams on the half shell. It is not surprising, therefore, that shellfish constitute one of the weakest links in our defense against enteric infection, whether bacterial or viral.

In 1958-1959, a study was undertaken to evaluate the interim standards for shucked oysters. This study (2450) and subsequent work by Kelly et al. of the USPHS Shellfish Sanitation Laboratory (2451, 2452) have indicated that the entire coliform group of organisms is not a meaningful indicator of pollution in shucked oysters at the market. Instead, they recommended the adoption of a new interim standard based on densities of *Escherichia coli*. A satisfactory sample of shucked oysters should show a fecal coliform density (MPN) of not more than 78 per 100 ml and a plate count of not more than 100,000 per ml (Class Ia); except that a fecal coliform density up to and including 230 per 100 ml and/or a plate count up to and including 500,000 per ml will be acceptable in occasional samples (Class Ib). Unsatisfactory samples (Class II) are those that exceed 230 fecal coliforms per 100 ml or a plate count of 500,000 per ml.

While the bacterial quality of shucked oysters is not directly related to water-quality criteria for shellfish growing areas, an understanding of the entire problem should lead to a better evaluation of this beneficial use of water.

The role of oysters as vehicles for the transmission of hepatitis from sewage-polluted waters was demonstrated firmly during an epidemic in Sweden in 1955-56 (2453). Outbreaks of infectious hepatitis in Pascagoula, Mississippi, and in Troy and Mobile, Alabama, in January 1961, were definitely traced to oysters harvested from sewage-polluted waters near Pascagoula (2454). Finally, there is strong epidemiological evidence that much of the endemic occurrence of hepatitis along the eastern seaboard is attributable to the consumption of raw or partially cooked clams. Crovari (2455) has demonstrated that mussels may be regarded as possible vehicles for the dissemination of poliovirus. Mussels will accumulate poliovirus from seawater and passage of the virus through this mollusk does not affect its pathogenicity. It appears, therefore, that shellfish are capable of transmitting enteric viruses as well as bacteria.

SWIMMING AND BATHING WATERS

To be acceptable to the public and the regulatory authorities, waters that are used for swimming and bathing must conform to three general conditions: (a) they must be esthetically enjoyable, i.e., free from obnoxious floating or suspended substances, objectionable color, and foul odors; (b) they must contain no substances that are toxic upon ingestion or irritating to the skin of human beings; and (c) they must be reasonably free from pathogenic organisms (333).

Stream standards that have been promulgated by various state and interstate agencies (see Chapter III) seldom attempt to define the first two conditions in any but general terms, i.e., the standards are qualitative and descriptive without being quantitative. Occasionally limits are set for temperature, pH, color, and turbidity inasmuch as these parameters are easy to determine. For a further discussion of the effects of potential physical and chemical pollutants on bathing-water quality, see the specific substances as listed in Chapter VI.

The third condition given above, viz, that swimming and bathing waters be reasonably free from pathogenic organisms, has been subject to strict and definitive bacterial standards in many states and regions. It is agreed by most investigators that the bacterial quality of water for bathing need not be as high as that for drinking, but that natural bathing water should be maintained reasonably free of bacteria of known sewage origin. Present knowledge and technical procedures are not sufficient to permit the development of precise quantitative standards to distinguish between bathing beaches that are safe and those that are not safe. Despite this limitation, many state and interstate agencies have promulgated bathing-water standards.

Garber (2456) has collated and evaluated the bacterial standards for bathing water from several state and municipal agencies. He and others have noted that the coliform concentration of acceptable bathing areas vary widely from 50 to 3000 bacteria per 100 ml (330, 333, 334, 335, 2456, 2457). This observation is confirmed by a review of Chapter III of this report and its appendices. Moreover, standards are sometimes expressed in terms of the arithmetical mean, the geometrical mean, or the median of monthly samples. Occasionally, the maximum concentration is specified. Frequently, the percentage of samples that may exceed a stated concentration is indicated.

Perhaps the most restrictive standards are those of the states of Utah and Washington (see Chapter III) which limit the median coliform content of a representative number of samples to 50 per 100 ml (336). Utah further specifies that not more than 5 percent of samples should exceed 100 coliform bacteria per 100 ml. Several states limit the mean coliform density to 240 per 100 ml (see Chapter III and appendices). The most widely utilized criterion is patterned after the ORSANCO objective that the arithmetical mean coliform density not exceed 1000 per 100 ml and that this concentration not be exceeded in more than 20 percent of the samples in any one month. Some agencies add the further restriction that the average of the samples taken on any day shall not exceed 2400 coliforms per 100 ml. For proper interpretation, bacterial results should always be supplemented by sanitary surveys and local epidemiological statistics (334, 335, 337, 338, 339, 340, 341, 342, 345, 2456, 2457, 2458, 2459, 2460, 2461, 2462).

It is interesting to compare the promulgated standards with coliform concentrations that actually exist in bathing waters. Scott (2463) reported results of a comprehensive survey in 1955 and 1956 of bathing waters along the entire Connecticut coastline and also along some tidal rivers. A total of 1150 samples were examined. The total mileage of shoreline was divided into four classes, with the following results: coliform densities were equal or less than 50 per 100 ml along 27.6 percent of the shoreline, equal or less than 500 per 100 ml along 80.1 percent, and equal or less than 1000 per 100 ml along 94.5 percent. Concentrations exceeding 1000 per ml were found only at 5.5 percent of the shoreline. On the basis of these results, a standard of 1000 coliforms per 100 ml for a mean monthly density would not appear to be unduly restrictive.

THE RATIONALE OF BACTERIAL STANDARDS FOR SWIMMING AND BATHING WATERS

Insofar as this survey has been able to determine, all of the bacterial standards for bathing waters as promulgated by state, interstate, and local agencies have been established arbitrarily on the basis of esthetic considerations and ability of compliance. None are founded on sound epidemiological evidence that bacterial infections have been spread by contact with contaminated water. Yet, there must be some rationale for determining bacterial criteria for bathing waters, based on the *probability* of infection. Certainly the *possibility* of infection exists, especially in swimming pools and fresh natural waters where some of the water may be swallowed. The possibility is more remote in saline water, but it may still exist.

Two approaches have been used to assess the relationship between the bacterial quality of bathing water and the incidence of illnesses in swimmers, as compared with non-swimmers. One approach, described by Streeter (141), is based on the incidence of typhoid and paratyphoid in a region, the morbidity-mortality ratio, the relationship between these diseases and other enteric infections, the ratio of coliforms to pathogens, the frequency of swimming, the assumption that 10 ml of water will be swallowed by each bather each day, and the probability that this ingestion will cause illness. Streeter calculated that the chance of contracting typhoid fever from swimming daily for a 90-day season in the Ohio River (if it contained 1000 coliform bacteria per 100 ml) would be 1 to 950. Furthermore, the chances of contracting diarrhea-enteritis would be about 1 in 50. Streeter concluded that in this area, bathing in this water would involve no great hazard for the individual bather, although a standard of 1000 coliforms per 100 ml is admittedly a compromise between that which would be desirable and that which is practicable.

The second approach involves an epidemiological and statistical study of populations that have been bathing or swimming in contaminated waters in contrast with those who have not been in such waters or have been exposed only to water of excellent bacterial quality. If Streeter's assumptions are correct, then a statistical study of the population along the Ohio River should reveal a higher incidence of enteric infection among those who swam in polluted water than among other populations. A study of this type was made for the U.S. Public Health Service by Stevenson and his associates (532, 1655, 1656). They undertook a series of field studies of selected population groups swimming in waters of different bacterial quality, to determine the relationship between incidence of illness among swimmers and the coliform density of the water. Table 5-11 summarizes some of the basic data collected (1655).

The results indicated that illness occurred more frequently among swimmers than non-swimmers. This observation is not surprising in view of the fact that water is an abnormal habitat for man, regardless of its quality. The results also showed that when total illnesses among swimmers and non-swimmers were compared, there appeared to be no significant correlation between illness incidence and quality of the water in the areas studied.

TABLE 5-11

SUMMARY OF DATA FROM STUDIES OF BATHING WATER QUALITY

(After Stevenson) (1655)

Beach	Coliform Concentration MPN per 100 ml			No. of Illnesses per 1000 Person-Days	
	Median	Minimum	Maximum	Among Swimmers	Among Non-Swimmers
Lake Michigan, at Chicago, I	91	9.1	3,500	7.1	3.7
Lake Michigan, at Chicago, II	190	23	24,000	8.3	5.6
Ohio River, Kentucky	2700	230	160,000	8.8	7.4
Pool, Kentucky	< 3	--	--	13.8	--
Tidal Water, New Rochelle, N.Y.	610	< 30	460,000	5.3	3.3
Tidal Water, Mamaroneck, N.Y.	253	< 30	460,000	6.2	3.3

It might also be of interest to note that among swimmers, eye, ear, nose, and throat ailments represented more than half of all the illnesses recorded, gastrointestinal disturbances about 20 percent, and skin irritations the remainder. Eye, ear, nose, and throat ailments represented an even higher percentage, 68 percent, of pool-swimmer illnesses.

Stevenson actually found a specific correlation between illness incidence and the quality of water in two instances. In the first case, rates were measured for several days following 3-day periods of high and low bacterial concentrations at one beach on Lake Michigan. It was observed that illness frequency was significantly higher among swimmers when the water had an average coliform density of 2300 per 100 ml than when the average density was 43 per 100 ml. In the second instance, swimming in the Ohio River when the "median coliform density" was 2700 per 100 ml appeared to have caused a significant increase in gastrointestinal illness, although the total number of such illnesses was very small (1657). Stevenson points out that these two cases do not constitute conclusive evidence of correlation between illness and bathing-water quality, because the numbers of individuals and days involved were so few.

A subsequent study was conducted by USPHS personnel on Long Island Sound to determine the relationship between illness and bodily exposure to contaminated salt water (2149). This investigation gave no evidence that variation in water quality of the sort encountered is capable of producing marked differences in the amount of illness experienced by swimmers. The data from this study were of sufficient internal consistency to indicate that significant effects would have shown up had they existed. Similar results from Poland were reported by Buczowska (2464).

In 1939 and again in 1955, the Joint Committee on Bathing Places of the Conference of State Sanitary Engineers and the Engineering and Sanitation Section of the American Public Health Association surveyed the state health departments to determine the incidence of disease attributable to swimming pools and bathing places. In 1939, there were several vague reports of sleeping sickness, sinusitis, intestinal upsets, eye inflammation, swimmer's itch, and impetigo believed to be associated with water contacts. In New Jersey, four cases of typhoid fever in 1923 and two cases in 1934 were believed to be due to bathing in grossly polluted streams. In Indiana, 144 cases of dysentery were believed to have been caused by bathing in water polluted by septic tank effluents. In 1955, the information submitted from 45 states and one territory revealed that there had been no authenticated cases of illness attributable to swimming pools and bathing places. "All in all, the 1939 and 1955 reports reveal surprisingly little reliable data to indict bathing places in the spread of disease. Whether or not intensive epidemiological investigations might produce additional information is a question" (2465).

Actually, an intensive epidemiological investigation was conducted by Moore, and his Committee on Bathing Beach Contamination, in England and Wales (2466, 2467, 2468, 2469, 2470, 2471). Extensive bacteriological and epidemiological studies were made over a period of five years in relation to more than 40 popular bathing beaches, the waters of the great majority of which were subject to contamination with sewage. The median presumptive coliform counts varied from 40 to 25,000 per ml, and as many as 40 percent of the samples contained over 10,000 coliforms per 100 ml. In addition to coliform bacteria, determinations were made for members of the salmonella group, of which 33 different species were isolated.

The general conclusions of the committee were that bathing in sewage-polluted sea water carries only a negligible risk to health and where the risk is present it is probably associated with chance contact with intact aggregates of infected fecal material. In the entire study there were four cases of paratyphoid fever that could possibly have been attributed to bathing in infected sea water. In each case, however, the bathing area was grossly contaminated with visible fecal material. The committee indicated that unless the water is so fouled as to render the bathing beach esthetically revolting, it would seem that public-health requirements are reasonably well met by the present British policy of improving grossly insanitary beaches and preventing as far as possible the pollution of the waters with undisintegrated fecal matter.

In contrast with the thorough epidemiological work of Brown and his committee, the literature contains several reports of enteric disease reputedly caused by bathing in polluted waters (2472, 2473, 2474, 2475). None of these reports, however, are supported by reputable epidemiological evidence. Indeed, Brown himself gives a thorough review (2467) of all historical information relating illness to bathing.

As a result of studies such as those described in this section on the rationale of bathing-water standards, it has been recommended that some of the strict bacterial

requirements for such waters might be relaxed (1522, 1655, 1658, 1659, 1660).

OTHER INFECTIONS FROM BATHING WATERS

There have been several reports of skin eruptions, respiratory infections, and outbreaks of other non-enteric diseases traced to water contacts. No attempt is made to cover all of these illnesses in detail, but mention of a few cases should suffice to indicate that these hazards exist. For more detail, see Chapter VII.

Abrasions of the skin that occur while swimming may become infected and produce granulomatous lesions. In Colorado at a warm mineral pool, 262 cases were found to be due to the tuberculosis-type organism *Mycobacterium balnei* (2476, 2477, 2478). Earlier in Sweden, about 80 cases of a similar lesion were traced to swimming pools (2479, 2480). Greenberg and Kupka (2481, 2482) have reviewed the literature relative to transmission of mycobacteria, lupus-like organisms, and other tubercular bacteria through swimming pools and contaminated waste water.

Schistosome dermatitis, or "swimmer's itch," has affected bathers in rivers, ponds, and lakes that support snails. The causative agents are the cercariae, the free-swimming larval stage of certain parasitic worms of the family Schistosomatidea. The snails serve as an intermediate host. While not related to man-made pollution, cercarial dermatitis is definitely attributable to bathing in water of less-than-desirable quality (2465, 2483, 2484).

Outbreaks of leptospirosis have occurred in the United States and most other parts of the world as a result of swimming in ponds, slow-moving streams, and even swimming pools contaminated by domestic or wild animals. This disease, like schistosome dermatitis, is due to natural sources of contamination and not to man's activities (2485, 2486, 2487, 2488, 2489, 2490, 2491).

Cases of primary tuberculosis in children have been attributed to immersion and near drowning in sewage-contaminated water (2492, 2493). A Russian source claims that tularemia may be caused by bathing in, as well as drinking, infected water (2494). Some aquatic plants, such as smartweed *(Polygonum hydropiperoides)* and a marine alga *(Lyngbya majuscula)*, have been reported as causative agents of dermatitis on bathers (2287, 2495).

Although the viruses of poliomyelitis have been isolated frequently from sewage and contaminated water, there is no evidence that this disease has ever been transmitted through bathing waters (2465, 2468, 2470, 2496, 2497, 2498). Kelly and Sanderson (2499) isolated ECHO-type enteroviruses from unchlorinated wading pools in Albany, N. Y., but failed to detect viral agents in chlorinated swimming pools.

BOATING AND ESTHETIC ENJOYMENT

It is difficult to draw a sharp line dividing waters that are used for boating and esthetic enjoyment from those that are used for swimming, bathing, and water-contact sports, and from those that are used for fishing and wildlife. These three beneficial uses are normally compatible and frequently concurrent. In general, however, the water-quality criteria for boating and esthetic enjoyment are not as rigid or restrictive as those for actual water contact by humans, fish, or wild animals. Of the three conditions listed for swimming, for example, only the first applies to boating and esthetic enjoyment.

Conditions of water quality that affect boating and esthetic enjoyment are visible floating, suspended, or settled solids arising from the disposal of sewage or garbage; sludge banks; slime infestation; heavy growths of attached plants or animals; blooms or high concentrations of plankton; discoloration or excessive turbidity from sewage, industrial wastes, or even natural sources; the evolution of dissolved gases, especially hydrogen sulfide; visible oil or grease, including emulsions; excessive acidity or alkalinity that leads to corrosion or delignification of boats and docks; surfactants that foam when the water is agitated or aerated; and excessive water temperatures that cause high rates of evaporation and cloudiness over the water.

Biological growths that are often attributable to certain industrial wastes sometimes produce infestations of slimes (2500, 2501, 2502) which interfere with boating and esthetic enjoyment. *Sphaerotilis natans* is especially troublesome in streams below industries that discharge wastes with a high carbon-nitrogen ratio (see Chapter VII).

Excessive inorganic nutrients (nitrates, phosphates, carbonates, silicates) hasten the eutrophication of lakes and also fertilize flowing streams with the resultant production of heavy plankton blooms (2012, 2166, 2262, 2288, 2503). In addition, attached water plants such as water hyacinth, watermilfoil, and water chestnut may clog water channels and interfere with various recreational uses of water (589, 858, 889, 891, 893, 2495, 2503, 2505). Amphipods and polychaetes, some of which utilize the organic detritus from sewage and industrial wastes, foul harbor structures and form thick masses on bottoms of ships (2506). For further details about corollary biological pollutants, see Chapter VII.

Several state and interstate agencies have promulgated bacterial standards for recreational waters that are not used for swimming and bathing. Such standards are similar to those for water contact except that they permit monthly average MPN values as high as 5000 coliforms per 100 ml during the recreational season. There does not appear to be any epidemiological evidence to support these standards. Apparently they have been adopted arbitrarily on the basis of common decency and economic attainment.

Surfactants are esthetically objectionable when they contribute to foam. Water containing alkyl benzene sulfonates (ABS) in excess of 1.0 mg/l is likely to foam when it passes over dams or riffles in a stream; indeed, the threshold for foaming in tap water may be as low as 0.5 mg/l (2063). For further detail, see Chapter X. Hexadecanol, on the other hand, apparently causes no interference with the normal recreational uses of lakes to which it has been applied for suppression of evaporation.

Boats themselves may be a source of esthetic degradation of recreational waters. Weekend surveys of a small boat harbor at Eaton's Neck near New York, free from any sources of pollution other than from boats, disclosed

significant coliform concentrations in the water; but the most disturbing fact was the repeated observance of refuse, fecal matter, and toilet paper of boat origin (2507).

Considerable attention has been devoted in the past decade to the recreational use of domestic water supply reservoirs (2508, 2509, 2510, 2511, 2512). The American Water Works Association has issued a statement of policy (2513) that the recreational use of "equalizing" and "terminal" reservoirs, and the adjacent marginal lands, is inimical to the basic function of furnishing a safe and potable water supply and should be prohibited. Limited recreational use of "upstream" reservoirs is considered permissible under appropriate sanitary regulations except for those upstream reservoirs from which the water is delivered directly to the consumers with disinfection only. The California State Department of Public Health has published a comprehensive survey of the hazards associated with the recreational use of domestic water supply reservoirs (2514).

WATER POWER AND NAVIGATION

The pollutants in water that interfere with its beneficial use for water power and navigation are: (a) substances such as acid, alkali, and excessive salinity that accelerate corrosion, cavitation, or delignification, (b) debris, silt, and other suspended solids that block channels and intake devices or settle in reservoirs to reduce their useful storage, (c) organic matter that generates putrescible odors and corrosive hydrogen sulfide gas in deep reservoirs, (d) algae, fungi, worms, barnacles, and other corollary pollutants that clog passageways, cling to vessels, or accelerate corrosion, (e) marine borers that destroy wharves and docks, and (f) floating oil that poses a potential fire hazard.

Limiting and/or threshold concentrations for each of these categories of pollutants are seldom, if ever, found in the literature. Substances that accelerate corrosion include dissolved oxygen, hydrogen ions, sulfides, chlorides, free carbon dioxide, high temperatures, and certain types of bacteria and algae. To minimize corrosion by natural waters it is desirable to keep the concentration of each of these factors as low as possible, but no definitive limits can be set. As for silt, debris, and other suspended solids, it is desirable to keep all such material out of natural surface waters, but such a goal is impossible of attainment when natural sources contribute the bulk of such pollution. The formation of putrescible odors and hydrogen sulfide can be minimized by the maintenance of aerobic conditions (346, 1756) (see BOD, Dissolved Oxygen, Hydrogen Sulfide, and Oil in Chapter VI; and also Impoundments and Low-Flow Augmentation in Chapter II).

Much of the control over navigable waters in the U.S.A. resides with the Secretary of the Army, acting through the Corps of Engineers, in the enforcement of the River and Harbor Act of 1899. Section 13 of that Act specifies that: "It shall not be lawful to throw, discharge, or deposit, or cause, suffer, or procure to be thrown, discharged or deposited, either from or out of any ship, barge, or other floating craft of any kind, or from the shore, wharf, manufacturing establishment, or mill of any kind, any refuse matter of any kind or description whatsoever *other than that flowing from streets and sewers and passing therefrom in a liquid state,* into any navigable water of the United States, or into any tributary of any navigable water from which the same shall float or be washed into such navigable water; and it shall not be lawful to deposit, or cause, suffer, or procure to be deposited material of any kind in any place on the bank of any navigable water, or on the bank of any tributary of any navigable water, where the same shall be liable to be washed into such navigable water, either by ordinary or high tides, or by storms or floods, or otherwise, where navigation shall or may be impeded or obstructed: Provided, . . . that the Secretary of War, whenever in the judgment of the Chief of Engineers anchorage and navigation will not be injured thereby, may permit the deposit of any material above mentioned in navigable waters, within the limits to be defined and under conditions to be prescribed by him, provided application is made to him prior to depositing such material . . ." (emphasis added).

The original purpose of this act was to prevent shoaling and other interference to navigation caused by solids. In 1936, however, the Appellate Court sustained a decision that oil interfered with navigation by causing a hazard. As a consequence, this law can be used to prevent the discharge of oily substances from vessels in navigable waters or their tributaries and to compel treatment of wastes from refineries and other establishments.

The Secretary of the Army is charged also with the enforcement of Section 3 of the Oil Pollution Act of 7 June 1924 which reads in part, that: "Except in cases of emergency imperiling life or property, or unavoidable accident, collision, or stranding . . . it shall be unlawful for any person to discharge, or suffer, or permit the discharge of oil by any method, means, or manner into or upon the coastal navigable waters of the United States from any vessel using oil as fuel for the generation of propulsion power, or any vessel carrying or having oil thereon in excess of that necessary for its lubricating requirements and such as may be required under the laws of the United States and the rules and regulations prescribed thereunder. The Secretary is authorized and empowered to prescribe regulations permitting the discharge of oil from vessels in such quantities, under such conditions, and at such times and places as in his opinion will not be deleterious to health or sea food, or a menace to navigation or dangerous to persons or property engaged in commerce on such waters, and for the loading, handling, and unloading of oil."

This act is explicit in its application to vessels which may discharge oil to the coastal navigable waters and all inland waters navigable in fact in which the tide ebbs and flows. The 1899 Act is much broader in its scope, for it includes "any tributary of any navigable water," a phrase that embraces almost every stream in the United States.

The control over wastes "flowing from streets and sewers and passing therefrom in a liquid state" is excluded from Section 13 of the River and Harbor Act of 1899. Such control is exercised by the several states and by the U.S. Public Health Service, as described in Chapter III.

CHAPTER VI

POTENTIAL POLLUTANTS

Any substance that may enter or be contained in ground or surface waters is deemed for the purposes of this report to be a "potential" pollutant—potential in the sense that, if concentrated sufficiently, it can adversely and unreasonably affect such waters for one or more beneficial uses; and yet, if diluted adequately, it will be harmless to all beneficial uses. In view of this definition, every known substance is a potential pollutant and should be included in this investigation of water-quality criteria. Such was the intent of the first two editions of this compendium and such should be the ultimate goal of future revisions thereof.

In the three literature surveys that led to this edition, however, there were practical limits to the extent and scope of the work. The tremendous number of readily available references and the time required to abstract, catalogue, and evaluate them resulted in the fact that only the more-common and most-publicized pollutants are included. For the less-common minerals and compounds, few or no references were uncovered, although it is possible that significant literature exists and could be revealed in a specialized search. Indeed, this second edition includes hundreds of potential pollutants that were not mentioned in the first edition. Where no data were found, the substance has been omitted from this chapter. Later revisions of the report, however, may show the substance in question to be of significance to water-pollution-control agencies. It is hoped that the data contained herein will be of considerable value to the WPC boards in their case-by-case studies and that the framework here established can be used for subsequent revisions and expansions of this report.

The potential pollutants in this chapter are listed alphabetically by their correct or most-common names, with physical, inorganic, organic, and biological substances blended into the alphabetical listing. For chemical substances, the correct name was taken from the latest Merck Index (364) or the 43rd Edition of the Handbook of Chemistry and Physics (911), but where other names are in common use an attempt has been made to cross-reference. For example, CH_3SH is listed as Methanethiol but cross-referenced as Methyl Mercaptan.

This second edition differs from the first and the Addendum in that biological pollutants, radioactive substances, pesticides, and surface-active agents are covered in separate chapters (VII to X inclusive). Nevertheless, this chapter remains the key one for the compendium because every potential pollutant listed in the following chapters is cross-indexed herein. When in doubt as to where to find an exotic compound that may be a pesticide and also a surface-active agent, for example, the reader is advised to check first the alphabetical listing in Chapter VI.

Where literature relating to a potential pollutant is extensive and where more than one beneficial use is involved, a standard outline has been followed. This outline gives a general statement describing principal characteristics, common uses, and likely sources of the pollutant; followed by cross-references to related substances, bibliographical reference numbers; and finally a description of the effects upon certain beneficial uses. Where the literature is sparse or where it refers almost exclusively to one beneficial use, the outline has been discarded in favor of conventional paragraphs.

Many of the abstracted references deal with the effects upon beneficial uses of mixed wastes, involving many substances of unknown concentration, e.g., sulfite waste liquors, for which the overall strength is expressed as mg/l of total solids or BOD, or possibly in terms of the specific gravity or Baumé scale. Such wastes are treated as collective terms and listed directly as pollutants; hence the report contains such terms as "dairy wastes," "gas-plant wastes," and "metal-plating wastes." This listing of certain collective terms to the exclusion of others should not be construed as an attempt to single out a few industrial wastes for special mention; rather, it is a natural outgrowth of the cataloguing of wastes in terms of their constituent pollutants. Any industrial waste that is not listed, per se, was omitted because the polluting substances could be isolated and listed separately.

Cross referencing has been applied in many instances throughout this chapter, but as such it is far from being complete. The reader is assumed to understand the subject sufficiently well to use his own ideas of cross-referencing. When investigating the subject of "acetates," for example, the reader should check also on lead acetate, sodium acetate, zinc acetate, and similar listings.

In the literature used for this survey, the concentrations of substances are expressed in many ways, e.g., as parts per million, milligrams per liter, milliequivalents per liter, molar solutions or decimals thereof, normal solutions, or as volume dilutions of liquids and gases. Such variety in expressing concentrations is confusing to the reader and for that reason an attempt has been made in this report to convert all results to milligrams per liter. Some of these conversions are only approximate, for the original data may have been incomplete, e.g., in converting dilutions by volume the temperatures must be known in order to evaluate specific gravity precisely. In other instances, especially for mixed wastes of unknown constituents, conversion has been impossible and the original data have been presented without change. In a similar way, an attempt has been made to express all temperatures in degrees centigrade.

Just as a dictionary or an encyclopedia must abbreviate many facts and omit others, so has this chapter of the report been condensed to give only the most salient information relating to each potential pollutant. In a thorough case-by-case study, a water-pollution-control engineer will find the material as abstracted herein to be

inadequate and he may wish to investigate the extensive bibliography. To supplement this report, the WPC boards should have in their libraries a standard chemical handbook such as the latest edition of "The Handbook of Chemistry and Physics" (911) or "The Merck Index" (364), "Standard Methods For the Examination of Water and Wastewater" (469), the A.W.W.A.'s "Water Quality and Treatment" (152), a textbook of the chemical process industries such as that by Shreve (189), a book on water biology such as "The Microscopy of Drinking Water" (16), references dealing with insecticides such as those by Frear (2997) and by Rudd and Genelly (3005), and complete files of A.C.S., A.W.W.A. and W.P.C.F. journals, to mention only a few. It is recommended furthermore, that each board subscribe to "Water Pollution Abstracts," published monthly by the Water Pollution Research Board of Great Britain, which contains the finest and most complete abstracts of water-pollution literature on a worldwide basis.

The remainder of this chapter contains the alphabetical listing of potential pollutants.

ABIETIC ACID $C_{19}H_{29}COOH$

A widely available organic acid, prepared by the isomerization of resinous substances, this yellow amorphous powder is employed in the manufacture of esters for use in lacquers and varnishes, and in the production of metal resinates, soaps, plastics, and paper sizes. Sodium abietate from Kraft mill wastes is reported by Van Horn et al. (190, 344) to have a minimum lethal concentration of 3.0 mg/l for shiners in 120 hours at 18°C. Kawabe and Tomiyama (3240) found that pitch abietic acid was the most toxic of the ether-soluble components of the caustic soda digestion liquor at a pulp mill when tested against *Scenedesmus obliquus* and *Carassius auratus.*

ABS

(see Chapter X)

ACETALDEHYDE CH_3CHO

This flammable liquid with a pungent odor is miscible with water. It is used extensively in the manufacture of plastics, dyes, synthetic rubber, and other chemical products. The oral LD_{50} for rats has been reported as 1.93 grams/kg of body weight.

According to Garrett and Daugherty (1441, 2959, 2960) the 24-hour TL_m for the marine pin perch *(Lagodon rhomeboides)* was 70 mg/l and the maximum concentration at which no deaths occurred was 60 mg/l. Toward the bluegill sunfish *(Lepomis machrochirus)* in soft water at 18-20°C, the 96-hour TL_m was 53 mg/l (2936). In similar water, a 50 percent reduction in growth of the diatom, *Navicula seminulum,* occurred when the concentration of acetaldehyde reached 249 mg/l (2936). To suppress oxygen utilization by synthetic sewage, a concentration of 230 mg/l was required (2923).

ACETAMIDE CH_3CONH_2

Highly soluble in water, this crystalline compound is used in the plastics industry and in chemical manufacturing. The oral LD_{50} for rats is reported as 30 grams/kg of body weight (364). Wallen et al. (2940) tested its toxicity toward the mosquito-fish *(Gambusia affinis)* in highly turbid water at 21-24°C. They found the 24-and 48-hour TL_m concentrations to be 26,300 mg/l while the 96-hour was 13,300 mg/l. Turbidity was not significantly clarified by this compound.

ACETATES

(see also Acetic Acid, Amyl Acetate, Butyl Acetate, Mercuro-Organic Compounds, Lead, Sodium, Zinc, and the other cations that form acetates)

Acetates are the salts or esters of acetic acid. They occur in numerous chemical industries and in the manufacture of synthetic textiles. Acetates appear to be much less toxic than undissociated acetic acid. Lockhart (3241) reports the taste threshold of the acetate ion to be 360 mg/l when sodium acetate is used and 1020 mg/l when potassium acetate is used.

In Lake Erie water after 48 hours exposure at 25°C, the threshold concentration of sodium acetate for *Daphnia magna* appears to be about 5,200 mg/l (352). Another value as high as 5,800 mg/l has been reported (353). This salt is probably toxic only when in concentration sufficient to exert an unfavorable osmotic pressure (352). The threshold of toxicity of sodium acetate at 15-18°C for *Polycelis nigra* (a flatworm) occurs at 8,850 mg/l (354). Phenyl mercury acetate and pyridyl mercuric acetate, however, appear to be much more toxic to aquatic organisms (see Mercuro-Organic Compounds).

Von Oettinger (3242) has compiled the following toxicological data for several acetates when the compounds were fed orally to animals:

Compound	*Animal*	*LD_{50} in grams/kg of body weight*
Ethyl acetate	rats	5.62
Isopropyl acetate	rats	6.75
Allyl acetate	rats	0.13
Butyl acetate	rats	4.13
Butyl acetate	mice	7.06
Octyl acetate	rats	3.00
Benzyl acetate	rats	3.69
Benzyl acetate	rabbits	2.64
Heptyl acetate	rats	8.35

ACETIC ACID CH_3COOH

(see also Acetates, Acidity, Acids-Organic)

In the pure state, acetic acid is a colorless, pungent, biting liquid, highly miscible with water. It occurs in beet-sugar, winery, vinegar, soured-fruit, wood-distillation, textile, or chemical wastes.

The oral LD_{50} for rats has been reported as 3.31 grams/kg of body weight and for mice 4.96 grams/kg (3242, 3243). Rats fed 100 to 5000 mg/l of acetic acid in their drinking water for 9 weeks showed a decrease in appetite and growth (2980).

Fish can stand 5000 mg/l of acetic acid for a short exposure; indeed this compound is sometimes used in the treatment of diseased fish (359). For longer exposures, however, much lower concentrations are lethal. Acetic acid can be toxic to fish without lowering the pH value to 5.0, for toxicity appears to be attributable primarily to the undissociated acid in solution; hence pH is not a reliable index of acetic-acid pollution (361).

Hardness appears to be antagonistic to the toxicity of acetic acid.

Lethal concentrations have been reported as 50 mg/l in 24 hours for brook trout and 114 mg/l in 24 hours for minnows (359). For creek chub at 15-21°C in well-aerated water, the lethal concentration ranged from 100 to 200 mg/l (1442). Some goldfish have been killed at concentrations as low as 100 mg/l during prolonged exposure (313), but the 24-hour MLD has been given as 286 mg/l (359) and as 423 mg/l (2920). For the bluegill sunfish, the 96-hour TL_m in soft water at 18-20°C was reported to be 75 mg/l (2936). Toward fingerling channel catfish at 25°C the 72-hour TL_m was approximately 270 mg/l, all fish were killed at 629 mg/l, and none at 15.8 mg/l (2979, 2981). In turbid water, Wallen et al. (2940) found the 24-, 48-, and 96-hour TL_m concentrations toward the mosquito-fish *(Gambusia affinis)* all to be 251 mg/l.

For lower organisms, Palmer and Maloney (2041) reported that 2.0 mg/l of acetic acid had no effect on six species of algae (two blue-green, two green, and two diatoms). In a 5-day exposure in soft water at 20°C, 74 mg/l of acetic acid caused a 50-percent reduction in the growth of *Navicula seminulum,* a diatom (2936). The threshold concentration for immobilization of *Daphnia magna* in Lake Erie water after prolonged exposure at 25°C was 150 mg/l (358), but a threshold value as low as 80 mg/l has been reported (353). Ellis (313) indicates that the lethal concentration of acetic acid in hard water was 125 mg/l, with killing times of 24 to 72 hours.

ACETONE CH_3COCH_3

A volatile, inflammable, fragrant, colorless liquid that is miscible with water, acetone is used as a solvent for many organic compounds and in the manufacture of numerous substances. It may occur in wastes from many chemical industries, gas plants, coal-tar processes, paint manufacturing, or from the fermentation process by which it is produced. The oral LD_{50} for rats has been reported as 9.75 grams/kg of body weight (3244).

In stabilized tap water with adequate oxygen saturation, an acetone concentration of 792 mg/l had no effect on minnows after 24 hours (362), but in tap water sunfish were killed by concentrations of 14,250 mg/l (313, 363). Wallen et al. (2940) exposed mosquito-fish *(Gambusia affinis)* to acetone in highly turbid water at 23-27°C, for which the 48-and 96-hour TL_m values were both 13,000 mg/l.

The threshold concentration for immobilization of *Daphnia magna* in Lake Erie water with prolonged exposure at 25°C was 9,280 mg/l according to Anderson (358). The threshold concentrations for immobilization of several aquatic invertebrates in Lake Erie water at 20-25°C were reported (2955) as follows:

Organism	*Threshold Concentration in mg/l*
Daphnia magna, young	8,300
Daphnia magna, adult	12,900
Leptodora kindtii	5,300
Cyclops vernalis	13,600
Diaptomus oregonensis	6,500
Miscellaneous fish	14,250

ACETONITRILE CH_3CN

(see also Cyanides, Hydrogen Cyanide)

Sometimes known as methyl cyanide, acetonitrile is a colorless liquid used as a solvent for many organic compounds. It is miscible with water, but it does not appear to disocciate appreciably into methyl and cyanide ions. Consequently, it does not lead to the appreciable formation of HCN in water. For this reason, it is not expected that acetonitrile will be highly toxic.

In stabilized tap water kept saturated with oxygen, 17.1 mg/l of acetonitrile had no affect on minnows during a 24-hour exposure (362). Henderson et al. (2958) conducted bioassays using fathead minnows, bluegills, and guppies in hard and soft water saturated with oxygen at 25°C. The median toxic limits for commercial acetonitrile are shown below:

Kind of Fish	*Type of Water*	*TLm in mg/l*		
		24-hour	*48-hour*	*96-hour*
Fathead minnow	hard	1150	1050	1000
Fathead minnow	soft	1050	1000	1000
Bluegill sunfish	soft	1850	1850	1850
Guppy	soft	1650	1650	1650

Adult bluegills exposed up to four weeks in water containing 100 mg/l of acetonitrile did not pick up any organoleptic properties that were apparent to a taste panel (2958).

ACETYLENE $HC{\equiv}CH$

This colorless flammable gas is used for welding and as a raw material for a great variety of organic syntheses. It is soluble in water at 18°C to the extent of 1,000 cc per liter (approximately 1,000 mg/l) and in such a saturated solution, sunfish were not killed by exposure for one hour (363). In stabilized tap water saturated with oxygen, Schaut (362) found that 17.1 mg/l of acetylene had no effect on minnows after one-hour exposure. Thus, minnows and sunfish appear to be able to withstand short-time exposures to this gas.

For longer exposures, however, it was found that brook trout fingerlings died in 33 hours or less in well-oxygenated water at 10° to 14°C containing 200 mg/l of acetylene, and goldfish died in 24 to 48 hours in water containing 400 mg/l (247). The State of Washington Department of Fisheries (2091) found that the critical level for fingerling chinook salmon in brackish water during a 3-day exposure was less than 3500 cc of acetylene per liter and for young rainbow trout in fresh water between 3000 and 5000 cc per liter.

ACIDITY

1. General. Strictly speaking, acidity cannot be a direct or specific pollutant, for it is merely a measure of the effects of a combination of substances and conditions in water. It may be defined as the power of a water to neutralize hydroxyl ions and it is expressed in terms of the calcium carbonate equivalent of the hydroxyl ions neutralized. Acidity is usually caused by the presence of free carbon dioxide, mineral acids such as sulfuric, weakly dissociated acids such as phosphoric that affect the buffering action, and salts of strong acids and weak bases. It is determined by titrating with 0.02N sodium

hydroxide to the methyl-orange end-point. If the initial pH value of a sample of water is less than about 4.1 or 4.2, the amount of 0.02N sodium hydroxide required to reach the methyl-orange end-point (at about pH 4.3) is a measure of the "mineral-acid acidity" and the amount required to reach the phenolphthalein end-point is a measure of the "total acidity" (469). Acidity should not be confused with pH value, for one water with a pH of 6.0 may have a low total acidity whereas another highly buffered water with a pH of 7.0 may have a high total acidity.

Acidity in surface or ground waters may be attributable to natural causes, such as humic acids extracted from swamps or peat beds, or to industrial wastes such as pickling liquors, effluent from the manufacture of explosives, acid-mine drainage, or sulfite waste liquors.

2. Cross References. Alkalinity, pH, and specific mineral and organic acids.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The U.S. Public Health Service Drinking Water Standards of 1962 (2036) do not specify limiting values for acidity. To the practical waterworks engineer, however, excessive acidity is frequently detrimental in that it must be neutralized to mitigate corrosion.

There has been a tendency to regard acid-mine streams as bacteriologically safe because of their extreme acidity. Joseph and Shay (1443), however, found *E. coli* in all of the acid-mine stream samples that they tested, and reported that although *E. coli* counts are rapidly reduced when polluted water is discharged into acid streams, a small number of viable bacteria persists even at a pH as low as 2.0.

Excessive acidity of the water promotes the solution of zinc from galvanized pipes and other equipment, possibly to a degree harmful to the subsequent uses of the water (1444).

b. Industrial Water Supplies. Limits for acidity in industrial waters are seldom encountered inasmuch as such restrictions are generally expressed in terms of pH values (see pH and Chapter V).

c. Irrigation Water. Excessive acidification of soils containing nickel may render the nickel soluble, causing severe injury or death of plants (1445). In acid nutrient solutions, beryllium is highly toxic to plants (1446). Ligon and Pierre (1447) found that increasing the acidity of culture solutions from a pH of 6 to a pH of 4.5 resulted in a drop in yield of corn of about 50 percent. In general, however, moderate acidity in irrigation water is beneficial to alkali soils, for it helps to neutralize carbonates and prevent the precipitation of calcium (see Chapter V-Irrigation).

d. Fish and Other Aquatic Life. The pH values of ocean waters generally lie between 8.1 and 8.3; consequently acidity is not a factor to be considered in marine waters (346). In fresh waters, on the other hand, the pH values and acidities of natural streams vary widely, depending upon the soil and vegetation of the watershed. Brook trout were found to live comfortably in different streams with pH values ranging from 4.1 to 9.5, but in all cases the streams were substantially unpolluted and acidity, if any, was due to organic acids of natural origin (346).

Mineral-acid pollution, however, can be quite detrimental to fish at pH values considerably higher than those which fish can tolerate in unpolluted waters containing only acids of natural origin. Ellis (313) has investigated the toxicity of certain mineral and organic acids toward goldfish, and a summary of his results is shown in Table 6-1.

TABLE 6-1

TOXICITY OF ACIDS TO GOLDFISH

(After Ellis) (313)

Acid	*Amount added in mg/l*	*Resulting pH value*	*Period of survival*
Sulfuric	134*	4.3*	6.2 to 96 hours
Sulfuric	134	4.5	Over 4 days
Hydrochloric	166	4.0	4.5 to 6.5 hours
Hydrochloric	159	4.5	Over 4 days
Nitric	750	3.4	0.5 to 0.8 hours
Nitric	200	4.9	Over 4 days
Acetic	100	6.8	48 hours to over 4 days
Acetic	10	7.3	Over 4 days
Lactic	654	4.0	6 to 43 hours
Lactic	430	4.6	Over 4 days
Oxalic	1,000	2.6	0.4 to 0.5 hours
Oxalic	200	5.3	Over 4 days
Citric	894	4.0	4 to 48 hours
Citric	625	4.5	Over 4 days

* In soft water. All other results were obtained in hard water.

From these and other data, Ellis concluded that any acid in a concentration sufficient to give a pH value less than 4.0 would be toxic to goldfish, but above pH 4.0 the toxicity depends on the specific action of the particular acid. Lactic acid, for example, was non-toxic in four days at pH 4.6 whereas acetic acid was toxic at pH 6.8.

Table 6-1 shows the amount of acid added and the resulting pH, but not the resulting acidity, which is a function of the buffering effect of the water. Thus, when 134 mg/l of sulfuric acid were added to soft water, the resulting pH value was 4.3 and survival was limited; but in hard water the same amount of acid gave a pH of 4.5 and extended survival. Synergism and antagonism of other substances affect markedly the toxic action of acidity; or, more likely, the toxic action of many substances is altered by the acidity of the water.

Stiemke and Eckenfelder (270) tested the reaction of bluegill fingerlings to solutions of various acids in distilled water. Acid was added at periodic intervals of time until all fish were killed. The results of their investigations are summarized in the table below.

Acid	*No. of Fish Tested*	*First Fish Died At*		*Last Fish Died At*	
		pH	*Acidity mg/l*	*pH*	*Acidity mg/l*
Hydrochloric	9	3.30	25	3.00	50
Nitric	6	3.30	25	3.15	36
Sulfuric	9	3.45	20	3.00	52
Phosphoric	3	3.11	78	3.11	78
Acetic	9	3.75	85	3.65	140
Propionic	6	3.94	55	3.84	90
Lactic	3	3.50	37	3.41	50
Hydrochloric and sulfuric	3	3.45	31	3.05	54
Sulfuric and acetic	3	3.45	57	3.20	80
Hydrochloric and acetic	3	3.50	62	3.47	78

In contrast to the foregoing results, Leitch (1015) reported that more than 4.0 mg/l of mineral acids will kill trout. Parsons (2797) surveyed a stream polluted by acid drainage from strip mines. In sections of the

stream where the pH dropped to 4.0 and the total acidity was 100 mg/l, no fish life and very few plankton existed.

e. Shellfish Culture. Loosanoff and Tommers reported that increased acidity, occurring either naturally as a result of the influx of acid swamp waters or as a result of industrial pollution, reduced the rate of pumping among oysters, thus interfering with their feeding activities. At a pH of 7.75 the oysters appeared to pump normally; at pH 6.5 the pumping rate was significantly lower; and at pH of 4.14 the pumping rate was only 10 percent of normal (1448).

ACIDS, ORGANIC, GENERAL

(see also pH, Acidity, and the specific acids)

Hammerton (360) reported that lactic, acetic, oleic, stearic, and palmitic acids were all destructive to portland cement, but that oxalic acid was not. Anderson (358) claims that in Lake Erie water at 25°C all of the organic acids tested, with the exception of benzoic and tannic acid, were non-toxic to *Daphnia magna* until they reduced the pH value to 6.0 or less.

ACRIDINE $C_6H_4CHN\ C_6H_4$

Related to pyridine and quinoline, and like them a weak organic base, acridine is a solid substance that is only slightly soluble in boiling water. It may occur in wastes from coal-tar and gas plants, and possibly in dye wastes. It has been reported to kill perch in a concentration of 0.7 mg/l (365) and *Daphnia* in a concentration of 5.0 mg/l (313). Yearling trout lost equilibrium in 60 minutes at a concentration of 1.5 mg/l at 12°C (3245). A concentration of 5 mg/l was lethal to rainbow trout in two hours and to the bluegill sunfish in 13 hours. The sea lamprey withstood this concentration for 13 hours but with obvious distress (2976).

ACROLEIN

(see Chapter IX)

ACRYLONITRILE CH_2CHCN

(see also Cyanides, Hydrogen Cyanide)

A flammable liquid used in the manufacture of plastics and synthetic rubber, and as a pesticide fumigant for stored grain (364), acrylonitrile is moderately soluble in water (3246). It does not appear to dissociate appreciably in water and consequently it does not form measurable HCN, the toxic cyanide principle. For this reason, it is not anticipated that acrylonitrile will be highly toxic to aquatic life (2958). Bandt (3247), however, states that acrylonitrile breaks down in water to yield HCN.

Daugherty and Garrett (1441) indicate that concentrations of 20 mg/l in sea water are deleterious for marine fish such as pin perch. According to Renn (2968) the tolerance level for mixed fresh-water fish is between 10 and 18 mg/l as N(38 to 68 mg/l as acrylonitrile). Bandt (3247) reports that the lower tolerance limit for fish is about 20 to 25 mg/l whereas all fish are killed within 24 hours by 100 mg/l. Garrett (2959, 2960) found the 24-hour TL_m for pin perch to be 24.5 mg/l. A concentration of 30 mg/l gave 100 percent mortality.

Henderson et al. (2958) conducted bioassays using three species of fish in both hard and soft water saturated with oxygen at 25°C. Their results as shown below are expressed as median toxic limits based on commercial acrylonitrile.

Kind of Fish	*Type of Water*	*TL_m in mg/l*		
		24-hour	*48-hour*	*96-hour*
Fathead minnow	hard	32.7	16.7	14.3
Fathead minnow	soft	34.3	21.5	18.1
Bluegills	soft	25.5	14.3	11.8
Guppy	soft	44.6	33.5	33.5

Adult bluegills exposed up to four weeks in water containing 5 mg/l of acrylonitrile did not pick up any organoleptic properties that were apparent to a taste panel (2958).

Cherry et al. (3246) studied the effect of acrylonitrile on the mixed biota in stream purification. They found that concentrations of 10 to 25 mg/l did not interfere with mixed cultures but at 50 mg/l the growth was predominantly fungal.

ACTINOMYCETES

(see Chapter VII)

ADIPONITRILE $NC(CH_2)_4CN$

(see also Cyanides, Hydrogen Cyanide)

Henderson et al. (2958) conducted bioassays using three species of fish in both hard and soft water saturated with oxygen at 25°C. For commercial adiponitrile they found the following median toxic limits:

Kind of Fish	*Type of Water*	*TL_m in mg/l*		
		24-hour	*48-hour*	*96-hour*
Fathead minnows	hard	835	835	820
Fathead minnows	soft	1350	1300	1250
Bluegill sunfish	soft	1250	815	720
Guppy	soft	1200	830	775

This compound does not appear to dissociate appreciably in water and consequently not much HCN can be formed to become toxic. Adult bluegills exposed up to four weeks in water containing 100 mg/l of adiponitrile did not pick up any organoleptic properties that were apparent to a taste panel (2958).

AEROSOL O. T.

(see Chapter X)

ALBUMIN

Egg albumin is a complex protein, soluble in water and dilute salt solutions, with a molecular weight of approximately 43,000. In stabilized tap water, Schaut (362) found that a concentration of 17.1 mg/l of albumin was not harmful to minnows during 24 hours of exposure, but the dissolved-oxygen content was lowered 28 percent, indicating that biochemical decomposition occurred.

ALBUMINOID AMMONIA or ALBUMINOID NITROGEN

(see also Ammonia and other nitrogen compounds)

Albuminoid nitrogen is defined in the 10th Edition of Standard Methods for the Examination of Water and

Sewage (469) as "the nitrogen equivalent of ammonia formed or liberated from nitrogenous matter by the action of alkaline permanganate in water after expulsion of ammonia nitrogen by distillation." Its significance is difficult to determine or to define, for it merely represents a portion of the organic nitrogen that is readily released by a chemical reaction. Formerly determined and reported in most analyses of polluted waters, albuminoid ammonia (or albuminoid nitrogen if the results are reported as N rather than NH_3) is seldom used in modern analytical work in the U. S., and consequently most of the references thereto are in out-dated or foreign publications.

ALCOHOLS, GENERAL

(see also Allyl Alcohol, Amyl Alcohol, Benzyl Alcohol, Butyl Alcohol, Ethyl Alcohol, Cetyl Alcohol, Methyl Alcohol, Octyl Alcohols, Phytosterol, Propyl Alcohol, and other specific alcohols)

Under this heading are abstracted articles covering several alcohols for comparative purposes. Most of the criteria relating to specific alcohols are listed under the designated compound.

According to Welch and Slocum (3248) the acute oral toxicity of primary alcohols toward rats is as follows: methyl, 9.1 mg/kg of body weight; ethyl, 7.4 mg/kg; propyl, 3.3 mg/kg; butyl, 2.75 mg/kg; amyl, 3.3 mg/kg; hexyl, 4.1 mg/kg; and heptyl, 6.6 mg/kg.

Toxicity tests with creek chub, a fish considered to be average in tolerance, at 15 to 21° C in well-aerated water, revealed the "critical ranges" shown in the following table. Critical range is defined as the range in concentration below which all four test fish lived for 24 hours and above which all died (1442).

Alcohol	*Critical Range in mg/l*
n-propyl alcohol	350–500
n-amyl alcohol	350–500
mixed primary isoamyl alcohols	400–600
isopropyl alcohol	900–1100
n-butyl alcohol	1000–1400
tertiary amyl alcohol	1300–2000
tertiary butyl alcohol	3000–6000
ethyl alcohol	7000–9000
methyl alcohol	8000–17000

Hodgson (2956) determined the concentrations of primary alcohols required to stimulate the movement of the water beetle *(Laccophilus)*, with the following results:

Alcohol	*Critical Range in mg/l*
methyl	115,000
ethyl	198,000
n-propyl	192,000
n-butyl	3,410
n-pentyl	644
n-hexyl	112

ALCOHOL SULFATES

(see Chapter X)

ALDEHYDES

(see Acetaldehyde, Benzaldehyde, Formaldehyde, Furfural, and Vanillin)

ALDRIN

(see Chapter IX)

ALGICIDES

(see specific compound in Chapter IX)

ALIPHATIC AROMATIC SULFONATES

ALIPHATIC SULFONATES

(see Chapter X)

ALKALINITY

1. General. Like acidity, alkalinity is not a specific polluting substance, but rather a combined effect of several substances and conditions. It is a measure of the power of a solution to neutralize hydrogen ions and it is expressed in terms of an equivalent amount of calcium carbonate. Alkalinity is caused by the presence of carbonates, bicarbonates, hydroxides, and to a lesser extent by borates, silicates, phosphates, and organic substances. It is determined by titrating with 0.02N sulfuric acid to the phenolphthalein and methyl-orange endpoints, the former measuring the so-called caustic alkalinity and the latter the total alkalinity. Like acidity, alkalinity is related to pH but high alkalinities should not be confused with high pH values. Thus, a relatively pure water with a pH value of 7.0 will have a low total alkalinity whereas a buffered water at pH 6.0 will have a high total alkalinity. For a more thorough discussion of alkalinity and an evaluation of the hydroxyl, carbonate, and bicarbonate components thereof, see Standard Methods For the Analysis of Water and Wastewater (469).

Some natural waters, especially those in the southwestern U. S., are highly alkaline while others, such as those in western Washington or the New England states, are low in alkalinity. The alkalinities of streams are frequently increased by the addition of municipal sewage and many industrial wastes, too numerous to list herein.

2. Cross References. Acidity, pH, Hardness, Dissolved Solids, Carbonates, Bicarbonates, Hydroxides, and other specific substances that may affect akalinity.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. In itself, alkalinity is not considered to be detrimental to humans but it is generally associated with high pH values, hardness, and excessive dissolved solids, all of which may be deleterious.

b. Industrial Water Supplies. Alkalinity is detrimental in many industrial processes, especially those involving the production of food and beverages. It is particularly frowned upon in the production of carbonated and acid-fruit beverages because it neutralizes the natural taste-producing substances and makes the beverage more susceptible to bacterial action (179). The ranges of recommended threshold values of total alkalinities in industrial water supplies are presented in Table 6-2.

In contrast, alkalinity is desirable in many industrial waters, especially if it serves to inhibit corrosion by creating a favorable calcium-carbonate balance. In oilfield work, Nelson (349) recommends that the alkalinity of unaerated water should be at least 20 mg/l.

c. Irrigation Water. Excessive alkalinity in irrigation water is detrimental in that it adds to the total salinity and is frequently accompanied by high pH values. In conventional chemical analyses of irrigation waters, however, alkalinity is frequently not listed (see Chapter V).

d. Stock and Wildlife Watering. High alkalinities in water are reported to have been detrimental to stock. When the caustic alkalinity reaches 50 mg/l, trouble with diarrhea in chickens begins (1019), and at a total alkalinity of 170 mg/l animals were reported to develop diarrhea (1020).

e. Fish and Other Aquatic Life. Doudoroff and Katz (361) cite references indicating that none of the strong alkalies, such as calcium, potassium, and sodium hydroxide, has been shown clearly to be lethal to fully developed fish in natural waters when its concentration is insufficient to raise the pH well above 9.0. Interference with normal development and other damage to fish life sometimes may occur, however, at lower pH values. When caused almost entirely by bicarbonates, alkalinity does not seem to have any harmful effect upon plankton and other aquatic life (1021).

TABLE 6-2

RANGES OF RECOMMENDED THRESHOLD VALUES OF TOTAL ALKALINITIES IN INDUSTRIAL PROCESS WATERS

Industry and Process	*Recommended Threshold Values in mg/l*	*Reference*
Brewing		
Light beer	75-80	152, 173
Dark beer	80-150	152, 173
Carbonated beverages	30-85	173
Carbonated beverages	50	152, 180
Carbonated beverages	51.3	1016
Carbonated beverages	60-100	1017
Carbonated beverages	85	185
Carbonated beverages	100	184, 188
Carbonated beverages	125	179
Carbonated beverages	128.5	186
Carbonated beverages	170	185
Food products	30-250	173
Fruit juice	100	164
Laundrying (diapers)	60	993
Pulp and paper making		
Groundwood pulp	150	244
Kraft paper, bleached	75	351
Kraft paper, unbleached	150	351
Fine papers	45-75	551, 350
Soda and sulfate pulp	75	245
Rayon manufacture	50	152
Rayon manufacture	75	550, 405
Tanning	128.2	1018
Tanning	135	152

Stiemke and Eckenfelder (270) found that the average lethal doses of various alkaline solutions toward bluegill fingerlings were as follows:

Sustance	*Alkalinity as Calcium Carbonate in mg/l*	*pH*
Sodium hydroxide	70	10.55
Ammonium hydroxide	31	9.60
Sodium carbonate	120	----
Potassium iodide and sodium hydroxide	57	----

According to Warrick et al. (599) high alkalinity is antagonistic toward the toxicity of copper sulfate to fish. The relative toxicity of 25 mg/l of copper sulfate to brown trout fry varied inversely with the alkalinity, with all fish dying in 2.5 hours when the alkalinity was only 6.0 mg/l whereas some fish survived after 12.5 hours when the alkalinity was 248 mg/l.

It is generally recognized that the best waters for the support of diversified aquatic life are those with pH values between 7 and 8, having a total alkalinity of 100 to 120 mg/l or more (3249, 3250). This alkalinity serves as a buffer to help prevent any sudden change in pH value, which might cause death to fish or other aquatic life. In Michigan, the addition of lime to Stoner Lake increased the alkalinity from 6 to 15 mg/l, improved the biological productivity, and even caused some extensive phytoplankton blooms.

ALKENYL DIMETHYL ETHYL AMMONIUM BROMIDE

ALKYL ARYL COMPOUNDS

ALKYL BENZENE SULFONATE

ALKYL DIMETHYL COMPOUNDS

ALKYL SULFATE

ALKYL SULFONATE

(See Chapter X)

ALLYL ALCOHOL $CH_2{=}CHCH_2OH$

A colorless liquid with a pungent mustard-like odor, allyl alcohol is miscible with water. It is used in war gases, resins, and plasticizers. It is very irritating to mucous membranes and skin (364). The oral LD_{50} is reported as 40 mg/kg of body weight for dogs (364) and 100 mg/kg for rats (3251). When fed in the drinking water of rats, retardation of weight gain began at 250 mg/l, but other effects were minor even at 1000 mg/l (3252). According to Woelke (2989), allyl alcohol is lethal toward bivalve larvae at 2.5 mg/l. Hubault (3253) reported the threshold of harmfulness of allyl alcohol toward rudd to be 10 mg/l.

ALUM

(see Aluminum Sulfate, Aluminum Ammonium Sulfate, and other aluminum compounds that are sometimes called alum)

ALUMINA

(see Aluminum Oxide)

ALUMINUM Al

1. General. One of the most abundant elements on the face of the earth, aluminum occurs in many rocks and ores but never as a pure metal in nature. Although the metal itself is insoluble, many of its salts are readily soluble. Other aluminum salts, however, are quite insoluble and consequently aluminum is not likely to occur for long in surface waters because it precipitates and settles or is absorbed as aluminum hydroxide, aluminum carbonate, etc. In streams the presence of aluminum ions may result from industrial wastes or more likely from wash water from water-treatment plants. This section of the report deals with references to aluminum ions in water, where no mention is made of the salts from which

the pollution was derived. For the salts, see subsequent pages and cross references.

2. Cross References. Aluminum Ammonium Sulfate, Aluminum Chloride, Aluminum Nitrate, Aluminum Oxide, Aluminum Potassium Sulfate, Aluminum Sulfate.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. As one of the most abundant elements, aluminum is a constituent of all soils, plants, and animal tissues. It occurs in fruits and vegetables in concentrations up to 3.7 mg/kg and in some cereals up to 15 mg/kg. The total aluminum in the normal diet has been estimated at 10 to 100 mg per day (2121). Very little of the ingested aluminum is absorbed in the alimentary canal and consequently it is almost entirely evacuated in the feces. The total aluminum content of an adult human body is only about 50 to 150 mg (2121). Aluminum in public supplies is not considered a public-health problem, at least when it is present as a result of treatment processes. No evidence has ever been found to prove that the use of aluminum in water supplies, cooking utensils, or baking powders is harmful to human beings (32, 36, 152, 155, 353, 633). Evidence has been uncovered to indicate that salts of aluminum in food lower the toxicity of calcium fluoride occuring in combination with natural phosphates (1022, 3254).

b. Industrial Water Supplies. Aluminum compounds in water in concentrations as low as 0.05 mg/l may cause trouble in industries such as laundries and mineral water plants (36). Aluminum in the concentrations commonly found in water has no effect on the brewing of beer (175). For rayon manufacturing and processing, the recommended maximum permissible concentration is 0.25 mg/l (405, 550).

c. Irrigation Water. Aluminum is generally a minor constituent of irrigation waters, and is of little importance to irrigation (268); however, aluminum in high concentrations in reclaimed effluents could be toxic to crops (269). Scofield has found that aluminum, like calcium, reduces the injurious effect of sodium in soil, and when present in soil or irrigation water may be beneficial (348).

The following concentrations of aluminum have been reported to be harmful to various plants grown in solution cultures:

Concentration of Aluminum, in mg/l	*Plants*	*Effect*	*Reference*
1.0	Corn, barley and sorghum	Injury to roots, stalk, and tops in this order	1447
1.0-4.56	Corn	Reduced yields	1447
1.0-100	Flax	Abnormal growth	1458
2.0	Barley	Depressed growth	1447
7 or less	Sorghum	Depressed growth	1447
14	Corn	Depressed growth	1447

On the other hand, McLean and Gilbert as reported by Ligon and Pierre (1447) found that 3.4-13.6 mg/l of Al^{+++} stimulated the growth of rye and oats; and 13.6 mg/l reduced yields of corn by only 4-7 percent. Aluminum in small amounts was reported to reduce the symptoms of manganese poisoning in flax grown in solution culture (1458).

Ligon and Pierre (1447) suggested that some of the conflicting results of independent studies might be attributable to differences in the pH values of the culture solutions used. In their experiments, the pH was maintained at 4.5 to keep aluminum in solution; at this pH, without aluminum, the yield of corn was 50 percent lower than that of corn grown at pH 6.0. At pH 4.5, aluminum at a concentration of 1.0 mg/l further reduced the yields by about 25 percent; at concentrations of 2.28 and 4.56 mg/l, aluminum further reduced the yields by 39 and 59 percent respectively.

d. Stock and Wildlife Watering. An average daily dose of 2 mg aluminum has not harmed rats (353). According to Maynard (995), aluminum has not been proved to be essential for animals. Much higher levels of aluminum than those found in food or water have been fed continuously to rats, dogs, pigs, and man without observable harm. However, in excessive amounts, there is the risk that aluminum will interfere with phosphorus metabolism, causing secondary symptoms of phosphorus deficiency.

e. Fish and Other Aquatic Life. The following concentrations of aluminum have been reported lethal to aquatic animals in the time specified:

Concentration of Aluminum, mg/l	*Compound Used*	*Time of Exposure*	*Type of Fish*	*Reference*
0.07	----	----	sticklebacks	2941
0.10	$Al(NO_3)_3$	1 week	sticklebacks	1460
0.27	$AlCl_3$	50 hours	eels	1459
0.3	$Al(NO_3)_3$	1 day	sticklebacks	1460
0.5	$AlCl_3$	----	fish	1023
2.7	$AlCl_3$	3.6 hours	eels	1459
5.0	----	5 minutes	trout	604
17.8	$AlCl_3$ (in sea water)	short	redfish	1461

The following concentrations of aluminum have not harmed fish in the period specified:

Concentration in mg/l	*Type of water*	*Time of Exposure*	*Type of Fish*	*Reference*
0.25	acid	----	fish	1023
0.27	----	50 hours	young eels	1459
1.0	----	5 minutes	trout	604
8.9	seawater	----	fish & oysters	1461

f. Water for Bathing. Alum in swimming pools in a concentration equivalent to 0.1 mg/l aluminum may cause irritation of human eyes; and 0.5 mg/l is acutely irritating (1024).

ALUMINUM AMMONIUM SULFATE $AlNH_4(SO_4)_2 \cdot 12H_2O$

Occurring generally as the colorless crystalline compound with 12 molecules of water, aluminum ammonium sulfate is highly soluble in water and may be found in wastes from dye works and cloth-printing industries.

The threshold concentration of this compound for immobilization of *Daphnia magna* in Lake Erie water was found to be 190 mg/l (358). According to Ellis (313) trout succumbed to 523 mg/l of aluminum ammonium sulfate in tap water within 10 hours. The highest concentration of this salt tolerated by young eels for more than 25 hours was reported (1459) to be about 12 mg/l.

ALUMINUM CHLORIDE $AlCl_3$ and $AlCl_3 \cdot 6H_2O$

This salt of aluminum, highly soluble in water, is used in dyeing fabrics, manufacturing parchment paper, refining operations, and disinfection.

When aluminum chloride was dissolved in tap water at pH 7.2-7.4, concentrations of aluminum in excess of 0.5 mg/l were fatal to various fishes. Young eels died

within 3.6 hours in a solution of aluminum chloride containing 2.7 mg/l of aluminum but they tolerated 0.27 mg/l for 50 hours (1459). Wallen et al. (2940) studied the effect of aluminum chloride on the mosquito-fish *(Gambusia affinis)* in highly turbid water at 20-21°C. They found a 96-hour TL_m value of 133 mg/l. This dosage reduced the turbidity from 230 mg/l to less than 25 mg/l.

The following concentrations of aluminum chloride have been reported to be lethal to various test fish in sea water:

Concentration of $AlCl_3$ in mg/l	*Type of Water*	*Test Organism*	*Effect*	*Reference*
88	sea water	redfish	killed quickly	1461
88	sea water	other fish	killed more slowly	1461
132	sea water	all fish tested	most killed in a few hours, all in a few days	1461
176	sea water	all fish tested	all killed in a few hours	1461

The foregoing information shows a wide variation in the toxic level of $AlCl_3$ toward fish. In a similar manner there is no consistent pattern of toxicity toward lower forms of aquatic life. Using water from the River Havel from which the test organisms were recovered, Bringmann and Kuhn (2158) studied the threshold effects of aluminum chloride on various organisms during an exposure of 4 days at 23-27°C. They found that *Daphnia* withstood a concentration of 1000 mg/l as aluminum whereas *Scenedesmus* exhibited toxic effects at 1.5-2.0 mg/l of aluminum. In contrast, the threshold concentration of aluminum chloride ($AlCl_3$) for immobilization of *Daphnia magna* in Lake Erie waters at 25°C was found to be less than 6.7 mg/l, for a 64-hour period (598).

Oysters do not appear to be sensitive to the action of $AlCl_3$ in seawater (2977).

ALUMINUM NITRATE

$Al(NO_3)_3$ and $Al(NO_3)_3 \cdot 9H_2O$

This highly soluble salt of aluminum is used in the manufacture of the incandescent filaments, as a mordant in printing fabrics, and in the tanning and finishing of leather.

Aluminum nitrate has been found to be toxic to *Polycelis nigra* (a flatworm) in concentrations equivalent to 110 mg/l of aluminum (608). The threshold concentration of aluminum nitrate for death of stickleback in tap water is reported to be 0.55 mg/l (598). Using aluminum nitrate in very soft water, Jones found the lethal concentrations limit of aluminum for stickleback to be 0.07 mg/l. In different concentrations of aluminum from this compound, the average survival times of this fish were one day at 0.3 mg/l and one week at 0.1 mg/l (1459, 1460, 2920, 2941).

ALUMINUM OXIDE

Al_2O_3

This white amorphous powder, also known as alumina or aluminum trioxide, readily absorbs water from the air, but is insoluble in water. It is used as an abrasive, for paint filler, and in paper mills.

Alumina as a colloidal residue has been blamed for an increase in cancer incidence, but residual salts of alumina appear to be without harmful effects (1025).

The following concentrations of aluminum oxide have been suggested (152, 162) as limits permissible in water for use in boilers:

Pressure (psi)	0-150	150-250	250-400	Over 400
Al_2O_3 (mg/l)	5	0.5	0.05	0.01

For rayon (viscose) pulp production, the concentration of alumina should not exceed 8.0 mg/l (152).

The constant intake of water containing almost 3 grains per imperial gallon (about 42.9 mg/l) of aluminum oxide was believed to be the cause of death of a number of cattle (1026).

ALUMINUM POTASSIUM SULFATE

$AlK(SO_4)_2$

Readily soluble in water, aluminum potassium sulfate may be found in wastes from dye works, tanneries and leather works, canneries, paper mills, and explosives works. The salt generally occurs as $AlK(SO_4)2 \cdot 12H_2O$ or as $Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$.

The threshold concentration of anhydrous aluminum potassium sulfate for immobilization of *Daphnia magna* in Lake Erie water was found to be 206 mg/l (358). The following concentrations of $AlK(SO_4)_2$ have been reported to have killed fish within the period of time specified:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
10	hard	4 days	goldfish	353
100	hard	12-96 hours	goldfish	598, 2920
250	—	—	minnows	313
544	tap	6 hours	salmon	313
544	tap	15 hours	trout	313
1000	hard	1-10 hours	goldfish	313

In contrast to the foregoing tabulation, Ellis (313) reports that goldfish withstood 100 mg/l of $AlK(SO_4)_2$ in hard water for 100 hours without detriment. The maximum concentration of aluminum potassium sulfate tolerated by young eels for more than 25 hours was reported to be about 13 mg/l (1459).

ALUMINUM SULFATE (ALUM)

$Al_2(SO_4)_3$ and $Al_2(SO_4)_3 \cdot 18H_2O$

1. General. Aluminum sulfate is used in water treatment as a coagulant for colloidal and suspended solids, including turbidity and organisms, with doses of from 5 to 70 mg/l generally being used (587). Aluminum sulfate has been found partially effective for inactivating viruses of infectious hepatitis (885) and polio (724), for removal of cysts of amoeba (743), and for removal of color (837), but it appears to have no effect on odors in water (1028). It has been reported that a study made in 1907 of the effects of various metallic sulfates on fish, germinated seeds of peas, wheat, and rape, yeasts, and various soluble enzymes indicated that aluminum sulfate was poisonous (921).

2. Cross References. Aluminum, Aluminum Ammonium Sulfate, Aluminum Potassium Sulfate.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The USPHS Drinking Water Standards of 1962 (2036) do not prescribe limits for alum in water. Solutions of 1 to 5 mg/l aluminum sulfate did not increase the solubility of lead sulfate or carbonate beyond their solubility in distilled water. However, where lead service pipes are used, high con-

centrations of aluminum sulfate may cause an increase in the lead content of the water (1029).

(b) Industrial Water Supplies. Aluminum sulfate is undesirable in water for use in the paper and textile industries (257). Excessive quantities of alum in water used for paper have a deleterious effect on the strength of the paper product (1030). For use in dyeing rayon, a residual content of alum of less than 0.4 mg/l is desirable (852).

c. Irrigation. As aluminum sulfate, 10 mg/l of aluminum caused no apparent injury to oat plants but 25 mg/l or more significantly reduced growth (1462).

d. Fish and Other Aquatic Life. In fresh water, 7 mg/l of aluminum sulfate has been reported to be fatal to *Fundulus* within 5 days and 14 mg/l within 36 hours (1459). Schaut (362) reported that 17.1 mg/l of $Al_2(SO_4)_3 \cdot 18H_2O$ was harmless to minnows in stabilized tap water during one hour of exposure. Sanborn (1030) found that 100 mg/l of $Al_2(SO_4)_3 \cdot 18H_2O$ did not affect goldfish, sunfish, and bass during seven days of exposure but that 250 mg/l killed them in 8 to 24 hours. Wallen et al. (2940) studied the effect of aluminum sulfate on the mosquito-fish (*Gambusia affinis*) in turbid water at 19-22° C. They found the 96-hour TL_m to be 235 mg/l, at which concentration the turbidity was reduced from 180 mg/l to less than 25 mg/l, owing to the coagulating action of the alum.

The threshold concentration of aluminum sulfate for immobilization of *Daphnia magna* in Lake Erie water was found to be 106 mg/l (358).

AMINES AND AMINO COMPOUNDS

(See also Aniline, Benzene Derivatives, Benzylamine, Diethanolamine, Diethylamine, Dimethylamine, Ethylamine, Hydroxylamine, Methylamine, Rosin Amines (in Chapter IX), Triethylamine, Trimethylamine, and Urea).

Compounds derived from ammonia by the replacement of hydrogen by one or more univalent hydrocarbon radicals are known as amines; for example, methylamine is CH_3NH_2, dimethylamine is $(CH_3)_2NH$, and trimethylamine is $(CH_3)_3N$. Several of the references uncovered in this survey refer to specific amines. In such cases, these compounds are generally listed separately in this volume. Other references cover multiple amines in such a manner that they can best be shown as a group under this section.

Gillette, Miller, and Redman (1442) ran toxicity tests with creek chub, a fish considered to be average in tolerance, at 15-21° C in well-aerated water. Toxicity was expressed in terms of a "critical range," defined as the range of concentration in mg/l below which all four fish lived for 24 hours and above which all died. For the amines and related compounds, the critical ranges were reported to be as follows:

Substance	*Critical Range, in mg/l*
Diamylamine	5-20
Bis-benzylethylenediaminediacetate	5-20
Monomethylamine	10-30
Di-sec-butylamine	15-40
Tri-n-butylamine	20-40
Di-isobutylamine	20-40
Mono-sec-butylamine	20-60
Mono-isobutylamine	20-60
Di-n-propylamine	20-60
Di-n-butylamine	20-60
Nonic 218 (trade name)	20-60
Dimethylamine	30-50
Monoethylamine	30-50
Ethylenediamine	30-60
Monoamylamine	30-50
1, 3-dibutylthiourea	30-100
Mono-n-butylamine	30-70
Tri-n-propylamine	30-70
Di-iso-propylamine	40-60
Mono-n-propylamine	40-60
Monoethylethanolamine	40-70
Monoisopropylamine	40-80
Triethylamine	50-80
Diethylamine	70-100
Diethylethanolamine	80-120
1, 3-diethylthiourea	100-300
Ethyldiethanolamine	160-200
Diethylnitrosoamine	900-1100
Buramine (trade name)	1000-1500
Endothal (trade name)	1600-3200
Diethylaminohydrochloride	4000-6000
Ethylenethiourea	6000-8000
1, 3-dimethylurea	7000-15,000
Urea	16,000-30,000

According to Smith et al. (2978) the oral DL_{50} for rats and the 95-percent confidence limits for several amino compounds are as follows:

Compound	*LD_{50} in gm/kg of body weight*	*95 percent confidence limits*
Ethylamine	0.40	0.29-0.56
Diisopropylamine	0.77	0.61-0.94
Dibutylamine	0.55	0.48-0.62
Hexylamine	0.67	0.62-0.74
2-Ethylbutylamine	0.39	0.34-0.45

4-AMINO-M-TOLUENE SULFONIC ACID $C_7H_6NH_2SO_3H$

Wallen et al. (2940) studied the effect of this compound on the mosquito-fish (*Gambusia affinis*) in highly turbid water at 22-24°C. They found the 24-, 48-, and 96-hour TL_m concentrations to be 425, 410, and 375 mg/l respectively. The turbidity was reduced from 650 mg/l to 220 mg/l and the maximum dosage lowered the pH to only 6.3.

AMINOTRIAZOLE

(see Chapter IX)

AMMATE (AMMONIUM SULFAMATE)

(see Chapter IX)

AMMONIA NH_3

1. General. In the pure form, ammonia is a colorless gas with a very pungent odor, made synthetically from atmospheric nitrogen and hydrogen, or obtained during the manufacture of coal gas. In surface or ground waters, however, it generally results from the decomposition of nitrogenous organic matter, being one of the constituents of the complex nitrogen cycle. It may also result from the discharge of industrial wastes from chemical or gas plants, from ice plants, or from scouring and cleaning operations where "ammonia water" is used. Rivers known to be unpolluted have very low ammonia concentrations, generally less than 0.2 mg/l as N (2972).

Being soluble in water to the extent of 100,000 mg/l at 20°C, ammonia gas reacts with water, liberating much heat, to form ammonium hydroxide. This, in turn, dissociates readily into ammonium and hydroxyl ions, thereby tending to raise the pH value of the solution. The combined reversible equations are as follows:

$$NH_3 + H_2O \rightleftharpoons NH_4OH \rightleftharpoons NH_4^+ + OH^-$$

Inasmuch as the dissociation constant for ammonium hydroxide is 1.8×10^{-5} at 25°C (911), the ratio of ammonium ions to ammonium hydroxide molecules is a function of pH as follows:

$$\frac{[NH_4^+]}{[NH_4OH]} = \frac{1.8 \times 10^{-5}}{[OH^-]}$$

At pH 6, this ratio is 1800 to 1, at pH 7 it is 180 to 1, and at pH 8 it is 18 to 1. For common ranges of pH, therefore, it is evidence that most of the ammonia in water exists in the form of ammonium ions. Furthermore, all of the common ammonium salts are also readily soluble in water, yielding NH_4^+ and an anion. In the case of the chloride, nitrate, and sulfate, the solution of ammonium salt results in a decrease in pH since part of the ammonium ions combine with hydroxyl ions in the water.

Despite the fact that the ammonium ion predominates, except at high pH values, the data in much of the literature are in terms of ammonia (NH_3). In recent work, however, the term "ammonia nitrogen" (expressed as N) is gaining prominence. The test for ammonia nitrogen, as outlined in Standard Methods for the Analysis of Water and Sewage (469), measures all of the nitrogen in the form of NH_3, NH_4OH, and NH_4^+.

The results given below pertain only to literature dealing with the terms ammonia or ammonium ion. Where concentrations are expressed in terms of the hydroxide or as salts of ammonia, the data are listed separately under the respective heading.

2. Cross References. Albuminoid Ammonia, Ammonium Hydroxide, Various Ammonium Salts, Chapter IX (Pesticides) and Chapter X (Surface-Active Agents)

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. No limits on ammonia are set by the USPHS Drinking Water Standards of 1962 (2036) or the WHO International Standards (2328); but the WHO European Drinking Water Standards (2329) set a recommended limit of 0.5 mg/l as NH_4^+. According to Laufer (170) a generally accepted limit for free ammonia for sanitary purity of water supplies is between 0.05 and 0.10 mg/l. Although free ammonia is often of vegetable origin and without hygienic significance, its concentration in water in excess of 0.10 mg/l renders the water suspect of recent pollution (855).

In discussing the use of tinned vs. untinned copper tubing for distribution of domestic water, Royden et al. (1042) feel that untinned copper should not be used if the ammonia content of the water exceeds 5.0 mg/l.

Although the ingestion of "aqua ammonia" or "ammonia water" containing 28 to 29 percent ammonia in water has been reported to be fatal (364) there appears to be no physiological harm in concentrations of ammonia that might occur in natural or polluted waters. In fact, ammonia is used in waterworks to form chloramines for sterilization and residual action in water.

The odor threshold for ammonia has been reported as 0.037 mg/l (1756), but for the brewing of coffee the taste threshold is 34 mg/l (2059).

b. Industrial Water Supplies. The slight concentrations of ammonia in natural waters appear to be of no industrial significance, although Hammerton (360) reports that ammonium salts are destructive to concrete made from portland cement. For steeping of barley in malting processes, the concentration of ammonium salts should be low (240).

c. Irrigation Waters. Ammonia is one of the minor constituents of irrigation water, usually occurring in low concentrations, and of little importance to irrigation practice (268). Because it changes rapidly to nitrites and nitrates, ammonia is actually a fertilizer for most crops. Indeed, ammonium salts constitute a major source of nitrogen fertilization.

d. Stock and Wildlife. It has been reported (353) that the ingestion of 50 to 80 ml of 0.5 percent ammonia by rabbits at intervals of one to two days for periods up to 17 months caused chronic acidosis and tissue changes. On the other hand, 23,850 mg/kg of ammonia (fed as ammonium persulfate) in the diet of dogs had no apparent effect in 16 months.

e. Fish and Other Aquatic Life. According to many references, (1043, 1464, 3057, 3332, 3333) the toxicity of ammonia and ammonium salts to aquatic animals is directly related to the amount of undissociated ammonium hydroxide in the solution, which in turn is a function of pH as explained under "General" above. Thus, a high concentration of ammonium ions in water at a low pH may not be toxic, but if the pH is raised toxicity will probably increase (361). Ellis found that the toxicity of a given concentration of ammonium compounds toward fish increased by 200 percent or more between pH 7.4 and 8.0.

The presence of carbon dioxide, up to concentrations in the range of 15 to 60 mg/l, appears to reduce the toxicity of ammonia presumably by lowering the pH value (139, 140, 142, 143, 3321). Inasmuch as CO_2 is excreted by the fish, the pH value at the gill surface will be lower than in the bulk of the solution, thereby reducing the proportion of un-ionized ammonia at the gill (3321).

The toxicity of ammonia to fish is increased markedly at low tensions of dissolved oxygen (144, 2096, 2518, 2640, 2952, 3334). A concentration of non-ionized ammonia equivalent to 0.2 mg/l of nitrogen decreased the survival time of trout at a level of dissolved oxygen that otherwise had no lethal effect (2096). The concentration of excreted carbon dioxide at the gill surface varies with the oxygen content of the water; hence as the oxygen tension is reduced, the concentration of excreted CO_2 is also reduced and the pH value of the water in contact with the gill surface rises, leading to an increased toxicity of an ammonia solution. This mechanism explains the increased toxicity of ammonia at low oxygen tensions (3321).

The following concentrations of ammonia have been reported to be toxic or lethal to fish in the time specified:

Concentration NH_3 in mg/l	*Time of Exposure*	*Type of Fish*	*Reference*
0.3-0.4	--	trout fry	1465
0.3-1.0	--	fish	1044
0.6*	100-200 minutes	rainbow trout	88
0.7	390 minutes	rainbow trout	2970
1.0-2.0	--	fish	1465
1.2	193 minutes	*Squalius cephalus*	2970
2.0	--	fish	1045
2.0-2.5	1-4 days	goldfish	313
2.5	1-4 days	goldfish	1466
2.0-7	--	fish	1460, 1466
2.9	13 hours	*Cichla ocellaris*	145
3.1 (soft water, 30°C)	96-hour TL_m	bluegill sunfish	2936
3.4 (soft water, 20°C)	96-hour TL_m	bluegill sunfish	2936
5.0	--	rainbow trout	2977
5-7 (distilled water, 20°C)	6 hours	minnows	2942
6-7 (hard water, 20°C)	6 hours	minnows	2942
7-8	1 hour	sunfish	363
13	--	fish	346
17.1	1 hour	minnows	362
17-136	15-16 minutes	eel, roach, trout	1467
23.7 (hard water, 30°C)	96-hour TL_m	bluegill sunfish	2936
24.4 (hard water, 20°C)	96-hour TL_m	bluegill sunfish	2936
75.7	less than 4 minutes	trout	146

*un-ionized ammonia

Brockway (1044) states that an ammonia concentration of 1.0 mg/l decreases the ability of the hemoglobin to combine with oxygen and hence the fish may suffocate. A concentration as low as 0.3 mg/l causes a noticeable drop in the oxygen content of the blood of fishes. In the foregoing table, the work of Ellis (313) is most widely quoted and concentrations of 2.5 mg/l have been considered harmful in the pH range from 7.4 to 8.5. Ellis reports, however, that concentrations of 1.5 mg/l are not harmful to most varieties of fish and Schaut (362) claims that minnows were not harmed by a one-hour exposure to 4.3 mg/l. Solutions containing mixtures of ammonium and cyanide ions are more toxic to fish than solutions containing either ion alone (1468).

With respect to other aquatic life, the following results were reported by the Academy of Natural Sciences of Philadelphia (2936):

Organism	*Time of Exposure*	*Type of Water*	*Temperature °C*	*Concentration of NH_3 as N in mg/l producing noted effect*
Physa heterostropha (snail)	96-hour	soft	20	90 (a)
		hard	20	133.9 (a)
		soft	30	94.5 (a)
		hard	30	133.9 (a)
Navicula seminulum (diatom)	5-day	soft	22	420 (b)
		hard	22	420 (b)
		soft	28	320 (b)
		hard	28	420 (b)
		soft	30	410 (b)
		hard	30	350 (b)

(a) 96-hour TL_m
(b) 50-percent reduction in division (growth)

Algae, which thrive on high nitrate concentrations, appear to be harmed or inhibited when the nitrogen is in the form of ammonia. Gusseva (584) reported that 0.4 to 0.5 mg/l of ammonia nitrogen caused a complete disappearance of *Aphanizomenon*. The lethal concentration of ammonia for *Daphnia* has been reported at 8 mg/l (2977). Stammer (147) reports the following toxicity threshold values for indicator organisms of a saprophytic system, in terms of free ammonia:—Organisms of the oligosaprobic and β-mesasaprobic zones, 0.08 to 0.4 mg/l; those of the α-mesasaprobic zones 0.3 to 4.3 mg/l; and those of the polysaprobic zone 3.2 to 220 mg/l.

AMMONIA LIQUOR FROM COKE-GAS PLANTS

1. General. The concentrations of eight constituents of ammonia waste liquors have been reported (1466) as follows: phenols—10 mg/l; higher tar acids—10 mg/l; ammonia as ammonium hydroxide—10 mg/l; ammonium salts—500 mg/l; thiocyanate—200 mg/l; cyanide—10 mg/l; pyridine—400 mg/l; and naphthalene—5 mg/l.

2. Cross References. Ammonia, Ammonia Salts, Cyanides, Naphthalene Organic Acids, Phenols, Pyridine, and related compounds.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. In the extracts from ammonia waste liquors, the following threshold odors were determined (1469):

Phenolic wastes	1.0 mg/l
Acidic wastes	10.0 mg/l
Neutral wastes	1.0 mg/l
Basic wastes	3.0 mg/l

b. Irrigation Waters. Ammonia gas liquors containing 1.5-2.0 percent nitrogen may be used as nitrogenous fertilizer, especially in combination with other balanced fertilizers. The small concentrations of phenol and thiocyanate may cause temporary damage to crops, but their disinfecting action may possibly have a beneficial effect on plant diseases. The waste liquors should be applied in early spring or after grasses have been cut (148, 1470, 1471).

c. Fish and Other Aquatic Life. Ammonia liquors contain free ammonia, sulfide, and cyanide, all of which are relatively high in toxicity to aquatic life. They also contain phenol, which has a medium toxicity, and substances with relatively low toxicity such as thiosulfate and the higher tar acids (1466).

Schaut (362) studied the affect of ammoniacal liquor from a coke-gas plant on minnows in stabilized tap water. At a dilution of 1:2400, the minnows were not affected after one hour of exposure, but at a dilution of 1:240 the reaction of fish was similar to that of 0.5 mg/l of sodium cyanide, i.e., it excited the fish, caused them to stop eating, and killed 25 percent in a 24-hour period.

The waste liquor, as tested, contained 1100 mg/l of phenol, 60 mg/l of cyanide as HCN, and 600 mg/l of HCNS as well as ammonia. At a dilution of 1:500,000 it gave a faint medicinal taste and at a dilution of 1:240, a faint yellow color.

AMMONIUM ACETATE CH_3COONH_4

(see also Ammonia, Acetates)

Used in textile dyeing, for preserving meat, and as a mild diuretic for animals, this compound is highly soluble in water. According to Wallen et al. (2940), the 24-hour, 48-hour, and 96-hour TL_m values for mosquito-fish *(Gambusia affinis)* in turbid water at pH 7.6-8.4 were all 238 mg/l. This concentration reduced the turbidity from 1400 mg/l to less than 25 mg/l.

AMMONIUM ALUM

(see Aluminum Ammonium Sulfate)

AMMONIUM ARSENATE $(NH_4)_2HAsO_4$

(see also Ammonia, Arsenic)

This highly soluble salt was found to have no effect upon the sea lamprey, rainbow trout, or bluegill sunfish when exposed for 24 hours at a concentration of 5 mg/l in Lake Huron water at pH 7.5-8.2 (2976).

AMMONIUM CARBONATE $(NH)_2CO_3$

(see also Ammonia)

This colorless crystalline or amorphous substance is used in baking powders, for wool scouring, in tanning and textile mills, and in many manufacturing processes. It has been reported in gas-manufacturing wastes (363) and ice-plant effluents (808).

Being highly soluble in water, this salt dissociates into ammonium ions that may combine with hydroxyl ions to form NH_4OH. It has the added detrimental effect that the carbonate ion offsets the tendency of the ammonium ion to lower the pH (see Ammonia). The carbonate has a buffering action that influences toxicity of the ammonium ion. For example, 240 mg/l of ammonium carbonate in distilled water killed goldfish in about 3.5 hours whereas 5350 mg/l of ammonium chloride did not kill in 12 hours (1043). The following information on the toxicity of ammonium carbonate toward fish has been noted:

Concentration of $(NH_4)_2CO_3$ in mg/l	*Remarks*	*Reference*
5.5–7.0	Lethal toward fish	808
10	Tolerated by goldfish for over 100 hours	313
35	Threshold effect toward fish	1466
48	Killed goldfish in 6 days in hard water	313
100	Killed goldfish in 4-10 hrs. in hard water	313
155–197	Fatal to shiners and carp in a few hours	313
238	96-hour TL_m for mosquito-fish in turbid water	2940
600–800	Killed orange-spotted sunfish in one hour	363

AMMONIUM CHLORIDE NH_4Cl

(see Ammonia)

This highly soluble colorless salt is used extensively in industry for dyeing, tanning, washing, and other operations. One source of ammonium chloride in streams is the weathering of granite or other rock; hence the presence of ammonium ion in water is not necessarily an indication of human pollution (1472).

The toxicity of this compound, like that of other ammonium salts, depends on the resulting pH of the water. Unlike the carbonate, however, this salt in high concentrations tends to lower the pH value, thereby counteracting the toxicity of ammonium hydroxide. In distilled water, 5,350 mg/l of ammonium chloride did not kill goldfish in 12 hours but a solution of 2,695 mg/l in distilled water became toxic when sodium hydroxide or other alkalies were added, the increase in toxicity being proportional to the increase in hydroxyl ions (1043). In other experiments tabulated below, ammonium chloride was less toxic in distilled water (where the pH was lowered) than in tap water or hard water that provided buffering action to maintain a high pH.

The effect of ammonium chloride toward fish has been reported as follows:

Concentration of NH_4Cl in mg/l	*Time of Exposure*	*Type of Fish*	*Remarks*	*Reference*
6	96-hour TL_m	bluegill sunfish	standard dilution water	2935
38	7.5 hours	rainbow trout	not lethal	2907
160	30 hours	rainbow trout	limiting concentration	346
180	--	shiners and carp	no effect in tap water	313
268	6-days	goldfish	killed in hard water	313
300	6 hours	minnows	minimum lethal dose in hard water	2943, 2944
314		trout	toxic threshold	1046
490	96-hour TL_m	mosquito fish	turbid water	2940
535	4.75 hours	bluegills	killed in tap water	313
535	18 days	bluegills	killed in distilled water	313
700-800	1 hour	sunfish	killed	363
1570	--	rainbow trout	lethal dose	2920
4000-4500	6 hours	minnows	minimum lethal dose in distilled water	2943, 2944

According to Anderson (598) the threshold concentration for immobilization of *Daphnia magna* in Lake Erie water at 25° C. was found to be 91 mg/l in 64 hours. Ellis (313) found that 535 mg/l killed *Daphnia magna* in six hours. Fairchild (2946) studied the effects of varying oxygen tensions on *Daphnia* exposed to ammonium chloride for 100 hours at 23° C., with the following results:

Dissolved oxygen, mg/l	*Threshold toxicity value, mg/l*
6.4	247
3.14	174
1.48	100

For Lake Erie water at 20-25° C., threshold concentrations of ammonium chloride were reported (2955) as follows:

Organism	*Threshold concentration (mg/l) for immobilization*
Daphnia magna, young	92
Leptodora kindtii	3.1
Cyclops vernalis	86
Mesocylops leukarti	75
Diaptomus oregonensis	5

Hodgson (2956) found that the median concentration of ammonium chloride required to stimulate the movement of a water beetle *(Laccophilus maculosis)* was 2460 mg/l.

AMMONIUM CHROMATE $(NH_4)_2CrO_4$

According to Wallen et al. (2940) the 96-hour TL_m of this highly soluble salt toward the mosquito-fish *(Gambusia affinis)* in highly turbid water was 240 mg/l. The addition of this concentration did not materially lower the turbidity.

AMMONIUM DICHROMATE $(NH_4)_2Cr_2O_7$

This highly soluble salt is used in lithography, photoengraving, in special mordants, pyrotechnics, and porcelain finishes (364). According to Wallen et al. (2940), the 96-hour TL_m toward the mosquito-fish *(Gambusia affinis)* in turbid water was 136 mg/l. This concentration did not lower the turbidity appreciably.

AMMONIUM FERROCYANIDE $(NH_4)_4Fe(CN)_6$

(see also Ammonia, Iron Salts, and Cyanides)

According to Shelford (363) orange-spotted sunfish were killed in one hour by 150 to 200 mg/l of this freely

soluble compound which the author states is a constituent of some gas-manufacturing wastes.

AMMONIUM FLUORIDE NH_4F

This highly soluble compound is used in textile dyeing and printing, for preserving wood, for etching glass, and as a mothproofing agent (364). According to Simonin (3271) 200 mg/l will kill the fish, *Tinca vulgaris*, in less than 48 hours. For the related compound, ammonium fluosilicate, the lethal dose was found to be only 50 mg/l, and for ammonium bifluoride it was 100 mg/l.

AMMONIUM HYDROXIDE NH_4OH

(see Ammonia)

This substance is dealt with separately from ammonia and the ammonium salts only because some the references report data in terms of ammonium hydroxide. Whenever ammonia gas or ammonium salts are dissolved in water, or when ammonium ion is formed in the decomposition of organic matter, the undissociated compound NH_4OH results. The proportion of ammonium ion and undissociated base is a functions of pH, as explained under Ammonia and the toxicity of the resulting solution depends primarily on the concentration of NH_4OH, i.e., the undissociated compound is the toxic principle.

In as much as toxicity varies considerably with pH and hence is a function of the buffering action of the water used, it is difficult to compare the results of many investigators. Toxic concentrations of ammonium hydroxide toward fish have been reported as follows:

Concentration of NH_4OH in mg/l	*Time of Exposure*	*Type of Fish*	*Remarks*	*Reference*
4-5	—	goldfish	lethal	2920
6.25	24 hours	brook trout	lethal	313, 359
10	—	suckers, shiners, carp, trout	lethal	808
10	24 hours	creek chub	not lethal, 15-21°C	1442
13	24 hours	suckers, shiners, carp	lethal	313, 359
15	48-hour TL_m	bluegill sunfish	Philadelphia tap water, 20°C	2093
17.5	48-hour TL_m	fathead minnows	—	3335
18.5	48-hour TL_m	bluegill sunfish	Philadelphia tap water, reoxygenated, 20°C	2093
20	15 minutes	suckers, shiners, carp	lethal	313
30	24 hours	small fish	lethal	313, 359
30	24 hours	creek chub	lethal, 15-21°C	1442
30	—	perch	lethal	2920
37	96-hour TL_m	mosquito-fish	turbid water	2940

In contrast, it has been reported (359) that a concentration of 9.4 mg/l has not harmed suckers, shiners, and carp in 24 hours of exposure.

According to Anderson (358) the threshold concentration of ammonium hydroxide for the immobilization of *Daphnia magna* in Lake Erie water at 25°C was found to be less than 8.75 mg/l.

AMMONIUM MOLYBDATE $(NH_4)_6Mo_7O_{24}\cdot H_2O$

(see Ammonia, Molybdenum)

This highly soluble salt is used for decorating ceramics and in photography. Bringmann and Kuhn (2158) tested ammonium molybdate in River Havel water from which the test organisms had been recovered. They found no adverse response by *Daphnia* held for 48 hours at 23°C nor by *E. coli* incubated at 27°C even at 1000 mg/l of molybdenum. For *Scenedesmus* held for 4 days at 24°C, however, the median toxic threshold effect occurred at 54 mg/l of molydenum.

AMMONIUM NITRATE NH_4NO_3

(see Ammonia)

This soluble odorless compound is used as a fertilizer, in pyrotechnics, for explosives and matches, and in chemical industries. Ellis (313) quotes references to the effect that a concentration of ammonium nitrate of 800 mg/l in tap water killed bluegills in 3.9 hours but in distilled water not until 16 days. Goldfish were killed in distilled water by 4.545 mg/l in 90 hours. This salt dissolves readily and dissociates into its ions but since the ammonium ion combines with the hydroxyl ion of the water, the pH is lowered markedly in unbuffered water. Since it is the undissociated NH_4OH that is the toxic principle, it is easy to understand why a solution in tap water is more toxic than in distilled water.

AMMONIUM PERSULFATE $(NH_4)_2S_2O_8$

(see also Ammonia)

Highly soluble in water, this compound is used in dyeing, electroplating, photography, and many other applications (364). Using water of the River Havel from which the test organisms were recovered, Bringmann and Kuhn (2158) found that the median threshold effect of this compound toward *Daphnia* held for 48 hours at 23°C occurred at 120 mg/l, measured as persulfate. Toward *Scenedesmus* during a 4-day exposure at 24°C, the median threshold effect occurred at 33 mg/l of S_2O_8, but toward *E. coli* at 27°C, there was no measurable effect at 1000 mg/l of persulfate.

AMMONIUM PICRATE $C_6H_2ONH_4(NO_2)_3$

(see Ammonia, Picric Acid, Explosives)

This substance is used in the manufacture of explosives and fireworks. Being freely soluble in water, it may occur in waters from munitions works. According to Ruchhoft and Norris (655) 0.5 mg/l of ammonium picrate in drinking water can be detected by taste and 1.0 mg/l is objectionable.

AMMONIUM SULFAMATE (AMMATE)

(see Chapter IX)

AMMONIUM SULFATE $(NH_4)_2SO_4$

(see Ammonia)

Used widely as a fertilizer and in many industrial processes, this crystalline salt dissolves readily in water with a resulting decrease in pH; hence its toxic effect upon fish is analogous to that of ammonium nitrate and ammonium chloride but less severe than that of ammonium carbonate which has a buffering action.

Ellis (313) quotes a reference to the effect that 66 mg/l in tap water killed bluegills in 3.5 hours, but in distilled water the same concentration required 17 days to kill the bluegills (Note: this is the pH effect on ammonium hydroxide concentration. See Ammonia). In his own experiment Ellis found that 264 mg/l in hard water killed goldfish in six days or less. According to Shelford (363) orange-spotted sunfish were killed in one hour by 420 to 500 mg/l.

LeClerc and Devlaminck (2944) report the minimum lethal concentration in 6 hours toward minnows in hard water at 18°C to be 400-500 mg/l; but in distilled water at 16°C, the minnows did not succumb until the concentration reached 4000-5000 mg/l. Wallen et al. (2940) found the 96-hour TL_m for mosquito-fish (*Gambusia affinis*) in turbid water at 20-21°C to be 1290 mg/l. The ammonium sulfate reduced the turbidity from 240 mg/l to less than 25 mg/l.

Fairchild (2946) studied the effects of varying oxygen tension on the toxicity of ammonium sulfate toward Daphnia exposed for 100 hours at a temperature of 23° C. Results showed a toxicity threshold of 288 mg/l when the dissolved oxygen was 6.6 mg/l; but the toxicity level of the ammonium sulfate dropped to 116 mg/l when the dissolved oxygen was only 1.52 mg/l. Threshold concentrations for other organisms were reported (2955) as shown below:

Organism	*Concentration in mg/l for Threshold Immobilization*
Daphnia magna, young	106
Daphnia magna, adult	152
Diaptomus oregonensis	13

AMMONIUM SULFIDE $(NH_4)_2S$

(see also Ammonia, Sulfides)

According to Ellis (313), 100 mg/l of ammonium sulfide in hard water killed goldfish in 72 hours, but with only 10 mg/l the fish survived more than 100 hours of exposure. He reports that this substance may occur in chemical, beet-sugar, or gas-plant wastes. Wallen et al. (2940) found the 96-hour TL_m toward the mosquito-fish in turbid water at 21°C to be 248 mg/l. The addition of this compound had very little effect upon the turbidity.

The solution of this colorless crystalline substance in water results in a dissociation into ammonium ions and sulfide ions. The former react in part when the hydroxyl ions to form NH_4OH while the latter react with hydrogen ions to form HS^- and H_2S, depending on the pH of the solution. Thus, toxicity is controlled by the resulting combinations in the water, rather than by the added salt itself.

AMMONIUM SULFITE $(NH_4)_2SO_3 \cdot H_2O$

This highly soluble compound is used as a reducing agent in photographic work. Wallen et al. (2940) tested its toxicity toward the mosquito-fish in highly turbid water at 20-21°C. They found the 24-hour, 48-hour, and 96-hour TL_m values to be 240 mg/l. The addition of this compound lowered the turbidity from 220 to less than 25 mg/l.

AMMONIUM THIOCYANATE NH_4SCN

(see also Ammonia, Cyanides)

This highly soluble substance is used in textile processes, pyrotechnics, and in chemical process industries (364). It may also occur in gas-plant wastes (313, 363).

According to Shelford (363), orange-spotted sunfish were killed in one hour by 280 to 300 mg/l, and Demyamenko (431) reports that a concentration of 200 mg/l is deadly to fish. In highly turbid water at 16 to 23°C, Wallen et al. (2940) found the 96-hour TL_m toward the mosquito-fish (*Gambusia affinis*) to be 114 mg/l, although the 24-hour TL_m was as high as 910 mg/l. The addition of this salt did not lower the turbidity. The lethal limit for 24 hours exposure has been reported (2977) at 1600 mg/l for goldfish and tench.

Larvae of chironomus are killed by 50 mg/l of this salt, but *Carinogammarus* and *Asellus aquaticus* withstand 100 mg/l without noticeable harm (2977). The concentration of NH_4SCN required to lower the BOD of sewage is in excess of 5000 mg/l (2923).

AMMUNITION PLANT WASTES

(see Explosives, Trinitrotoluene)

AMYGDALIN $C_{20}H_{27}NO_{11}$

(see also Hydrocyanic acid)

This substance, a cyanogetic glucoside, is widely distributed in nature, being found in bitter almonds, leaves of cherry laurel, peach, cherry, apricot, quince, plum kernels, other hard-nut fruits, cotoneaster, and many other plants. The enzyme *emulsin* hydrolyzes amygdalin to glucose, benzaldehyde, and hydrocyanic acid. According to Schaut (362), about 7 mg/l of amygdalin is toxic to young carp and minnows.

Chlorination of water containing amygdalin may produce a very toxic median when chlorine and the hydrolysis product HCN are brought together. Apparently chlorine does not destroy amygdalin or the enzyme, but it may retard enzymatic action.

AMYL ACETATE $CH_3COOC_5H_{11}$

Isoamyl acetate, a colorless neutral liquid with a pear-like odor, is soluble in 400 parts of water. It is used extensively in organic chemical industries, varnishes, lacquers, and dyeing (364). The threshold odor is reported at a concentration of only 0.0006 mg/l (1756).

Toxicity tests with creek chub, a fish considered to be average in tolerance, at 15-21°C in well-aerated water indicated that all four test fish lived for 24 hours at concentrations of 50 mg/l or less, and all died at concentrations exceeding 120 mg/l (1442). Using highly turbid water with mosquito-fish (*Gambusia affinis*) as the test organism, Wallen et al. (2940) found the 24-, 48-, and 96-hour TL_m values all to be 65 mg/l.

For *Daphnia* exposed for two days at 23°C, the median threshold of harmful effect occurred at 120 mg/l of amyl acetate; for *Scenedesmus* at 180 mg/l during 4 days exposure at 24°C; but for *Escherichia coli*, 1000 mg/l at 27°C had no apparent effect (2977).

AMYL ALCOHOL $C_5H_{11}OH$

Normal amyl alcohol is a liquid used in organic syntheses and as a solvent for resins, varnishes, lacquers, fats, and oils. It is slightly soluble in water and occurs in chemical wastes and some distilling wastes. The oral toxicity toward rats has been reported (3248) as 3.3 mg/kg of body weight.

The toxicity of n-amyl alcohol toward fish has been reported as high as 1000 mg/l (2920) and as low as 1.0 mg/l (313). Ellis (313) found that concentrations of iso-amyl alcohol of 100 mg/l in hard water killed gold-

fish in 82 hours, but as little as 1.0 mg/l killed in 161 hours. For the gudgeon, a small European fresh-water fish, the lethal range of n- amyl alcohol has been reported (2977) as 350 to 500 mg/l; but for tertiary amyl alcohol, the lethal range is 1300 to 1500 mg/l.

In Lake Erie water, according to Anderson (358) the threshold concentration of n-amyl alcohol for immobilization of *Daphnia magna* at 25°C after prolonged exposure was 881 mg/l. Bringmann and Kuhn (2158) reported the threshold toxic effect on *Daphnia* as 440 mg/l, during two days exposure at 23°C, the threshold effect occurred at 280 mg/l, but for *Escherichia coli* at 27°C, 1000 mg/l showed no effect.

AMYLENE

(see Pentene)

ANILINE $C_6H_5NH_2$

(see also Dyes)

Known also as phenylamine, this highly soluble colorless oily liquid occurs in dye, varnish, rubber, chemical, and gas-plant wastes. The oral LD_{50} in dogs is reported as 500 mg/kg of body weight (364). In humans, acute doses of aniline may cause cyanosis, headache, or mental confusion, while chronic doses may produce anemia, anorexia, loss of weight, cretaneous lesions, and bladder trouble. The maximum allowable concentration in water has been given as 5.0 mg/l, and serious poisoning may result from the ingestion of 0.25 ml of the pure liquid (364).

Toward fish, a wide range of toxic levels has been reported. Burgess (2962) cites 100 mg/l for trout, 200 mg/l for minnows, and 1000 mg/l for goldfish. In a concentration of 1020 to 1120 mg/l, aniline killed orange-spotted sunfish in one hour (363), but at a concentration as low as 250 mg/l it was reported to be deadly to fish in an unspecified time (431).

For lower organisms, the threshold concentration for immobilization of *Daphnia magna* during prolonged exposure in Lake Erie water at 25°C was 279 mg/l (358). In contrast, Bringmann and Kuhn (2158) claim that the median threshold effect toward *Daphnia* occurred at 0.4 mg/l during a 2-day exposure at 23°C. For *Scenedesmus* at 24°C for 4 days, the threshold effect occurred at 10 mg/l, while for *E. coli* at 27°C no effect was apparent at concentrations up to 1000 mg/l.

ANILENE HYDROCHLORIDE

(see Benzene derivatives)

ANILENE SULFATE $(C_6H_5NH_2)_2 \cdot H_2SO_4$

(see also Dyes)

This crystalline powder is highly soluble in water. It may occur in wastes from textile and chemical industries. According to Bringmann and Kuhn (2158) the threshold of toxicity toward *Daphnia* occurred at 0.6 mg/l during a 2-day exposure at 23°C. For *Scenedesmus* held 4 days at 24°C, the threshold concentration was 32 mg/l, but for *E. coli* at 27°C, no effect was observed at concentrations up to 1000 mg/l.

ANIMAL AND VEGETABLE OILS

(see also Oil-petroleum)

Large quantities of grease and fat are found in wastes from packing plants, slaughter houses, rendering plants, refineries, cotton seed processing plants, textile mills, milk-processing plants, and chemical works (917). Free oil was the most harmful constituent of the wastes from a quinine-manufacturing plant in India (758).

In many wastes containing oils, such as maize and soya-bean oil, tastes are imparted to fish flesh, owing to the unsaturated fatty acids (616). According to Eldridge and Purdy (983) soluble oils used in machining operations coat stream surfaces, prevent aeration, and create a nuisance. Such wastes have B.O.D. values ranging from 1300 to 12,000 mg/l. The Michigan Stream Control Commission considers 15 mg/l of such oil to be an upper limit.

Schaut (362) reports that saponified grease liquors from an animal oil plant, diluted 1:240 with stabilized tap water, gave a fragrant odor and a noticeable turbidity. Minnows in this dilution showed no distress in five days of exposure. Waste liquor from the sulfonation of animal oils, at 1:240 dilution, gave high turbidity and surface oil spots, with a strong oily odor, and minnows placed in such a dilution would not eat. They recovered their appetities in fresh water. At a dilution of 1:2400, no deleterious effect was observed during a one-hour exposure.

ANTHRACENE $C_{14}H_{10}$

Used as an important source of dyestuffs, this solid hydrocarbon is insoluble in water although it can be dissolved in alcohol and other organic solvents. Even if it should be discharged in industrial wastes, it may be expected to precipitate from solution. In Lake Huron water during a 24-hour exposure at 12°C, a concentration of 5.0 mg/l had no effect on rainbow trout, the bluegill sunfish, or the sea lamprey (2976).

ANTIBIOTICS

(see Chapter IX)

ANTIMONY Sb

(see also Antimony Potassium Tartrate, Antimony Trichloride, Antimony Trifluoride, Antimony Trioxide)

This silvery-white metal, also known as stibium, is seldom found pure in nature. It occurs chiefly as the sulfide (stibnite) or as the oxides, cervantite (Sb_2O_4). and valentinite (Sb_2O_3). The metal is used for alloys and other metallurgical purposes. The salts, primarily sulfides and oxides, are employed in the rubber, textile, fireworks, paint, ceramic, and glass industries (2121). The trichloride is a dye and it may occur in textile wastes. Antimony forms salts with a + 3 and + 5 valence. The trichloride, sulfate, potassium tartrate, and pentachloride are soluble in water but the antimony tends to be precipitated as Sb_2O_3 or Sb_2O_5. The sulfides are also insoluble. Consequently, any dissolved antimony that might be discharged to natural waters would soon precipitate and be removed by sedimentation and/or adsorption.

There is no evidence that antimony is an essential element in human nutrition, but it has been found to be toxic. Indeed, it has been used since ancient times as an emetic. Doses as low as 100 mg have been fatal. The symptoms are similar to those of arsenical poisoning. Since 1949, antimony potassium tartrate has been given intravenously for the treatment of schistosomiasis. On the other hand, it has been known to produce severe electrocardiographic abnormalties, skin eruptions, and even pneumonia (2121).

The compounds of antimony are toxic, and are classed as acutely moderate or chronically severe. According to this categorizing, moderate toxicity includes injury to internal organs, and severe toxicity includes seriously debilitating effects or death (2325). It has been reported that 97.2 mg of antimony has caused the death of an adult, and 48.6 mg the death of a child. Antimony metal, up to 100 mg/day per 100 gm body weight fed to rats, allowed normal growth but resulted in a consistent injury to the heart. Antimony added to the food of rabbits caused a progressive increase in both the hemoglobin and in the total red cells, and when fed to cats was accompanied by a decrease in white cells (2121).

It has been reported (3344) that antimony can be concentrated by certain forms of marine life to over 300 times its concentration in the surrounding waters.

ANTIMONY POTASSIUM TARTRATE $K(SbO)C_4H_4O_6 \cdot \frac{1}{2}H_2O$

Known as "tartar emetic", this soluble salt of antimony is used as a mordant in the textile and leather industry (364), and for ant baits or thrip control. The death of man may result from ingestion of 150 mg of tartar emetic by mouth. Daily doses of 20 mg of tartar emetic have limited the growth of rats. A single dose of 115 mg tartar emetic per kg of body weight has been fatal to rabbits within 26 to 72 hours; and 120 to 125 mg per kg has been fatal within 24 to 36 hours (353). The minimum lethal oral dose for rats is reported at 300 mg/kg. Rats fed up to 100 mg/kg daily for 12 months reportedly grew normally, but heart injury resulted (2121). Horses can take 5.8 grams, and cattle 3.8 grams three times daily without apparent harm (353).

Tartar emetic has been employed in medical use, particularly for the treatment of certain tropical parasitic diseases, but is no longer recommended because of the frequency and severity of toxic reactions, less toxic antimony compounds being available (364). It is also used in veterinary medicine. Effects on the heart have been frequently noted as a toxic manifestation of tartar emetic, the mortality from cardiac arrhythmia being reported as 50 percent between the years 1952 and 1954. Seven out of 12 patients manifested considerable electrocardiographic abnormalities while undergoing tartar emetic therapy. A more recent survey of eleven patients led to the conclusion that the cardiac disturbance is a combined effect of functional disorder of the autonomic system caused by an inhibitory effect of antimony on the cerebral cortex and hyperexcitability of the myocardium induced by antimony (2121).

The 96-hour TL_m of fathead minnows has been estimated at 20 mg/l in soft water and 12 mg/l in hard water, as antimony (2125, 2154). Tartar emetic is reported to begin hindrance of protozoan food intake at 15 mg/l, of cellular division in green algae at 3.5 mg/l, and of movement of *Daphnia* at 9 mg/l (3343). A concentration of 5 mg/l had no effect on sea lampreys in Lake Huron water during a 24-hour exposure (2976).

Using water from the River Havel from which the test organisms were recovered, Bringmann and Kuhn (2158) studied the threshold effects of antimony ammonium tartrate on various organisms. For *Daphnia* exposed for 48 hours at 23°C, the median threshold effect occurred at 9 mg/l of antimony; for *Scenedesmus* at 24°C for 4 days, the median toxic threshold was 3.5 mg/l as antimony; and for *Escherichia coli* at 27°C, it was 33 mg/l as antimony.

ANTIMONY TRICHLORIDE $SbCl_3$

This liquid substance is used in an acid solution as a mordant for patent leather and in dyeing (364). It has been encountered in poisoning cases. Anderson (598) has reported a threshold immobolization concentration for *Daphnia magna* in Lake Erie water at 25°C of 37 mg/l. The 96-hour TL_m for the fathead minnow has been estimated at 9 mg/l in soft water and 17 mg/l in hard water, both as antimony, based on exploratory tests only (2154). In Lake Huron water at pH 7.5-8.2, saturated with dissolved oxygen, and at 13°C, rainbow trout, bluegill sunfish, and the sea lamprey were not affected during a 24-hour exposure to 5 mg/l of $SbCl_3$ or $SbCl_5$ (2976).

ANTIMONY TRIFLUORIDE SbF_3

This soluble trivalent salt of antimony is soluble in water. It is used for dyeing in the textile industry and also for the manufacture of pottery and porcelains (364). At a concentration of 200 mg/l it killed fish *(Tinca vulgaris)* in less than 24 hours. Given orally to adult guinea pigs, 100 mg/kg of SbF_3 was fatal (3271).

ANTIMONY TRIOXIDE Sb_2O_3

This oxide of antimony, used as a paint pigment and a mordant, is insoluble in water and consequently not likely to be found for long in polluted streams. The oral LD_{50} in rats is greater than 20 gm/kg (364). In chronic studies, dosages up to 4 mg daily have been well tolerated by rats (2121). When it was administered in food at a 2 percent level, reduced growth rates were observed in rats and rabbits (149). Single doses of 2 to 3 grams of antimony trioxide, antimony pentoxide (Sb_2O_5), and sodium metantimonate ($2NaSbO_3 \cdot 7H_2O$), cause no indication of poisoning in rats. Daily doses of 250 to 500 mg of antimony trioxide, antimony pentoxide, and sodium metantimonate had no effect on the growth of rats. Daily doses of 150 mg/kg of antimony trioxide have been apparently harmless to rabbits over a 4-week period (353). The 96-hour TL_m toward the fathead minnow in hard or soft water, based on exploratory tests only, was found to be over 80 mg/l, as antimony (2154).

ARAMITE

(see Chapter IX)

ARESKAP

ARESKET 300

ARESKLENE 400

(see Chapter X)

AROMATIC SOLVENTS

(see Chapter IX)

ARQUAD C

ARQUAD D

(see Chapter X)

ARSENIC As

1. General. Found to a small extent in nature in the elemental form arsenic occurs mostly as the arsenides of true metals or as pyrites. It is generally recovered as a by-product from the processing of other ores, e.g., copper, lead, zinc, tin. Upon smelting of these ores, arsenic is oxidized and liberated in the flue dust, chiefly as arsenic trioxide. It also forms a tetraoxide and pentoxide. Hence, arsenic may have valences of +3, +4, and +5.

Arsenic is used in metallurgy to increase hardening and heat resistance, in glassware and ceramics, in tanneries, in dye manufacture, and in chemical industries. Its major use, however, has been for pesticides (insects, weeds, fungi) and as a wood preservative. It is also used in the manufacture of arsenical organic compounds for therapy (364, 2121). Elemental arsenic is insoluble in water but many of the arsenates are highly soluble. Indeed, arsenic compounds occur naturally in some waters of the western U. S.

The following material has been derived from the general literature on arsenic. In addition, however, the reader should consult the information given under specific arsenic compounds.

2. Cross References. Ammonium Arsenate, Arsenic Trioxide, Lead Arsenate, Sodium Arsenate, Sodium Arsenite, Selenium, Chapter VIII, Chapter IX.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Although arsenic compounds are constantly taken into the body as a constituent of food, there is no evidence that arsenic is an essential element in human nutrition (2121). Normal human blood contains 0.2 to 1.0 mg/l of arsenic. It is found naturally in many foods such as vegetables and fruit, and shellfish may contain over 100 mg/kg. It also occurs as a residue on sprayed fruits and vegetables, for which the U. S. Food and Drug Administration has set a limit of 3.5 mg/kg. The Food Standards Committee for England and Wales put into effect on August 1959 a new regulation for arsenic limiting ready-to-drink beverages to 0.1 mg/l and food to 1.0 mg/kg (150).

Arsenic is notorious for its toxicity to humans. Ingestion of as little as 100 mg usually results in severe poisoning and as little as 130 mg has proved fatal (2121). One reference (3336) reports a lethal dose at 325 mg of sodium arsenite or 187 mg of As. Furthermore, arsenic accumulates in the body, so that small doses may become fatal in time. A single dose may require ten days for complete disappearance and this slow excretion is the basis for the cumulative toxic effect. Chronic arsenosis is of slow onset, and may not become apparent for 2 to 6 years. Small eruptions occur on the hands and on the soles of the feet, sometimes developing into arsenical cancers. Liver and heart ailments may also supervene (155, 996). In water taken over a long period of time the following concentrations of arsenic have been reported as poisonous to human beings:—0.21 mg/l (997), 0.3-1.0 mg/l (155), and 0.4-10.0 mg/l (996, 997). On the other hand, the following concentrations have been reported to be safe in drinking water:—0.05 mg/l (152, 353, 897), 0.10 mg/l (32, 155, 460, 1000, 1001), 0.15 mg/l (353, 579, 997), 0.15-0.25 mg/l (1000), 0.2 (152, 633), and 1.0 mg/l temporarily for several months in a part of Los Angeles (152).

Arsenic has been suspected as a causal factor for Haff's disease among human beings, but no proof has been found that there is any relationship between them (998, 999). Arsenical pollution of wells is reported to have caused deaths of humans in South Africa; in one case the water contained 12 mg/l of arsenic (1048).

Several incidents have demonstrated that arsenic in water may be carcinogenic. There have been several instances where cancers of the skin, and possibly the liver, have been attributed to arsenic in the domestic water supply (3337, 3338, 3339).

In 1942 for the first time, the USPHS Drinking Water Standards prescribed a maximum permissible concentration of 0.05 mg/l for arsenic and this mandatory limit is still in effect (2036). In 1962, however, a recommended limit of 0.01 mg/l was added to the mandatory limit of 0.05 mg/l. The WHO International (2328) and European (2329) Drinking Water Standards prescribe a maximum allowable concentration of 0.2 mg/l for arsenic.

b. Irrigation. Arsenic is a normal constituent of most soils, with concentrations ranging up to 500 mg/kg. Although very low concentrations of arsenates actually stimulate plant growth, the presence of excessive soluble arsenic in irrigation waters will reduce the yield of crops, the main effect appearing to be the destruction of chlorophyll in the foliage. Plants grown in water containing one mg/l of arsenic trioxides showed a blackening of the vascular bundles in the leaves. Beans and cucumbers are very sensitive, while turnips, cereals, and grasses are relatively resistant. Old orchard soils in Washington that contained 4 to 12 mg/kg of arsenic trioxide in the top soil were found to have become unproductive.

The root growth of lemon plants grown in solution cultures was stimulated by 1 mg/l of arsenic dosed as either an arsenate or an arsenite; however, 5 mg/l of arsenate arsenic and 10 mg/l of arsenite arsenic were definitely toxic to root growth and top growth (3340). Hewitt (1473) found that 23 mg/l of arsenate was slightly injurious to sugar beets in sand culture.

c. Stock and Wildlife Watering. In New Zealand, sickness and death among cattle have been caused by arsenic of natural origin in water supplies (1002, 1049). The lethal dose of arsenic for animals is believed to be about 20 mg per animal pound (1003). Fowl and pigs have died after eating one feed of contaminated pig meal containing 6.5 mg of arsenic per ounce (1004).

On the other hand, selenium poisoning among cattle, dogs and chickens has been treated by feeding arsenic in concentrations of 12 to 15 mg/l in water (1005); and 5 mg/l arsenic (as Na_2HAsO_4) in drinking water has counteracted selenium poisoning among pigs, dogs, and rats (921).

In a brief outline of the toxic effects of some common poisons, Wadsworth (3108) gives the following toxic doses for arsenic:

Animal	*Toxic dose, in grams*
Fowl	0.05– 0.10
Dogs	0.10– 0.20
Swine	0.5 – 1.0
Sheep, goats, horses	10.0 –15.0
Cows	15–30

For rats and mice, 96-hour LD_{50} doses of arsenic trioxide varied from 15.1 to 214 mg/kg based on oral administration (3341). Another reference (2999) reported the LD_{50} for female rats was 298 mg/kg based on calcium arsenate, or 112 mg/kg as arsenic. The minimum lethal dose of arsenic trioxide for various laboratory animals ranged from 5 to 100 mg per kg of body weight, solutions being more lethal than powders (364). Doses of 324 mg have killed experimental chickens in 24 hours (1004).

d. Fish and Other Aquatic Life. (see also Arsenic Trioxide, Sodium Arsenate, Sodium Arsenite). The folfollowing concentrations of arsenic have been reported as toxic to fish:

Concentration of Arsenic, mg/l	*Time of Exposure*	*Type of Fish*	*Reference*
1.1	--	fish	353, 465
1.1-2.2	2 days	pike perch	2977
2.2	3 days	bleak	2977
3.1	4-6 days	carp	2977
3.1	3 days	eels	2977
4.3	11 days	crabs	2977
7.6	10 days	bass	311
11.6	36 hours	minnows	617
15	--	crappies and bluegills	900
17.8	--	minnows	2962
60	16 hours	minnows	617
234	--	minnows	2962

In contrast, the following concentrations have been reported as tolerable within the period specified:

Concentration of Arsenic, mg/l	*Time of Exposure*	*Type of Fish*	*Reference*
0.7-1.1	48 days	pike perch	2977
0.76	--	fish	677
1.1-1.6	11 days	bleak	2977
1.5-5.3	1-7 days	fish	353
2.2	13 days	carp	2977
2.2	13 days	eels	2977
3.1	90 days	crabs	2977
5.3	24-148 hours	fish	313, 778, 1009
6.0	232 hours	bass	900
7.6	30 days	trout	1012
13.0	1 hour	minnows	353

With respect to lower forms of aquatic life, arsenic concentrations of 3-14 mg/l have not harmed mayfly nymphs and 10-20 mg/l has been harmless toward dragon and damsel flies (574). Concentrations of 2-4 mg/l of arsenic are reported not to interfere with the self-purification of streams (579). Bacteria grow even in the presence of 10,000 mg/l of potassium arsenate and algae are not killed at 1000 mg/l of arsenate (2977). The fermentation and propagation of yeast are stopped at 300 mg/l of arsenate (2977).

Arsenic-76 occurs in the effluent from nuclear reactors (3342) and may be concentrated to a limited extent in the aquatic food chain. For more detail, see Chapter VIII.

The Mersey and Severn River Boards in England have adopted working standards limiting the total concentration in effluents of all heavy metals, including arsenic, to 1.0 mg/l (1756, 2950).

4. Summary. On the basis of the foregoing information, the following limits for arsenic are indicated for these beneficial uses:

a. Domestic water supplies	0.05 mg/l
b. Irrigation	1.0 mg/l
c. Stock and wildlife watering	1.0 mg/l
d. Fish and other aquatic life	1.0 mg/l

ARSENIC TRIOXIDE As_2O_3

(see also Arsenic, Lead Arsenate, Sodium Arsenate, Sodium Arsenite)

This compound is the most common oxide of arsenic and owing to the method of recovery of arsenic from ores of other metals, arsenic trioxide serves as the primary material for other arsenic compounds. It is also called "white arsenic" and arsenous acid. It is the primary ingredient of arsenical pesticides. It is sparingly and slowly soluble in cold water.

The following concentrations of arsenic trioxide have been harmful to fish or other aquatic life.

Concentration of Arsenic Trioxide in mg/l	*Time of Exposure, days*	*Type of Organism*	*Reference*
1.96	--	Chironomus	574, 1008
2 to 3	--	fish food organisms	1007
2.5	--	fish food organisms	311
2.5 to 4	--	fish food organisms	313
5.3	8	pink salmon	2091
10	--	fish	1011
10	10	bass	311
10	--	bass, bluegills, crappies	3005
16	3 to 16	mussels	313
40	--	flatworms	353, 313

Surber and Meehan (1008) carried out a comprehensive study of the toxicity of arsenic trioxide to many different fish food organisms. Their results indicated that important fish food organisms can tolerate 2.0 mg/l of arsenic trioxide. For detailed information on the percentage survival of various forms, see the original paper.

The following concentrations of arsenic trioxide have not been harmful to some aquatic organisms in the time specified:

Concentration of Arsenic Trioxide in mg/l	*Time of Exposure*	*Type of Organism*	*Reference*
1.0	--	fish	677
1.9	--	chironomus larvae	574
2	--	food organisms	353, 1008
2	--	food organisms	313
2-6	--	bass, bluegills, crappies	3005
2-7	1-7 days	fish	353
3-14	--	mayfly nymphs	574
5	--	some zooplankton	574
5	24 hours	trout, bluegills, sea lamprey	2976
5	--	fish	353
5-10	15-30 minutes	coho salmon	2988
7	24-148 hours	fish	1009, 778, 313
8	--	mussels	313
10	1 month	trout	1012
10-20	--	insect larvae	574
17.1	1 hour	minnows	353

ATA

(see Chapter IX)

ATABRINE

(see Quinacrine Hydrochloride)

BARIUM Ba

1. General. This yellowish-white alkaline-earth metal is rapidly decomposed by water to form barium ions. It is found in nature as barytes, $BaSO_4$, witherite, $BaCO_3$, and other ores. Many of its salts are soluble, but the carbonate and sulfate are highly insoluble (911); consequently it is to be expected that any barium ions discharged to natural waters will be quickly precipitated and removed by adsorption or sedimentation. Barium ions are not normally present in natural surface or ground waters in measurable concentration although they have been detected in a few springs and in effluents from areas where barytes or witherite are mined (2972). For sea water, the concentration of barium has been reported (3345) as 0.0062 mg/l.

Barium and its salts are used in the metallurgical industry for special alloys, in the paint industry, as a constituent of cements designed to withstand salt water, in the ceramic and glass industries, and in several other applications (2121).

2. Cross References. Barium salts, Chapter VIII.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Neither the WHO International nor WHO European Drinking Water Standards (2328, 2329) prescribes a recommended or maximum allowable limit for barium; and until 1962 there was no limit in the USPHS Drinking Water Standards. At that time, however, a mandatory limit of 1.0 mg/l was established, presumably because of possible toxic effects of barium on the heart, blood vessels, and nerves (2062).

The fatal oral dose of barium for man is reported to be 550 to 600 mg, although a case is recorded where a man ingested about 7 grams of $BaCl_2$ and recovered eventually (2121). Barium salts are considered to be muscle stimulants, especially for the heart muscle. By constricting blood vessels, barium may cause an increase in blood pressure. On the other hand, there is no evidence that barium accumulates in the bone, muscle, kidney, or other tissue. It is excreted more rapidly than calcium. Hence there is no danger of a cumulative effect (2121).

Stokinger and Woodward (2325) have calculated a safe concentration of barium in drinking water on the basis of the threshold limiting values in industrial atmospheres, an estimate of the amount absorbed into the blood stream, and daily consumption of two liters of water. On this basis a limiting concentration of 2.0 mg/l was computed; but to provide a safety factor and to allow for possible accumulation in the body, a limit of 1.0 mg/l was recommended.

b. Irrigation. Barium is present in trace amounts in most soils and up to 1.0 percent in some. It is apparently not utilized by most plants except possibly in trace quantities, and indeed it is considered to be poisonous to most plants. An exception is the Brazil nut which may contain over 1000 mg/kg (2121).

c. Fish and Other Aquatic Life. The lethal concentration limit of barium for stickleback (*Gasterosteus aculeatus*) has been given as 400 mg/l (353, 2941). The lethal concentration limit is defined as the concentration to which the substance must be reduced before definite toxic effect disappears (2941). Barium is concentrated by goldfish from solution by a factor of approximately 150 (2435). The Mersey and Severn River Boards in England have established effluent standards that restrict barium alone or in combination with all heavy metals to a total concentration of 1.0 mg/l (2950). For further details relative to the toxicity of barium toward aquatic life, see Barium Acetate, Barium Carbonate, Barium Chloride, Barium Fluoride, and Barium Nitrate.

4. Summary. On the basis of quite limited information, the following tentative limits for barium are suggested:

a. Domestic water supplies ..... 1.0 mg/l
b. Fish and other aquatic life ..... 5.0 mg/l

BARIUM ACETATE $Ba(C_2H_3O_2)_2 \cdot H_2O$

(see also Barium)

Highly soluble in water, this salt is used as a mordant in dyeing. The oral LD_{50} in rabbits is reported as 236 mg/kg of body weight (364). In Lake Huron water at 12°C, 5 mg/l of barium acetate had no effect on rainbow trout, bluegill sunfish, or the sea lamprey during a 24-hour exposure (2976).

BARIUM CARBONATE $BaCO_3$

(see also Barium)

Occurring in nature as the mineral witherite, this compound is soluble in water only to the extent of 22 mg/l at 18° C (911). The oral LD_{50} for rats is reported as 800 mg/kg of body weight (364). Wallen et al. (2940) tested its toxicity toward the mosquito-fish (*Gambusia affinis*) in highly turbid water at 17-20°C. They reported the 96-hour TL_m as greater than 10,000 mg/l but it is not evident that more than 22 mg/l went into solution.

BARIUM CHLORIDE $BaCl_2 \cdot 2H_2O$

(see also Barium)

This readily soluble salt is used as a mordant for acid dyes, for weighting and dyeing fabrics, in paint manufacture, and in tanning operations. The oral LD_{50} for dogs is 90 mg/kg of body weight (364).

Toward fish, the following effects have been reported:

Concentration of $BaCl_2$ in mg/l	*Time of Exposure*	*Type of Fish*	*Remarks*	*Reference*
50	72 hours	young silver salmon	harmed nervous system	2091
158	72 hours	young silver salmon	killed 90 percent	2091
282	23 hours	young silver salmon	total kill	2091
520	over 50 hours	young eels	tolerated at 20°C	1459
1640	96 hours	mosquito-fish	TL_m in turbid water at 20°C	2940
2083	36 hours	young eels	TL_m at 20°C	1459
2480	9 hours	minnows	lethal at 15°C	1459
3200	48 hours	mosquito-fish	TL_m in turbid water at 20°C	2940
3260	24 hours	*Orizias*	lethal concentration	1459
4400	24 hours	mosquito-fish	TL_m in turbid water at 20°C	2940
5000	12-17 hours	goldfish	lethal in dist. water	313
8-10,000	—	goldfish	lethal at 20.5°C	353, 364, 2920

For fish-food organisms, Anderson (598) reports the threshold concentration for immobilization of *Daphnia magna* in Lake Erie water at 25°C to be 29 mg/l in 64 hours. Using water of the River Havel from which the test organisms had been recovered, Bringmann and Kuhn (2158) found the toxic threshold for *Daphnia* at 23°C to be 170 mg/l as barium or 200 mg/l as $BaCl_2$ during a 48-hour exposure. For *Scenedesmus* the toxic threshold at 24°C was 34 mg/l as barium or 40 mg/l as $BaCl_2$ during a 96-hour exposure; while *E. coli* were not affected by 1000 mg/l as barium at 27°C.

The following threshold concentrations for aquatic invertebrates in Lake Erie water at 20-25°C were reported (2955):

Organism	*Threshold Concentration For Immobilization, mg/l*
Daphnia magna	29
Leptodora kindtii	12
Cyclops vernalis	123
Other fish	5000

According to Bijan and Deschiens (3346) barium chloride was lethal to aquatic life as follows:

Type of Organism	*Scientific Name*	*Lethal Concentration in mg/l*
Snail	*Bulinus contortus*	14.3
Snail	*Planorbis glabratus*	11.1
Goldfish	*Carassius auratus*	200
Aquatic plant	*Elodea canadensis*	10.0

BARIUM FLUORIDE BaF_2

(see also Barium)

This highly soluble salt is used in heat treating metals, in embalming, and as an opacifier for vitreous enamels. The oral LD_{50} for guinea pigs is 350 mg/kg of body weight (364). The lethal dose in 48 hours toward the tench, *Tinca vulgaris,* is reported (3271) to be 10,000 mg/l.

BARIUM NITRATE $Ba(NO_3)_2$

(see also Barium)

Readily soluble in water, this compound is used in the vacuum-tube industry, for green signal flares, and for pyrotechnics (364).

The lethal concentration of barium nitrate toward stickleback at 15-18°C has been reported as 400 mg/l as barium, or 760 mg/l as barium nitrate (598, 1460, 2920). The average survival times for higher concentrations were one week at 500 mg/l, four days at 1000 mg/l, two days at 2500 mg/l, and only one day at 3000 mg/l, all as barium (1460). Mathews et al. (3347) found that the maximum concentration of barium nitrate that did not affect fish was 25 mg/l. In Lake Huron water at 12°C, 5.0 mg/l had no effect on rainbow trout, bluegill sunfish, or the sea lamprey during a 24-hour exposure (2976). According to Bijan and Deschiens (3346), two species of snails (*Bulinus contortus* and *Planorbis glabratus*) were killed by 20 mg/l of barium nitrate, goldfish by 200 mg/l, and the aquatic plant, *Elodea canadensis,* by 10 mg/l.

BARIUM SULFIDE BaS

(see also Barium)

This slightly soluble gray powder is used as a depilatory, in luminous paints, and for vulcanizing rubber (364). A concentration of 5.0 mg/l in Lake Huron water at 12°C had no effect on rainbow trout, bluegill sunfish, or the sea lamprey during a 24-hour exposure (2976).

BARON

(see Chapter IX)

BEET-SUGAR WASTES

(see also Saponin, B.O.D.)

The introduction of beet-sugar wastes suddenly into a stream has caused mass killing of fish, the inhibiting of diatom growth, the stimulation of sewage fungus, and the destruction of normal benthal organisms (457). The lethal effect is attributed to a combination of the deoxygenating effect of the B.O.D. and the toxicity of the beet saponins (458). Cole reported that a 2-percent solution of Steffen's waste was fatal for fish (311).

BENOCLOR

(see Chapter IX)

BENZALDEHYDE C_6H_5CHO

(see also Amygdalin)

This aromatic liquid aldehyde, highly soluble in water, is used extensively in the chemical industry in the synthesis of compounds containing aromatic groups. It is also employed as a perfume and flavoring material under the name of *oil of bitter almonds.*

Ruchhoft (459) reports that in chlorinated water the lowest concentration of p-hydroxybenzaldehyde (HOC_6H_4CHO) that usually caused a taste in water is 0.002 mg/l. According to Klein (1756), a concentration of 0.003 mg/l of benzaldehyde causes a faint odor in water.

In stabilized tap water saturated with oxygen, Schaut (362) found that 17.1 mg/l of 85 percent benzaldehyde caused minnows to stop eating, but they did not die.

BENZENE C_6H_6

(see also Benzene Derivatives, Cyclohexane)

Benzene, the simplest of the aromatic compounds, is used extensively as a commercial solvent and for the synthesis of other organic substances. It occurs in wastes from chemical plants, dyeing and other textile operations, and many other industrial processes. At normal temperatures, it is a volatile, flammable, colorless liquid with an ethereal odor. It is soluble in water to the extent of 820 mg/l at 22°C. The oral LD_{50} for rats is reported as 5600 and 5700 mg/kg of body weight (364, 3348).

According to Avramenko (3349) the benzene content of drinking water should be kept below 4.5 mg/l to avoid taste, but Kirkor (460) claims that the minimum detectable concentration of benzene in water is 0.5 mg/l. Chlorination and the presence of other substances, however, may alter this figure.

Toward fish, the toxicity of benzene has been reported as low as 5 mg/l and as high as 395 mg/l. According to LeClerc (2942), the minimum lethal dose of benzene toward minnows during a 6-hour exposure was 5 to 7 mg/l in distilled water at 18°C and 6 to 7 mg/l in hard water at 16°C. Using the bluegill sunfish in Philadelphia tap water at 20°C, Turnbull et al. (2093) found the 24- and

48-hour TL_m values both to be 20 mg/l. At 60 mg/l all fish died within two hours. At 34 mg/l all died within 24 hours. The lethal concentration for rainbow trout has also been reported as 10 to 20 mg/l and the toxicity threshold as 5 mg/l (2977). Several other investigators (461, 462, 463, 2961) reported that concentrations of benzene as low as 20 mg/l killed fresh-water fish in one hour or longer. According to Shelford (363) the lethal concentration for sunfish is 35-37 mg/l. Using highly turbid water at 20-22°C and the mosquito-fish (*Gambusia affinis*), Wallen et al. (2940) found the 24- and 48-hour TL_m to be 395 mg/l and the 96-hour TL_m to be 386 mg/l. The addition of benzene did not help to clarify the turbid water.

Photosynthetic activity of the giant kelp (*Macrocystis pyrifera*) was not affected by exposure to 10 mg/l of benzene over a period of 96 hours (2108).

BENZENE DERIVATIVES

(see also Benzene, Cyclohexane, Phenol, Halogenated Hydrocarbons, Halogenated Phenols, and their cross-references; also Chapter IX)

Sollman (1474) tested the toxicity of phenols, quinones, and other benzene derivatives to goldfish in glass aquaria containing two liters of tap water at room temperature to which the goldfish had been adjusted. The chemical under investigation was added and symptoms or fatality were observed during one to three days. Death after 48 hours was so exceptional that this time was selected for fatality calculation. The "approximate fatal concentration" was taken as the geometric mean between the largest concentration survived for 48 hours and the smallest concentration that was fatal in this time for practically all fish. The results of these tests are represented below.

Substance	*Approximate Fatal Concentration, in mg/l*	*Number of Fish Used*
Hydroquinone	0.287	27
p-methylaminophenol (metol, elon)	0.5	4
2, 2, 4-trimethyl (α-phenyl-isopropyl) 1, 2, dihydroquinoline	1.8	4
p-aminophenol	2.0	4
2-mercaptobenzothiazol (captax)	2	4
hydroquinone monobenzyl ether (algerite alba)	2.5	5
n-phenyl-β-naphthylamine (algerite powder)	4.4	6
aniline hydrochloride	5.5	3
p-isopropoxy diphenylamine (iso)	5.7	3
p-phenylenediamine	5.74	6
pyrocatechol	14	3
pyrogallol	18	5
p-hydroxyphenylglycine	20	2
phenol	28.9	8
4-chloro-2, 5-dihydroxy diphenyl sulfone	35	4
4, 5-dimethyl-2-mercaptothiazole	56	3
resorcinol	57.4	3
p-octyl diphenylamine	>40	4
2, 4-diaminophenol hydrochloride (amidol)	80	3
hydroquinone monomethyl ether	200	4
phloroglucinol	630	4

BENZENE HEXACHLORIDE

(see Chapter IX)

BENZOIC ACID C_6H_5COOH

1. General. Benzoic acid is a white crystalline solid which occurs naturally in resin, gum benzoin, and to a limited extent in cranberries. It may occur in wastes from chemical or gas plants, food-processing plants, or textile processes.

2. Cross References. See Sodium Benzoate, Phenol, Salicylic Acid.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Ruchhoft (459) quotes references to the effect that, in chlorinated water, the lowest concentrations of the following substances producing tastes are:

Substance	*Concentration in mg/l*
m-hydroxybenzoic acid	1.0
p-hydroxybenzoic acid	0.01
o-hydroxylbenzyl alcohol	less than 0.001

b. Fish and Other Aquatic Life. Ellis (313) states that 200 mg/l of benzoic acid is lethal to goldfish in 7 to 96 hours and Shelford (363) shows that concentrations of 550 to 570 mg/l killed orange-spotted sunfish in one hour. Wallen et al. (2940) tested the effect of benzoic acid on the mosquito-fish (*Gambusia affinis*) in highly turbid water at 19-21°C. They found the 24-, 48-, and 96-hour TL_m values to be 240, 225, and 180 mg/l respectively. The benzoic acid precipitated the turbidity. The threshold concentration for immobilization of *Daphnia magna* during prolonged exposure with Lake Erie water at 25°C was 146 mg/l (348).

According to Doudoroff and Katz (361) benzoic acid can be toxic to fish without lowering the pH value to 5.0. Toxicity is attributed to the undissociated acid in solution; hence pH is not a reliable index of dangerous pollution.

The introduction of the COOOH group into the phenol molecule to form HOC_6H_4COOH in either the meta or para position decreases the toxicity to goldfish but in the ortho position, toxicity is increased. Gersdorff (464) reports that relative toxicities, i.e. minimal products of concentration and survival time, were as follows at 27° C:

Substance	*Relative Toxicity*
p-hydroxybenzoic acid	0.22
m-hydroxybenzoic acid	0.35
phenol	1.00
benzoic acid	1.6
o-hydroxybenzoic acid	2.5

BENZONITRILE C_6H_5CN

This flammable liquid with the odor of almond is slightly soluble in cold water. At a concentration of 5 mg/l it caused no effect upon rainbow trout, bluegill sunfish, or sea lamprey exposed for 24 hours in Lake Huron water at 12°C (2976). Henderson et al. (2958) found the TL_m concentrations at 25°C toward three species of fish to be as follows:

Fish	*Type of Water*	*TL_m in mg/l* 24-hour	48-hour	96-hour
Fathead minnow	Hard	116	78	78
Fathead minnow	Soft	240	180	135
Bluegill sunfish	Soft	78	78	78
Guppies	Soft	400	400	400

Adult bluegills exposed for four weeks in water containing 35 mg/l of benzonitrile did not pick up any organoleptic properties that were apparent to a taste panel but there was a sweetish solvent odor in the test aquaria (2958).

BENZOPYRENE

(see Chapter X)

BENZYL ALCOHOL $C_6H_5CH_2OH$

Known also as phenylmethanol, this liquid with a faint aromatic odor is highly soluble in water. It is used in perfumery, in flavoring, as an insect repellant and as an organic solvent (364). According to Bringmann and Kuhn (2158) the median threshold effect of benzyl alcohol toward *Daphnia* held at 23°C for two days occurred at 360 mg/l; toward *Scenedesmus* during a 4-day exposure at 24°C the threshold was 640 mg/l; and toward *E. coli* at 27°C no effect was evident for concentrations up to 1000 mg/l.

BENZYL BROMIDE $C_6H_5CH_2Br$

(see also Benzyl Chloride)

This compound of bromine with toluene is a lachrymatory liquid that is insoluble in, but slowly decomposed by, water (364, 3292). Studying the relation of chemical structure to the irritant responses in marine fish, Hiatt et al. (3350) found that 0.05 mg/l of benzyl bromide produced no irritant response but 0.1 mg/l caused violent irritant activity.

BENZYL CHLORIDE $C_6H_5CH_2Cl$

(see also Benzyl Bromide)

This refractive liquid with an unpleasant irritating odor is produced by chlorinating toluene (364). It is insoluble in water (3292). Hiatt et al. (3350) found that 1.0 mg/l of benzyl chloride produced no irritant response in marine fish but 10 mg/l caused a slight irritant activity.

BENZYLAMINE $C_6H_5CH_2NH_2$

This liquid is miscible with water, producing a strong alkaline reaction, apparently from dissociation and the formation of ammonium ions. It is used industrially for organic synthesis (364). Bringmann and Kuhn (2158) found that the toxic threshold for *Scenedesmus* occurred at 6 mg/l during a 4-day exposure at 24°C. For *Daphnia* the threshold was 60 mg/l during 2 days at 23°C, but *E. coli* at 27°C showed no adverse response at concentrations up to 1000 mg/l.

BERYLLIUM Be

1. General. A relatively rare element, found chiefly in the mineral beryl, this substance is not likely to occur in natural waters. Although the chloride and nitrate are very soluble in water and the sulfate moderately so, the carbonate and hydroxide are almost insoluble in cold water (364). Beryllium is used primarily in metallurgy to produce special alloys, in the manufacture of X-ray diffraction tubes and electrodes for neon signs, and in nuclear reactors (2121).

2. Cross References. Cadmium, Zinc.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Pomelee, in a comprehensive review article, reported that as late as 1953 there was no indication that beryllium in any form is harmful when taken internally through the digestive tract (1478). On the other hand, beryllium has been incriminated in pulmonary ailments of workers exposed to beryllium dusts (2121).

Following the development about 1947 of spectrochemical techniques for detecting beryllium in air, there has been a substantial increase in the number of cases of beryllium poisoning reported in the literature. These cases have occurred as an occupational disease among individuals exposed in industry. The toxicity of beryllium to animals has been demonstrated in the laboratory, and it has been determined that poisoning among human beings has been caused by exposures to beryllium in the atmosphere at concentrations of less than 1 to 100 micrograms per cubic meter of air. The symptoms of berylliosis include skin and lung diseases of variable severity. The reactions of individuals to a given exposure level are said to vary widely, but apparently all persons will show a reaction if time and level of exposure are great enough (1477).

Absorption of beryllium from the alimentary tract is slight (about 0.006 percent of that ingested) and excretion is fairly rapid (2121).

b. Irrigation Waters. In nutrient solution, at acid pH values, beryllium is highly toxic to plants. At pH values above 11.2, however, beryllium appears to be beneficial to plants with magnesium deficiency (1446). Solutions containing 15 to 120 mg/l of beryllium delayed germination and retarded the growth of cress and mustard seeds in solution culture (1479).

c. Stock and Wildlife Watering. Rats were healthy after 2 years on a diet that included about 6.0 mg of beryllium sulfate per day, i.e., 3.6 mg of beryllium per day, or 18 mg of beryllium per kg of body weight per day. If it is assumed that this dosage is applicable to cows, then a cow could drink 250 gallons per day of water containing 6000 mg/l of beryllium without ill effects. Two dogs showed no ill effects after 19 months of daily ingestion of 10 mg of beryllium sulfate daily per kg of body weight. Of three dogs fed one gram of beryllium sulfate daily per kg of body weight, two showed no ill effects after 9 and 19 months; but one lost weight during a 9-month period and was killed for examination. No evidence of tissue damage was found (1478).

d. Fish and Other Aquatic Life. Pomelee (1478) described a series of experiments to determine the effect of beryllium on fish and snails. In all of these experiments beryllium sulfate was complexed with sodium tartrate to keep beryllium in solution at pH 7-9. Beryllium in concentrations up to 28.5 mg/l was not toxic to goldfish, minnows, or snails; this concentration was reached after a period of three weeks during which beryllium sulfate and sodium tartrate were added daily to the aquaria.

When lagoon wastes from a beryllium plant were added to the aquarium water at the rate of 100-300 mg/l daily, no symptoms of injury to the fish could be detected at the end of 15 days, when the aquarium liquid contained less than 3 mg/l of Be, less than 1.0 mg/l of Fe, and less than 2100 mg/l of sulfate at pH 8.0. For five more days, 1000 ml of lagoon waste was added daily, but on the sixth day the fish became sluggish. Goldfish, transferred to fresh water at that time, recovered; minnows and snails left in the waste solution died after 4

days. A 5-percent waste solution was not toxic to fish, but a 10-percent solution was toxic. It has been suggested that the toxicity might be attributable to the high salt concentration of the lagoon water rather than to the presence of berryllium (1478).

Tarzwell and Henderson (2125, 2154) tested the toxicity of three beryllium salts toward fathead minnows and bluegills in hard and soft waters with the following 96-hour TL_m results in terms of beryllium:

	Concentration of Beryllium in mg/l			
	Fathead Minnows		*Bluegills*	
Compound	*Soft*	*Hard*	*Soft*	*Hard*
Beryllium chloride	0.15	15	--	--
Beryllium sulfate	0.2	11	1.3	12
Beryllium nitrate	0.15	20	--	--

These results demonstrate that beryllium is considerably more toxic in soft water than in hard water. According to Hodgson (2956) a barium nitrate concentration of 30,000 mg/l is required to evoke a stimulatory reaction in the water beetle, *Laccophilus*.

e. Swimming and Other Recreational Uses. No information is available concerning the specific effect of beryllium in swimming waters. However, Pomelee has stated that water-soluble beryllium salts can cause ulcers if they enter a break in the skin (1478).

BHC

(see Chapter IX)

BICARBONATES HCO_3

(see also Alkalinity, pH, Carbonates, Hydroxides, Chapter V-Irrigation)

The concentration of bicarbonates in natural and polluted waters is a function not only of the bicarbonates added thereto but also of the temperature, pH, and concentration of other dissolved solids. A full discussion of carbonate equilibria is beyond the scope of this report and the reader is referred to the work of Langelier (692). For this study of water-quality criteria, however, it is important to note that bicarbonates tend to reach an equilibrium with carbonates in accordance with the reactions:

$$H^+ + CO_3^{--} \rightleftharpoons HCO_3^-$$

$$\frac{[H^+][CO_3^{--}]}{[HCO_3^-]} = K$$

where K is reported (911) to be 4.4×10^{-11} at 25°C. Thus, at pH 7 the ratio of bicarbonate ions to carbonate ions will be 2270 to 1, but at pH 10 it will be only 2.27 to 1 and at pH 11, the ratio will be 1 to 4.4. The concentration of bicarbonates therefore, depends on the pH value and the concentration of carbonates.

At medium and low pH values, a second equilibrium reaction occurs, for bicarbonates tend to unite with hydrogen ions to form H_2CO_3 which, in turn, releases free CO_2, thus

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

The equilibrium equation for this reaction is

$$\frac{[HCO_3^-][H^+]}{[H_2CO_3]} = K_1$$

where K_1 is reported (911) to be 3.5×10^{-7} at 18°C. Thus at pH 8 the ratio of carbonic acid to bicarbonate ions is only 0.0286, but at pH 7 it is 0.286 and at pH 6 it is 2.86.

Bicarbonates may reach water from many natural sources, including absorption of CO_2 from the air and the decomposition of organic matter, or they may be discharged by innumerable industrial processes, for bicarbonates are among the most commonly used salts.

Other than the fact that excessive bicarbonates add to the salinity and total solids content of water, and through the complex operations of the carbonate equilibria tend to form carbonates and scale at high temperatures, bicarbonates in water are seldom considered to be detrimental. Hibbard (250) recommends the following concentrations of bicarbonates:

Use	*Concentration mg/l*	*Remarks*
Domestic water supplies		
Drinking	150	desirable or permissible
Cooking	150	desirable or permissible
Washing	60	desirable or permissible
Laundering	none	desirable or permissible
Industrial water supplies		
Textile	200	undesirable or objectionable
Sugar	100	undesirable or objectionable
Pottery	200	desirable or permissible
Steam Boilers	100	undesirable or objectionable

In concentrations of 257 mg/l or less, sodium bicarbonate caused a white shell in ice manufacturing (229). For limitations in boiler feed water, see Chapter V. High bicarbonate contents are reported to affect the stability of vitamins in the manufacture of preserves and to cause swelling of skins in tanneries (2368).

In a general discussion of alkali waters in Montana, Cobleigh (1059) points out that carbonates and bicarbonates in drinking waters react with gastric acids sometimes to the benefit of the drinker. He refers to a modified list of USGS data which gives 700 mg/l as the concentration of bicarbonates unhealthful to most people. Lockhart et al. (3241) report the taste threshold in distilled water of the bicarbonate ion (added as $NaHCO_3$) to be 770 mg/l.

The 10-year weighted average analyses of Colorado River water, according to Kelley (1060), show 172 mg/l of bicarbonate. It did not appear to Kelley that the use of this water for irrigation would seriously affect the underground supply, growth of citrus trees, or condition of the soil in southern California. Harley and Lindner (1061), on the other hand, found that irrigation waters in north central Washington containing 200 mg/l and more of bicarbonates caused a marked decline in the vigor of apple and pear trees after a number of years.

Chapter V of this report includes a discussion of "residual sodium carbonate", defined as carbonates plus bicarbonates minus the sum of calcium and magnesium, all in milliequivalents per liter. Where the residual sodium carbonate is high, i.e., over 2.5 milliequivalents per liter, calcium and magnesium ions tend to be precipitated as carbonates, leaving a high ratio of sodium to calcium ions. In irrigation water, bicarbonates in themselves do not cause difficulty, but by aiding in the precipitation of calcium carbonate they adversely affect the sodium ratio.

In U. S. waters that support a good fish fauna, 5 percent of such waters have less than 40 mg/l of bicarbonate, 50 percent have less than 90 mg/l, and 95 percent have less than 180 mg/l (310).

BIOCHEMICAL OXYGEN DEMAND

(see also Dissolved Oxygen, Oxygen Consumed)

As in tests for alkalinity, acidity, color, turbidity, and specific conductance the determination of biochemical oxygen demand (B.O.D.) does not reveal the concentration of a specific substance. Instead it measures the effect of a combination of substances and conditions. The rate at which B.O.D. is exerted generally follows the unimolecular pattern as shown by equations in Chapter II.

As a parameter of the detrimental effects of organic matter upon a surface water, the 5-day B.O.D. value alone means very little. In itself, B.O.D. is not a pollutant and exercises no direct harm. Only by depressing the dissolved-oxygen content to levels that are inimical to fish life and other beneficial uses does B.O.D. exert an indirect effect. Where reaeration, dilution, and/or photosynthetic action offset or minimize this depletion, B.O.D. does not interfere with the reasonable uses of the water.

B.O.D. is important only insofar as it produces septicity or decreased dissolved oxygen, or subsequent growth of saprophytic bacteria which increase the turbidity or other undesirable characteristics of the streams. In a slow, sluggish stream, a 5-day B.O.D. of 5 mg/l might be sufficient to produce deoxygenation resulting in anaerobic conditions, whereas a swift mountain stream can easily handle 50 mg/l of 5-day B.O.D. without appreciable depletion of dissolved oxygen. Each stream must be considered in its own right, and until the reaeration characteristic of the stream is known the limiting values of B.O.D. cannot be set.

Many state and interstate agencies include B.O.D. limitations in stream standards while others specify that effluents shall not exceed a given concentration of B.O.D. or that B.O.D. reduction by treatment shall reach or exceed a stated efficiency. For details of these state and interstate standards, see Chapter III of this report and the appendices thereto.

BLAST

(see Chapter X)

B.O.D.

(see Biochemical Oxygen Demand)

BORANES

(see Boron)

BORAX

(see Sodium Borate)

BORIC ACID

(see Boron)

BORON

1. General. Never found in nature in its elemental form, boron occurs as sodium borate (borax) or as calcium borate (colemanite) in mineral deposits and natural waters of southern California and in Italy. Elemental boron is used in nuclear installations as a shielding material (neutron absorber). It is also used in metallurgy to harden other metals (364, 2121).

Boric acid and boron salts are used extensively in industry for weatherproofing wood, fireproofing fabrics, manufacturing glass and porcelain, production of leather and carpets, cosmetics, photography, artificial gems, and many other purposes. Boric acid is used as a bactericide and fungicide. Finally, boron in the form of boron hydrides or borates is used in high-energy fuels (354, 2121).

Boron may be substituted for carbon in many organic compounds, e.g., boron trichloride, boron tribromide. It may also be synthesized directly with hydrogen to form boranes, such or diborane, B_2H_6, a gas with a nauseating odor; pentaborane, B_5H_9, a volatile liquid with a sweetish odor; and a decaborane, $B_{10}H_{14}$, a crystalline solid with a bitter-chocolate odor. The boranes are used as rocket fuels and may be encountered in other situations where high-energy fuels are desired.

2. Cross References. Sodium Borate, Sodium Perborate, Chapter V—Irrigation Waters.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Although boron is essential in the nutrition of higher plants, there is no evidence that it performs any vital function in human or animal nutrition (2121). It is present in the ordinary human diet to the extent of 10 to 20 mg/day, with fruits and vegetables as the largest contributors. In food or in water it is rapidly and completely absorbed by the human system, but it is also promptly excreted in urine (2121).

The ingestion of excessive doses of borates may cause nausea, cramps, convulsions, coma, and other symptoms of distress. The fatal dose for adults has been reported as 5 to 20 grams (364) and as 20 to 45 grams (2121). Normal adults were fed 3 grams of boric acid daily for 11 to 16 days without apparent toxic effects (3265).

Boron in drinking water is not generally regarded as a hazard to human beings (633). Goudey and others have reported that boron concentrations up to 30 mg/l are not harmful in drinking water. Above this concentration, it may interfere with digestion because of its preservative action on foods (353, 1055, 1056). Quantities up to 0.5 grams per day of either borax or boric acid have no immediate effect of any kind on healthy individuals (997). Hoskins, however, has recommended a boron limit of 20 mg/l in drinking water (1057).

b. Irrigation. The problem of boron in irrigation water is covered extensively in Chapter V under "Irrigation." Boron is an essential element in the nutrition of higher plants, yet concentrations of boron in irrigation waters in excess of 0.5 mg/l may be deleterious for certain crops. Crops such as asparagus, date palms, sugar beets, alfalfa, onions, turnips, cabbages, lettuce, and carrots can tolerate boron concentrations of 2.0 to 4.0 mg/l. Crops such as potatoes, tomatoes, peas, wheat, corn, oats, and lima beans can grow well at 1.0 to 2.0 mg/l of boron. Among the sensitive crops are pecans, artichokes, plums, pears, apples, cherries, grapes, peaches, oranges, avocados, grapefruits, and lemons, which can tolerate no more than 0.5 to 1.0 mg/l of boron (2391).

Plant roots take up small quantities of dissolved boron from the soil solution Boron adsorbed on the soil is not utilized by plants (3352). The absorbed boron is moved to the leaves, where the water is lost by transpiration. Boron remains in the leaf and tends to accumulate in the tip and margin. As the process continues, the boron concentration becomes sufficiently high to be toxic to the leaf tissue. This type of injury is found only on mature leaves, thus differing from boron-deficiency symptoms that appear only on the new growth (3266).

c. Stock and Wildlife Watering. The lethal dose of boric acid for animals varies from 1.2 to 3.45 grams per kg of body weight, according to the species (2121). Concentrations of 2500 mg/l of boric acid in drinking water have been detrimental to animals only insofar as growth was inhibited (2121). A dairy cow received 16 to 20 grams of borax daily over a 40-day period without ill effects, but the concentration of boron in the milk rose from 0.7 to 3.0 mg/l. The synthetic boranes are far more toxic to animals than natural boron compounds, for example the LD_{50} for decaborane administered orally to rats was reported as 64.3 mg/kg (3267, 3268).

d. Fish and Other Aquatic Life. LeClerc and Devlaminck (2942, 2943, 2944) reported the minimum lethal dose for minnows exposed to boric acid for six hours at 20°C to be 18,000 to 19,000 mg/l in distilled water and 19,000 to 19,500 mg/l in hard water. Wallen et al. (2940) tested the effect of boric acid and sodium borate in highly turbid water on the mosquito-fish (*Gambusia affinis*), with the following results:

	Temperature		*Concentration in mg/l*		
Chemical	*Range*	*pH Range*	*24-hour* TL_m	*48-hour* TL_m	*96-hour* TL_m
Boric acid	20-23°C	5.4-7.3	18,000	10,500	5,600
Sodium borate	22-26°C	8.6-9.1	12,000	8,200	3,600

Wurtz (1054) has reported the results of a study of the effects of boric acid on one rainbow trout and one rudd. A solution of 2,000 mg/l of boric acid was harmless to both fish; 5,000 mg/l caused only a slight darkening of the skin of the trout. The trout became immobile and lost its balance in a few minutes in concentrations up to 80,000 mg/l but recovered rapidly when it was transferred to fresh water, even after immersion in the boric-acid solution for 30 minutes. The rudd appeared unharmed by concentrations up to 80,000 mg/l for short periods; however, it died after 18 hours in a 6,250 mg/l solution of boric acid. A roach in 6,250 mg/l solution also died, after 46 hours.

Boric acid can be toxic to fresh water fish without lowering the pH to 5.0. Thus, pH is not a reliable index of dangerous pollution by boric acid (361).

Turnbull et al. (2093) found the 24-hour TL_m of boron trifluoride toward the bluegill sunfish in Philadelphia tap water at 20°C to be 15,000 mg/l.

To produce a 50-percent inhibition of the 5-day oxygen utilization of synthetic sewage, Herman (2923) found that over 1000 mg/l of boric acid was required.

BREWERY WASTES

(see also B.O.D., Sugars, Detergents, Soaps)

For a thorough discussion of the nature of brewery wastes, the reader is referred to a standard text on chemical processes and industrial wastes (189, 346). The principal deleterious effect of such wastes is their high B.O.D. It has been reported (465) that yeast wort is harmless to fish in a dilution of 1:40.

BROMINE Br_2

A dark reddish-brown fuming liquid, elemental bromine is relatively soluble in water. It is used for medicinal compounds, dyestuffs, and antiknock compounds for gasoline motors. It has also been used for sterilization of swimming-pool water. Sources of molecular bromine in water are chemical industries and salt-works effluents. Bromine, like other halogens, is antiseptic and disinfectant; hence it may possibly interfere with bacterial and other natural purification processes.

A concentration of 10 mg/l of bromine in soft water has killed *Daphnia magna* (313). Jones (2920) reported that 20 mg/l of bromine killed goldfish at 18-23°C. Hiatt (3350) indicated that 1.0 mg/l of bromine showed no irritant response from marine fish, but 10 mg/l caused violent irritant activity.

BSM-11, BUFFEN 30, BUTROL

(see Chapter IX)

BUTADIENE $CH_2{=}CHCH{=}CH_2$

A colorless gas, 1, 3-butadiene is insoluble in water. It is used as a polymer component in the synthesis of rubber. According to Garrett (2959), the 24-hour TL_m for the marine pinperch (*Lagodon rhomebodies*) is 71.5 mg/l. No deaths occurred at 50 mg/l.

BUTANONE

(see Methyl Ethyl Ketone)

BUTYL ACETATE $CH_3COOC_4H_9$

Normal butyl acetate is a liquid highly soluble in water. It is used in the manufacture of plastics, lacquer, artificial leather, and photographic films (364). The oral LD_{50} for rats has been reported as 4.13 grams/kg of body weight and for mice 7.06 grams/kg (3242).

Bringmann and Kuhn (2158) found that the median threshold effect of n-butyl acetate toward *Daphnia* during a two-day exposure at 23°C occurred at a concentration of 44 mg/l. For *Scenedesmus* at 24°C for 4 days, the median effect occurred at 320 mg/l; but for *E. coli* at 27°C, no effect was apparent at concentrations less than 1000 mg/l.

BUTYL ALCOHOL C_4H_9OH

Normal butyl alcohol, a colorless liquid, is used extensively in industry, being prepared from cornstarch or from acetylene. It may occur in many types of wastes, including those from the paint, varnish, and chemical industries. The oral LD_{50} of n-butyl alcohol for rats has been reported as 4.36 mg/kg of body weight (364) and as 2.75 mg/kg (3248). According to Ettinger et al. (2172, 3269) the median response to the odor threshold of n-butyl alcohol occurred at a concentration of about 0.2 mg/l.

Toward fish, the deleterious concentrations reported in the literature vary widely. Ellis (313) quotes several references to the effect that butyl alcohol in a concentration of 20 mg/l killed goldfish in hard water in 15 to 96 hours; 29 mg/l killed minnows and goldfish; and 250 mg/l killed goldfish in 7 to 20 hours. A concentration of 400 mg/l is reported to have killed fish in 51 minutes. For gudgeon, a European fresh-water fish, the lethal range for n-butyl alcohol is 1000 to 1400 mg/l; but for tertiary butyl alcohol it is 3000 to 6000 mg/l (2977).

The concentration of n-butyl alcohol required to stimulate the movement of 50 percent of a group of water beetles (*Laccophilus*) was reported as 3410 mg/l (2956).

BUTYRIC ACID $CH_3CH_2CH_2COOH$

This oily liquid with an unpleasant rancid odor is miscible with water. It is used to decalcify hides, for varnishes, and in the manufacture of esters used in artificial flavoring (364). According to Smyth et al. (2978) the oral LD_{50} for rats is 8.79 gm/kg of body weight, while von Oettingen (3270) reports a minimum lethal dose for animals of 3.6 to 3.7 gm/kg.

For trout, the lethal concentration has been given as 400 mg/l whereas the threshold for harmful effect is about 100 mg/l (2977). Using water of the River Havel from which the test organisms had been recovered, Bringmann and Kuhn (2158) found the median threshold effect of butyric acid toward *Daphnia* occurred at 60 mg/l after two days of exposure at 23°C; and toward *Scenedesmus* at 200 mg/l after 4 days exposure at 24°C.

CADMIUM Cd

1. General. In the elemental form, cadmium is insoluble in water. It occurs in nature largely as the sulfide salt, greenockite or cadmium blend, often as an impurity in zinc-lead ores. Cadmium is used in metallurgy to alloy with copper, lead, silver, aluminum, and nickel. It is also used in electroplating, ceramics, pigmentation, photography, and nuclear reactors. Cadmium salts are sometimes employed as insecticides and antihelminthics (364, 2121, 2129).

Although the chloride, nitrate, and sulfate of cadmium are highly soluble in water, the carbonate and hydroxide are insoluble; hence, at high pH values, cadmium will be precipitated. Cadmium salts may be found in wastes from electroplating plants, pigment works, textile printing, lead mines, and chemical industries. Welsch and Lieber (2130) reported ground-water contamination by cadmium to the extent of 3.2 mg/l on Long Island, N. Y., as a result of wastes from electroplating industries. High concentrations of cadmium have been reported in Missouri mine waters and one spring there had 1000 mg/l of cadmium (2132).

This section deals with literature in which cadmium or cadmium ion, per se, have been reported. Where cadmium salts are referred to, they are covered under the appropriate heading.

2. Cross References. Cadmium Salts.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Cadmium poisoning of humans has resulted from the consumption of foods or liquids prepared and left in cadmium-plated containers (2121, 2129). As a result, several health departments have forbidden the use of cadmium-plated food containers and pipes. Cadmium-contaminated ice cubes in cold drinks have caused acute gastritis symptoms within one hour (921). Consumption of cadmium salts causes cramps, nausea, vomiting, and diarrhea. Oral ingestion of cadmium has been reported as the cause of a number of human deaths (2129). A single dose of about 8.9 grams of cadmium chloride caused the death of a boy within 1.5 hours (353).

Cadmium tends to concentrate in the liver, kidneys, pancreas, and thyroid of humans and animals (2134). Once it enters the body, it is likely to remain. Normally, many plant and animal tissues contain about 1 mg of cadmium per kg of tissue (2121) but there is no evidence that cadmium is biologically essential or beneficial. The daily intake of cadmium in man can vary from 4 to 60 micrograms, depending on the foods chosen. The major sources appear to be seafoods and grains (2137).

Human beings have been sickened by 13 to 15 mg/kg of cadmium in popsicles, 67 mg/l in punch, 300 mg/l in a cold drink, and 530 mg/kg in gelatin (353). As little as 14.5 mg of cadmium taken orally has caused nausea and vomiting; yet in another instance, as much as 330 mg has not caused permanent injury (353, 1062). Four members of a family whose drinking water contained 0.047 mg/l of cadmium had no history of adverse effects (2132). For further evidence of the action of cadmium on mammals, see Stock and Wildlife Watering below.

Prior to 1950, the USSR established the maximum permissible concentration of cadmium in domestic supplies at 0.1 mg/l (460). The 1962 Drinking Water Standards of the USPHS (2036) set a mandatory limit of 0.01 mg/l for cadmium and the WHO European Standards (2329) prescribe a tolerance limit of 0.05 mg/l. No limit is set in the WHO International Standards (2328).

b. Irrigation. A cadmium concentration of 28 mg/l in a nutrient solution was reported to be injurious to sugar beets grown in sand culture (1473).

c. Stock and Wildlife Watering (see also Cadmium Chloride). The fatal dose of cadmium for dogs has been reported to be 0.15 to 0.3 grams per kilogram of body weight; it has also been reported at 0.3 to 0.6 grams. The fatal dose of cadmium for rabbits appears to be 0.3 to 0.5 grams (353). Ingestion of cadmium in drinking water or food caused a decrease in the hemoglobin content of the blood of rats (1063). Cadmium at a concentration of 5 mg/l in the drinking water increased the mortality among rats on a diet containing 11 mg/l of selenium (1481).

Byerrum and his colleagues (2138, 2142, 2145, 2146, 2147, 2150) conducted extensive tests on rats and dogs fed cadmium, in the form of cadmium chloride, in dosages ranging from 0.1 to 10.0 mg/l of cadmium in drinking water for one year. There were no differences between the treated animals and the controls in water consumption, food intake, or body weight, nor were any pathological changes in blood or other tissues noted. The cadmium content of livers, and kidneys increased in direct proportion to the cadmium intake. Calculated as micrograms of cadmium per gram of tissue, kidneys retained two to three times the amount of cadmium retained by the liver. Some rats, fed 50 mg/l of cadmium

for three months, showed a reduction in growth rate, water consumption, and blood hemoglobin. Moreover, teeth were bleached.

The emetic property of cadmium was found to occur among dogs at cadmium concentrations as low as 5.0 mg/l during warm weather when large volumes of water were consumed. Dogs received up to 10 mg/l of cadmium for four years without pathological changes.

Byerrum (2138) concluded that, owing to the accumulation and retention of cadmium in the liver and kidney, a limit should be placed on this substance in drinking water. He suggested that the limit should not be above 0.1 mg/l and preferably lower.

d. Fish and Other Aquatic Life. Most of the quantitative data on toxicity of cadmium toward fish and other aquatic organisms are given under the specific cadmium salt. Expressed as cadmium, these data indicate that the lethal concentration for fish varies from about 0.01 to about 10 mg/l depending on the test animal, the type of water, temperature, and time of exposure. Schweiger (2151) found that 0.03 mg/l of cadmium was not harmful to one- and two-year-old tench, carp, rainbow trout and char, nor to the crustacea, worms, and insect larvae on which they fed. Concentrations of 0.05 to 0.10 mg/l of cadmium in distilled water produced distress syndromes in the water snail, *Australorbis glabratus* (2733).

Cadmium acts synergistically with other substances to increase toxicity. Cadmium concentrations of 0.03 mg/l in combination with 0.15 mg/l of zinc from galvanized screens caused mortality of salmon fry (2153). Cadmium can form a complex with cyanide in plating baths, but in dilute solutions the complex is almost completely dissociated and highly toxic. Synergism of the toxic cadmium and cyanide ions liberated in the dissociation is indicated (2157).

CADMIUM CHLORIDE $CdCl_2 \cdot 2\frac{1}{2}H_2O$

1. General. This soluble salt of cadmium occurs generally in the crystalline form with $2\frac{1}{2}$ molecules of water. It is used in dyeing and printing of calico, and in photography (364).

2. Cross References. Cadmium and other cadmium salts.

3. Effects Upon Beneficial Uses.

a. Stock and Wildlife Watering. After their teeth had become calcified, the use by rats of drinking water containing 40 mg/l of cadmium chloride was followed by an increase in the incidence of caries (1064). In the form of cadmium chloride, 50 mg/l of cadmium in the food or water caused a decrease in blood hemoglobin and in the pigmentation of the tooth enamel of rats (921). A concentration of 16 mg/l of cadmium chloride in the diet of rats caused bleaching of the teeth; 31 mg/l affected the rate of growth; and 250 mg/l was fatal (353).

For rabbits, 0.15 to 0.3 grams cadmium chloride per kilogram body weight has been lethal (353). For cats, 125 mg/l of cadmium chloride in the diet has been fatal, but 62.5 mg/l had no effect on growth, and a daily dose of 0.56 mg had no cumulative effect (353).

b. Fish and Aquatic Life. The threshold concentration of cadmium chloride for immobilization of *Daphnia magna* in Lake Erie water at 25°C was found to be less than 0.0026 mg/l (353, 598). In water of the River Havel, the threshold concentrations for detrimental effects of cadmium added as cadmium chloride have been reported (2158) for three organisms as follows:

Test Organism	*Threshold Concentration of Cd in mg/l*
Daphnia magna	0.1
Scenedesmus	0.1
Escherichia coli	0.15

Goldfish in distilled water have been killed by a concentration of cadmium chloride of 0.0165 mg/l in 8.5 to 18 hours (313). The highest concentration of cadmium chloride tolerated by young eels for more than 50 hours was about 0.18 mg/l, and at a concentration of 1.83 mg/l the average survival time was only 18.4 hours at 21°C (1459). The lethal concentration of cadmium chloride for three mature small fresh-water fish (Orizias), exposed for 24 hours to the test solution, was reported to be about 45 mg/l (1459). In exploratory tests, Tarzwell and Henderson (2125, 2154) found the 96-hour TL_m of $CdCl_2 \cdot 2\frac{1}{2}H_2O$ toward fathead minnows to be 0.9 mg/l in soft water and 5.0 mg/l in hard water. The wide range of toxicities between distilled water and hard water suggests a definite antagonistic action of calcium, magnesium, and perhaps other salts toward cadmium toxicity.

CADMIUM NITRATE $Cd(NO_3)_2 \cdot 4H_2O$

The toxicity threshold of cadmium for the flatworm *Polycelis nigra* has been reported to be 2.7 mg/l (608). For sticklebacks the toxic threshold concentration of cadmium nitrate has been reported to be 0.42 mg/l in tap water (598). Jones found the lethal concentration limit of cadmium to sticklebacks at 15-18°C to be 0.2 mg/l as cadmium when cadmium nitrate was used as the test compound (1460, 2920). The fish survived 0.3 mg/l (as cadmium) for one week, 0.7 mg/l for four days, 3.0 mg/l for two days, and 7 mg/l for only one day. In fresh water, 6 mg/l of cadmium nitrate has been fatal to the fish *Fundulus* within 36 hours (1459). Toward the guppy, the LD_{50} concentration of cadmium nitrate was found to be 0.056 mg/l, as cadmium (2921).

CADMIUM SULFATE $CdSO_4 \cdot 2\frac{2}{3}H_2O$

This highly soluble salt of cadmium may be found in wastes from lead mines and chemical industries. The sulfates of cadmium and zinc have an additive toxic effect upon fish; sulfates of cadmium and copper are synergistic in their effect (353) (see also Chapter V).

The following concentrations of cadmium sulfate have killed minnows in distilled water (991).

Concentration in mg/l	*Time of Exposure in minutes*
1,042	180
5,200	102
10,400	78

A 50-percent reduction in the 5-day biochemical oxidation of sewage treated with cadmium sulfate was caused by 55 mg/l of cadmium (2922) and by 142 mg/l of cadmium (2923).

CALCIUM Ca

1. General. Elemental calcium does not occur in nature because it oxidizes readily in air and reacts with water to release hydrogen gas. Calcium salts and calcium ions, however, are among the most commonly encountered substances in water. They may result from the leaching of soil and other natural sources or they may be contained in sewage and many types of industrial wastes.

Under this heading are grouped the references that pertain to calcium or calcium ions. Where the literature deals with calcium salts, the references are grouped under the specified salt. The effects of calcium are also to be noted under the heading of "Hardness".

2. Cross References. Hardness; Calcium salts, Chapter V, and Chapter VII (Insects).

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The human body requires approximately 0.7 to 2.0 grams of calcium per day as a food element (36, 152), an amount considerably in excess of the calcium concentration of normally consumed quantities of even hard water. According to many people, calcium deficiency is the most common nutritional lack in the U. S. (3362). It is assumed that in the adult, the calcium requirement in the diet should equal the calcium excretion, which is about 10 mg per kg per day (3362). Some investigators believe that calcium in water can be used by the body as a supplement to the calcium in the diet (1065); however, the nutritional value of calcium in water has not yet been established and is still questionable (623, 653). Concentrations up to 1800 mg/l of calcium in drinking water have been reported to be harmless (353). The calcium concentration of vegetables cooked in water of high calcium content has been found to be higher than that of vegetables cooked in low-calcium water (921, 1066, 1067).

Excessive calcium and magnesium in drinking water have been implicated as factors predisposing to the formation of concretions in the body, such as kidney or bladder stones (3365). On the other hand, there is also evidence of adverse physiological effects from an insufficiency of calcium in water. Urovsk disease, a severe type of rickets, occurs in regions where the concentration of calcium in drinking water is low (3366, 3367). Morris et al. (3368, 3369) report an inverse correlation between the calcium content of waters and cardiovascular disease, i.e., high calcium is associated with a low incidence of heart attacks, but Schroder and Duran (3370) failed to find a similar relationship in Japan. While several effects of calcium in drinking water and physiological reactions have been suspected, no definite causal relationship has been proved as yet. So far as can be determined at the present time, calcium limits are desirable for domestic supplies not because of a hazard to health, but because calcium may be disadvantageous for other household uses, such as washing, bathing, and laundering, and because it tends to cause incrustations on cooking utensils and water heaters.

Hibbard (250) has recommended the following limiting concentrations of calcium in waters for domestic use:

Drinking and cooking	30 mg/l
Washing	10 mg/l
Laundry	0 mg/l

The Spanish Royal Decree of 1920 fixed the limit of calcium in potable waters at 150 mg/l, as CaO (631, 997). Baylis (499) has recommended the following limiting calcium concentrations for the several classes of water: Class A, the ideal, 50 mg/l, Class B, minimum aim, 75 mg/l, Class C to D, permissible with restrictions, 100-150 mg/l.

The USPHS Drinking Water Standards (2036) of 1962 and the WHO European Standards (2329) of 1961 do not contain any limits for calcium; but the WHO International Standards (2328) of 1958 indicate that 75 mg/l is a permissible limit and 200 mg/l an excessive limit in drinking water.

b. Industrial Water Supplies. (see also Hardness). Calcium in the water used for preparing developer solutions for photography may cause the precipitation of calcium sulfite, resulting in spots on the films unless the precipitate is allowed to settle out of solution (242). Calcium in brewery water causes the precipitation of calcium phosphate (166). Nordell (2338) points out that high calcium can cause undesirable effects by (a) forming scale, (b) reacting with alkaline solutions to form precipitates and curds, especially in washing operations using soap, (c) interfering with the preparation of emulsions, where the calcium tends to break the emulsions, (d) interfering with the processing of other colloids, (e) causing difficulties in electroplating rinsing operations, and (f) upsetting certain fermentation processes.

On the other hand, calcium is beneficial in water as one of the factors that tend to inhibit corrosion of cast iron and steel (3363, 3364).

The following concentrations of calcium have been recommended as limits for various industrial uses:

Use	*Threshold Concentration in mg/l*	*Reference*
Brewing, light beer	100–200	152
Brewing, dark beer	200–500	152
Soda and sulfate pulping	20	245
Sugar mfg.	20	250
Textile mfg.	10	250

c. Irrigation Water. Calcium in irrigation water has been covered in detail in Chapter V. Calcium is essential for normal plant growth and for the maintenance of good tilth in the soil, and is desirable in water for irrigation (268, 278, 635, 3371, 3372).

d. Stock and Wildlife Watering. Stander (3373) has suggested an interim threshold limit of 1,000 mg/l of calcium in water used by livestock. For more details, see Hardness.

e. Fish and Other Aquatic Life. Calcium in water reduces the toxicity of many chemical compounds to fish and other aquatic fauna; for example, mature fish have been killed by 0.1 mg/l of lead in water containing only one mg/l of calcium, but have not been harmed by this amount of lead in water containing 50 mg/l of calcium. A concentration of 50 mg/l of calcium has cancelled the toxic effect upon some fish of 2 mg/l of zinc; 0.7 mg/l of lead; and 10 mg/l of lead (573). Aluminum has been found to be toxic to river crabs in water containing little calcium (575).

The data of various independent investigators indicated that calcium chloride and nitrate, when added to distilled or soft waters, could be toxic to fish at concen-

trations between 300 and 1000 mg/l as calcium. Other available data concerning lethality of higher concentrations of calcium salts, generally calcium chloride, in various waters indicate that fish have survived from one to three days at concentrations of 2500 to 4000 mg/l of calcium (1459).

According to a reference cited by Hart et al. (310), of the U.S. waters supporting a good mixed fish fauna, ordinarily about 5 percent have less than 15 mg/l of calcium; 50 percent have less than 28 mg/l; and 95 percent have less than 52 mg/l.

Marine fish have been shown to concentrate Ca-45 from sea water at a factor of 10 or 20 to 1 (3374, 3375). Furthermore, marine fish discriminate against strontium in favor of calcium (3374). *Daphnia* and *Cyclops* also concentrate Ca-45 from fresh water, but when transferred to non-radioactive solutions, they quickly excrete the Ca-45. See also Chapter VIII.

For a flatworm, *Polycelis nigra,* the threshold of toxicity of calcium has been reported as 2600 mg/l when calcium is present as calcium chloride; and 1200 mg/l when calcium is present as calcium nitrate (608).

The lethal threshold concentration of calcium for stickleback is reported to be 800 mg/l (353, 2941).

f. Shellfish Culture. Thinness of shells of fresh-water mussels is associated with a deficiency of calcium in the water (331).

CALCIUM CARBONATE $CaCO_3$

(see also Calcium, Carbonates)

Commonly found in nature in the form of limestone or calcite, this salt is difficultly soluble, having a solubility product of 0.87×10^{-8} at 25°C (911). Its concentration in natural or treated waters is intimately linked with the complex carbonate equilibria (see Carbonates). In addition to its natural sources, calcium carbonate in molecular or dissociated form may be contained in innumerable industrial wastes. The references described below pertain only to calcium carbonate molecules and not to the effects of calcium or carbonate ions, which are reported elsewhere.

The taste threshold of calcium carbonate has been reported as 50 to 200 mg/l (621). Water to be used in making ice should be treated if the total concentration of calcium and magnesium carbonates is more than 70 mg/l (168). Calcium carbonate in water appears to be necessary to permit complete utilization of food by aquatic animals (1068). For the effects of adding lime on the productivity of lakes, see Calcium Hydroxide.

Wallen et al. (2940) studied the effect of adding calcium carbonate to highly turbid waters containing the mosquito-fish (*Gambusia affinis*) at 19-21°C. They found that the 96-hour TL_m was greater than 56,000 mg/l of calcium carbonate. At this dosage, the turbidity was reduced from an initial value of 260 mg/l to 35 mg/l.

CALCIUM CHLORIDE $CaCl_2$

1. General. This salt of calcium is highly soluble in water. Calcium chloride is used in brewing and in manufacturing mineral waters. It is found in wastes from bromine and salt works, oil wells, and surface run-off from roads treated against dust. Calcium chloride in natural waters is invariably associated with larger quantities of other calcium and magnesium salts, which together may render the water too highly mineralized for drinking and domestic purposes, but do not alone render water hazardous to people.

2. Cross References. Dissolved Solids, Specific Conductance, Distilled Water.

3. Effects on Beneficial Uses.

a. Domestic Water Supplies. The taste threshhold of calcium chloride in drinking water is said to be 150 to 350 mg/l (621). According to Lockhart et al. (3241) the taste threshhold of calcium ion is 125 mg/l and of the chloride ion 222 mg/l; hence the chloride ion controls and $CaCl_2$ can be tasted at 347 mg/l. In order to prevent unpleasant salty tastes and disturbances of appetite, it has been recommended that water should not contain more than 500 mg/l of calcium chloride (284). On the other hand, it has also been stated that small quantities of calcium and magnesium chloride, even concentrations of 40 to 50 mg/l, are objectionable in domestic water supplies because of taste and hardness (32).

b. Industrial Uses. The following are reported maximum concentrations of calcium chloride permissible in brewing waters:

Use	*Concentration in mg/l*	*Reference*
Brewing, light and dark	200	173
Brewing	100	170
Brewing, pale ales, I	30	170
pale ales, II	35-55	170
mild ales	55-110	170
stout	110-165	170

c. Irrigation Use. In high concentrations, calcium chloride in nutrient solutions will reduce plant growth. Gauch and Wadleigh (3376, 3377) showed that an increase of one atmosphere in the total osmotic concentration as the result of adding $CaCl_2$ (about 1775 mg/l) to the nutrient solution reduced the dry weight of red kidney bean plants by 15.5 percent while 3550 mg/l caused a 26 percent reduction. On the other hand, the guayule plant was shown to be very tolerant of $CaCl_2$, making satisfactory growth in the presence of 3 atmospheres of osmotic pressure from added $CaCl_2$ (about 5390 mg/l) (3378). One atmosphere (1775 mg/l) of added $CaCl_2$ reduced the dry weight of rice straw, rich grains, and rice roots at maturity (3379). Two atmospheres of added $CaCl_2$ injured four varieties of table grapes grown in sand culture (3380). It should be recognized that the foregoing concentrations of one and two atmospheres are far higher than any likely to be found in irrigation waters.

d. Stock and Wildlife Watering. The following concentrations of calcium chloride have produced the noted effects on animals:

Concentration of $CaCl_2$ in mg/l	*Animal*	*Effect*	*Reference*
8,325	rats	decreased water consumption	2398
10,000	rats	interfered with production of normal litters	287, 640
10,000-15,000	cows	moderate effect on nerves and appetite	2980
10,000-25,000	rats	interfered with reproduction	2980
15,000	rats	decreased growth rate	287, 640
15,000-20,000	chickens	interfered with growth	2980
20,000	rats	interfered with lactation	287, 640
20,000-25,000	sheep	tolerated for six weeks	2980
25,000	rats	caused death	287, 640

In Lake Erie water at 20-25°C, threshold concentrations of $CaCl_2$ for immobilization of aquatic organisms were reported (2955) as follows:

Organism	*Threshold Concentration in mg/l*
Daphnia magna, young	920
Cyclops uernalis	1730
Mesocyclops leukarti	1440
Whitefish fry	22080
Pickerel fry	12060

The following concentrations of calcium chloride have been reported to have killed or injured fish:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
555	tap	1 week	rock bass	313
2,775	distilled	2-4 days	minnows	313, 598
5,000	--	142 hours	shiners	645
7,752	distilled	22-27 hours	goldfish	313, 2920
8,400	synthetic	24-hour TL_m	bluegills	1459
9,500	standard	96-hour TL_m	bluegill sunfish	2933, 2934, 2935
10,000	--	3 hours	tench	645
10,000	--	--	fish	1070
10,000	--	--	sunfish	1070
10,000	--	27.6 hours	shiners	645
10,000	--	48.8 hours	bream	645
10,650	--	96-hour TL_m	bluegill sunfish	3381, 2936
11,100	--	50 hours	young eels	1459
12,060 (threshold)	--	--	pickerel	353
12,500	--	--	fish	598, 431
13,400	turbid	96-hour TL_m	mosquito-fish	2940
13,900	--	24 hours	Orizias	1459
22,080 (threshold)	--	--	whitefish	353

A concentration of 277 mg/l of calcium chloride in distilled water was not harmful to minnows over a period of five to seven weeks (313). A gradually increasing concentration of calcium chloride up to 9500 to 13,500 mg/l has resulted in the death of fish within 12 days (644).

CALCIUM FLUORIDE CaF_2

(see also Calcium, Fluorides)

Known also as fluorspar, this mineral occurs widely throughout the world. It is soluble to the extent of about 15 mg/l at 18°C (364). Fluorspar is the primary source of fluorine and its compounds. It is also used as a flux in steel manufacture, and it finds many uses in glass, ceramics, and chemical industries. According to Simonin (3271) the oral LD_{50} for guinea pigs is more than 5.0 grams/kg of body weight. The lethal dose required to kill the fish, *Tinca vulgaris*, is reported by Simonin (3271) to be 30,000 mg/l, which is far greater than the solubility.

CALCIUM HYDROXIDE $Ca(OH)_2$

1. General. Known also as calcium hydrate and slaked lime, calcium hydroxide is moderately soluble in water, to the extent of about 1700 mg/l at 15°C (364). Calcium hydroxide may be found in wastes from tanneries, canneries, leather and leatherette works, chemical and paint plants, glue factories, and other industrial processes.

2. Cross References. Calcium, Hydroxides, Carbonates.

3. Effects on Beneficial Uses.

a. Domestic Water Supplies. The solvent action of 0 to 25 mg/l of calcium hydroxide was fairly constant, but 1000 mg/l dissolved considerable quantities of lead salts from pipes. In concentrations normally found in water supplies, however, calcium hydroxide would probably not increase plumbosolvency (1029).

b. Stock and Wildlife Watering. A concentration of 1800 mg/l of calcium hydroxide produced no ill effects through several generations of rats (284, 287).

c. Fish and Other Aquatic Life. The following concentrations of calcium hydroxide have been reported as toxic to fish in the time specified.

Concentration in mg/l	*Time of Exposure*	*Type of Fish*	*Reference*
18	--	fish	808
92	26 minutes	trout	2977
<100 (pH 11.1)	--	goldfish	2920
100	3-5 hours	goldfish, bass, sunfish	1030
100 (unadjusted pH)	3-7 days	bass	1030
100-200	--	carp	1071
198	10 minutes	trout	2977
200 (unadjusted pH)	30-80 minutes	goldfish, bass	1030
500 (unadjusted pH)	20-53 minutes	goldfish	1030
700	26 minutes	trout	313

Goldfish, bass, and sunfish have survived 50 mg/l for seven or more days (1030).

Using highly turbid water at 21-23°C and the mosquito-fish (*Gambusia affinis*) as the test organism Wallen et al., (2940) found the 24-hour, 48-hour, and 96-hour TL_m values to be 240, 220, and 160 mg/l of calcium hydroxide respectively. At these dosages, initial turbidities of 800 mg/l were reduced to 200 mg/l or lower.

The application of lime to soft-water lakes has increased their productivity, presumably by raising the alkalinity and providing larger reservoirs of immediately available carbon dioxide in the form of bicarbonates (2173, 2174). A high content of lime in an impounding reservoir in Germany encouraged the development of hornweed (*Ceratophyllum demersum*) which tended to block waterworks intake screens (3382).

CALCIUM HYPOCHLORITE $Ca(OCl)_2$

(see also Calcium, Chlorine)

Calcium hypochlorite is discharged occasionally in trade wastes. It has been reported that concentrations of calcium hypochlorite from 0.5 to 10 mg/l have killed trout and other fish (359).

CALCIUM MOLYBDATE $CaMoO_4$

(see Molybdenum)

CALCIUM NITRATE $Ca(NO_3)_2$

(see also Calcium, Nitrates)

Calcium nitrate is very soluble in water and may be found in some chemical wastes. It is used in manufacturing matches, explosives, fireworks, etc.

A concentration of 3,280 mg/l in tap water is reported as the toxicity threshhold for stickleback (598). In distilled water 6,061 mg/l has killed goldfish in 43 to 48 hours (313). For stickleback in soft water, the lethal concentration limit for calcium nitrate was found to be 800 mg/l as calcium. The average survival time was one week at a concentration of 1000 mg/l (as calcium), four days at 1500 mg/l, two days at 3000 mg/l, and only one day at 4500 mg/l (1460). In contrast, Traina (3381) found the 96-hour TL_m for the bluegill sunfish at 20°C to be 10,000 mg/l of calcium nitrate.

CALCIUM SILICOFLUORIDE $CaSiF_6 \cdot 2H_2O$

This anhydrous salt is almost insoluble in cold water. Its oral LD_{50} for guinea pigs is reported (364, 3271) as 250 mg/kg of body weight, while for the fish, *Tinca vulgaris*, the lethal dose is given as 50 mg/l (3271).

CALCIUM SULFATE $CaSO_4$

1. General. Known also as gypsum or plaster of Paris, calcium sulfate is soluble to the extent of about 2,500 mg/l in cold water (364). It is used in paint manufacture and in paper mills, among other industrial processes.

2. Cross References. Calcium, Sulfate, and Chapter V—Irrigation.

3. Effects on Beneficial Uses.

a. Domestic Water Supplies. High calcium sulfate concentrations in water are disadvantageous for most household uses, but for drinking purposes, 300 mg/l or more is not harmful (1072). The taste threshold of calcium sulfate has been reported to be 250 to 900 mg/l (621). Calcium sulfate significantly increases plumbosolvency; raising the calcium sulfate concentration from 25 to 250 mg/l increased by 10 percent the amount of lead dissolved from lead pipes (1073).

b. Industrial Water Supplies. Calcium sulfate in small amounts is beneficial in mash water for pale beer (171, 174, 175, 1074). The permissible amount of gypsum varies, according to different authorities, from 300 to 1750 mg/l (1074).

The following maximum concentrations of calcium sulfate have been recommended for brewing:

Type of beer	*Concentration in mg/l*	*Reference*
Light brews	100-200	173, 152
Dark brews	200-250	173
Dark brews	200-500	152, 170
Pale ales, I	200-340	170
Pale ales, II	340-540	170
Mild ales	70-200	170

In general, calcium sulfate is beneficial in the brewing industry, for it helps to maintain the acidity of the wort and therefore causes more complete coagulation of albuminous matter. It also reduces the solubility of the bitter substances of the hop (2344).

c. Irrigation. Gypsum in irrigation waters improves or restores the permeability and tilth of soil with an unfavorable sodium ratio (271, 283). See also Chapter V—Irrigation.

d. Stock and Wildlife Watering. A saturated solution of calcium sulfate, used as the sole source of drinking water for rats, permitted satisfactory growth (287). A concentration of 2,400 mg/l of calcium sulfate permitted normal growth and reproduction among rats (284).

e. Fish and Other Aquatic Life. Trama (3381) reported that a saturated solution of calcium sulfate at 20°C did not produce significant mortalities among bluegills; but a later report from the same organization (2936) indicated that this concentration was the 96-hour TL_m at 18-20°C in soft water for the bluegill sunfish. It was also reported (2936) that 3200 mg/l caused a 50-percent reduction in the rate of growth of the diatom, *Navicula seminulum*. Using highly turbid water at 21-25°C and the mosquitofish (*Gambusia affinis*) as the test animal, Wallen et al. (2940) indicated that the 96-hour TL_m was greater than 56,000 mg/l, a concentration much larger than the solubility.

f. Shellfish Culture. Calcium sulfate in sea water in concentrations up to 633 mg/l was found to be toxic to Japanese oysters (811).

CAMPHOR $C_{10}H_{16}O$

This ketonic derivative of a saturated dicyclic hydrocarbon is a gumlike crystalline compound that is obtained by steam distillation of wood, twigs, and leaves of the camphor tree. It is used in medicine as a diaphoretic, stimulant, and sedative, in the manufacture of celluloid, and in pyrotechnics. Kirkor (460) reports that the minimum detectable concentration of camphor that can be tasted in water is 1.9 mg/l. In Lake Huron water at 12°C, the sea lamprey was not effected by 5.0 mg/l of camphor during a 24-hour exposure (2976).

CAPTAN 50-W

(see Chapter IX)

CARBOHYDRATES

(see Sugars)

CARBOLIC ACID

(see Phenol)

CARBON C

(see also Carbon Dioxide, Carbonates, Bicarbonates, Chapter VIII)

Elemental carbon occurs in three forms: (a) as diamonds, (b) as graphite, and (c) as coal, lampblack, or other amorphous substances. Animal and wood charcoals are used chiefly as "activated carbons" for clarifying, deodorizing, decolorizing, and filtering; while gas black and lamp black are used as pigment for rubber tires, for printing and drawing inks, for electrical and insulating apparatus, and for phonograph records (364). Granular or powdered carbon may be present in rinse waters, floor drainage, or other wastes from many of these processes.

A study of carbon-plant wastes at St. Marys, Penna. (7) showed that their B.O.D. was negligible, that they were practically saturated with oxygen, that the pH values were near normal, and that alkalinities were not excessive. The only pollutant in these wastes appeared to be the high suspended solids content arising from the powdered carbon. Such wastes, however, imparted a black discoloration to the stream, added turbidity, and precipitated films of carbon on rocks and stream beds, thereby interfering with normal aquatic life.

Activated carbon apparently has the power to inactivate the virus of infectious hepatitis (885) and it has been used to control algae in reservoirs, at dosages of 0.26 lb per 1,000 sq ft of surface. Both of these effects upon biological life are probably attributable to surface-action phenomena rather than to toxicity.

The radioisotope, C-14, has gained prominence owing to its long half-life and its production in the testing of

atomic weapons. See Chapter VIII for information on concentration factors.

Organic carbon, the carbon oxidized by dichromate or another strong oxidizing agent, is frequently determined in polluted waters and benthic deposits. As in the case of B.O.D., this test measures one significant criterion of the strength of a waste, but it is not per se a potential pollutant.

CARBONATES CO_3^{--}

1. General. The concentration of carbonates in natural and polluted waters is a function not only of the substances added thereto but also of the temperature, pH, cations, and other dissolved salts. A full discussion of carbonate equilibria is beyond the scope of this report and the reader is referred to the work of Langelier (692). For a review of the relationship between carbonates, bicarbonates, and pH, see Bicarbonates in Chapter VI. Inasmuch as many of the carbonates are quite insoluble in water, generally more so than the chlorides, nitrates, or sulfates, there is a tendency for certain carbonate salts to be removed from polluted waters by precipitation and adsorption. For these reasons, carbonates are less widely present than chlorides and sulfates in western irrigation waters (275).

2. Cross References. Alkalinity, pH, Bicarbonate, Hydroxides, Chapter V—Irrigation.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The USPHS Drinking Water Standards of 1962 (2036) place no restrictions on carbonates in natural waters, nor in chemically treated waters as was done in the 1946 standards. Hibbard (250) recommends that the concentration of carbonate ion in drinking and cooking water be kept below 20 mg/l (33.3 mg/l as alkalinity). In his discussion of alkali waters in Montana, Cobleigh (670) points out that carbonates and bicarbonates in drinking waters react with gastric juices sometimes to the consumer's benefit. He presents a modified list of USGS data that gives 350 mg/l of carbonates as the concentration unhealthful to most people. According to Lockhart (3241) the taste threshold of carbonate in distilled water, when added as sodium carbonate, is 44 mg/l.

b. Industrial Water Supplies. Excessive carbonates interfere with acid and carbonated beverages (see Alkalinity), with brewing, with ice making, and with boiler water (see Chapter V). For boiler feed water, the permissible carbonate concentration is a function of pressure in the boiler, as follows (152):

Pressures, psi	*Carbonate Concentration in mg/l*
0-150	200
150-250	100
250-400	40
Over 400	20

For brewing, carbonate concentrations should not exceed 50 to 60 mg/l (see Chapter V).

c. Fish and Other Aquatic Life. For the effects of ammonia, potassium, and sodium carbonates on fish and *Daphnia*, see the appropriate cation in this chapter. Hedgepeth (1085) claims that the presence of free carbonates may be responsible for the absence of *Diaptomus franciscanus*, a crustacean, from certain ponds near San Francisco. In general, it may be expected that carbonates, in themselves, are not detrimental to fish life but their buffering action and effect upon pH may contribute to the toxicity of high pH value.

CARBON CHLOROFORM EXTRACT

The 1962 USPHS Drinking Water Standards (2036) set a recommended limit of 0.2 mg/l on carbon chloroform extract based on analytical techniques developed at the Robert A. Taft Sanitary Engineering Center (3004, 3353, 3354, 3355, 3356). In this procedure, a sample of water varying from 100 to 75,000 gallons, depending on the source of the sample and the anticipated amount of extractable material, is filtered through 1200 to 1500 grams of granular activated carbon at a filter rate of 2 to 10 gpm per square foot. The carbon is then air-dried and extracted with chloroform. This solvent does not recover all of the adsorbed material nor are all organic substances adsorbed, but the materials recovered by this technique are representative of the taste- and odor-producing components of waste water.

CCE concentrations of Ohio River water have been reported to range from 0.1 to 0.36 mg/l, and the raw water at Nitro, West Virginia had CCE values of 0.17 to 3.05 mg/l (3357). In contrast, only 0.024 mg/l of CCE material could be recovered from the Columbia River (3102). Gasoline can cause taste and odor if the hydrocarbons are present in amounts of a few micrograms per liter (3102). Water from clean sources rarely exceeds 0.05 mg/l of CCE substances while water that exceeds 0.20 mg/l usually is of poor quality from a taste and odor standpoint (3358). Middleton and Lichtenberg (3356) report that CCE concentrations of 0.1 mg/l or less occurred in the following percentages of samples from five major rivers:

Ohio River	50 percent
Mississippi River	72 percent
Missouri River	90 percent
Colorado River	92 percent
Columbia River	96 percent

According to Derby et al. (2062) analysis of available data indicates that water supplies containing over 0.2 mg/l of CCE represent an exceptional and unwarranted exposure of the water consumer to ill-defined chemicals.

CARBON DIOXIDE CO_2

1. General. A colorless, odorless, non-combustible gas, constituting about 0.04 percent of normal air (330), carbon dioxide is highly soluble in water. At one atmosphere of partial pressure, pure water will absorb 1688 mg/l of CO_2, (911) but at 0.0004 atmospheres the dissolved CO_2 will be only about 0.7 mg/l. The source of free carbon dioxide in water is seldom that from the air phase, however, for CO_2 is a product of aerobic or anaerobic decomposition of organic matter and it is intimately bound in the complex carbonate equilibria (see Bicarbonates and Carbonates).

Dissolved carbon dioxide reacts with water to form carbonic acid,

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

which, in turn, dissociates thusly

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

such that

$$\frac{[H^+][HCO_3^-]}{[H_2CO_3]} = 3.5 \times 10^{-7} \text{ at } 18°C$$

Thus, at pH 8 the ratio of carbonic acid to bicarbonate ions is only .0286 but at pH 7 it is 0.86 and at pH 6 it is 2.86. Below pH 4.3, almost all of the bicarbonate is converted to carbonic acid.

The tests for carbon dioxide as described in Standard Methods for the Examination of Water and Wastewater (469) do not distinguish between dissolved CO_2 and carbonic acid, and the direct test or the computation of "free CO_2" indicates the sum of these two forms. In this report, therefore, references to carbon dioxide should be understood to embrace carbonic acid as well.

2. Cross References. Alkalinity, Acidity, Bicarbonates, Carbonates, and pH.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Free carbon dioxide in domestic water is of significance only in connection with corrosion and aggressiveness of water, for it appears to have no direct physiological effect (155, 1080). In fact, soft drinks and beer are highly charged with CO_2. Such carbonated water showed no bactericidal action against *E. coli* and *E. typhosa* after an exposure of 40 minutes (1081), and a concentration of less than 100 mg/l produces no taste in water (855). Poultry fed for 30 days with highly carbonated water showed no ill effect (2980). High concentrations in domestic water systems are detrimental, however, in that they accelerate the corrosion of iron and steel, and they promote the solution of lead (155, 855, 1082).

b. Industrial Water Supplies. Restrictions on the content of free CO_2 do not appear directly in many standards of industrial waters (see Chapter V), but requirements of pH, acidity, and alkalinity indirectly restrict the carbon-dioxide content. The T.A.P.P.I. specifications for chemical composition of process waters for the manufacture of various types of paper state that free CO_2 should not exceed 10 mg/l. According to Ruppert (3769) beer prepared with water containing free CO_2 shows an undesirable bitterness.

According to Schmassmann (856), free carbon dioxide is the constituent of natural waters that is most likely to damage calcareous building materials such as cement. The presence of free CO_2 in water increases the solvent action on calcium carbonate in cement and concrete (196), this action being proportional to the concentration of carbon dioxide. Dr. Ruth Terzaghi (197) reports that free carbon dioxide in concentrations exceeding 20 mg/l will attack concrete sufficiently rapidly to require protective action (see Chapter V-Concrete Mixing and Concrete Corrosion). Molinari (2356) confirms this observation for subsurface waters that contained up to 41.2 mg/l of free CO_2.

c. Fish and Other Aquatic Life. The concentrations of dissolved CO_2 and carbonic acid in water have a marked effect on fish. In their migration, fish tend to respond to slight gradients in carbon-dioxide tension (1083) and to avoid concentrations of 1.0 to 6.0 mg/l (367). According to Wells (1075) it is doubtful if any freshwater fishes can continue to live throughout the year in water with an average CO_2 content as high as 12 mg/l, and concentrations of 20 mg/l will quickly prove fatal to the more-sensitive species. The lethal limit for trout has been reported as 45 mg/l and for tench as 200 mg/l (2977). According to Surber (3359), concentrations of CO_2 between 55 and 78.5 mg/l interfered with the hatching of trout eggs; but Alderdice and Wickett (3360) report that mortality to chum salmon eggs is independent of CO_2 below 125 mg/l.

Lagler (308) indicates that free carbon dioxide in excess of 20 mg/l may be harmful to fish in normal fresh water, but when the dissolved-oxygen content drops to 3 to 5 mg/l, lower CO_2 concentrations may be detrimental. This observation has been supported by the Water Pollution Research Board of England (2907, 3321) and the National Council for Stream Improvement (2500).

The presence of carbon dioxide may, at times, have a beneficial effect insofar as fish are concerned. It has been shown by several investigators (139, 140, 143, 2518, 3057) that CO_2 lowers the pH and consequently the unionized ammonia in certain waters. Herbert (143) reported that 30 mg/l of CO_2 reduced the toxicity of ammonia to trout by lowering the pH value. On the other hand, lowering of the pH value would increase the toxicity of cyanides. The sensitivity of fish to carbon dioxide appears to decrease directly with an increase in temperature (1084).

In U.S. waters that support good fish fauna, ordinarily 5 percent have less than 0.1 mg/l, 50 percent have less than 1.5 mg/l and 95 percent have less than 5.0 mg/l of free carbon dioxide (310). According to Doudoroff and Katz (361), concentrations of 100 to 200 mg/l of CO_2 can be rapidly lethal to more-sensitive fish in presence of adequate dissolved oxygen, and 50 to 100 mg/l or even lower may be lethal after prolonged exposure or with less oxygen. Some delicate marine species, such as herring, are even more sensitive than freshwater forms. Carbonic acid can be toxic even at pH values which are not in themselves harmful; hence pH is not a reliable index of dangerous CO_2 pollution (361).

CARBON DISULFIDE CS_2

In its commercial form, this colorless, clear, foul-smelling, flammable liquid is miscible with water to the extent of one ml in 530 ml (364). It is used extensively in industry as a solvent for fats, oils, waxes, and resins, for varnishes and lacquers, and for disinfection. It has been reported in gas manufacturing wastes and chemical plant effluents (313, 362). In public waters in the U.S.S.R. the maximum permissible concentration of carbon disulfide is 1.0 mg/l, according to Kirkor (460).

Shelford (363) states that concentrations of 100 to 127 mg/l in tap water killed orange-spotted sunfish in

one hour. Ellis (313) quotes a reference to the effect that a brief 7-minute exposure to 5,000 mg/l caused trout to die two days later. The threshold of toxicity for perch and bleak is reported (2977) at 35 mg/l.

CARBONIC ACID

(see Carbon Dioxide)

CARBON MONOXIDE CO

This colorless, odorless, highly poisonous gas is soluble in water to the extent of 28.4 mg/l under a total pressure of one atmosphere. It may occur in wastes from quenching operations, gas manufacturing, or plants where exhaust gases are scrubbed with water (313, 363).

Experiments on various species of fish have revealed the following lethal characteristics of carbon monoxide in water:

Lethal Concentration in mg/l	*Time of Exposure in Minutes*	*Type of Fish*	*Reference*
1.2	--	Varied fish	2920
1.5	60 to 370	Minnows and sunfish	1075
47.5	45 to 330	Minnows and sunfish	1075
75	60	Orange-spotted sunfish	363
146	10 to 240	Minnows and sunfish	1075
1,160	60	Straw-colored minnows	313
1,160	115	Blunt-nosed minnows	313
1,160	300	Green sunfish	313
1,160	340	Orange-spotted sunfish	313
11,314	595	Black bullhead	313

Doudoroff and Katz (361) state that toxic concentrations of carbon monoxide vary, with levels as low as 0.1 to 1.0 mg/l. They maintain that the presence of more than a trace of this gas should be considered as a hazard to fish life.

CARBON TETRACHLORIDE CCl_4

This colorless nonflammable liquid with a strong odor is soluble in water to the extent of 800 mg/l at 20°C. It is used extensively in industry as an organic solvent, as a fire extinguisher, for dry cleaning of clothing, and as an antihelminthic in human and veterinary medicine. Death has occurred from ingestion of 5 ml (about 8 grams), inhalation of its fumes has caused numerous respiratory symptoms and even death, and dermatitis results from repeated skin contacts (364, 3292). According to Wadsworth (2971), the toxic oral dose for sheep is 1.0 ml and for cows 5.0 ml. The Soviet Union has established the maximum permissible concentration of CCl_4 in surface waters at 5.0 mg/l, based on its organoleptic properties (3107).

CCE

(see Carbon Chloroform Extract)

CEMETERIES

According to van Haaren (1482) analyses of the surface and ground waters of a number of cemeteries have shown severe pollution. Ten years are required for the decomposition of a corpse.

CERIUM Ce

(See also Chapter VIII—Radioactivity)

Metallic cerium does not occur free in nature, the principal source of this element being monazite sand (a phosphate). Cerium salts are used in textile dyeing and printing, in glass manufacture, as an abrasive, in the manufacture of arc lamps and neon tubes, and medicinally as antiemetics. There is conflicting opinion about the possible deleterious effects of cerium salts, but there is no evidence of severe toxicity (364, 2121).

Cerium forms salts with valences of +3 and +4. Among the ceric salts (+4), the ammonium nitrate, ammonium sulfate, and sulfate are highly soluble in water, but the oxide and fluoride are insoluble. Most of the cerous salts (+3) are soluble, except for the carbonate and fluoride. It is probable, therefore, that ceric or cerous salts discharged in industrial wastes will remain in solution except at high pH values until they are oxidized and precipitated as CeO_2.

The lethal limit of cerous chloride to small mature fresh-water fish (*Orizias*) during a 24-hour exposure was reported (1459) to be 190 mg/l. Using water of the River Havel, from which the test organisms were taken. Bringmann and Kuhn (2158) found that the toxic threshold of cerous chloride toward *Scenedesmus* at 24° C during a 4-day exposure was only 0.15-0.20 mg/l as cerium; and for *Escherichia coli* the threshold was at 0.75-1.5 mg/l as cerium. Yet, for *Daphnia* at 23°C and a 48-hour exposure, a concentration of 1000 mg/l of cerium showed no deleterious effect. In similar tests with cerous sulfate, *Scenedesmus* exhibited threshold effects at 0.14 mg/l of cerium whereas *Daphnia* was unaffected at 1000 mg/l of cerium. The lethal dose of cerous fluoride required in 48 hours for the fish, *Tinca vulgaris* was reported as 30,000 mg/l (3271).

Cerium is accumulated from the water environment by many forms of aquatic life. Morgan (2800) found concentration factors for cerium-141 of 15,170 for *Ochromonas*, 5100 for *Platymonas*, 7400 for *Chlamydomonas* (all flagellates), 28,500 for the diatom *Nitzschia*, and 280-1740 for marine bacteria. It has been reported (3272, 3273) that particulate cerium became associated with *Nitzschia* cells at a faster rate than ionic cerium. For further information about concentration factors, see Chapter VIII—Radioactivity.

CESIUM Cs

(see also Chapter VIII)

This silvery-white ductile metal reacts violently with air or water; consequently it is not found pure in nature. It is obtained from the ore pollucite (cesium aluminum sodium silicate). Cesium forms salts similar to those of sodium or potassium but they have found little use in industry except in the manufacture of photoelectric cells (364, 2121).

Until it occurred as an important constituent of radioactive fall-out, as Cs-137, cesium was virtually ignored as an element. Relatively little is known about its properties or toxicities. It appears to behave very much like potassium in human and animal metabolism, and consequently has a low level of toxicity.

There is considerable literature dealing with the accumulation of Cs-137 and Cs-134 from the water environment into aquatic organisms. A few of these concentration factors are presented in the following table:

Organism	Concentration Factor	Reference
Bacteria	15-116	3386
Algae (9 species)	52-1530	3388
Algae	28-2180	3385
Algae	34,200	3385
Aquatic organisms, mixed	50-10,000	3387
Carp	3000	3387
Sunfish	9500	3387
Oysters (soft tissue)	50	3375

Pendleton (3387) claims that the principal route through which Cs-137 in hazardous amounts could reach man is by way of the aquatic food chain. The presence of potassium in water has a slight depressing effect on the concentration of cesium by water (3388).

Cesium-137 is absorbed readily by rocks and soils; hence it is not likely to travel far through ground water (1976).

CETYL ALCOHOL $CH_3(CH_2)_{14}CH_2OH$

(see also Protozoa and Insects, in Chapter VII)

Known also as hexadecanol, this white crystalline substance has long been used as an emollient in cosmetics, as an emulsifier, and in the manufacture of sulfated alcohol for textile soaps (364). It is insoluble in water. Indeed, tests with a sensitivity of 0.005 mg/l could not detect it in solution (3274). Recently, cetyl alcohol and related compounds have been used experimentally to form a monomolecular film on water which tends to minimize evaporation. Reports indicate that evaporation may be decreased by 9 to 65 percent, depending on temperature, winds, wave action, and the extent and thickness of the film (3275, 3276, 3277). Scores of articles have appeared since 1954 describing this procedure and its effects on evaporation. Only those publications that deal directly with the effect of cetyl alcohol on beneficial uses of water are relevant to this report.

In general, there appear to be four properties of water that may be altered by the application of cetyl alcohol to lakes, viz temperature, surface tension, bacterial densities, and dissolved oxygen. In the field tests reported through 1961, there appeared to be no change in dissolved solids (3278), no mortality or modification of the fish population or upon the ability of anglers to catch fish (2291), no deleterious effect on plankton or benthic organisms (2291), and no evidence of increased taste and odor (3275).

By reducing evaporation and the natural cooling action attendant thereto, a monomolecular film tends to increase the temperature of the lake waters, especially near the surface. In comparison with controls, temperature rises of 1° to 3°C have been reported (2293, 2684, 3279, 3280). The surface tension of lakes treated with cetyl alcohol drops from about 72 dynes per cm to as low as 30 dynes per cm (2293, 2294). Large water striders were observed to fall through the altered water films and drown (2293). There is also some evidence that the lower surface tension may interfere with the emergence of midge flies (2683, 2684, 3278), a factor that might influence the fish population in the long term.

Bacterial densities in lakes treated with cetyl alcohol have been observed to increase markedly (2290, 2292, 2293, 2294, 3281, 3282) owing to the bacterial decomposition of this compound. No detrimental effect of this increase has been reported. The monomolecular film may reduce the transfer of gases into or out of the waters of a lake (3283) but any change in dissolved oxygen appears to be limited to about 2 percent of saturation (2291).

Berger (3274) reported tests in which fathead minnows were exposed for 5 days to emulsified cetyl alcohol in concentrations of 1, 10, 100, and 500 mg/l. All fish survived but at 500 mg/l there was sufficient depletion of oxygen that fish were distressed. This substance caused no mortality among fish in aquaria maintained on a diet containing 50 percent hexadecanol for periods up to 91 days. Trout planted in a treated pond showed the same growth at the end of one year as those planted in an untreated pond (3278).

CETYL AMMONIUM COMPOUNDS

CETYL PYRIDINIUM CHLORIDE

(see Chapter X)

CHEMICAL WARFARE AGENTS

(see also Hydrogen Cyanide, Explosives, Chapter VIII, Chapter IX)

Acts of sabotage or war might be aimed at impairing the potability or other usefulness of water supplies. Various chemical poisons have been known for a long time, but many have only limited practical value because very large quantities are needed to contaminate an entire water supply to a harmful degree; for example, about 600 lbs of chemical per million gallons of water would be needed to produce a lethal concentration HCN (1483).

More recently, however, various chemical poisons have been produced which are lethal to man at dosages of less than one mg per kilogram of body weight. Among these agents are mustard gases, fluoroacetate compounds, and various organic phosphates (nerve gases) that may cause death in only a few seconds. These poisons make more practical the possibility of contaminating an entire water supply, but still 50 to 500 lbs of chemical per million gallons of water would be needed (1483).

Greenberg (1484) has pointed out that contamination of large supplies of water, such as city reservoirs, with war gases is not very likely inasmuch as enormous amounts would be required to render the water unsafe to drink. The most effective war gases are the blister gases and nerve gases about which few published data are available. It has been estimated that in order to contaminate a 189 million gallon reservoir, 79,000 lbs of mustard gas or 36,000 lbs of lewisite would be required (1484).

There are numerous agents of chemical warfare that may pollute natural waters as a result of enemy action or accidental discharges from manufacturing plants. Normally, however, such agents would not enter into the deliberations and case-by-case studies of water-pollution control boards. For a thorough review of the polluting potential of numerous chemical-warfare agents, reference is made to the work of Sanchis (466).

CHENOPODIUM OIL

Oil of chenopodium is derived from the goosefoot plant. Schaut (362) found that 5 to 10 mg/l of this oil

killed minnows in 24 to 48 hours. A concentration of 17.1 mg/l gave a strong, pleasant odor in tap water but this concentration affected minnows in four hours and killed all in 24 hours.

CHLORAMINES NH_2Cl, $NHCl_2$ and NCl_3

1. General. Chloramines are formed when water containing ammonia, ammonium hydroxide, or ammonium ions is chlorinated, thus

$$NH_4^+ + HOCl \rightleftharpoons H^+ + H_2O + NH_2Cl \text{ (monochloramine)}$$
$$NH_2Cl + HOCl \rightleftharpoons H_2O + NHCl_2 \text{ (dichloramine)}$$
$$NHCl_2 + HOCl \rightleftharpoons H_2O + NCl_3 \text{ (trichloramine)}$$

The proportion of mono-, di-, and trichloramine in any solution is a function of temperature, pH, and the concentrations of ammonium ions and hypochlorous acid (1087). Trichloramines will not be formed except at low pH values. At the normal range of pH (6.0 to 8.0) monochloramine and dichloramine apparently react to produce nitrogen gas thus: $NH_2Cl + NHCl_2 \rightarrow N_2 + 3H^+ + 3Cl^-$

Not only do mono- and di-chloramines exist in treated water supplies where they are used to provide disinfection without chlorinous tastes, but they also occur in chlorinated effluents from municipal sewage works and in wastes from many industrial processes that employ chlorine for disinfection and bleaching. Hence, chloramines are likely to occur in polluted waters.

2. Cross References. Ammonia, Chlorine, Chloramine-T.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. A discussion of the use and merits of chloramines as compared with free chlorine as disinfectants for treated waters is beyond the scope of this report, and the reader is referred to the work of Moore (1087) and others at Harvard University and to that of Butterfield and Wattie (490, 494).

Where chlorination of water results in the formation of taste-bearing chloro-organic compounds, the use of ammonia to form chloramines appears to result in a taste reduction (956, 1028, 1086)

b. Fish and Other Aquatic Life. According to Westfall (311, 673), chloramine is more toxic than free chlorine to warm-water fish; with 0.4 mg/l killing adult fish and 0.06 mg/l being lethal to trout fry. Ellis (313) reports that 0.4 mg/l killed sunfish and bullheads, 0.76 mg/l, hardy minnows; and 1.2 mg/l, large carp. According to Merkens (717) 0.08 mg/l of residual chlorine, mostly in the form of chloramine, killed about half of a batch of rainbow trout in 7 days at saturated dissolved oxygen.

The Department of Fisheries of the State of Washington conducted extensive experiments on the effects of chlorine and chloramines on chinook, pink skeeria, and silver salmon in fresh and saline waters (2091). The critical level for a 72-hour exposure of these three species was less than 0.1 mg/l of chlorine. Chloramines were not formed in salt water by the concentrations and ratios of chlorine and ammonia (1:3) used in these experiments; but in aerated fresh water, chloramines were found to be more toxic than chlorine, and dichloramine was more deadly than monochloramine under the conditions of the experiment. In contrast, the Water Pollution Research Board of England (1816) found monochloramine slightly less toxic than free chlorine toward rainbow trout at pH 7.0.

Using water of the River Havel from which the test organisms had been recovered, Bringmann and Kuhn (2158) found that the threshold concentration of chloramines for *Daphnia* exposed for 48 hours at 23°C was 4 mg/l, and for *Scenedesmus* during a 96-hour exposure at 24°C it was 10 mg/l.

CHLORAMINE-T $CH_3C_6H_4SO_2N\ NaCl$

(see also Chloramines, Chlorine)

This soluble compound releases HOCl when dissolved in water and hence it is used as a bactericidal agent. Ellis (313) quotes a reference to the effect that 5 mg/l of chloramine-T in Nile River water killed *Daphnia* and *Cyclops*.

CHLORATES

(see Potassium Chlorate and Sodium Chlorate)

Chlordane

(see Chapter IX)

CHLORIDES Cl^-

1. General. Chlorides are found in practically all natural waters. They may be of natural mineral origin, or derived (a) from sea-water contamination of underground supplies, (b) from salts spread on fields for agricultural purposes, (c) from human or animal sewage, or (d) from industrial effluents, such as those from paper works, galvanizing plants, water softening plants, oil wells, and petroleum refineries.

The Ohio River Valley Water Sanitation Commission has set chloride standards for river water (3389, 3390). On the main stem of the Ohio River, it is recommended that the chloride concentration never exceed a monthly average of 125 mg/l at the most probable minimum monthly average 10-year flow, and that the maximum concentration at any time never exceed 250 mg/l. The primary concern is economic damage rather than public health. For public supplies, the water is classified as "acceptable" if the chloride concentration is less than 125 mg/l, "doubtful" if between 125 and 250 mg/l, and "unsatisfactory" above 250 mg/l. For industrial needs, the corresponding limits are less than 50, 50-175, and over 175 mg/l respectively.

2. Cross References. Tastes, Chloride Salts under the appropriate cation, Dissolved Solids.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Chlorides in drinking water are generally not harmful to human beings until high concentrations are reached, although chlorides may be injurious to some people suffering from diseases of

heart or kidneys. Restrictions on chloride concentrations in drinking water are generally based on palatability requirements rather than on health.

Chlorides in water may impart a salty taste at concentrations as low as 100 mg/l (152, 1080), although in some waters 700 mg/l may not be noticeable (152) (see also Tastes). According to the Kettering Laboratory (3389, 3391, 3392), the most taste-sensitive people can detect chloride from calcium salts at 96 mg/l and from sodium salts at 121 mg/l (based on work by Whipple in 1907). For average individuals, the taste threshold is about 400 mg/l. Lockhart et al. (3241) reported the taste thresholds of NaCl KCl, and $CaCl_2$ to be 345, 650, and 347 mg/l, corresponding to chloride concentrations of 210, 310, and 222 mg/l respectively. The fact that a taste is detectable, however, does not mean that it is objectionable; indeed, many people prefer water that is partly mineralized to the flat taste of distilled water.

The tolerance of chlorides by human beings varies with climate and exertion, and chlorides lost through perspiration may be replaced by chlorides in either the diet or drinking water. From hot dry areas have come reports that chloride concentrations up to almost 900 mg/l have not been harmful (1073), and Rudolfs maintains that even 1000 mg/l is harmless (855). According to a Kettering Laboratory survey (3392), laxative effects of chloride are generally apparent only when an individual has been accustomed to a lower concentration of the salt. Within a few days, an individual adjusts physiologically to a higher level of salt intake. In general, it is the cation (calcium, magnesium, sodium, or potassium) associated with the chloride that produces a harmful effect. It has been reported in Russia (3393, 3394) that under conditions of artifically induced thirst, test subjects drank 40-60 percent less water containing 1000 mg/l of chloride than they did of tap water.

Chloride limits, both those in use as standards and those recommended for use as possible standards, vary over a wide range. It is believed that the source of the chlorides may be a more important factor for drinking water than their quantity and that any sudden increase in the chloride content of a supply should be suspected as a possible indication of pollution and should be investigated further.

The USPHS Drinking Water Standards of 1962 (2036) recommend that chlorides do not exceed 250 mg/l. In other agencies or other countries, the criteria for chlorides have been given at values ranging from 5 mg/l to 600 mg/l (250, 499, 627, 630, 631, 632, 929, 944, 992, 1088, 1457). The WHO International Standards of 1958 set a permissible limit of 200 mg/l and an excessive limit of 600 mg/l (2328). For the WHO European Drinking Water Standards (2329), the recommended limit is 350 mg/l and no tolerance limit is specified.

b. Industrial Water Supplies. In Chapter V it is to be noted that several industrial processes have recommended threshold concentrations for chloride ions or chloride compounds. Some of these values are tabulated below, but for more complete information, see Chapter V.

Industry	*Recommended Threshold Concentration, in mg/l*			
	Chlorides	*$CaCl_2$*	*$MgCl_2$*	*NaCl*
Brewing	60–100	100–200	50–200	275–500
Carbonated beverages	250	---	---	---
Dairy industry	30	---	---	---
Food canning and freezing	---	---	---	1000–1500
Food equipment washing	250	---	---	---
Paper making:				
Groundwood pulp	75	---	---	---
Soda pulp	75	---	---	---
Kraft pulp	200	---	---	---
Steel manufacture	175	---	---	---
Sugar making	20	---	---	---
Textile processes	100	---	---	---

Chlorides appear to exert a significant effect on the rate of corrosion of steel and aluminum. The Bethlehem Steel Corporation reported that there was evidence of corrosion attack on rolled surfaces when the chloride content of quenching waters reached 185 mg/l. Many producers of rolled stainless steel consider 50 mg/l the chloride limit for roller cooling waters. Corrosion of all metals used in the water-handling system has been reported at 45-50 mg/l of chlorides (3389).

Research by several independent investigators (2361, 3305, 3395, 3396) has shown that chloride ions have an immediate effect on corrosion of steel at concentrations as low as 3.0 mg/l, and for stainless steel at 10 mg/l. Indeed, in the stainless steel portion of a power reactor, chloride concentrations over 0.5 mg/l were found to accelerate stress corrosion (3395). For aluminum, one reference (3397) indicates that 5.0 mg/l of chloride caused some pitting and increased the corrosion rate slightly; but another article (3305) reports that there was no substantial increase in the corrosion rate of aluminum in aerated distilled water at 26°C at chloride concentrations as high as 300 mg/l.

c. Irrigation Use. Chlorides are considered to be among the most troublesome anions in irrigation water. They are generally more toxic than sulfates to most plants, including lemons, alfalfa, fruit trees, and potatoes; but sulfates are more toxic than chlorides to sugar beets (273).

The following concentrations of chloride can be harmful to plants (see also Chapter V—Irrigation):

Concentration in mg/l	*Concentration in meq./liter*	*Reference*
100-150	3-4	1089
200	6	1090, 275
177-355	5-10	271
225	--	3398
250	--	618
350	--	219, 921
1260	35.5	1091
1500	--	921

The following concentrations of chloride have been reported as not harmful: less than 71 mg/l (264); less than 177 mg/l (271); 62 mg/l (1060).

d. Stock and Wildlife Watering. Chloride concentrations in excess of 4000 mg/l have been reported to cause injury to livestock (932). Chloride concentrations of 1500 mg/l are reported to be safe for cattle, sheep, swine, and chickens (3391). A limiting concentration of 3000 mg/l has been proposed in South Africa (3373). For further details of the effect of dissolved solids on livestock, see Chapter V.

e. Fish and Aquatic Life. Hart et al. (310) cite data indicating that among U.S. waters supporting a good fish fauna, ordinarily the concentration of chlorides is below

3 mg/l in 5 percent; below 9 mg/l in 50 percent and below 170 mg/l in 95 percent of such waters. The following concentrations of chloride have been reported to be harmful to fish:

Concentration in mg/l	*Type of Fish*	*Reference*
400	trout	932
2000	some fish	1092
4000	bass, pike, perch	932
4500-6000	carp eggs	1489
8100-10,500	small bluegills	2692

On the other hand, 2000 mg/l of chloride has been reported as not harmful for some fish (1092). Chlorinity is closely related to the total salinity and its effects on osmosis; hence it is evident that fresh-water fish cannot tolerate excessive changes in salinity but similarly, most salt-water fish are vulnerable to waters of low salinity.

The Aquatic Life Advisory Commission of ORSANCO concluded that it is impossible to generalize on the effects of chloride concentrations on aquatic life, for each mixture of chlorides with other salts must be evaluated separately (3390).

4. Summary. On the basis of the foregoing information, it appears that the following concentrations of chloride will not be normally deleterious to the specified beneficial uses:

a. Domestic water supply	250 mg/l
b. Industrial water supply	50 mg/l
c. Irrigation	100 mg/l
d. Stock and wildlife	1500 mg/l

CHLORINATED BENZENES

(see Chapter IX)

CHLORINATED CAMPHENE

(see Chapter IX, Toxaphene)

CHLORINATED HYDROCARBONS

CHLORINE

1. General. Not to be confused with chlorides, which are present in almost all natural waters, chlorine is not a normal constituent of such waters. It is the elemental form of a greenish-yellow gas that dissolves readily in water. At 20°C, one volume of water will dissolve 2.3 volumes of chlorine gas, or about 7,000 mg/l (364). When it dissolves in water, chlorine hydrolyzes immediately and completely, according to the reaction:

$$Cl_2 + H_2O \rightarrow HOCl + H + Cl^-$$

At concentrations of total chlorine below 1,000 mg/l, no measurable quantity of chlorine gas exists in solution as Cl_2, all chlorine being present as HOCl or its dissociated ions. The HOCl is a weak acid and dissociates according to the equation.

$$HOCl \rightleftharpoons H^+ + OCl^-$$

where the dissociation constant is given as 3.3×10^{-8} at 20°C. Thus it can be seen that the ratio between HOCl and OCl^- is a function of pH, with the percentage of HOCl being 96 percent at pH 6, 75 percent at pH 7, 22 percent at pH 8, and only about 3 percent at pH 9. This relationship of HOCl and pH is particularly significant inasmuch as the undissociated form appears to be the primary toxic principle and the bactericidal agent in the use of chlorine for disinfection (1087).

Free available chlorine, i.e. HOCl and OCl^-, and combined available chlorine, i.e. the chloramines, may appear briefly in surface or ground waters as a result of discharges from municipal sewage-treatment works or industrial processes. In the chlorination of sewage and treatment-plant effluents before discharge to a water course it is customary to add chlorine in an amount equivalent to only a fraction of the chlorine demand, and hence free available chlorine is seldom found in such effluents. Combinations of chlorine with ammonia, organic matter, and cyanides may be present, however, and may prove to be detrimental to fish life and other beneficial uses. Industrial processes most likely to contain free or combined chlorine are those employing bleaching operations, e.g., textile mills and paper pulping operations, or those using chlorine for the control of organisms in cooling waters (346).

2. Cross References. Calcium Hypochlorite, Chloramines, Cyanides, Chlorides, Halogenated Hydrocarbons, Halogenated Phenols, Insecticides, and other chlorinated compounds.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Chlorine is used extensively in the U.S. and elsewhere as a bactericidal agent in municipal water works. Extensive literature pertaining to the effectiveness of chlorination against viruses, viable bacteria, spores, amoeba, cysts, ova, and other forms of biota is available, but it is not within the province of this report to deal with methods of treatment or the quality of treated water. It is relevant to note, however, instances where free chlorine, per se, might be regarded as a pollutant in raw water sources.

In normal concentrations, chlorine does not render water abnormally corrosive to zinc (977) nor does it increase the solubility of lead salts (1029). In pure water, residual chlorine concentrations as high as 2.0 mg/l have not been found to cause objectionable tastes and odors (1086) but where certain organic substances, such as phenols, are present, very small concentrations of chlorine can produce undesirable tastes and odors. Rohlich and Sarles (759) quote references to the effect that chlorine in dosages up to 0.1 mg/l did not produce odor with phenol when the latter was present up to 0.05 mg/l, and that the critical range for chlorophenol tastes was for chlorine dosages from 0.175 to 0.4 mg/l with phenol from 0.002 to 0.17 mg/l. According to Campbell (2059) the taste threshold of chlorine in redistilled water was 5.2 mg/l.

It is generally agreed that the small amounts of chlorine present in chlorinated water are dissipated by reaction with saliva and gastric juices as soon as the water is swallowed (152). Muegge (1467) and Hudson (3303) report that 50 to 90 mg/l of chlorine in drinking water have been used by humans without adverse effect, but Muegge (1467) also cites an instance where 90 mg/l produced strong physiological reactions. According to Watson and Kibler (1096), and another reference (1098), chlorinated drinking water may cause asthma, colitis, and eczema. When patients drink distilled water instead of

chlorinated water, their condition improved, but when they returned to chlorinated water the symptoms reappeared.

b. Industrial Water Supplies. Many industrial waters are chlorinated to control bacteria and other slime-producing organisms (222, 436, 1099, 1100), but in the food-processing industries, chlorine may cause tastes in the canned or frozen products (225) or may be detrimental in corroding cans (223). Traces of chlorine in water used for metal-plating baths will affect the smoothness or brightness of the deposits (214). For the manufacture of fine papers, T.A.P.P.I. recommends that the residual chlorine not exceed 2.0 mg/l (350), but in earlier work under T.A.P.P.I. sponsorship Fleming et al., (552) set the limit at 0.3 mg/l for high-grade paper and 0.8 mg/l for lower-grade products.

c. Irrigation. No injury to land plants occurs when irrigation water contains 50 mg/l or less residual chlorine (152, 1089); concentrations of 100 to 150 mg/l injured or retarded some plants and did not affect others; 200 to 300 mg/l injured tops of plants but did not harm roots; and 500 to 1000 mg/l retarded growth of seedlings and killed tops and roots of plants. Roots from tomato cuttings in chlorinated water were not affected by 5.0 mg/l but were retarded in growth at 10 mg/l. Cut flowers were not affected by concentrations up to 10 mg/l but aquatic plants were harmed by concentrations of 3.0 mg/l or more (1089).

d. Stock and Wildlife Watering. According to Blabaum and Nichols (1468), white mice can grow from weanlings to adulthood and thrive on water containing 200 mg/l of free available chlorine. Weight gain and growth of mice using 100 and 200 mg/l of free available chlorine were equal to those of controls.

e. Fish and Other Aquatic Life. Free available chlorine in water may be toxic to fish and other aquatic life at low concentrations, and combinations of chlorine with cyanides, phenols, and other substances may be even more detrimental (see cross references). It is known that the toxicity of chlorine to bacteria is due primarily to undissociated HOCl and consequently lethality increases with decreasing pH values. It is probable that the toxicity to fish is also affected by pH value but so far as it is known this question has not been explored thoroughly (346). The following concentrations of free chlorine have been reported to be toxic or harmful to fish:

Concentration in mg/l	*Time of Exposure*	*Type of Fish*	*Remarks*	*Reference*
0.03	--	rainbow trout	killed	2920, 2962
0.05	23 days	young salmon	critical level	2091
0.08	7 days	rainbow trout	half killed	717, 1816
0.11 to 0.13	days	fish	harmful	603
0.15 to 0.2	12 to 16 days	carp	25% killed	346, 776
0.2 to 0.3	--	fish	harmful	1101
0.25	5 hours	fingerlings	killed	361
0.3	2 hours	trout	killed	361, 311
0.3 to 1.0	--	fish	killed	1102, 311
0.8	47 minutes	small trout	killed	346
0.8	4 hours	golden shiners	killed	3361
1.0	1 hour	trout	killed	361
1.0	4 days	many types	killed	346, 776
1.0	--	goldfish	killed	361
1.0	--	trout and goldfish	killed	1012
2.0	--	goldfish	killed	2920, 2962
2.0 to 4.0	--	fish	30% killed	912
3.0	24 hours	green sunfish	28% killed	3361

In contrast, fish have been reported to have survived the following concentrations of free chlorine:

Concentration in mg/l	*Time of Exposure*	*Type of Fish*	*Remarks*	*Reference*
0.1	2.5 hours	small trout	not harmed	346
0.1	--	fish	not harmed	1101
0.25	42 hours	goldfish	no effect	361
0.3	2 hours	minnows	no effect	362
0.5	--	trout and goldfish	survived	1012
1.0	--	minnows	survived	1103
1.0	--	goldfish	recommended threshold	1103, 633
1.0	100 hours	eels	not effected	776
1.0	--	carp	not harmful	361
1.0	48 hours	fish	max. conc. survived	1058
2.0	48 hours	green sunfish	no mortality	3361
3.8	48 hours	tadpoles	no mortality	3361
5.0	--	goldfish	survived	384

The wide discrepancy in the foregoing tabulations is evidence of the fact that pH, temperature, dissolved oxygen, and the synergism and antagonism of other pollutants markedly affect the toxicity of free chlorine toward fish. The Water Pollution Research Board of England (1816) found that the toxicity of chlorine in ammoniated water at pH 7.0 was proportional to the titrable chlorine residual and not to the chlorine added. Rainbow trout was the test fish. It was also determined that free chlorine was slightly more toxic than monochloramine.

According to Hiatt et al. (3350), marine fish exhibit a slight irritant activity at 1.0 mg/l of chlorine and violent irritant activity at 10.0 mg/l.

The tolerances of other aquatic organisms toward free chlorine appear to vary widely. Most algae have been controlled by 0.25 to 3.0 mg/l (587, 1490, 2255) and 5 to 10 mg/l killed *Synura* (587). For *Chironomus* (blood worms) dosages as high as 15 to 50 mg/l may be required (1490). Minute crustacea, rotifers, and diatoms were killed by 1.0 mg/l but worms, molluscs, mites, and larvae were not affected (1102). In soft water, 0.5 mg/l killed *Daphnia* in 72 hours (313), 1.0 mg/l is reported to "control" *Daphnia* (385) and in Nile River water, *Daphnia* and *Cyclops* were killed by 2.0 mg/l (313). Larvae of *Chironomus* were killed by 2.6 mg/l in 1.5 hours (100 percent); by 1.3 mg/l in 3.2 hours (100 percent); and 85 percent by 0.65 mg/l in 24 hours (568). Fresh-water mussels, snails, and sponges in cooling systems were killed, but not dislodged, by 2.5 mg/l.

In sea-water conduits, exposure for 8 hours per day to sea water containing 10 mg/l chlorine did not kill all adult mussels and anemones. Most barnacles were killed by exposure of 4 hours per day to this concentration; and tunicates and bryozoa were all killed by exposure for one hour. With continuous chlorination to a residual of 2.5 mg/l, all organisms were killed in 5 to 8 days, but with 1.0 mg/l a few barnacles and all anemones were alive after 15 days (976).

Chang et al. (2812) found that free-living nematodes ingest pathogenic bacteria and viruses, and that these pathogens can remain viable within the nematodes for 24 to 48 hours. Furthermore, the nematodes are highly resistant to free chlorine in water, not being affected by 3.0 mg/l in 120 minutes or by 15 to 45 mg/l in a one-minute exposure at 25°C. Even with an initial chlorine concentration of 95-100 mg/l, 50-60 percent of the nematodes survived a 5-minute contact and 10-20 percent survived a 15-minute contact.

According to Clendenning and North (2106, 2865), the photosynthetic capacity of bottom fronds of the giant kelp, *Macrocystis pyrifera*, was not affected in 5 days

exposure to 1.0 mg/l of chlorine in sea water; but at 5 to 10 mg/l there was a 10-15 percent reduction in photosynthesis after two days and 50-70 percent reduction after 5 to 7 days.

The Mersey River Board in England (2950) has proposed that the free chlorine residual of wastes discharged to the River Alt and the River Bollin should not exceed 1.0 mg/l.

f. Shellfish Culture. Clams and oysters will withstand short exposures to free chlorine, and such treatment has been used in purification of shellfish. In Massachusetts, clams are conditioned for 24 hours in sea water containing a residual of 0.3 to 0.5 mg/l. In England, shellfish are exposed briefly to water containing 5.0 mg/l of free chlorine, then placed in chlorine-free water (1100). The USPHS Manual of Recommended Practice For Sanitary Control of the Shellfish Industry (527) requires that water used to condition shellfish carry a residual of not less than 0.05 mg/l at all times. Experimental evidence indicates that most oysters are sensitive to initial chlorine concentrations varying from 0.01 to 0.05 mg/l, i.e., their pumping activity is reduced, and effective pumping cannot be maintained at chlorine concentrations over 1.0 mg/l (1104).

g. Bathing Waters. Free residual chlorine in natural waters should not be considered detrimental to bathing inasmuch as this bactericidal agent is used in swimming pools and many state health departments require residual chlorine concentrations in such pools. Mood et al. (1105) report that a slight increase in eye irritation is obtained by increasing the chlorine residual from 0.05 to 0.50 mg/l, but within the pH range of 7.0 to 8.0, the pH has a greater effect upon eye irritation than does the chlorine residual in concentrations between 0.05 and 0.50 mg/l.

CHLOROAURIC ACID $AuCl_3 \cdot HCl \cdot 4H_2O$

This acid gold trichloride, a yellowish crystalline solid very soluble in water, is used in photography, gold plating, and the manufacture of ruby glass (364). The lethal concentration of $AuCl_3 \cdot HCl$ for three mature small fresh-water fish (*Orizias*) exposed for 24 hours to only 20 ml of test solution, was reported to be about 14 mg/l (1459). With this same substance, Jones (1460) found the lethal limit to be 0.4 mg/l, as gold, for stickleback. The average survival times at other concentrations were as follows: one week at 0.6 mg/l, and only 4 days at 1.0 mg/l as gold.

CHLOROBEN

(see Chapter IX, Chlorinated Benzenes)

CHLOROFORM $CHCl_3$

This colorless, volatile, but non-inflammable heavy liquid of ethereal odor and sweetish taste is used as an anesthetic, counterirritant, solvent, cleansing agent, and antiseptic. Stickleback will avoid solutions of 100 to 200 mg/l of chloroform in tap water and at 500 mg/l they become anesthetized (467). At this latter concentration, the respiration of the fish decreased rapidly in 10 minutes and stopped in 20 minutes. At a concentration as low as 10 mg/l, fish struggled for 20 to 30 minutes then sank to the bottom. When placed in fresh water for 90 minutes, however, they revived (468).

CHLOROMETHANE

(see Methyl Chloride)

CHLOROPHENOLS

(see Chapter IX)

CHLOROPHENYL DIMETHYL UREA (CMU)

(see Chapter IX)

CHLORPLATINIC ACID $H_2PtCl_6 \cdot 6H_2O$

A brownish-yellow crystalline solid, easily soluble in water, this acid salt is used in platinum plating, photography, indelible ink, mirrors, and as a catalyzer (364). To three mature small fresh-water fish (*Orizias*), exposed for 24 hours to only 20 ml of test solution, the lethal concentration of H_2PtCl_6 was reported to be about 33 mg/l (1459).

CHLORTHION

(see Chapter IX)

CHROMATES

(see Chromium)

CHROMIC ACID

(see Chromium)

CHROMIUM

1. General. This section deals with chromium ions in their various forms, i.e. as chromous ion (Cr^{++}), as chromic ion (Cr^{+++}), as chromite ion (CrO_3^{---}) or (CrO_2^-), as chromate ion (CrO_4^{--}), and as dichromate ion ($Cr_2O_7^{--}$). In the chromic or chromite condition the chromium is trivalent, while in the chromate and dichromate form it is hexavalent. All chromous compounds tend strongly to be oxidized to the chromic condition. Hexavalent chromium can be reduced to the trivalent form by heat, by organic matter, or by reducing agents. Confusion in terminology frequently arises from the fact that chromium trioxide, a hexavalent form, is also called "chromic acid" or "chromic acid anhydride", thereby confusing it with the trivalent chromic ion. In the following abstract of literature, care has been taken to avoid this confusion of terms.

Of the trivalent chromic salts, the chloride, nitrate and sulfate are readily soluble in water, but the hydroxide and carbonate are quite insoluble. Of the hexavalent chromate salts, only sodium, potassium, and ammonium chromates are soluble. The corresponding dichromates are also quite soluble.

Hexavalent chromium salts are used extensively in metal pickling and plating operations, in anodizing aluminum, in the leather industry as a tanning agent, in the manufacture of paints, dyes, explosives, ceramics, paper, and many other substances. Trivalent chromium salts, on the other hand, are used much less extensively, being employed as mordants in textile dyeing, in the ceramic and glass industry, and in photography (364,

1106, 2121). Chromium compounds may be present in wastes from many of the foregoing industries or they may be discharged in chromium-treated cooling waters (152). Rather extensive literature is available to describe the effects of chromium as a corrosion inhibitor in cooling systems and further numerous references deal with the effect of hexavalent and trivalent chromium upon biological sewage treatment processes and sludge digestion (346), but such literature is not within the province of this survey.

2. Cross References. Chapter VIII (Radioactivity)

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The USPHS Drinking Water Standards of 1962, as well as those of 1946, set a mandatory limit of 0.05 mg/l for hexavalent chromium, but none for the trivalent form (2036). The WHO International (2328) and WHO European (2329) Drinking Water Standards also prescribe a limit of 0.05 mg/l for hexavalent chromium. The 1942 standards in the U.S. were even more severe in permitting no hexavalent chromium whatsoever, but this restriction was relaxed in 1946 to allow certain ground-water supplies that were slightly affected by chromates to qualify (1106). Also, at that time, a concentration of 0.05 mg/l was the lowest amount that was analytically determinable.

There is no evidence that chromium salts are essential or beneficial to human nutrition. When administered orally, chromium salts are not retained in the body but are rapidly and completely eliminated (2121). Although the salts of trivalent chromium are not considered to be physiologically harmful (997), there is evidence that large doses of chromates leads to corrosive effects in the intestinal tract and to nephritis (2129). The toxic dose for man is reported by Rothstein (2129) to be about 0.5 grams of potassium bichromate.

On the basis of physiological effects, it is difficult to understand why the USPHS and WHO have placed such a low limit on hexavalent chromium and why they made this limit mandatory. On Long Island, according to Davids, and Lieber (1106), a family used water for over three years from a well that was polluted with hexavalent-chromium wastes, with no apparent ill effects. When first discovered, hexavalent chromium was present in a concentration in 1.0 mg/l, and in May 1951 the water contained 25.0 mg/l. One physical exam of the entire family (man, wife, and two children) revealed no abnormalities and the family has refused to abandon the supply, or to submit to further examinations. The persistence of this family may eventually prove to be a valuable source of information concerning the physiological effects of hexavalent chromium in drinking water (1106).

Pomeroy (2924) used as his only fluid for drinking for 15 days water to which potassium dichromate had been added at a concentration of 10 mg/l as chromium. During that time he ingested 235 mg of chromium. Three periods of nausea were noted. The experiment was continued for two more weeks at concentrations of 2.5 to 5.0 mg/l. At a concentration of 5 mg/l, mild nausea resulted from drinking freely on an empty stomach, but under similar conditions, concentrations of 2.5 and 3.5 mg/l failed to produce any symptoms. On the basis of the animal experiments described below (under Stock and Wildlife Feeding), it would appear that man could drink water containing 5.0 mg/l of hexavalent chromium without deleterious physiological effects.

Chromium is not commonly encountered in natural foods, although traces enter cooked food from stainless-steel utensils. The amount of chromium so ingested is not known to have any physiological effects.

Chromium salts impart color to water, but this effect is not discernible below about 1.5 mg/l. Similarly, the taste threshold for the most sensitive person is about 1.5 mg/l.

b. Irrigation. Chromium is present in trace amounts in soils and in plants, but there is no evidence that chromium is essential or beneficial for plant nutrition (2121). Sedova (2925) reported that concentrations of trivalent or hexavalent chromium in excess of 1.0 mg per kg of soil inhibited nitrification. On the other hand, the addition of 5 mg of chromium per kg of soil resulted in a slight increase of the nitrogen content of peas (2926). Chromium is picked up by plants from the soil, for vegetables grown on soil irrigated with wastewaters containing chromium had 3 to 10 times more chromium than those grown on similar soils devoid of chromium (2925).

According to Klintworth (1493) chromium is toxic to plants at all concentrations. In sand culture, concentrations of 3.4 to 17.3 mg/l of trivalent chromium in nutrient solutions were slightly toxic to various crops. The chromate ion was slightly more toxic than the chromic ion at equivalent concentrations (1473). Added as potassium dichromate, 5 mg/l of chromium in a nutrient solution caused slight chlorosis among oat plants, 10 mg/l caused marked chlorosis, and 15 to 50 mg/l markedly reduced the growth of the plants. A concentration of only 2 mg/l intensified the injury caused by nickel (1462).

c. Stock and Wildlife Watering. It has been reported (353) that one gram of trivalent chromium per day, as $CrPO_4$, over a period of 17 weeks did not cause illness, loss of weight, or tissue damage to cats. For hexavalent chromium salts, according to Gross and Heller (1108) the maximum non-toxic level in drinking water for white rats is 500 mg/l. Also, drinking water containing 500 mg/l of potassium chromate does not affect utilization of food by rabbits, but 10,000 mg/l of zinc chromate markedly interfered with digestion. A concentration of 5 mg/l of chromium (valence not specified) in the drinking water caused an increase in mortality among rats on a diet containing 11 mg/l of selenium (1481). As much as 100 mg/kg of chromium, fed as Na_2CrO_4, had no effect on the growth, feed conversion, or mortality of chicks (2927).

Byerrum and his colleagues (2138, 2145, 2147, 2150, 2928, 2929) conducted extensive experiments with rats and dogs to determine the long-term effects of trivalent and hexavalent chromium in drinking water. Controls received distilled water while other rats received chromium from K_2CrO_4 at dosages up to 11 mg/l of chromium for a year. Other tests were run with 25 mg/l of chromium from potassium chromate and 25 mg/l from chromic chloride. No significant differences in weight,

food intake, water consumption, or blood analyses were observed between the experimental groups and the controls. At chromium concentrations of 5 mg/l and lower, little chromium was found in any tissue, indicating that it was not being retained in the body. At 7.7 mg/l and higher, however, all tissues, especially the spleen showed an appreciable increase. The group receiving 25 mg/l of hexavalent chromium had an average tissue chromium content about eight times that of the group that was given trivalent chromium. Even at 25 mg/l, however, there was no significant difference in weight gain or food consumption. Over a 4-year period with dogs, no pathological changes were noted, even up to 11 mg/l of chromium.

d. Fish and Other Aquatic Life. The toxicity of chromium salts toward aquatic life varies widely with the species, temperature, pH, valence of the chromium, and synergistic or antagonistic effects, especially that of hardness. Fish are relatively tolerant of chromium salts, but lower forms of aquatic life are extremely sensitive, as the following information reveals. There appears to be no evidence to lead to a conclusion that hexavalent chromium is more toxic toward fish than the trivalent form. Toward fish, the toxicities of hexavalent chromium compounds have been reported as follows:

Concentration of Chromium mg/l	*Compound Used*	*Type of Fish*	*Remarks*	*Reference*
5	--	fish	toxic	1109
5.2	$K_2Cr_2O_7$	brown trout	toxic	346
7.1	$K_2Cr_2O_7$	carp	not harmed	2930
9	CrO_4	fish	toxic limit	353
10	$Na_2Cr_2O_7$	--	only slightly hazardous	1492
10	--	silver salmon	fresh water, toxic	2931
17.8	--	silver salmon	sea water, toxic	2931
20	$K_2Cr_2O_7$ and K_2CrO_4	rainbow trout	toxic at 18°C	617, 1110, 2190
35.3	$K_2Cr_2O_7$	goldfish	not harmed	313
45	$K_2Cr_2O_7$	bluegills	tolerated in hard water for 20 days	1459
50	$K_2Cr_2O_7$	trout	killed within 33 hrs.	1459
50	--	bluegills	toxic limit for 30-day exposure	1111
52	$K_2Cr_2O_7$	young eels	tolerated for 50 hrs.	1459
52	CrO_3	goldfish	toxic in 30 minutes	313
52	CrO_3	goldfish	survived 4 days	313
68	$K_2Cr_2O_7$	bluegills	tolerated in hard water for 5 days	1459
70	--	bluegills	toxic limit for one week's exposure	1111
75	$K_2Cr_2O_7$	bluegills	died within 4 days	1459
83	$K_2Cr_2O_7$	bluegills	tolerated for 10 days	1459
100	K_2CrO_4	trout	24-hour TL_m	2932
100	$K_2Cr_2O_7$	trout	fatal after 6 hrs.	1459
103	$K_2Cr_2O_7$	bluegills	96-hour TL_m	2933, 2934, 2935
104	CrO_3	goldfish	toxic in 6 to 84 hrs.	353, 2190
110	$K_2Cr_2O_7$	sunfish	96-hour TL_m	2937, 2938
113	$K_2Cr_2O_7$	sunfish	96-hour TL_m	2936
130	$K_2Cr_2O_7$	young eels	tolerated over 50 hours	1459
135	$K_2Cr_2O_7$	sunfish	96-hour TL_m (hard water)	2936
145	$Na_2Cr_2O_7$	bluegills	24-hour TL_m	1317
148	$Na_2Cr_2O_7$	bluegills	toxic limit	353
170	K_2CrO_4	sunfish	96-hour TL_m	2937, 2938
177	$K_2Cr_2O_7$	goldfish	toxic in 3 days	313
180–362	--	several species	toxic	1459
196	--	large-mouth bass	TL_m	2939
200	$K_2Cr_2O_7$	mummichogs	tolerated in sea wtr. for over a wk.	1459
213	$Na_2Cr_2O_7$	bluegills	48-hour TL_m	2093
300	Na_2CrO_4	bluegills	24-hour TL_m	1317
520	K_2CrO_4	young eels	killed in 5-12 hrs.	1459

In highly turbid water, Wallen et al. (2940) found the 96-hour TL_m values for several chromate and dichromate compounds toward the mosquito-fish (*Gambusia affinis*) to be as follows:

Compound	*Temperature Range*	*pH Range*	*96-hour TL_m in mg/l For Compound*	*96-hour TL_m in mg/l As Chromium*
Ammonium chromate	19-23°C	7.5-7.8	240	82
Ammonium dichromate	18-20	5.7-7.4	136	56
Potassium chromate	17-21	7.6-8.1	400	104
Potassium dichromate	21-23	5.4-6.7	280	99
Sodium chromate	20-22	7.7-8.6	420	135
Sodium dichromate	24-27	6.0-7.9	264	92

For trivalent chromium, toxicities and survival times for fish have been reported as follows:

Concentration of Chromium, mg/l	*Compound Used*	*Type of Fish*	*Remarks*	*Reference*
1.2	$Cr_2(SO_4)_3$	Sticklebacks	Lethal limit	1460, 2941
1.3	$Cr_2(SO_4)_3$	Sticklebacks	Survived only 1 wk.	1460
2.0	$Cr_2(SO_4)_3$	Sticklebacks	Survived only 2 days	1460
2.4	$Cr_2(SO_4)_3$	Sticklebacks	Lethal limit	598
5.0	$Cr_2(SO_4)_3$	Sticklebacks	Survived only 1 day	1460
5.2	$KCr(SO_4)_2$	Young eels	Survived an average of 18.7 hours	1459
40	$Cr_2(SO_4)_3$	Minnows	Survived in distilled water only 6 hours	1459, 2942, 2943, 2944

From the foregoing tables, it appears that trivalent chromium might be more toxic than the hexavalent form despite apparent beliefs to the contrary. On the other side of the ledger, Olson (2945) reports that young chinook salmon were exposed for 12 weeks to either 0.2 mg/l of hexavalent or trivalent chromium. The hexavalent form showed reduced growth and increased mortality whereas the trivalent form had no observable effect.

Towards other organisms, toxicities of hexavalent chromium have been reported as follows:

Concentration of Chromium, mg/l	Compound used	Type of organism	Remarks	Reference
0.016	$Na_2Cr_2O_7$	*Daphnia magna*	toxic threshold	353
0.05	--	*Daphnia magna*	killed in 6 days	1112
<<0.10*	$Na_2Cr_2O_7$	*Daphnia magna*	toxic threshold	358
<<0.10*	Na_2CrO_4	*Daphnia magna*	toxic threshold	358
0.21	$K_2Cr_2O_7$	Protozoan (*Microregma*)	threshold effect	3343
0.21	$K_2Cr_2O_7$	Diatom (*Navicula*)	softwater TL_m, 22°C	2936
0.25	$K_2Cr_2O_7$	Diatom (*Navicula*)	hardwater TL_m, 22°C	2936
0.51	Na_2CrO_4	*Daphnia magna*	toxic threshold	2946
0.7	$K_2Cr_2O_7$	*Daphnia*	threshold effect	2158
0.7	$K_2Cr_2O_7$	*Scenedesmus*	threshold effect	2158
0.7	$K_2Cr_2O_7$	*E. coli*	threshold effect	2158
1.4	Na_2CrO_4	*Gammarus pulex*	total mortality	2947
17.3	$K_2Cr_2O_7$	Snail	softwater TL_m, 20°C	2936
25.0	--	Midge fly larvae	not toxic	2688
40.6	$K_2Cr_2O_7$	Snail	hardwater TL_m, 20°C	2936
148	CrO_4	*Polycelis nigra*	toxic threshold	354

*<<signifies "very much less than"

For trivalent chromium, Anderson (598) reports that the toxic threshold for *Daphnia magna* in Lake Erie water at 25°C is very much less than 1.2 mg/l (3.6 mg/l of $CrCl_3$). Bringmann and Kuhn (3343) indicate that the thresholds of toxicity occurred at 5 mg/l for *Scenedesmus*, 37 mg/l for *Microegma*, and 42 mg/l for *Daphnia*. With a flatworm, *Polycelis nigra*, Jones (608) found the toxic threshold in 48 hours of exposure to be 75 mg/l of chromium. Thus, toward *Daphnia magna*, hexavalent chromium appears to be more toxic than the trivalent form; but toward *Polycelis nigra* the opposite is true.

The toxicity of sodium chromate toward *Daphnia magna* increases as the dissolved oxygen tension of the water is lowered (2946).

Algae concentrate radioactive chromium by factors of 100 to 500 or more (2442). For further details of concentration factors, see Chapter VIII. Radioactivity.

According to Ingols and Kirkpatrick (1491) the toxicity of chromium toward bacteria is controlled by (a) the valence of the chromium, (b) the type of organism, (c) the amount of organic matter present, and (d) the presence or absence of dissolved oxygen. The chromate is much more toxic under anaerobic conditions than the chromic ion whereas, in general, the chromic ion is more toxic under aerobic conditions than the chromate ion. It must be recognized, of course, that the chromate ion is reduced to the chromic form at low concentrations under aerobic conditions.

Trivalent chromium has been reported to lower the 5-day BOD by 50 percent as a concentration of 62.5 mg/l (2922) and at 117 mg/l (2923). Hexavalent chromium at 100 mg/l lowered the 5-day BOD by 50 percent (2922). Trivalent chromium is somewhat more toxic to microorganisms than the hexavalent form in the range of 1 to 10 mg/l (2948), probably because the trivalent salts are sufficiently acid to depress the pH somewhat (2949).

Clendenning and North (2106, 2107, 2865, 2866) tested the effect of hexavalent chromium, supplied as $K_2Cr_2O_7$, on the rate of photosynthesis of the giant kelp, *Macrocystis pyrifera*. At 1.0 mg/l of chromium, photosynthesis was not diminished by two days contact, was reduced 10-20 percent after 5 days, and 20-30 percent after 7-9 days. The concentration of chromium required to cause a 50 percent inactivation of photosynthesis in 4 days was estimated at 5.0 mg/l.

The Severn and Mersey River Boards in England have adopted working standards that limit the total concentration of all heavy metal, including chromium, to 1.0 mg/l (1756, 2950).

4. Summary. On the basis of the foregoing information, it appears that the following concentrations of chromium, trivalent or hexavalent, will not interfere with the specified beneficial uses:

a. Domestic water supply	0.05 mg/l
b. Stock and wildlife watering	5.0 mg/l
c. Fish life	1.0 mg/l
Other aquatic life	0.05 mg/l

CITRIC ACID $C_3H_4OH\ (COOH)_3$

(see also Sodium Citrate)

One of the acids most widely distributed in nature, occurring particularly in citrus fruits, citric acid is a colorless, crystalline substance which is highly soluble in water. It is used extensively in the beverage and food industries and in pharmaceutical preparations. The acid may be obtained by extraction from citrus juices, precipitation as calcium salt, and freeing by dilute sulfuric acid; but it is also produced commercially by controlled fermentation of sucrose (189).

Ellis (313) found that, in hard water, 625 mg/l of citric acid was not lethal to goldfish in 100 hours but 894 mg/l killed them in 4 to 28 hours at 18-23°C. In soft water, on the other hand, 120 mg/l killed *Daphnia magna* in 24 to 72 hours. Anderson (358) showed that the threshold concentration for immobilization of *Daphnia magna* after prolonged exposure, using Lake Erie water at 25°C, was 153 mg/l. The difference between the foregoing values for *Daphnia* is probably attributable to hardness and other constituents in the Lake Erie water.

There appears to be an antagonistic effect of calcium and magnesium on the toxicity of citric acid, inasmuch as the weak acid is only slightly dissociated in water and inasmuch as calcium precipitates the dissociated citrate ion. Doudoroff and Katz (361) report that citric acid can be directly lethal to fully developed fish only when it lowers the pH value to 5.0 or lower; hence toxicity varies with the buffering of the water.

CMU (MONURON)

(see Chapter IX)

COAL-TAR PRODUCTS

(see Tar, Oil, Hydrocarbons, Phenol, Naphthalene)

COAL-TAR PRODUCTS PLANT WASTES

(see also Naphthalene, Phenanthrene, Pyridine, Xylene, Quinoline, Thiophene, Acridine, Cresylic Acid, Cresols, Phenols)

Containing a mixture of many substances, this waste gave a slight medicinal taste when chlorinated, at a dilution of 1:25,000. Without chlorination, a dilution of 1:2400 gave a slight odor of naphthalene. According to Schaut (362) minnows in a 1:2400 dilution tended to regurgitate their food but otherwise they were normal.

COBALT Co

1. General. Cobalt and its salts are used for making alloys, in nuclear technology, as pigment in the china and glass industry, and as binders in the tungsten-carbide tool industry. Cobalt salts may be divalent or trivalent. Solutions containing cobaltous ions (Co^{++}) are relatively stable but cobaltic ions (Co^{+++}) are powerful oxidizing agents and consequently they are unstable in natural waters.

The following material refers only to the literature related to the cobalt ion, per se, without reference to the accompanying anions. For cobalt salts, see the cross references.

2. Cross References. Cobalt Chloride, Cobalt Nitrate, Cobalt Sulfate, Chapter VIII.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. It has been reported (353, 2121) that cobalt has a relatively low toxicity to man, and that traces of cobalt are essential to nutrition. The ingestion of soluble salts in sufficient concentration, however, produces nausea and vomiting by local irritation (364). A maximum safe concentration of cobalt in drinking water cannot be established or estimated on the basis of present knowledge (3284). The ingestion of 0.1 to 0.25 mg per day does not appear to have any adverse effect whereas single daily doses of 25 mg per day over a period of a week or longer affect the hemoglobin content of blood (3284). No literature relative to taste or color thresholds was uncovered.

The existence of 0.09 mg/l of copper, 0.08 mg/l of cobalt, and 0.03 mg/l of nickel in a public water supply containing 80 to 90 mg/l of total solids, 4.4 mg/l of chlorides and with a pH value of 3.8 to 4.5 caused pitting of aluminum teakettles; but without the trace elements no pitting occurred (561).

b. Irrigation. In concentrations of 0.1 and 0.27 mg/l cobalt in culture solutions caused specific symptoms of injury to tomato plants. Cobalt sulfate in concentrations of 2.0 mg/l of cobalt stunted the growth of plants and caused withering (2977). A concentration of 10.7 mg/l caused severe injury and subsequent death of the plants. The presence of manganese in the culture solutions reduced somewhat the symptoms of cobalt injury (1499). It has not yet been proved that cobalt is essential for all green plants (1498).

A concentration of one mg/l of cobalt was toxic to flax grown in water culture, but the symptoms were reduced somewhat in the presence of small amounts of molybdenum (1458). Concentrations of 5.9, 14.7, and 29.5 mg/l of cobalt were very toxic to sugar beets, tomatoes, and other crops in sand culture (1473).

c. Stock and Wildlife Watering. Animals vary in their ability to tolerate cobalt. Indeed, trace amounts of cobalt appear to be essential in the diet. The minimum daily requirement of cobalt for growing lambs is reported to be 0.1 mg (1497) and for sheep it is probably 0.07 to 0.08 mg (1498). Forage containing 0.1 mg/kg of cobalt will supply the needs of cattle and sheep (3285).

The daily addition of 500 mg of cobalt, as chloride, sulfate, or carbonate, to the diet of calves for 30 days caused loss of appetite and weight. A loss of appetite among lactating cows has followed single doses of from 1 to 5 grams of cobalt sulfate. Two young bulls have tolerated daily doses of 10 grams for 90 and 127 days (1494). Cobalt in excess of 0.9 mg per kg of body weight produced toxic effects in dairy calves (1495), yet the lethal dose of cobalt in a dog is reported (353) to be more than 30 mg per kg of body weight. Toxic symptoms were observed among young rats receiving more than 0.6 mg of cobalt per day. A daily dose of 2 mg caused death in two weeks. In drinking water for rats, 100 mg/l caused tissue damage (353), a concentration of 200 mg/kg in the daily diet was toxic to rats, and 500 mg/kg caused death in 3 to 5 weeks (1495). Levels of dietary cobalt in excess of 5 mg/kg caused linear depression of chick growth and levels in excess of 50 mg/kg resulted in chick mortality (3286). In the drinking water, a concentration of 5 mg/l of cobalt caused increased mortality among rats fed a ration containing 11 mg/l of selenium (1481). The daily intake of a small amount of cobalt is reported to produce a striking polycythemia in a number of different species of animals (1496). There appears to be a relationship between the beneficial and detrimental effects of cobalt in the diet and the content of choline and vitamin V_{12} (3287).

d. Fish and Other Aquatic Life. (see cross references). Trace amounts of cobaltous ion appear to stimulate the growth of some organisms. A definite growth response in the protozoan ciliate, *Tetrahymena,* was produced by 0.5 micrograms per liter of cobalt, while the optimum total growth and rate of growth occurred at 8 micrograms per liter (3288). The addition of 1 to 5 mg/l of cobalt, as cobalt chloride or cobalt sulfate, to a medium used in the propagation of actinomycetes gave conspicuous growth (3289). Cobalt concentrations of 1.0 mg/l were not harmful to one-year-old tench, carp, rainbow trout, and char; or to crustacea, worms, and insect larvae forming the food of these fish (2151).

At higher concentrations, however, cobalt ions have demonstrated pronounced toxic effects. Jones (2941) found that the lethal concentration for sticklebacks, *Gasterosteus aculeatus,* was 10 mg/l of cobalt ion. The concentration of cobalt required to cause a 50 percent decrease in oxygen utilization of sewage or synthetic media was found to be 25 mg/l and 29 mg/l by independent investigators (2922, 2923). Other toxic concentrations are reported under the specific cobalt salts, i.e., cobalt chloride, cobalt nitrate, and cobalt sulfate.

Organisms tend to concentrate cobalt from the surrounding medium. According to Morgan (2800), cobalt-60 is concentrated 1070 to 1500 times by the flagellate *Ochromonas* and 271 times by the diatom *Navicula.*

COBALT CHLORIDE $CoCl_2$

(see also Cobalt)

Cobalt chloride, or cobaltous chloride, is a highly soluble salt that is used for sympathetic ink, barometers, hydrometers, galvanoplating, ceramics, and as a feed supplement in salt licks (364).

Ellis (313) found that concentrations of 1,000 mg/l in hard water killed goldfish in 30 to 32 hours, but a concentration only 10 mg/l in soft water was fatal to some goldfish in 168 hours. In tap water, the lethal limit for stickleback has been given as 22 mg/l (598). The threshold concentration for immobilization of *Daphnia magna* in Lake Erie water at 25°C according to Anderson (598) was found to be less than 3.1 mg/l in 64 hours, and has been reported to be about 2.8 mg/l (353).

The lethal concentration of cobalt chloride for fish has been reported at 16 mg/l for *fundulus* within 5 days; at 4050 mg/l in 24 hours for fish; and at 5200 mg/l for three mature small fresh-water fish (*Orizias*) exposed for 24 hours to only 20 ml of the test solution. A concentration of 200 mg/l had no effect on mummichogs in sea water (1459).

Using water from the River Havel from which the test organisms were recovered, Bringmann and Kuhn (2158, 3343) studied the threshold effects of cobalt added as cobalt chloride ($CoCl_2 \cdot 6H_2O$) on various species. For *Daphnia,* the median threshold effect occurred at 5 mg/l of cobalt, for *E. coli* at 2.5 mg/l, for *Scenedesmus* at 1.0 mg/l, and for a protozoan (*Microregma*) at 0.5 mg/l.

COBALT NITRATE $Co(NO_3)_2$

This soluble salt is used in the manufacture of cobalt pigments and sympathetic ink, also in decorating porcelain (364). According to Jones (608) the threshold concentration for toxicity to *Polycelis nigra,* a flatworm, in 48 hours was 83 mg/l of cobalt.

The lethal concentration limit for cobalt nitrate toward sticklebacks was reported to be 10 mg/l as cobalt. In different concentrations of cobalt, the average survival times of the fish were as follows: one week at 15 mg/l, 4 days at 20 mg/l, two days at 50 mg/l, and one day at 150 mg/l as cobalt (1460). Another investigator (2920, 2941) reported the lethal concentration of this salt toward sticklebacks at 15 mg/l as cobalt.

COBALT SULFATE $CoSO_4$

This red crystalline substance, readily soluble in water, is used in decorating and plating with cobalt and for supplying cobalt deficiencies in cattle and sheep. Too much cobalt sulfate, however, may be deleterious. Two ewe lambs became anemic after receiving cobalt sulfate in a dose of 5 mg as cobalt daily for 10 months. Cattle, however, have tolerated 50 mg of cobalt daily without polycythemic symptoms (1497). Blood changes occurred in calves when cobalt sulfate was consumed at a daily rate of about 50 mg per 100 lbs. of body weight. At higher dosages, appetite, growth and coordination were also affected (1500). A loss of appetite among lactating cows has followed single doses of from one to five grams of cobalt sulfate. Two young bulls have tolerated daily doses of 10 grams for 90 and 127 days (1494).

Applied as cobalt sulfate, 10 to 15 mg/l of cobalt was only slightly injurious to oat plants. More than 15 mg/l was severely toxic and caused a marked reduction in growth. In a nutrient solution, the addition of 2 mg/l of cobalt, added as sulfate, intensified the toxic effect of 2.5 mg/l of nickel (1462).

COLOR

1. General. Color in water may be of natural mineral or vegetable origin, caused by metallic substances, such as iron and manganese compounds, humus material, peat, tannins, algae, weeds, and protozoa. Waters may also be colored by inorganic or organic soluble wastes from many industries including nailworks, mining, refining, explosives, pulp and paper, chemicals, and others. Returned irrigation water also contributes to color.

In water-works practice, the true color of water is considered to be only that attributable to substances in solution after the suspensoids have been removed by centrifuging, because an accurate determination of color in water containing suspended matter is not possible. The term "apparent color" is used for color that includes an effect from suspended matters. However, the color of water with low turbidity is substantially the same as that of clear water. (Filtration has a decolorizing effect and should not be used to remove suspended matter). The unit of color considered as a standard is the color produced by the platinum-cobalt method of measuring, with the unit being one mg/l of platinum in water. Results are conventionally expressed as units of color, and not mg/l. The method is described in Standard Methods for the Examination of Water and Wastewater (469). In field work, the color of water may be compared with that of glass discs calibrated to correspond with colors on the platinum scale. Nemerow et al. (3399, 3400) have attempted to improve the quantitation of color in streams by the development of a "Stream Colorimeter."

2. Cross References. See Chapter III for standards of state and interstate agencies, also Chapter V.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The 1962 USPHS Drinking Water Standards (2036) limit the color of acceptable water to 15 units. The 1958 International Standards for Drinking Water (2328) indicate that 5 units of color are "permissive" but 50 units are "excessive." For high quality domestic water, Lubratovich and Ruble (1746) recommend that color be limited to 5 units, while Bean (1745) would place the limit at 3 units of color. The stream standards promulgated by various state and interstate agencies (see Chapter III) limit the color in the raw water to be used as sources of drinking water to values ranging from none to 100 units. Some standards specify that color in the raw water shall not be in sufficient amount or of such character as to make the stream unsuitable as a source of water supply for drinking, culinary, or food-processing purposes, after reasonable treatment. In domestic water, color is undesirable from esthetic considerations and also because it may dull clothes, or stain food and fixtures.

b. Industrial Water Supplies. Color is undesirable in water for laundries (240, 339, 499, 505, 542, 546, 547, 548); ice-making (534); dairy products (549); bottled beverages (180, 181, 186); photography (242); textiles (212, 256, 257, 258, 405, 550); pulp and paper (212, 244, 245, 350, 351, 551, 2372, 2373); for nitrocellulose production (2405) and other uses. For color limits permissible in industrial processes, see Table 6-3.

c. Fish and Other Aquatic Life. A reddish-brown or yellow discoloration has been observed in sea waters where catastrophic mass deaths of fish and shellfish have been associated with the appearance of multitudes of certain poisonous plankton organisms, either algae or protozoa (red tide) (420, 539, 545). Eutrophication of lakes, with concomitant production of algae, generally leads to increased color (2012, 2503, 3351).

TABLE 6-3

COLOR LIMITS PERMISSIBLE IN WATER FOR INDUSTRIAL USE

Use	*Units of color*	*Reference*
Baking	10	159
Boiler feed water	2-80, according to pressure	159
Bottled beverages	5-10	186
Brewing	0-10	159
Dairy Industry	0	2344
Food Equipment Washing	5-20	(see Chapter V)
Food Processing	5-10	159
Food Products	10	173
Nitrocellulose Production	1	2405
Pulp and paper		
Alkaline pulps	5	245, 2372
High-grade paper	5	552, 551, 350
Special papers (tissue, filter, etc.)	5	350
Bleached kraft papers	25	351, 2372
Unbleached kraft papers	100	351, 2372
Groundwood papers	30	351, 2372
Tanning	10-100	159
Textiles		
Cotton	50	256
Rayon	5	550, 405
General	0-70	(see Chapter V)

CONDUCTANCE

(see Specific Electrical Conductance)

CONIINE $C_8H_{17}N$

This colorless, oily, liquid alkaloid with a peculiar mousy odor is very poisonous to humans, causing paral-

ysis of the motor endings of the nerves. It is obtained from the poison hemlock, especially the seeds, and in olden days was used for suicide. In humans, a dose of 30 to 60 mg will cause weakness, drowsiness, and labored respiration and a dose of 120 mg is reported to be fatal (364). At a concentration of 17.1 mg/l in stabilized tap water, however, it showed no effect upon minnows during five days of contact (362).

COPPER Cu

1. General. The effects of copper in water have been reported in terms of copper or of copper salts. This section summarizes literature on the effects of copper in solution without regard to the anion involved. The effects of specific copper salts are described under separate headings. Metallic copper is insoluble in water, but many copper salts are highly soluble as cupric or cuprous ions. References in this section to copper refer to the copper ions.

Copper salts occur in natural surface waters only in trace amounts, up to about 0.05 mg/l, so that their presence is generally the result of pollution, attributable to the corrosive action of the water on copper and brass tubing, to industrial effluents, or frequently to the use of copper compounds for the control of undesirable plankton organisms. Under special conditions, copper has been found in ground water in concentrations up to 12 mg/l. Metallic copper is used in many alloys, for cooking utensils, extensively in the electrical industry, and for pipes, roofing, and many purposes where its conductivity or corrosion resistance are important. Copper salts are used in textile processes, pigmentation, tanning, photography, engraving, electroplating, insecticides, fungicides, and many other industrial processes (364, 2121, 3293).

The chloride, nitrate, and sulfate of divalent copper are highly soluble in water, but the carbonate, hydroxide, oxide, and sulfide are not. Indeed, cupric ions introduced into natural waters at pH 7 or above will quickly precipitate as the hydroxide or as basic copper carbonate, $CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$, to be removed by adsorption and/or sedimentation. As a result, copper ions are not likely to be found in natural surface waters or ground water.

Because copper in concentrations high enough to be dangerous to human beings renders water disagreeable to taste, it is believed that copper is probably not a hazard in domestic supplies. However, copper in water may be disadvantageous or detrimental for certain industrial uses; and although, in trace amounts, copper may be beneficial or even essential for the growth of living organisms, in excessive quantities it has been found toxic to a wide variety of aquatic forms, from bacteria to fish (152, 553).

2. Cross References. Copper Chloride, Copper Sulfate, other copper salts, Chapter VIII—Radioactivity and Chapter IX—Pesticides (Paris Green, Omazene).

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Copper is found in traces in all plant and animal life, and it is believed to be essential for nutrition. The physiological function of copper appears to be involved in the metabolism of iron, for the utilization of iron by the blood-forming organs does not occur properly in the absence of copper (2121, 3293). The copper requirement is reported to be about 2 mg per day for children and about 3 mg per day for adults (152, 553). Common foodstuffs contain up to 10 mg/kg of copper and a few foods such as liver, yeast, and oysters exceed 20-50 mg/kg (3294). The daily intake of copper is generally about 2 to 5 mg/day, but for some people a deficiency exists.

Copper is not considered to be a cumulative systemic poison, like lead or mercury. Most of the copper ingested is excreted by the body and very little is retained. Doses of 60 to 100 mg taken by mouth cause symptoms of gastroenteritis, with nausea and intestinal irritation, but doses of 10 to 30 mg have not caused poisoning even when continued for many days. It is believed that a normal daily diet may provide as much as 20 mg, and that a man weighing 60 kilograms can take one gram of copper per day safely. Many authorities believe that only doses over 100 mg are likely to be injurious (152, 553, 2129). An outbreak of 18 cases of gastroenteritis occurred in a Liverpool, England factory canteen owing to the contamination of tea by copper from a corroded drinking fountain. Analysis showed the copper in this water to be 44 mg/l. Although exhibiting acute copper intoxication, all workers recovered (3295). Although copper has been suspected as a cause of haemachromatosis, a rare liver disease among middleaged men (554), chronic copper poisoning among human beings has never been proved, even among people in copper industries who have absorbed enough copper for their skin and hair to be colored green (36, 152, 553, 2121). There is no evidence that poisoning has ever occurred as a result of consumption of copper in water.

The limiting factor in domestic water supplies is taste. Threshold concentrations for taste have been generally reported in the range of 1.0-2.0 mg/l of copper, while as much as 5-7.5 mg/l makes the water completely undrinkable (152, 553, 555, 556, 3296, 3297, 3298, 3299, 3300). Using cupric chloride in distilled water and in hard spring water (to which carbon-dioxide was added to prevent precipitation of basic copper carbonate), Cohen et al. (3301) found the reactions of a taste panel. The median response for threshold taste was at 6.6 mg/l for distilled water and 12.7 mg/l of copper ion for spring water. Five percent of the panel, however, detected copper in distilled water at 2.6 mg/l and in hard spring water at 5.0 mg/l. It has also been reported (3299) that 1.0 mg/l of copper reacts with soap to produce insoluble green curds, and similar small concentrations cause blue-green stain on porcelain fixtures.

The limit for copper set by the USPHS Drinking Water Standards (2036) has varied markedly during the history of these standards. In 1925 the limit was set at 0.2 mg/l and it was mandatory. In 1942 the limit was raised to 3.0 mg/l and it was a recommended standard but no longer mandatory. In the most recent (1962) standards, the recommended limit was lowered to 1.0 mg/l. This reduction was made primarily because of the taste problem (2062).

The WHO International Drinking Water Standards (2328) set a "permissible" limit of 1.0 mg/l and an "excessive" limit of 1.5 mg/l of copper, but no maximum allowable (mandatory) limit is established. The WHO

European Standards (2329) have a recommended limit of 3.0 mg/l of copper after 16 hours of contact with new pipe in the distribution or plumbing system; but water entering a distribution system should have less than 0.05 mg/l of copper.

b. Industrial Water Supplies. In food-preserving industries, copper causes undesirable color reactions, forming tannates and sulfides. In the milk industry, metals such as copper are catalysts for the oxidation of fatty matter and for the hydrolysis of milk constituents, i.e. they cause fatty, oily, and fishy tastes in the products (223, 2344). Traces of copper in metal-plating baths affect the smoothness and brightness of the metallic deposits (214). No domestic water supply was found containing enough copper to affect photography (560), but 100 mg/l of copper sulfate added to water used for the preparation of developing solutions for photographic work has caused fogging (242). Aluminum utensils have been damaged by water containing a mixture of salts, including copper compounds (561, 562, 3302). There is evidence that dissolved copper can deposit on other metals, especially aluminum and the zinc coating of galvanized steel, thereby markedly accelerating their corrosion (3303, 3304, 3305). Copper is especially undesirable in the woolen industry for it is absorbed readily on wool, e.g. for 5 mg/l of copper in the water solution, the concentration on the treated fabric will be about 70 mg/kg. In the bleaching of wool, copper catalyzes the reaction with hydrogen peroxide, the bleaching agent, to the extent that holes are formed in the material (3306). Reclaimed rubber processed with river water containing 10-25 mg/l of copper deteriorated more rapidly than rubber processed with water containing no copper (1523).

The British Ministry of Agriculture, Fisheries, and Food has established limits for copper in foods and beverages (3294). For ready-to-drink beverages, the limit is 2 mg/l, but for cider and concentrated soft drinks it is 7 mg/l, and for soft-drink concentrates, 20 mg/l. For most foods, the limit is 20 mg/kg, but for yeast and yeast products, 60 mg/kg, and for solid pectin, 300 mg/kg.

c. Irrigation. Minute quantities of copper are beneficial or essential for plant growth (553, 558, 563, 564, 2926, 3307). However, 0.17 to 0.20 mg/l concentrations have been toxic to sugar beets and barley grown in nutrient solution (565). A concentration of 0.02 mg/l was adequate to supply the copper requirement of tomatoes grown in nutrient solution, but 2.0 mg/l was toxic (564). Copper in nutrient solution at a concentration of 0.1 mg/l was toxic to orange and mandarin seedlings (1524) and prevented the growth of water hyacinths (1525). A concentration of 0.5 mg/l or higher was very toxic to flax in water culture (1458). In sand culture, nutrient solutions containing 6.4 to 31.8 mg/l of copper were injurious to sugar beets, tomatoes, and potatoes, but not to oats and kale (1473). Solutions containing 425 mg/l of copper delayed germination and retarded the later growth of cress and mustard seeds in solution culture, while only 106 mg/l retarded growth (1479). In the presence of 0.01 mg/l of molybdenum, concentrations of copper in excess of 0.01 mg/l had a marked effect in decreasing the length of the main stem of tomato roots, and indeed concentrations of copper as low as 0.00001 mg/l had a measurable effect (3308).

In a nutrient solution, one mg/l of copper sulfate stopped the root growth of peas and barley (1479). A concentration of 2 mg/l (as copper) occasionally, but not always, caused slight chlorosis in oats, but no other injury; 10 mg/l caused severe chlorosis; and 20 mg/l stunted growth (1462). A copper sulfate concentration of 1000 mg/l, as copper, partly destroyed the germinating powers of various seeds (1479).

Tobia and Hanna (3309) investigated the chemical fate of copper salts in the soil, especially with respect to organic matter and carbonate content of the soil. They found that when copper sulfate was added to irrigation water at a concentration of 20 mg/l, it reached an equilibrium in the soil at 1.0 mg/l after 6 hours at 20°C. Copper retention in the soil appeared to be correlated more with organic matter and soil alkalinity than with the clay content of the soil, and organic matter appears to be more effective than carbonates in retaining copper.

d. Stock and Wildlife Watering. Small amounts of copper are beneficial for hemoglobin count and growth in rats (353, 553). Copper is beneficial for the growth of suckling pigs and has been used, as copper sulfate, to cure anemia among pigs (553). Copper deficiency in animals is associated with a severe anemia similar to that caused by an iron deficiency. It is generally understood that copper is an active agent in the synthesis of hemoglobin (2121). Traces of copper in the diet of cattle and sheep are necessary to prevent scouring and wasting diseases (566, 1049). The effects of copper and molybdenum are related, and the two elements are reciprocally antagonistic (566, 1049, 1498, 1527).

Daily copper doses of 2 to 4 mg have been harmless to the growth of rats; doses of 6 to 9 mg per day have been harmful; doses amounting to more than one mg per kg of body weight for 5 days are injurious to growth (353).

Hale (553) has reported that one gram daily of soluble copper salts is safe for dogs, but more can be fatal; and that all test sheep died when given 18 to 182.5 grams of copper in quantities as high as 2 grams daily, although two survived for 53 and 128 days respectively. Cartwright (567) has reported that among cattle toxicity develops on one gram of copper sulfate per pound of live weight per day; the symptoms of poisoning include loss of appetite, copper accumulation in the liver, jaundice and yellow discoloration of the entire animal. Added to the daily ration of sheep at a rate of 25 mg per day, copper has been reported as toxic (1526). The minimum toxic level of copper in the diet of chicks was found to be 500 mg/kg in the feed (3310, 3311).

Cunningham (566) has found that cattle can tolerate 5 grams of copper sulfate per day for at least 18 months without toxic effects; for British breeds of sheep, however, doses of 1.5 to 2.0 grams daily are fatal within 30 days; and a dose of 2.5 grams is said to be near the toxic level for small ewes. Cartwright (567) reports that among cattle, copper toxicity develops on a daily dose of one gram of copper sulfate per pound of live weight.

Chronic poisoning of sheep has been caused by 1.5 grams of copper sulfate daily for 30 to 80 days. One sheep was poisoned in 6 months by a daily dose of 0.5

grams but another sheep was not poisoned by one gram per day over a 4-year period (353).

As a therapeutic dose 60 mg of copper sulfate daily has been administered to anemic pigs (553). A dog could take 4 grams daily with only slight effect.

The only reference found concerning the effect of copper sulfate in water states that 2500 mg/l of $CuSO_4 \cdot 5H_2O$ in water was harmful to turkeys when such water was the only drinking water available; 630 mg/l or more sometimes caused the turkeys to stop drinking the water entirely (597).

e. Fish and Other Aquatic Life. (see also Copper Chloride, Copper Nitrate, and Copper Sulfate). The toxicity of copper to aquatic organisms varies significantly not only with the species, but also with the physical and chemical characteristics of the water, such as its temperature, hardness, turbidity, and carbon dioxide content (2407, 3312). In hard water, the toxicity of copper salts is reduced by the precipitation of copper carbonate or other insoluble compounds (553, 3312). Ellis (1076) has found that the toxicity of copper to fish varies greatly depending on the presence of magnesium salts and phosphates. In the control of Chironomus larvae, copper and chlorine together act synergistically to increase the toxicity of each acting alone (568). The sulfates of copper and zinc, and of copper and cadmium are synergistic in their toxic effect on fish (353, 1528, 2973). Synergism also exists between copper and mercury (3313), and between copper and pentochlorophenate (3314). On the other hand, sodium nitrite (311) and sodium nitrate (353) have been reported to decrease the toxicity of copper sulfate to fish, and copper has shown evidence of decreasing the toxicity of cyanide (2965).

Copper concentrations varying from 0.1 to 1.0 mg/l have been found by various investigators to be not toxic for most fish (353, 572, 1467, 1468, 1528, 1529, 3315). On the other hand, concentrations of 0.015 to 3.0 mg/l have been reported as toxic, particularly in soft water, to many kinds of fish, crustacea, mollusks, insects, phytoplankton, and zooplankton (74, 353, 572, 573, 574, 575, 1529, 2093, 2154, 2263, 2271, 2409, 2495, 2921, 2931, 2936, 2941, 2962, 3312, 3316, 3317, 3318, 3319, 3320).

Doudoroff (1528) found that, although fish could survive for 8 hours at 8 mg/l of zinc alone and 0.2 mg/l of copper alone, most fish died within 8 hours when exposed to a mixed solution containing only 1.0 mg/l of zinc and 0.025 mg/l of copper. The Water Pollution Research Board of England conducted similar experiments with rainbow trout in soft and hard waters. The results showed synergism at the higher concentrations in soft water, although the threshold concentration for the mixture of copper and zinc was about the same that would be expected on the assumption that there was no synergism (88). In hard water, no synergism between copper and zinc was evident (74). These English investigators also demonstrated that the toxic threshold concentrations of copper for rainbow trout were diminished as the dissolved-oxygen tension was lowered (74, 3321, 3322).

Barnacles and related marine fouling organisms were killed in 2 hours by 10 to 30 mg/l of copper (569); in 2 to 10 days, by 0.22 to 1.0 mg/l (570). The growth of attached young barnacles was inhibited by 0.2 to 0.3 mg/l (571). *Mytilus edulis,* a mussel, was killed in 12 hours by 0.55 mg/l (570). About 0.1 mg/l of copper in distilled water produced distress in the snail (*Australorbus glabratus*) within a few hours (2733, 2734). Lobsters died within one day after being transferred to wooden tanks lined with copper, although 80 percent had lived for two months in aluminum, stainless steel, or iron tanks (1530).

In contrast, Surber (2688) found that the larvae of the midge, *Cricotopus bicincus,* matured in Michigan rivers at a copper concentration of 2.2 mg/l, although most other common benthic invertebrates were absent from this environment.

Copper is concentrated by plankton from the surrounding waters by factors of 1000 to 5000 or more (2436, 3375, 3386). For further detail on concentration factors, see Chapter VIII, Radioactivity.

Copper concentrations as low as 0.1 to 0.5 mg/l have been reported toxic to bacteria and other microorganisms (353, 574, 576, 577, 3324). Copper concentrations as low as 0.01 to 0.5 mg/l have interfered with B.O.D. determinations (576, 578, 2949, 3323), and also the self-purification processes of streams. However, Rudolfs cites a report that 0.4 mg/l did not interfere with self-purification (579). Also, the concentration of copper required to cause a 50-percent reduction in 5-day B.O.D. has been reported at 8.4 mg/l (2923) and 35 mg/l (2922).

The Severn and Mersey River Boards in England have established working standards that limit effluents to a total concentration of heavy metals, including copper, not to exceed 1.0 mg/l (1756, 2950).

Copper has been used extensively, as copper sulfate, for the control of algae (see Copper Sulfate and also Chapter IX, Pesticides). Ingols (3325) maintains that copper chelated by citrate or lignin is just as effective in controlling algae as the free copper ion, but the chelated copper is not toxic toward fish.

Clendenning and North (2106, 2107, 2865, 2866) evaluated the effect of copper (from the chloride and sulfate salts) upon the rate of photosynthesis of the giant kelp, *Macrocystis pyrifera.* With 0.1 mg/l of copper, net photosynthesis was inhibited 50 percent in 2 to 5 days and 70 percent in 7-9 days. Visible injury appeared within 10 days. Copper was slightly less toxic than mercury, but more so than nickel, chromium, lead, or zinc.

f. Shellfish Culture. For setting, oyster larvae require a copper concentration about 0.05 to 0.06 mg/l (328), but concentrations above 0.1 to 0.5 mg/l were found to be toxic to some oysters (329). The 96-hour TL_m concentration of copper for oysters was estimated at 1.9 mg/l (3326). Oysters cultured in sea water containing 0.13-0.5 mg/l of copper deposited the metal in their bodies and became unfit as a food substance (3326).

4. Summary. On the basis of the foregoing information, the following threshold concentrations of copper are indicated for specfic beneficial uses:

a. Domestic supply	1.0 mg/l
b. Irrigation	0.1 mg/l
c. Fish and aquatic life:	
Fresh water	0.02 mg/l
Sea water	0.05 mg/l

COPPER ACETOARSENITE (PARIS GREEN)

(see Chapter IX)

COPPER CHLORIDE $CuCl_2 \cdot 2H_2O$

(see also Copper and other copper salts)

Used in the electroplating of aluminum, the manufacture of indelible inks, and as a mordant in dyeing and printing fabrics, cupric chloride is highly soluble in water, Cuprous chloride, on the other hand, is almost insoluble in water.

The rapid death of goldfish in dilute solutions of copper chloride (0.009 mg/l as copper) has been reported (1459). The highest concentration of $CuCl_2$ tolerated by young eels for more than 50 hours was 0.13 mg/l (1459). The lethal concentration of copper chloride for three mature small fresh-water fish (*Orizias*), exposed for 24 hours to only 20 ml of test solution, was reported to be 3.3 mg/l (1459). Ellis quotes references to the effect that concentrations of copper chloride of 0.0188 mg/l in distilled water killed goldfish in 3½ to 7 hours; 672 mg/l in distilled water killed minnows in 82 minutes (313).

The 96-hour TL_m of copper chloride toward the bluegill sunfish in soft water at 18-20°C was reported as 1.25 mg/l measured as copper (2936). In Lake Huron water, 5.0 mg/l of copper chloride caused death to the rainbow trout in 10 hours and to the sea lamprey in 12 hours; however, this same concentration had no effect on the bluegill sunfish in 24 hours (2976).

The threshold concentration for immobilization of *Daphnia magna* by cupric chloride added to Lake Erie water was found by Anderson to be 0.027 mg/l (598) or 0.096 mg/l (358). According to another source (353), the threshold has also been reported to be 0.08 mg/l. For cupric ammonium chloride, threshold concentration toward *Daphnia* was 0.039 mg/l (598). In a similar manner, threshold concentrations of other aquatic animals in Lake Erie water in the 20-25°C range were found to be as follows (2955):

Organism	*Threshold Concentration in mg/l For Immobilization*
Daphnia magna, young	0.027
Cyclops vernalis	2.7
Mesocyclops leuckarti	1.9
Diaptomus oregonensis	0.0024

COPPER NITRATE $Cu(NO_3)_2 \cdot 3H_2O$

Highly soluble in water, this copper salt is used in pyrotechnics, textile dyeing and printing, photography, insecticides, and the electroplating industry (364).

Using copper nitrate, Jones found the lethal concentration limit at 15-18°C for sticklebacks to be 0.015 mg/l as copper. At higher concentrations, survival times were as follows: four days at 0.06 mg/l and one day at 0.3 mg/l as copper (1460). The Washington Department of Fisheries (2931) reports the minimum lethal concentration of copper nitrate to be 0.18 mg/l (as copper) in fresh water and 0.56 mg/l (as copper) in sea water. In tests with young pink salmon, the same agency (2091) reported that loss of equilibrium and initial mortalities occurred in less than 66 hours at 0.178 mg/l. There was some indication that the long-term dangerous concentration or lethal threshold was about 0.10 mg/l. In Lake Huron water, 5 mg/l of copper nitrate caused death or obvious distress to rainbow trout in 11 hours and to the sea lamprey in 13 hours. There was no effect toward bluegills in 24 hours.

Ellis (313) quotes a reference to the effect that 0.0188 mg/l of cupric nitrate in tap water killed many tadpoles and interfered with the development of the survivors.

COPPER OXIDES CuO and Cu_2O

(see also Copper and other copper salts)

Copper oxide is only slightly soluble in water and is not stored to any appreciable extent in the human body (580). Up to 0.6 grams of copper oxide daily have been ingested by several investigators over long periods without harmful effects (558).

Wallen et al. (2940) tested cupric oxide (CuO) in highly turbid water at 19-20°C to determine the effect upon the mosquito-fish, *Gambusia affinis*. The 96-hour TL_m was found to be greater than 56,000 mg/l and the turbidity was changed only from 330 to 165 mg/l.

COPPER POTASSIUM CYANIDE

(see Potassium Cupric Cyanide)

COPPER SULFATE $CuSO_4$ and $CuSO_4 \cdot 5H_2O$

1. General. This most common salt of copper, highly soluble in water, is used extensively in industry for tanning, dyeing, electroplating, process engraving, pigment manufacture, and scores of other processes. It is also used in human medicine as a fungicide, bactericide, astringent, and irritant; and also in veterinary medicine as an emetic, as an antihelminthic, and for the treatment of anemia (364).

One of the most important sources of copper pollution in water is the use of copper sulfate for the control of undesirable plankton growth in reservoirs and streams.

Because of variable toxicity of copper to living organisms (see also copper) the killing concentrations of copper sulfate reported by independent workers vary over a wide range. For example, concentrations from 0.01 to 20 mg/l of copper sulfate have been used to control different aquatic fauna, from insect larvae to snails. Concentrations from 0.05 to 10.0 mg/l have been effective against different kinds of algae, protozoa, and weeds. Owing to its extensive use as an algicide, copper sulfate might well be listed as a pesticide in Chapter IX; however, its many other uses justify its inclusion as part of this chapter.

2. Cross References. Copper, other copper salts.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. See Copper.

b. Industrial Water Supplies. See Copper.

c. Irrigation. See Copper.

d. Stock and Wildlife Watering. See Copper.

e. Fish and Aquatic Life. The toxicity of copper sulfate to fish varies with the species and exposure period, and the physical and chemical characteristics of the water (see Copper). Goldfish have been reported to have been killed by as little as 0.002 mg/l (573), and yet they have survived concentrations of one mg/l in hard water for at least the three-day period of study (598). Ander-

son (598) gives 0.016 mg/l as the toxic threshold of copper sulfate for stickleback in tap water. Hale (385) and Hopkins (384) have found 0.14 to 2.0 mg/l to be limiting safe concentrations for many different kinds of fish. Briefly summarizing the data from numerous papers, concentrations from 0.002 mg/l to 200 mg/l or more have been reported lethal to various fish in different waters (74, 313, 353, 359, 363, 415, 573, 586, 598, 599, 600, 601, 602, 603, 604, 605, 606, 1459, 1531, 2091, 2093, 2154, 2314, 2920, 2921, 2936, 2987, 3050, 3327).

On the other hand, concentrations from 0.14 mg/l to 900 mg/l have been reported non-toxic for fish of various species in different kinds of water (313, 359, 415, 572, 592, 602, 605, 607, 2988, 3315).

The highest concentrations of copper sulfate tolerated by specific fish have been reported (384, 385, 1531, 1532, 1533, 2255, 2263) as follows:

Fish	*Concentration in mg/l*	*Fish*	*Concentration in mg/l*
Trout	0.14	Goldfish	0.5
Carp	0.33	Perch	0.67
Suckers	0.33	Black bass and bluegills	0.8
Catfish	0.40	Sunfish	1.35
Pickerel	0.40	Black bass	2.00

Wallen et al. (2940) studied the toxicity of pure chemicals to the mosquito-fish (*Gambusia affinis*) in highly turbid waters. At temperatures of 24-27°C, the 96-hour TL_m for copper sulfate was 75 mg/l. Furthermore high concentrations of this chemical lowered the turbidity from 750 mg/l to less than 25 mg/l.

Fish have been more resistant to copper sulfate in hard, alkaline waters than in soft, acid waters (553, 599, 600, 601, 602, 1011, 2936). Indeed, toward rainbow trout at 17-18°C, copper sulfate was found to be about ten times more toxic in soft water (12 mg/l as $CaCO_3$) (74). The sulfates of copper and zinc and those of copper and cadmium are synergistic in their toxic effect upon fish (353). On the other hand, sodium nitrite has an antagonistic effect on the toxicity of copper sulfate to goldfish; 5000 mg/l of sodium nitrite has decreased the toxic effect of 10 mg/l of copper sulfate, and the addition of 50 mg/l of sodium chloride has still further reduced it (311). Sodium nitrate has also been reported to have antagonistic action against copper sulfate (353).

Turnbull et al. (2093) studied the toxic effect of copper sulfate toward bluegill sunfish at 20°C using a synthetic test water containing 172 mg/l of dissolved solids, 101.2 mg/l of hardness (as $CaCO_3$), 72.0 mg/l of alkalinity (as $CaCO_3$), and pH 8.3. Similar tests were conducted in waters approximately twice and half as mineralized as the standard water. They found the 48-hour TL_m to be 7.0 mg/l of copper sulfate with the standard test water, only 3.0 mg/l in the half-strength water, but 44 mg/l in the double-strength water.

Among other aquatic fauna, *Chironomus* larvae have not been harmed by copper sulfate in concentrations of 5.0 mg/l (568) and 100 mg/l (440, 1079); *Polycelis nigra* has been killed in 48 hours in lake water by 0.47 mg/l (353, 608); *Daphnia magna* has been killed by 0.096 mg/l (353); 0.01 to 0.02 mg/l (573, 598), and 0.08 mg/l (358); and planarian worms have been killed by 1.0 mg/l (573). The addition of 1.0 mg/l of copper sulfate to a culture medium completely inhibited the growth of *Sphaerotilus* (2836).

Using water from the River Havel, from which the test organisms had been recovered, Bringmann and Kuhn (2158, 3343) found that the threshold dosages of copper sulfate that produced a noticeable effect were 0.15 mg/l (as copper) for *Scenedesmus*, 0.10 mg/l (as copper) for *Daphnia*, 0.08 mg/l (as copper) for *Escherichia coli*, and 0.05 mg/l (as copper) for *Microregma*.

Larvae of the gnat, *Simulium dentalosum* was unaffected by 10.0 mg/l of copper sulfate (3316). Also 0.065 mg/l (as copper) had no effect on the fertilization and cleavage of the eggs of the sea urchin, but noticeable abnormalities occurred at 0.16 mg/l (as copper) (3328).

Klock and Pearson (2314) found the first apparent significant response to copper sulfate occurred at 0.02 mg/l for the isopod, *Neosphaeroma oregonensis*, and at 0.32 mg/l for the mussel, *Mytilis edulis*, and the polychaete, *Mercierella enigmatica*.

According to Hale (553), concentrations of copper sulfate ranging from 0.5 to 20.0 mg/l have killed typhoid and cholera bacteria both in solution and in laboratory cultures. Concentrations of 1250 to 2500 mg/l of $CuSO_4 \cdot 5H_2O$ had a sterilizing effect on microorganisms in drinking water (597). Oxygen utilization in a 5-day BOD test was decreased 50 percent by 31 mg/l of copper sulfate (2923).

The concentration of copper sulfate required to control plankton in water-supply reservoirs varies from as little as 0.05 or 0.10 mg/l for some organisms to as much as 12 mg/l for very resistant species (2861). Most algae and protozoa succumb to dosages of 1.0 mg/l or less (1490). Duckweed and pondweed were controlled by 0.6 to 1.0 mg/l of $CuSO_4 \cdot 5H_2O$, but a few cattails and tules survived this residual concentration (1531). To kill snails in swampy areas and streams, 2.0 mg/l of copper sulfate has been recommended (2739). The following rates of application of copper sulfate have been recommended (3329) to control specific types of algae in farm ponds:

Anabaena	0.09 mg/l	*Navicula*	0.07 mg/l
Beggiatoa	5.0 mg/l	*Oscillatoria*	0.1-0.4 mg/l
Chara	0.2-5.0 mg/l	*Scenedesmus*	5.0-10.0 mg/l
Cladophora	1.0 mg/l	*Spirogyra*	0.05-0.3 mg/l
Cladothrix	0.2 mg/l	*Ulothrix*	0.2 mg/l
Conferva	0.4 mg/l	*Volvox*	0.25 mg/l

The amount of copper sulfate required to control algae varies with the temperature, increasing about 2.5 percent for each degree below 15°C (2253). Tests were conducted with 30 different cultures of algae to determine the concentration of $CuSO_4 \cdot 5H_2O$ required for control. None of the species was controlled by 0.25 mg/l of the salt, 13 percent mere controlled by 0.50 mg/l, 37 percent by 1.00 mg/l, and 53 percent by 2.0 mg/l (2987).

The constant application of copper sulfate to lakes and ponds may result in the accumulation of copper in bottom muds and subsequent changes in the bottom populations. In a group of Wisconsin lakes, however, when the maximum concentration of copper in muds was 438 mg/kg no differences in the density of benthal populations could be attributed to copper (1534).

In alkaline waters, copper sulfate was ineffective in killing weeds even at a high concentration of 10 mg/l, owing to the precipitation of copper by hydroxide and carbonate ions (2876).

COPPERAS

(see Ferrous Sulfate)

CO-RAL

(see Chapter IX)

COTTONSEED OIL MONOGLYCERIDE

(see Chapter X)

CREATINE $C_4H_2N_3O_2$

A white, crystalline, nitrogenous substance, creatine is found in the muscles, brains and blood of vertebrates. In decomposing, it exerts a BOD, but otherwise appears to have no detrimental effect. Schaut (362) found that 17.1 mg/l of creatine in stabilized tap water was not harmful to minnows during a 24-hour exposure, but the dissolved oxygen was lowered 6 percent.

CREATININE $C_4H_7N_3O$

(see also Creatine)

Creatinine is easily formed from creatine in aqueous solution in the presence of mineral acid, and as such might be considered as a decomposition product. Creatinine is normally excreted in urine at a daily rate of 25 mg per kg of body weight and it also occurs widely distributed in plants. Like creatine, it appears to be inimical only by its BOD. Schaut (362) reports that 17.1 mg/l of creatinine in stabilized tap water was not harmful to minnows during a 24-hour exposure, but the dissolved oxygen was lowered 32 percent.

CREOSOTING-PLANT WASTES

(see also Phenols, Cresols, Tar)

Wastes from creosoting plants contain mixtures of phenols, cresols, and other organic substances. The toxicity of the mixed wastes depends primarily on phenolic groups and it varies sharply with temperature and the content of dissolved oxygen. According to Ellis (611) as little as 6.0 mg/l of some components can kill fish in less than 10 hours. Smaller concentrations have cumulative effects on the taste of fish, being stored in the fatty tissues and giving a carbolic taste. Such wastes may accumulate also on stream beds and interfere with natural flora and fauna.

CRESOLS $CH_3C_6H_4OH$

1. General. Cresols are colorless oily liquids or crystalline solids obtained from the distillation and chemical treatment of coal tar or wood tar. There are three isomers, all highly soluble in water. A crystalline solid or liquid (m.p. 30°C), o-cresol is used as a solvent or disinfectant. The oral LD_{50} in rats is given as 1,350 mg/kg of body weight. A liquid above 12°C, m-cresol is used in photographic developers and explosives, as well as in disinfecting and fumigating compounds. Its oral LD_{50} in rats is 2020 mg/kg of body weight. A solid below 36°C, p-cresol has an oral LD_{50} in rats of 1800 mg/kg of body weight.

2. Cross References. Cresylic Acid, Phenol, Toluene, Xylenol.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The 1962 USPHS Drinking Water Standards (2036) limit the recommended concentration of "phenols" to 0.001 mg/l. Presumably the cresols are included in this category. The limit has been set on the basis of taste and odor, especially in chlorinated waters (for more details, see Phenol). Ruchhoft (459) quotes references to the effect that, in chlorinated waters, the following concentrations of cresols represent the taste threshold:

Compound	*Lowest Concentration, mg/l, Causing Taste in Chlorinated Water*
o-cresol	0.0001
m-cresol	0.0020
p-cresol	0.0010
o-cresol carbonate	0.0050

According to Cass and Lange (3401), the following thresholds of chlorinated cresols in water have been reported:

Compound	*Taste Threshold, mg/l in Chlorinated Water*		
	pH 4	*pH 7.4*	*pH not given*
o-cresol	0.00002	0.0005	–
m-cresol	0.002	0.005	–
tricresol (a mixture of o-, m-, p-)	–	–	0.0002

Nesmeyanova (3402) gives the taste threshold for cresols in chlorinated water as 0.0002 to 0.001 mg/l.

In odor-free water without chlorine, Hoak (2982, 3403) found the threshold odor concentrations to be as follows:

Compound	*Odor Threshold in mg/l*	
	at 30°C	*at 60°C*
o-cresol	0.071	0.025
m-cresol	0.333	0.100
p-cresol	0.0455	0.200

b. Fish and Other Aquatic Life. The reported effects of o-, m-, and p-cresol on fish are shown in the following tabulations:

Concentration in mg/l	*Type of Fish*	*Effect*	*Reference*
1. *Ortho cresol*			
2.3	salmonide embryos	24-hour TL_m, approx.	3404
4.0–5.0	minnows	detected and avoided	1535
10	perch	lower toxic limit, 15°C	3405
11.2	fingerling catfish	96-hour TL_m, 21°C	2981
15.4–15.8	tench, bleak	24-hour TL_m, approx.	3404
17	perch	immobilized in 10 min., 15°C	3405
20	minnows	immobilized in 54 min., 15°C	3405
24	fathead minnows	48-hour TL_m	3335
29.5	carp	24-hour TL_m, approx.	3404
55–65	sunfish	killed in one hour	363
60	minnows	killed, 17°C	2920
66.8	fingerling catfish	72-hour TL_m, 21°C	2981
70	roach	killed in 3 hours, 21°C	910
110	roach	killed in 5 hours, 9.5°C	910
2. *Meta cresol*			
6.5	salmonide embryos	24-hour TL_m, approx.	3404
10	perch	lower toxic limit, 15°C	3405
19	perch	immobilized in 10 min., 15°C	3405
20	minnows	immobilized in 38 min., 15°C	3405
21	tench, bleak	24-hour TL_m, approx.	3404
24.5	carp	24-hour TL_m, approx.	3404
3. *Para cresol*			
4.0	salmonide embryos	24-hour TL_m, approx.	3404
4.0–5.0	minnows	detected and avoided	1535
5.0	rainbow trout	killed, 17°C	2920
7.5	yearling trout	toxic limit, 1 hour, 13°C	3245
10	perch	lower toxic limit, 15°C	3405
10–15	perch	killed	365
15.4–15.8	tench, bleak	24-hour TL_m, approx.	3404
20	perch	immobilized in 10 min., 15°C	3405
20	minnows	immobilized in 21 min., 15°C	3405
20	minnows	killed, 17°C	2920
21.2	carp	24-hour TL_m, approx.	3403
30–90	sunfish	killed in one hour	363
100	minnows	killed in 30 min.	1535

Concentration in mg/l	Type of Fish	Effect	Reference
4. *Isomer not given*			
8–10	minnows	min. lethal dose, 6 hours, dist. water 20°C	2942
10.0–13.6	bluegill sunfish	96-hour TL_m, 20°C	2935
10–15	fish	killed	935
10–20	goldfish	killed in 3-5 days, hard wtrs.	313
21–14	minnows	min. lethal dose, 6 hours, hard water, 20°C	2942
17–20	fish	killed	313
22	mosquito-fish	96-hour TL_m, turbid water, 17-20°C	2940
26	brook trout	killed in 5.5 min., 22.5°C	3406

One observer, noting that 10 mg/l killed fish, also reported that 0.5 mg/l had no appreciable effect.

The toxicity of cresols to fish appears to vary with the dissolved-oxygen content of the water, with low oxygen being synergistic (1051). Thus, 6.2 mg/l of paracresol in tap water caused trout to float helplessly in 100 minutes when the dissolved oxygen was 10 mg/l; but when the dissolved oxygen was only 3.0 mg/l the trout succumbed in 13 minutes (313).

Southgate (610) studied the toxicity of mixtures of KCN and p-cresol to trout. These substances do not appear to be synergistic, but exert an independent action on fish. Furthermore, concentrations of p-cresol and phenol of equivalent toxicity are interchangeable and appear to exert their toxic action on trout in the same manner. Paracresol and 1, 2, 6-xylenol are only partly interchangeable. Sub-lethal concentrations of 1, 2, 6-xylenol increase the toxicity of p-cresol but sub-lethal concentrations of p-cresol do not appreciably affect the toxicity of xylenol.

Clendenning and North (2106, 2856, 2870) investigated the effects of cresols on the giant kelp, *Macrocystis pyrifera*. The concentration required to cause a 50-percent inactivation of photosynthesis in bottom kelp fronds during a four-day exposure was 5-10 mg/l. Para-cresol was slightly less toxic than o- or m-cresol.

Toward lower aquatic life, the following effects of cresols have been reported:

Concentration in mg/l	Isomer	Organism	Effect	Reference
6	p	*Scenedesmus*	toxic threshold, 24°C	2158
10	--	Crustacea	killed in two hours	609
10	p	*Microregma*	toxic threshold	3343
10	--	*Gammaridae*	not affected	609
12	p	*Daphnia*	toxic threshold, 23°C	2158
16	o	*Daphnia*	toxic threshold, 23°C	2158
20	m	*Microregma*	toxic threshold	3343
20-30	--	*Culex* larvae	reaction threshold	3404
25	--	*Gammaridae*	killed	609
28	m	*Daphnia*	toxic threshold, 23°C	2158
30-40	--	*Daphnia pulex*	reaction threshold	3404
40	o	*Scenedesmus*	toxic threshold, 24°C	2158
40	m	*Scenedesmus*	toxic threshold, 24°C	2158
50	o	*Microregma*	toxic threshold	3343
80-100	--	*Sayomia plum.* larvae	reaction threshold	3404
90-100	--	*Pyonocypris vidua*	reaction threshold	3404
100-120	--	*Culex* pupae	reaction threshold	3404
600	o	*E. coli*	toxic threshold, 27°C	2158
600	m	*E. coli*	toxic threshold, 27°C	2158
940	o	bacteria	inhibited oxygen utilization	2923
1000	p	*E. coli*	not affected, 27°C	2158

CRESOTIC ACID $CH_3C_6H_3OH\ COOH$

(see also Cresols)

Used in the manufacture of dyes, cresotic acids are white to slightly reddish crystals. Ruchhoft (459) quotes references to the effect that, in chlorinated water, the lowest concentrations of cresotinic (cresotic) acids giving taste are:

Compound	Concentration
o-Cresotinic acid	0.01 mg/l
m-Cresotinic acid	1.0 mg/l
p-Cresotinic acid	0.1 mg/l

CRESYLIC ACID

(see also Cresols, Xylenol, and Phenols)

This substance, which is a mixture of isomeric cresols and xylenols from coal tar, mine flotation wastes, and sheep and cattle dips, killed goldfish at a concentration of 0.1 mg/l in hard water in five days and at a concentration of 1.0 mg/l in 6 to 48 hours. In soft water, 0.1 mg/l killed *Daphnia* in 72 hours (313).

Chinook, silver, and pink salmon were exposed for three days in both sea water and fresh water to varying concentrations of refined and crude cresylic acid (2091). Concentrations of 3.12 to 6.98 mg/l gave 100 percent kills, and the critical levels were estimated to be as low as 1.65 mg/l. Using a commercial impure o-cresylic acid, Clemens and Sneed (2981) found its 96-hour TL_m toward fingerling channel catfish to be 11.2 mg/l at 21°C. No fish were killed by 4.0 mg/l and all succumbed to 15.8 mg/l. Using refined cresylic acid, the Washington State Department of Fisheries (2091) found that concentrations of 3.12 to 6.60 mg/l killed most of the chinook and silver salmon exposed for three days in both fresh water and sea water. Critical levels were given as 1.65 to 3.40 mg/l. With crude cresylic acid, the lowest concentrations causing a significant kill varied from 3.27 to 8.69 mg/l and the critical levels were estimated at 3.27 to 6.1 mg/l.

Commercial preparations of cresylic acid in sulfonated oil have been used as insecticides and larvacides. Knowles, et al. (612) found that 25 gallons per acre of this preparation killed 29 percent of goldfish and 25 percent of minnows in 24 hours.

CUPRIC CHLORIDE

(see Copper Chloride)

CYANIDES CN^-

1. General. Under this heading are grouped all of the references that cite cyanides as toxic or deleterious compounds and give concentrations in terms of the cyanide ion (CN^-) without specifying the salt. Cyanides occur in the effluents from gas works and coke ovens, from the scrubbing of gases at steel plants, from metal cleaning and electroplating processes, and from chemical industries.

Attention is invited particularly to the general discussion under "Hydrogen Cyanide" with reference to the fact that HCN is largely undissociated at pH values of 8 and less. Hence, when toxicities are expressed in terms of the cyanide ion it must be realized that most of the cyanide in water is in the form of HCN. It is apparent that HCN rather than the cyanide ion is the major toxic principle. It must also be recognized that toxicities may vary markedly with pH and that a given concentration that is innocuous at pH 8 may become detrimental if the pH is lowered to 6 or less. On the other hand, Southgate

(346) reported that within the range of 6.0 to 8.5, pH had no appreciable effect on the toxicity of cyanide. In natural streams, cyanides deteriorate or are decomposed by bacterial action, so that excessive concentrations may be expected to diminish with time. Renn (2968) found a 90-95 percent removal of cyanides in percolation of waste waters through soil columns. Removal was greater in soils rich with organic matter than in clean sand or sandy clay. According to Ware (3407) the biochemical degradation of cyanide is little affected by temperature in the range of 10° to 35°C, but at lower or higher temperatures, decomposition proceeds much more slowly.

2. Cross References. Ammonium Thiocyanate, Nitriles, Cyanogen Chloride, Hydrogen Cyanide, Potassium Cyanate, Potassium Cyanide, Potassium Ferricyanide, Potassium Thiocyanate, Sodium Cyanate, Sodium Cyanide, Sodium Ferri-cyanide, Sodium Thiocyanate.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The WHO International (2328) and WHO European (2329) Drinking Water Standards both set a maximum allowable limit of 0.01 mg/l for cyanides, as CN^-. For the first time in 1962, the U.S.P.H.S. Drinking Water Standards (2036) set a recommended limit of 0.01 mg/l and a mandatory limit of 0.2 mg/l.

According to Klein (1756) the odor threshold for hydrogen cyanide in water is 0.001 mg/l. The maximum safe total ingestion of cyanide by humans has been estimated at something less than 18 mg/day, part of which will come from the normal environment and industrial exposure (2963).

It has been postulated (731, 733, 1119) that there may be a relation between cyanide intake and the incidence of poliomyelitis and pellagra. In a report from the Kettering Laboratory of the College of Medicine, University of Cincinnati, April, 1952, Dr. J. S. Cass pointed out that symptoms of cyanide poisoning are quite different from those of poliomyelitis. According to Cass, the body normally takes in small amounts of substances which contain cyanides, e.g., members of the cabbage family. The CN ion, except for a small portion exhaled, is changed rapidly to the relatively non-toxic sulfur complex (SCN, thiocyanate) in the liver, and is eliminated irregularly and slowly in the urine. There is no evidence that CN ion is stored in the body. Small doses of CN, incapable in themselves of causing intoxication, can be disposed of indefinitely in the body, since the natural detoxifying mechanisms are practically inexhaustible, although slow.

b. Stock and Wildlife Watering. See Hydrogen Cyanide and Sodium Cyanide.

c. Fish and Other Aquatic Life. The toxicity of cyanides toward fish is affected by the pH, temperature, dissolved oxygen, and concentration of minerals. As noted above and also under Hydrogen Cyanide, HCN appears to be the major toxic principle rather than CN^-; hence the lower the pH the greater the proportion of undissociated HCN. The toxicity of cyanide is also increased at elevated temperatures, a rise of 10°C producing a two- to three-fold increase in the rate of lethal action (3034, 3408). Low tensions of dissolved oxygen also increase the toxic action of cyanides (2109). Southgate (2411) reports the median period of survival of trout at 17°C in a solution containing 0.105 mg/l of CN to be about 8 hours at 95 percent oxygen saturation; about 5 hours at 73 percent, and only 10 minutes at 45 percent saturation. Certain metals, such as nickel, may complex with cyanide to reduce lethality especially at higher pH values, but zinc and cadmium cyanide complexes are exceedingly toxic (2157).

When fish are poisoned by cyanide, the gills become considerably brighter in color than those of normal fish, owing to the inhibition by cyanide of the oxidase responsible for the transferring of oxygen from the blood to the tissues (346). The toxicities of cyanides toward fish have been reported as shown below; (but see also HCN, KCN, and NaCN).

Cyanides in mg/l	*Time of Exposure*	*Type of Fish*	*Remarks*	*Reference*
0.05	120-136 hrs.	brook trout	all killed	1120
0.05–0.10	--	fish	MLD	1121
0.05	5 days	trout	lethal	2977
0.07	74 hours	rainbow trout	overturned	1580, 1645
0.1	1 day	fish	overturned	3409
0.1	--	fish	MLD	1122, 1123
0.1–0.2	1 to 2 days	rainbow trout	MLD	346
0.12–0.18	96-hour TL_m	bluegill sunfish	hard and soft water	2936
0.126	170 minutes	trout	overturned	353
0.15	4.8 to 64 min.	trout	overturned	659
0.176	--	bluegill, sunfish	toxic limit	353
0.18	24-hour TL_m	bluegill sunfish	soft water	2093
0.2	11 min.	rainbow trout	MLD	3410
0.2	150 min.	fish	overturned	3409
0.2	--	fish	killed rapidly	1124
0.33	2.5 hours	adult chub	killed	1465
0.42	11 min.	trout	overturned	353
0.50	141 min.	adult chub	MLD	2970
1.0	20 min.	trout	all killed	1120
10.0	1.5 hours	carp	MLD	936

The following concentrations of cyanides were survived by fish:

Cyanides in mg/l	*Time of Exposure*	*Type of Fish*	*Remarks*	*Reference*
0.02	27 days	trout	survived	1120
0.084	--	trout	not toxic	353
0.20	--	adult chub	survived	2970
0.25	--	bluegills	survived	353
0.375	--	bullheads	survived	353
0.4	96 hours	bluegills	survived	353
0.5	96 hours	bullheads	survived	353

In its Third Progress Report, the Aquatic Life Advisory Committee of the Ohio River Valley Water Sanitation Commission (2109) reviewed much of the research on cyanides and recommended that concentrations of free cyanide in excess of 0.025 mg/l be considered unsafe in the waters of the Ohio River.

Toward lower organisms, cyanide does not appear to be as toxic as toward fish. Surber (2688) reported that larvae of the midge, *Cricotopus bicinctus*, survived electroplating wastes that had cyanide concentrations up to 3.2 mg/l. For *Daphnia magna* the toxicity level for a 48-hour exposure was given at 3.4 mg/l (2977). The amoeba, *Mayorella palestinensis*, was killed at a cyanide concentration of 130 mg/l (2969), but a significant inhibition of photosynthesis by *Chlorella* did not occur until the cyanide concentration reached 7300 mg/l. The organisms that exert BOD were found to be inhibited at 1.0 mg/l (2962) and at 60 mg/l (3411) although the effect is more one of delay in exertion of BOD than total reduction.

CYANOBUTADIENE

According to Daugherty and Garrett (1441, 2959, 2960) 1-cyano-1, 3-butadiene exhibited the following effect upon the marine pinperch, *Lagodon rhomeboides:*

Maximum concentration at which no deaths occurred	50 mg/l
Minimum concentration at which deaths occurred	60 mg/l
24-hour TL_m	71.5 mg/l

CYANOGEN CHLORIDE CNCl

(see also Hydrogen Cyanide)

Cyanogen chloride is formed when sewage effluents containing gas liquor are chlorinated, by action of the chlorine on the hydrogen cyanide. Concentrations of cyanogen chloride ranging from 1.0 to 0.08 mg/l have killed fish (361, 1129, 1130).

CYCLIC QUATERNARY AMMONIUM COMPOUND

(see Chapter X)

CYCLOHEXANE AND CYCLOHEXENE C_6H_{12} and C_6H_{10}

(see also Benzene)

These cycloparaffins, found in petroleum, are used as solvents for lacquers and resins, or for paint remover. They are slightly more toxic to fish than is benzene. The oral LD_{50} for rats has been reported (2980) to be 29,820 mg/kg of body weight. According to Hubault (462), 10 mg/l of cyclohexene was toxic in four hours at 11.5°C and 10 mg/l of cyclohexene in 3.16 hours at 9°C. These compounds produce a state of torpor in the fish.

The toxicity of these hydrocarbons is diminished by the addition of a methyl group to form methylcyclohexane or methylcyclohexene. Hubault (462) reports that 50 mg/l of methylcyclohexane was toxic to freshwater fish in 3.67 hours at 10.5°C. This is five times the toxic concentration of straight cyclohexane. In Lake Huron water at 12°C, cyclohexene at a concentration of 5 mg/l has no effect on rainbow trout or the sea lamprey during a 24-hour exposure (2976). Using turbid water at 21-24°C and mosquito-fish (*Gambusia affinis*) as the test organism, Wallen et al. (2940) found the 24-, 48-, and 96-hour TL_m values to be 15,500 mg/l.

CYCLOHEXANE CARBOXYLIC ACID $C_6H_{11}COOH$

(also known as Naphthenic Acid)

This odorless solid, slightly soluble in water, is extracted from petroleum. It is used in insecticide formulations in paper mills, as a clarifier for mineral oil, and as a solubilizer for vulcanized rubber (364). It is reported to be fatal to European perch in concentrations of 4 to 16 mg/l (463, 614, 615). Crayfish were killed in 18 to 60 hours by exposure to 5 to 50 mg/l of this acid (614, 615), and minnows were killed in 72 hours by a concentration of 5.0 mg/l. At low concentrations it imparts a taste to the flesh of fish (616).

Cairns et al. (2936, 2954, 2957) found the following 96-hour TL_m values for the bluegill sunfish (*Lepomis macrochirus*), a pulmonate snail (*Physa heterostropha*), and the diatom (*Navicula seminulum*) in hard and soft water:

Organism	*Temperature °C*	*96-hour TL_m in mg/l* Soft Water	Hard Water
Fish	18–20	5.6	7.1
	30	5.6	7.0
Diatom	22	43.1	79.8
	28	41.8	56.0
	30	43.4	28.2
Snail	20	6.6–7.5	11.8
	30	18–19	11.7

As this table shows, the toxicity of naphthenic acid is not greatly affected by temperature or hardness. Periodic low oxygen tensions, however, lowered the TL_m values for the snail and fish to 2.0 mg/l at 20°C in soft water.

2, 4-D

(see Chapter IX)

DAIRY WASTES

(see also Lactic acid, Sugars, Soaps, Synthetic Detergents, BOD, and Chapter X)

Dairy wastes contain soaps and detergents, lactic acid, sugars, whey, and other substances that may exert a deleterious effect on a stream, either directly or indirectly. In some instances, fresh milk wastes have been beneficial by stimulating the production of fish-food organisms, especially Chironomus larvae (601). At a dilution of 1:240, dairy wastes had no effect on minnows (362), but at 1:15 dilution, other milk wastes were on the verge of distressing fish (601). At a dilution of 1:35, whey has been shown to be toxic to fish in a few hours. Cole (311) reports that dairy wastes contain soaps that are toxic to bass at concentrations of 600 mg/l. Lactic acid can be toxic to fish at 654 mg/l, or even less at low dissolved-oxygen contents.

DALAPON

(see Chapter IX)

DDD

(see Chapter IX)

DDT

(see Chapter X)

DECABORANE

(see Boron)

DECYL BENZENE SODIUM SULFONA

(see Chapter X)

DELRAD (RADA)

(see Chapter IX)

DERRIS ROOT

(see Chapter IX, Rotenone)

DETERGENTS

(see Chapter X)

DEXTROSE $C_6H_{12}O_6$

(see also Sugars)

This crystalline sugar, which occurs widely in plants and animals, does not exhibit any toxic action or dele-

terious effects other than osmotic pressure and B. O. D. In stabilized tap water kept saturated with oxygen, Schaut (362) showed that 17.1 mg/l of dextrose had no effect on minnows during 24 hours of observation.

DIAZINON

(see Chapter IX)

DIBORANE

(see Boron)

DIBUTYL PHENYL PHENOL SODIUM DISULFONATE

(see Chapter X)

DICAPTHON

(see Chapter IX)

DICHLONE

(see Chapter IX-Dichloronaphthoquinone)

DICHLOROBENZENE $C_6H_4Cl_2$

Metadichlorobenzene is a colorless liquid, insoluble in water, and seldom used commercially. Orthodichlorobenzene, also a liquid and insoluble in water, is used as a solvent for waxes, for preserving plants, and for destroying insects such as termites. Paradichlorobenzene, a white crystalline solid with a characteristic odor, is used largely for killing moths, their larvae, and other insects. It is soluble in water to the extent of 70 mg/l at 25°C. Demyanenko (313, 431) reports that a concentration of 50 mg/l of p-dichlorobenzene was lethal to fish.

DICHLORODIPHENYLTRICHLOROETHANE

(see Chapter IX-DDT)

DICHLOROETHANE $C_2H_4Cl_2$

Known also as ethylene dichloride, this heavy liquid with a pleasant odor and sweet taste is highly soluble in water. It is used as an organic solvent in industry and in the manufacture of tobacco extract. The oral LD_{50} for rats is given as 770 mg/kg of body weight (364). According to Garrett (2959) the TL_m for the marine pinperch was 250-275 mg/l for 1, 1-dichloroethane and 150-175 mg/l for 1, 2-dichloroethane.

DICHLORONAPHTHOQUINONE

(see Chapter IX)

DICHLOROPHENOL

(see Halogenated Phenols, also Chapter IX)

DICHLOROPHENOXY ACETIC ACID (2, 4-D)

(see Chapter IX)

DICHLOROPROPENE $C_3H_4Cl_2$

This liquid with a chloroform-like odor is used as a soil fumigant for the control of nematodes (364). The threshold of toxicity toward *Daphnia* and *Scenedesmus* has been reported as 40 mg/l. For the protozoan *Microregma* the toxic level is 100 mg/l (2977).

DICHROMATES

(see Chromium)

DIELDRIN

(see Chapter IX)

DIETHANOLAMINE $(C_2H_4OH)_2NH$

This deliquescent solid forms a viscous liquid that is miscible with water with an ammoniacal odor and a strong alkaline reaction, apparently from dissociation and the formation of ammonium ion. It is used in the petroleum and rubber industries, for the manufacture of surface-active agents, and for organic synthesis (364).

According to Gillette et al. (1442) the critical range for creek chub, a fish of average tolerance, exposed for 24 hours in well-aerated water at 15-21°C, was 80 to 120 mg/l, i.e., all fish survived 80 mg/l but all died at 120 mg/l. Hence, the 24-hour TL_m was approximately 100 mg/l. Turnbull et al. (2093) used bluegill sunfish (*Lepomis macrochirus*) in aerated water at 20°C and found the 24-hour TL_m to be 2100 mg/l and the 48-hour TL_m to be 1850 mg/l. In highly turbid water with the mosquito-fish (*Gambusia affinis*), Wallen et al. (2940) found the 24-, 48-, and 96-hour TL_m values to be 1800, 1550, and 1400 mg/l respectively. High dosages of this compound raised the pH value to 10.4 but did not clarify the turbidity.

DIETHYLAMINE $(C_2H_5)_2NH$

This flammable liquid is miscible with water, producing a strong alkaline reaction, apparently from its dissociation and the formation of ammonium ions. It is used in the rubber and petroleum industry and for the manufacture of resins, dyes, and pharmaceuticals (364). The oral LD_{50} for rats has been reported as 540 mg/kg of body weight (364).

Gillette et al. (1442) found that creek chub, a fish of average tolerance, in well-aerated water at 15-21°C withstood 70 mg/l of diethylamine for 24 hours, but all died at 100 mg/l. The approximate 24-hour TL_m was about 85 mg/l. Bringmann and Kuhn (2158, 3343) reported that the toxic threshold for *Scenedesmus* during 4 days of exposure at 24°C was only 4 mg/l, while for *Daphnia* during two days at 23°C the threshold concentration was 100 mg/l and for *Microregma*, 70 mg/l. There was no apparent effect on *E. coli* at concentrations up to 1000 mg/l.

DIETHYLENE GLYCOL $C_4H_{10}O_3$

This colorless liquid with a sweetish taste is miscible with water. It is used in antifreeze solutions, as a finishing agent for textiles, as a solvent for vat dyes, and in the composition of many products (364). In water of 1500 mg/l turbidity at 23-25°C, Wallen et al. (2940) found the 96-hour TL_m toward the mosquito-fish (*Gambusia affinis*) to be over 32,000 mg/l.

DIISOBUTYL CRESOXYMETHOXY ETHYLDIMETHYL BENZYL AMMONIUM CHLORIDE

DIISOBUTYL PHENOXYETHOXY ETHYLDIMETHYL BENZYL AMMONIUM CHLORIDE

(see Chapter X)

DILAN

(see Chapter IX)

DIMETHYLAMINE $(CH_3)_2NH$

A gas at ordinary temperatures, dimethylamine is highly soluble in water with a strong alkaline reaction, apparently from dissociation and the formation of ammonium ions. It is used industrially in tanning operations, in the manufacture of detergents, and in vulcanizing rubber. The threshold odor concentration has been reported (3412) as 0.6 ml/l.

According to Gillette et al. (1442) the critical range for creek chub, a fish of average tolerance, exposed for 24 hours in well-aerated water at 15-21°C, was 30 to 50 mg/l, i.e. all fish survived 30 mg/l but all died at 50 mg/l. Corti (146) tested rainbow trout *(Salmo irideus)* at a dimethylamine concentration of 205 mg/l. The resulting pH was 10.3 and the temperature was 13°C. The fish showed their first reaction in 33 to 40 seconds, turned over in 3 to 4 minutes, and made their last movement at 10 to 13 minutes.

DIMETHYLBENZYLAMMONIUM CHLORIDE

(see Chapter X)

DIMETHYLDIOXANE $C_6H_{12}O_2$

According to Klimkina (3413) this compound, used in organic synthesis, may be found in effluents. He recommends a maximum permissible concentration in water of 0.005 mg/l, based on toxic effects to aquatic life. The odor threshold is 2.5 mg/l, but a concentration of 30 mg/l does not inhibit oxygen consumption in biochemical degradation of wastes.

DINITROBENZENE $C_6H_4(NO_2)_2$

(see also Nitrobenzene, Trinitrobenzene)

The meta-, ortho-, and para-forms of this compound are crystalline solids that are moderately soluble in water. The oral LD_{50} for p-dinitrobenzene in cats is given as 29.4 mg/kg of body weight (364). According to LeClerc (2942), the minimum lethal dose of dinitrobenzene (isomer not specified) toward minnows during a 6-hour exposure at 23°C was 10-12 mg/l in distilled water and 8-10 mg/l in hard water. The lethal level for fish has also been given as 2.0 mg/l (2977).

DINITROCRESOLS $(NO_2)_2C_6H_2OHCH_3$

Sparingly soluble in water, 4,6-dinitro-o-cresol is used as a selective herbicide and insecticide. The oral LD_{50} in rats is given as 30 mg/kg of body weight (364). According to LeClerc (2942) the lowest concentrations that caused minnows to overturn within six hours were 1.5-2.0 mg/l in distilled water at 23°C and 3.0-4.0 mg/l in hard water at 18°C. Using River Havel water, Bringmann and Kuhn (2158, 3343) found that the beginning of toxic effects appeared at 8 mg/l for *Daphnia* at 23°C, 30 mg/l for the protozoan *Microregma,* 36 mg/l for the alga *Scenedesmus* at 24°C, and 100 mg/l for *E. coli* at 27°C.

2,4-dinitro-6-methyl cresol (DNC) is used as a herbicide. At 1.0 mg/l, the sodium salt of DNC caused the death of fish and snails in aquaria (3009).

DINITROPHENOLS $(NO_2)_2C_6H_3OH$

(see also Phenol, Nitrophenol, Trinitrophenol)

The nitration of phenol with dilute nitric acid produces first the ortho or para nitrophenols, then 2,4-dinitrophenol, and finally 2,4,6-trinitrophenol, known as picric acid. These various substances may occur in chemical, dye, and ammunition-plant wastes. Grindley (617) reports that the threshold concentration of dinitrophenol, below which neutral solutions were not toxic to minnows, was 30 mg/l. In solutions of 200 mg/l in neutral water, minnows overturned in 22 minutes. LeClerc (2942) observed that the minimum concentration of m-dinitrophenol causing minnows to overturn within 6 hours at 22-23°C was 35-38 mg/l in hard water, but only 0.5-1.0 mg/l in distilled water.

In River Havel water, the thresholds of toxic effect of 2,4-dinitrophenol were found by Bringmann and Kuhn (2158) to be 6 mg/l for *Daphnia* at 23°C, 40 mg/l for *Scenedesmus* at 24°C, and over 100 mg/l for *E. coli* at 27°C. Hermann (2923) reported that 100 mg/l of this compound caused a 50-percent decrease in oxygen utilization in the BOD test. According to Batte et al. (2993), 10 mg/l caused complete mortality to *Lymnaeid* snails within 24 hours.

2,4-dinitro-6-*sec*-butylphenol (DNBP) and 2,4-dinitro-o-cyclohexylphenol are used as pesticides, especially as herbicides. Small trout have died from concentrations of 100 mg/l of DNBP and from 12 mg/l of its sodium salt. DNCHP in concentrations of 1 to 10 mg/l killed all fish (3009).

DIOCTYL ESTER OF SODIUM SULFOSUCCINIC ACID

(see Chapter X)

DIPTEREX

(see Chapter IX)

DISODIUM HYDROGEN PHOSPHATE

(see Sodium Phosphates)

DISODIUM SALT OF DIBUTYL PHENYLPHENOL DISULFONATE

(see Chapter X)

DISSOLVED OXYGEN

1. General. Dissolved oxygen in natural-waters cannot rightfully be designated as a primary pollutant, but it does come under the category of a corollary pollutant because excessive dissolved oxygen arising from algal growths may adversely or unreasonably affect such waters for one or more beneficial uses. It is not with a surplus of dissolved oxygen, however, that the sanitary engineer is normally concerned, but rather with a deficiency or complete absence thereof. Inadequate dissolved oxygen in surface waters may contribute to an unfavor-

able environment for fish and other aquatic life, as explained below, and the absence of dissolved oxygen may give rise to odoriferous products of anaerobic decomposition.

The content of dissolved oxygen in water at equilibrium with a normal atmosphere is a function of the temperature and salinity of the water, the ability of water to hold oxygen decreasing with increases in temperature or dissolved solids. Tables of saturated dissolved-oxygen values are available in textbooks and handbooks (330, 911). Natural waters, however, are seldom at equilibrium and seldom exactly saturated with dissolved oxygen, for temperatures are changing and physical, chemical, bio-chemical, or biological activities are utilizing or liberating oxygen. For a formulation and discussion of deoxygenation and reaeration in surface water, see Chapter II.

2. Cross References. B.O.D., Algae, Ozone and Nascent Oxygen, Chapter II, and Chapter V.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The presence of dissolved oxygen in municipal water supplies is seldom considered to be deleterious, for it has no adverse physiological effect and actually increases the palatability of the water. It is detrimental, however, in the corrosion of metal pipes and efforts are sometimes made to eliminate or decrease the dissolved-oxygen content of water to inhibit corrosion (1149). Methods of treatment for corrosion control are not within the province of this report, and since dissolved oxygen is a natural constituent of surface waters, its planned suppression does not appear to be a function of water-pollution control.

b. Industrial Water Supplies. For many industrial uses of water, zero dissolved oxygen would be desirable as a means of inhibiting corrosion (1149), especially in cooling waters. In practice, however, industries anticipate the use of normal surface waters containing dissolved oxygen, or ground waters that acquire dissolved oxygen in the process of handling. In packaged power reactors, the dissolved-oxygen content of water should not exceed 0.03 mg/l to prevent stress corrosion of stainless steel (3395). For boiler feed water, the limiting dissolved-oxygen concentrations have been given as 2.0 mg/l for boiler pressures of 0 to 150 psi; 0.2 mg/l for 150 to 250 psi; and 0.0 mg/l for 250 psi and up (152).

c. Fish and Other Aquatic Life. There is a great deal of literature pertaining to the minimum dissolved-oxygen concentration necessary to sustain healthy aquatic life, especially fish, and the concentration below which fish will be killed by short-time exposures. To tabulate all of this literature would be meaningless, for much of it is repetitious and much of it cannot be correlated. No general statement can be made to give the minimum dissolved-oxygen concentration required to support fish life, owing to the fact that the oxygen requirements of fish vary with the species and age of the fish, with prior acclimatization, with temperature, with the concentration of other substances in the water, and with several other factors. A thorough summary of the effects of low concentrations of dissolved oxygen toward fish is given by Southgate (346), and by the Aquatic Life Advisory Committee of ORSANCO (2109, 2408, 2414). Much of the following material is condensed from those sources. For a further discussion of this subject, see also Chapter V—Propagation of Fish and Other Aquatic Life.

Temperature affects not only the amount of available dissolved oxygen in water but also the rate at which fish utilize such oxygen. At 6.2°C, goldfish consumed 16.1 ml per kg per hour; at 15°C, 50.1 ml per kg per hour; and at 20.8°C, 90.4 ml of oxygen per kg of fish flesh per hour. As a result of such oxygen requirements, the minimum concentration at which fish are asphyxiated will be higher at higher temperatures, e.g. for trout 1.16 mg/l at 10°C and 2.14 mg/l at 18°C for relatively short exposures. For longer exposures, the asphyxial points would probably be much higher; yet it has been shown that fish may, under natural conditions, become acclimatized to low oxygen concentrations. Within certain limits, fish can adjust their rate of respiration to compensate for changes in the concentration of dissolved oxygen. It is generally agreed, moreover, that those species which are sluggish in movement (e.g., carp, pike, eel) can withstand lower oxygen concentrations than fish (such as trout or salmon) which are more lively in habit.

At 13°C, according to Jones (1543), the stickleback has no immediate appreciation of water of low D.O. content and will swim into it with no hesitation. If the fish remain in water of low D.O., respiratory distress develops, accompanied by uneasiness and random movement. When the random movement carries the fish back into oxygenated water the fish recover quickly. Over a wide range of dissolved oxygen (from less than 3.0 mg/l up to 6.6 mg/l at 13°C this reaction takes 1-3 minutes. At 3°C, however, the reaction is very slow, taking 5-6 minutes even when water contains as little as 0.3 mg/l of dissolved oxygen. At 20°C and with D.O. values below 2 mg/l, response is very prompt with stress developing so quickly that fish will usually not swim into the poorly oxygenated water. Results with trout and minnows were similar (1543). Whitmore, Warren, and Doudoroff (3447) also reported on avoidance reactions of juvenile chinook salmon, coho salmon, largemouth bass, and bluegills.

The lethal effect of low concentrations of dissolved oxygen appears to be increased by the presence of toxic substances, such as excessive dissolved carbon dioxide, ammonia, cyanides, zinc, lead, copper, or cresols (144, 2109, 2957, 2964, 3321, 3324, 3228). With so many factors influencing the effect of oxygen deficiency, it is difficult to estimate the minimum safe concentration at which fish will be unharmed under natural conditions.

Extensive studies have been made by Moore (865) in which fish of several species were confined in boxes of wire netting and lowered to varying depths in lakes. The concentration of dissolved oxygen and the temperature were noted at each depth, and the reactions of the fish were observed. His results, summarized in Table 6-4, show that the median fish will die at concentrations of 3.1 mg/l in summer and 1.4 mg/l in winter, and will live at 4.2 mg/l in summer and 3.1 mg/l in winter.

In a somewhat similar study, the Water Pollution Research Board of England (2952) found the minimum

oxygen tensions at which various fish would survive for 84 hours at three temperatures to be as follows:

Fish	Minimum Dissolved Oxygen Concentration in mg/l at 10°C	16°C	20°C
Rainbow trout	1.89	3.00	2.64
Perch	1.05	1.34	1.25
Roach	0.65	0.71	1.42
Mirror carp	0.48	1.73	3.74
Tench	0.35	0.54	---
Dace	---	1.14	---
Bleak	---	1.50	---

TABLE 6-4

THRESHOLDS OF DISSOLVED OXYGEN FOR FISH

Data After Moore (865)

Species	SUMMER: Lowest observed concentration at which fish survived for 24 hours	SUMMER: Highest observed concentration at which fish were killed in 24 hours	WINTER: Lowest observed concentration at which fish survived for 48 hours	WINTER: Highest observed concentration at which fish were killed in 48 hours
Pike	6.0 mg/l	3.1 mg/l	3.1 mg/l	2.3 mg/l
Black bass	5.5 mg/l	3.1 mg/l	4.7 mg/l	2.3 mg/l
Black crappie	5.5 mg/l	4.2 mg/l	1.5 mg/l	1.4 mg/l
Common sunfish	4.2 mg/l	3.1 mg/l	1.4 mg/l	0.8 mg/l
Yellow perch	4.2 mg/l	3.1 mg/l	4.7 mg/l	1.5 mg/l
Sunfish	3.3 mg/l	3.1 mg/l	3.5 mg/l	0.8 mg/l
Blk. bullhead	3.3 mg/l	2.9 mg/l	1.1 mg/l	0.3 mg/l
Median values	4.2 mg/l	3.1 mg/l	3.1 mg/l	1.4 mg/l

Ellis (247, 313) indicated that under average stream conditions, 3.0 mg/l of dissolved oxygen, or less, should be regarded as hazardous or lethal, and that to maintain a varied fish fauna in good condition the dissolved-oxygen concentration should remain at 5.0 mg/l or higher. For trout in soft water, the lower limit has been set as high as 6.0 mg/l (1155). Quoting other authorities, Ellis (1156) reports that sustained dissolved-oxygen concentrations of 2.0 mg/l will kill all kinds of fish, 3.5 mg/l will be lethal to most species, and few fish will be found at under 4.5 mg/l. In contrast, salmon parr have been reported (1157) to live at least five days at 2.2 mg/l of dissolved oxygen, and in winter, fish have been reported (865) to tolerate 1.0 to 2.0 mg/l of dissolved oxygen for a short time. Numerous other references describe the effect of lowered oxygen tensions on various species of fish under many conditions of exposure (2409, 2952, 3057, 3321, 3414, 3415, 3416, 3417, 3418, 3419, 3420, 3421, 3422, 3423, 3424, 3425, 3426, 3427, 3428, 3429, 3430, 3431, 3432, 3433, 3434, 3435, 3436, 3437, 3438, 3439, 3440, 3441.)

Several factors aside from the deoxygenating effects of pollutants influence the concentration of dissolved oxygen in surface waters. There is a diurnal variation owing to the photosynthetic action of algae during daylight hours and their respiration at night (2279, 2280, 2972). Indeed, heavy fish mortalities have arisen from the oxygen demand caused by the decomposition of algae (2283). There is also a variation of oxygen with the depth of water, especially in lakes and stagnant ponds. For this reason, fish may avoid the deeper, cooler waters and be forced to remain in shallow warm areas (3442). Dissolved-oxygen concentrations near the bottom muds of lakes and sluggish rivers may approach zero. Under such conditions, the hatching of fish eggs has been delayed, or the fish hatching from such eggs have been deformed (3443, 3444, 3445, 3446).

The Aquatic Life Advisory Committee of ORSANCO (2408) has recommended that the minimum permissible oxygen concentration for a well-rounded warm-water fish population be as follows: The dissolved-oxygen content of warm-water fish habitats shall be not less than 5 mg/l during at least 16 hours of any 24-hour period. It may be less than 5 mg/l for a period not to exceed 8 hours within any 24-hour period, but at no time shall the oxygen content be less than 3 mg/l. To sustain a coarse fish population, the dissolved-oxygen concentration may be less than 5 mg/l for a period of not more than 8 hours out of any 24-hour period, but at no time shall the concentration be lower than 2 mg/l.

Supersaturation with dissolved oxygen has also been reported as detrimental to fish. In ponds at 150 percent of saturation, the carp population seemed to have a higher percentage of disease than in ponds where the oxygen value was 100-125 percent of saturation (1544). There may also be a relationship between dropsy and gill damage by gas bubbles. In Westlake Park in Los Angeles fish died when oxygen reached 300 percent of saturation, the lethality being attributed to oxygen bubbles surrounding the gills (418). According to Cole (311) bass could tolerate 40.3 mg/l for only short periods, but Ellis (313) reports that a variety of hardy fish survived supersaturated solutions for at least 24 hours. Apparently fish require dissolved oxygen and not air bubbles (1158), the latter proving deleterious at times (see also Ozone and Nascent Oxygen).

Studies by Fairchild (2946) of the toxicity of nine inorganic salts to *Daphnia magna* indicated that a reduction in the level of dissolved oxygen below 4.5 mg/l reduced the resistance of this crustacean to the salts. In bottom deposits, the eggs of *Ascaris* developed to the larval stage in two months when the dissolved oxygen was 7 to 9 mg/l; but the eggs failed to reach the larval stage at 5.0 to 5.6 mg/l of dissolved oxygen (1727).

d. Shellfish Culture. Oysters show considerable resistance to oxygen deficiencies, according to Mitchell (326). Only when exposed for more than a week to very low concentrations of dissolved oxygen were oysters killed, and hence a temporary decrease in available oxygen is not considered by Mitchell to be a significant factor in oyster culture. Clark (327) studied the effect of dissolved-oxygen content on oyster larvae. Although the normal concentration was 7.9 mg/l, larvae developed adequately at 2.4 mg/l.

4. Summary. On the basis of the available information described above, it is not feasible to attempt to suggest an optimum dissolved-oxygen content of water for domestic, industrial, stock and wildlife, or recreational uses. For fish and other aquatic life, the recommendations of the Aquatic Life Advisory Committee of ORSANCO (as quoted above) appear to be logical.

DISSOLVED SOLIDS

1. General. In natural waters the dissolved solids consist mainly of carbonates, bicarbonates, chlorides, sulfates, phosphates, and possibly nitrates of calcium, magnesium, sodium, and potassium, with traces of iron, manganese and other substances. The mineral content of natural waters may be lowered artificially by dilution or raised by the addition of chemical wastes, dissolved salts, acids, alkalis, gas and oil-well brines, or drainage waters from irrigated land (313, 611, 618, 2032, 2180, 2340).

All salts in solution change the physical and chemical nature of the water and exert osmotic pressure. Some have physiological or toxic effects as well. Discussions of the effects upon the beneficial uses of water of "dissolved solids" under this heading are limited to the effects attributable only to total salt concentration, or osmotic pressure, without reference to the effects of the salt constituents separately. It should be pointed out, however, that possible synergistic or antagonistic interactions between mixed salts in solution may cause the effects of salts in combination to be different from those of salts separately, and that the presence of poisonous salts (such as nitrates, sulfides, and fluorides) can render dangerous for use waters whose total solids concentration is apparently within acceptable limits.

2. Cross References. Distilled Water, Specific Electric Conductance, Chapter V.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The 1962 USPHS Drinking Water Standards (2036) specify that the total dissolved solids should not exceed 500 mg/l if more suitable supplies are, or can be made, available. This limit was set primarily on the basis of taste thresholds. The 1958 WHO International Standards (2328) set the "permissible limit" at 500 mg/l and the "excessive limit" at 1500 mg/l, but no "maximum allowable limit" is given. The 1961 WHO European Standards (2329) do not include limits for total dissolved solids.

Many communities in the United States and in other countries use water containing from 2000 to 4000 mg/l of dissolved salts, when no better water is available (32, 620, 621, 622, 1545, 3469). Such waters are not palatable, may not quench thirst, and may have laxative action on new users. However, no harmful physiological effects of a permanent nature have been called to the attention of health authorities. On the other hand, there appears to be no proof that mineral waters have any beneficial or therapeutic value resulting from their mineral content (623, 624, 625), although life at spas and similar resorts may be undeniably healthful and wholesome for other reasons. One team of workers has found allergic diseases higher in frequency where water was low in dissolved salts (626).

Waters containing more than 4000 mg/l of total salts are generally considered unfit for human use, although in hot climates higher salt concentrations can be tolerated than in temperate climates (627). Waters containing 5000 mg/l or more are reported to be bitter and act as bladder and intestinal irritants (295). In one village in British Somaliland, the water contains 10,000 mg/l of salts, of which 4400 mg/l are sulfates (1546). Volhardt and Schutte (1547) described a series of experiments in the course of which eleven healthy young men on dry diets drank only 500 ml of sea water daily for 5-6 days, while continuing with their usual jobs. Although marked loss of weight and changes in the composition of blood and urine occurred, recovery from these disturbances was rapid nevertheless.

It is generally agreed that the salt concentration of good, palatable water should not exceed 500 mg/l; however, higher concentrations may be consumed without harmful physiological effects and may indeed even be more beneficial. Each water with a total salt concentration over 1000 mg/l should be judged on the basis of the local situation, alternative supplies, and the reaction of the local population.

An interesting influence of total dissolved solids on domestic water supply is discussed by Buswell and Upton (3470). When a fire hose is directed onto a high-voltage power line, the firemen are in danger of electrical shock. The current that will flow down the host stream will depend on the voltage of the power line and the specific electrical conductance of the water, which is a function of the dissolved solids.

b. Industrial Water Supplies. Dissolved solids in industrial waters can cause foaming in boilers and interference with clearness, color, or taste of many finished products. Water with extremely low dissolved solids is desirable for atomic power reactor cooling systems (3395). High contents of dissolved solids also tend to accelerate corrosion (3471). Concentrations that have been recommended for various industrial water uses are shown in the following tabulation. For further details, see Chapter V.

LIMITING CONCENTRATIONS OF DISSOLVED SOLIDS FOR INDUSTRIAL WATERS

Industry and Use of Water	*Limiting Concentration, mg/l*
Boiler feed water	50–3000 (according to pressure)
Brewing, light beer	500
Brewing, dark beer	1000
Brewing and distilling, general	500–1500
Canning and freezing	850
Carbonated beverages	850
Confectionery	50– 100
Dairy washwaters	850
Food equipment washing	850
Food processing, general	850
Ice manufacture	170–1300
Plastics, clear	200
Paper manufacture	
Fine papers	200
Groundwood papers	500
Kraft paper, bleached	300
Kraft paper, unbleached	500
Soda and sulfate pulp	250
High-grade paper products	80
Lower-grade products	150– 200
Rayon manufacture	100– 200

c. Irrigation Water. The total concentration of dissolved solids in irrigation waters generally varies from 150 to 1500 mg/l and in a few cases is found to be as high as 5000 mg/l, a concentration tolerated by only the most salt-resistant plants (285, 635). Some varieties of barley will produce grain satisfactorily even though irrigated over most of the life cycle with water containing 20,000 mg/l of salt (3472). High salinities may be deleterious to plants directly, or indirectly through their effects on the soil; but their harmfulness may be modified by a number of factors, such as the kind of crop, character of soil, drainage conditions, types of salts found in the water, climate, etc. (For a detailed discussion, see Irrigation, under Chapter V.)

There is abundant literature on the effects of dissolved salts on irrigation in general and upon specific crops for given soils under certain conditions of application (267, 271, 282, 636, 637, 638, 639, 1548, 1549, 1550, 1912, 2135, 2200, 2383, 2384a, 2385, 2387, 2391, 3472, 3474, 3475, 3476, 3477, 3478). To tabulate or abstract these references

beyond the scope of this report. Much of the work has been correlated or summarized by the Salinity Laboratory of the U.S. Department of Agriculture at Riverside, California, as reviewed in Chapter V.

Chapman, Wilcox, and Hayward (267) consider 1000 mg/l a concentration "approaching the limit for best crop growth." Irrigation waters in California have been classified (263, 269) according to salt content as follows:

Classification	*Concentration in mg/l*
Excellent	175 or less
Good	175–525
Permissible	525–1400
Doubtful	1400–2100
Injurious	2100 or more

In other references (246, 274) three classes have been given in this manner:

Class	*Suitability*	*Salt Concentration, mg/l*
I	suitable under almost all conditions	under 700
II	suitability dependent on crop, soil, climate, etc.	700–2100
III	unsuitable under most conditions	over 2100

d. Stock and Wildlife Watering. Livestock and poultry may be injured by drinking water that contains excessive dissolved solids. Continuous use of such water may cause general loss of condition, weakness, scouring, unthriftiness, reduced milk or egg production, bone degeneration, and death. However, animals can drink temporarily highly saline waters that would be harmful if used continuously. Animals can also adjust gradually to the use of waters with a higher solids concentration than that which they normally drink, although sudden change from slightly to highly mineralized waters causes acute distress and diarrhea of varying severity. The limits of tolerance depend upon the kind of salts present, the species of animal, its diet, age, physiological condition, season of year, climate, etc. (282, 284, 288, 290, 640, 2398). For further details, see Chapter V, Stock and Wildlife Watering.

The following tabulation shows the reported effects of various concentrations of total salts on animals:

Concentration in mg/l	*Animals*	*Reported Effects*	*Reference*
2,860	poultry	safe upper limit	282
3,000	livestock	injurious	641
3,600–5,756	cattle	deleterious	643
4,000	livestock	injurious	289, 290
4,000	rats	no deleterious effect	2398
4,290	pigs	safe upper limit	282
4,546–7,369	cows	no harmful effect	291
5,000	livestock	interim threshold limit	3373
5,000	cattle	tolerated	2397
5,700	horses	satisfactory	287
6,260	horses	thrived	2395
6,435	horses	safe upper limit	282
7,000	cattle	reduced weight	2398
7,150	dairy cattle	safe upper limit	282
7,150	cows	reduced milk production	2393
7,800	horses	safe upper limit	2395
7,865	horses	thrived	642
8,000	sheep	tolerated	2397
9,100	horses	harmful	287
9,100	cattle	lost condition	642
9,400	cattle	safe upper limit	2395
10,000	beef cattle	safe upper limit	282
10,000	rats	deleterious	2398
10,000	white rats	tolerated	290, 641
10,000	livestock	loss of weight	290, 295, 642
11,400	cows and sheep	satisfactory	287
12,900	adult dry sheep	safe upper limit	282
13,500	horses	sustained without work	642
14,700	sheep	thrived	642
15,000	farm animals, dry	satisfactory upper limit	288
15,000	lactating cows and pigs	injured	288
15,000	horses, cattle, sheep	fatal during long periods	284
15,600	sheep	safe upper limit	2395
18,000	cattle	thrived	642
18,600	sheep	used temporarily	2393

A maximum permissible concentration of dissolved solids in water of 2500 mg/l has been recommended by Tobisca (285), while Stander (3373) has suggested an interim threshold limit of 5000 mg/l. In Montana, the following arbitrary standards are used for livestock waters: up to 2500 mg/l, good; 2500 to 3500 mg/l, fair; 3500 to 4500 mg/l, poor; over 4500 mg/l, unsatisfactory (297).

e. Fish and Aquatic Life. It has been reported (310) that among inland waters in the United States supporting a good mixed fish fauna, about 5 percent have a dissolved-solids concentration under 72 mg/l; about 50 percent under 169 mg/l; and about 95 percent under 400 mg/l.

The blood of fresh-water fishes has an osmotic pressure approximately equal to six atmospheres, or about 7000 mg/l as sodium chloride, and fresh-water fish have been able to live well in sea water diluted to this level (307, 311, 313, 644). The antagonistic effects of mixtures of salts in sea water and brines makes it possible for fish to tolerate higher concentrations of individual salts in mixed than in pure solutions (307, 313, 645). In practical pollution work any effluents with an osmotic pressure greater than six atmospheres may be expected to be deleterious to fresh-water fish. Limiting concentrations of dissolved solids for fresh-water fish are not definitely known, but may range from 5,000 to 10,000 mg/l, according to species and prior acclimating (1552, 3068). The Aquatic Life Advisory Committee of ORSANCO (2414) described the many factors affecting tolerance to salinity but did not recommend limiting values.

Some fish are adapted to living in more saline waters, and a few species of fresh-water forms have been found in natural waters with a salt concentration of 15,000 to 20,000 mg/l (646). Fish can become acclimatized slowly to higher salinities than those to which they are accustomed (644), but fish in waters of low salinity cannot survive sudden exposure to high salinities, such as those resulting from discharges of oil-well brines (645, 647, 648).

Dissolved solids may influence the toxicity of heavy metals and organic compounds to fish and other aquatic life, primarily because of the antagonistic effect of hardness metals. Chromates, copper, cyanides, detergents, phenolic compounds, zinc and several other substances are generally more toxic in distilled water than in hard water of high dissolved solids. For details, see the specific substances in Chapters VI and X.

Clemens et al. (3479, 3480) investigated the toxicity of brine water from oil wells toward a variety of aquatic life, both fish and invertebrates. A wide range of the median threshold toxicity values was found.

f. Shellfish Culture. Inasmuch as oysters grow in brackish waters, decreases in salinity following floods or other heavy discharges of fresh water into shellfish-growing areas have interfered with feeding, fattening, and reproduction of oysters (320, 321, 649), and have sometimes resulted in heavy mortalities (322, 650, 651, 652). Although oysters can live within a wide range of salinities, the tolerable limits of salinity differ with the species; and under unfavorable conditions changes in salinity equivalent to a density change of 0.002 (approx. 1000

mg/l) may be significantly harmful, especially during the spawning season (316).

In the literature reviewed here the concentrations of dissolved solids in oyster-growing waters have been reported in the following different ways: (a) in terms of the density of the water (315, 316); (b) as a percentage of the salinity of sea water (320, 321, 650); (c) as grains per gallon (314); and (d) as parts per thousand or parts per million (318, 322). For the purpose of coordinating the available data on dissolved solids in water, all these types of reference have been converted, roughly, to mg/l. Density has been changed to mg/l of total solids on the basis of a sea-water density of 1.026 for 35,000 mg/l of salinity and 20°C. Sea water has been assumed to have an average salinity of 35,000 mg/l, and the conversion of percentages of salinity has been based on this figure. In the paragraph below, both the original references as given by the authors, and their approximate equivalents as mg/l are listed.

Oysters could tolerate a salinity as low as 5000 mg/l for only 20 days (322). Oysters stopped fattening at salinities below 20 percent of sea water (approximately 7000 mg/l) (321). The optimum salinity for *Ostrea virginica* is 25 to 29 percent (8750 to 10,150 mg/l); for *Ostrea angulata,* 28 to 35 percent (9800 to 12,250 mg/l) (320). Oysters are found in water with densities between 1.002 and 1.025 (2700 to 33,800 mg/l), thrive best at densities from 1.011 to 1.022 (14,800 to 30,000 mg/l) and cannot tolerate densities less than 1.007 (9500 mg/l) for more than short periods (315). The most favorable salt content of water for oyster culture is about 1800 grains per gallon (30,800 mg/l) composed largely of chlorides (314). Dissolved salts concentrations from 10,000 to 27,000 mg/l are satisfactory for oysters (318). Oysters can tolerate salinities between 20 and 50 percent of sea water (7000 to 17,500 mg/l) (319).

Oysters can withstand significant variations in salinity, living as they do in regions where such variation is normal. However, all species do not have the same salinity preference. In general the larger oysters, genus *Crassotrea,* prefer somewhat lower salinities than the flat oysters, *Ostrea.* The optimum salinity for the eastern oyster, *C. virginica,* and the Pacific oyster, *C. gigas,* is commonly said to be about 18,000 to 20,000 mg/l. The optimum for the Olympia oyster, *O. lurida,* seems to be somewhat higher (2415).

The gaper clam appears to be restricted to a minimum salinity of about 27,000 mg/l while the softshell clam is found almost to the fresh-water line (2417).

In his comprehensive review of the literature about oysters, Korringa (1448) pointed out that there is contradictory information concerning the influence of salinity differences on the development of oyster eggs. Some investigators think that eggs and larvae can withstand a wide range of salinities; others are equally certain that only salinities lower than that of the open sea are suitable for normal development. The salinity ranges found to be suitable for normal development have varied both with the species and the investigators.

g. Recharging Groundwater. The composition of waters stored in underground basins is affected by the source and nature of the recharge waters. Where return irrigation and waste waters are substantial contributors to recharge waters, the salinity has increased to the detriment of ground-water quality (1557). The dissolved-solids concentration of irrigation runoff may be as much as 15 times as great as that in the irrigation supply. The possibility of pollution through irrigation is now receiving wider recognition (1488, 1558).

h. Navigation. For prevention of marine borers, such as *Teredo navalis,* a total salt concentration not exceeding 5,000 mg/l is considered desirable (1486).

4. Summary. On the basis of this literature survey, dissolved solids up to the following limits should not interfere with the indicated beneficial uses:

a. Domestic water supply	1000 mg/l
b. Irrigation	700 mg/l
c. Stock watering	2500 mg/l
d. Fresh-water fish and aquatic life	2000 mg/l

DISTILLED WATER

(see also Dissolved Solids, Specific Electrical Conductance)

As processed water, distilled water deserves mention in this report only because solutions made up in distilled water have sometimes been used in studies of the biological effects of chemicals upon fish. The results of such experiments are not strictly comparable with those involving the use of natural or tap waters because the presence and composition of dissolved substances in water may significantly change the toxicity of many pollutants.

Distilled water is generally tasteless and may even be distasteful; dissolved minerals and gases render water palatable (653).

The effects of distilled water on fish and fish food organisms are variable (313); at times distilled water itself has been toxic to fishes (307), owing to the decrease in osmotic pressure. Solutions of copper sulfate and lead chloride have been found to be more toxic to fish-food organisms in distilled water than in tap water; the effect of mercury compounds, on the other hand, has been reported to be the same in both kinds of water (654). For the effects of copper, chromates, cyanides, detergents, phenols, zinc, and other substances in distilled water, see the specific substance in Chapters VI and X.

DISTILLERY WASTES

(see also B.O.D., Alcohols, Soaps, Synthetic Detergents)

Schaut (362) reported that a 1:240 dilution of a distillery waste (composition or strength not specified) gave high odor, color, and turbidity; and it depleted the oxygen content by 28 percent in one hour. However, minnows exposed at this dilution for one hour showed no visible effects. The major detrimental effects of this waste, insofar as aquatic life is concerned, seem to be its high B.O.D. and its effect upon temperature in the receiving waters (3448). There is also an increase in phosphates, which may increase productivity in the stream.

DI-SYSTON

(see Chapter IX)

DIURON

(see Chapter IX)

DNQ

(see Chapter IX-Dichloronaphthoquinone)

DODECYLACETAMIDODIMETHYLBENZYL AMMONIUM CHLORIDE

(see Chapter X)

DOWPON

(see Chapter IX)

DUPONOL 80

(see Chapter X)

DYES

(see also Nigrosine, Aniline, Chromates)

A complete description of the various types of dyes and their effects upon the color and turbidity of receiving waters is beyond the capacity of this survey. Certain dyes are known to be toxic to bacteria, aquatic organisms, and plant life. In fact, dyes have been used for weed killers and bacterial disinfectants, The following references were uncovered in the course of this survey, but the reader should recognize that these few brief comments only touch on the fringes of a potentially huge field of research.

Dyes used in the textile industry are frequently classified as direct, acid, basic, sulfur, vat, and miscellaneous dyes. The direct, acid, and basic dye wastes are all highly colored and commonly have a higher B.O.D. than that of domestic sewage (1097).

Direct dyes are readily soluble and can be used without a mordant. Where they are used, mordants and adjuvants may prove to be more toxic than the dye itself (611). Used mostly on cotton, rayon and vegetable fibers, direct dyes are azo compounds and are frequently derivatives of benzidine and tolidine. Because of their great solubility, direct dyes are difficult to remove from industrial wastes (1097).

Acid dyes, used on animal fibers such as wool, are salts of color acids and are also azo compounds of benzidine and tolidine. Basic dyes are salts of color bases capable of dyeing animal fibers directly but requiring a tannin mordant for vegetable fibers. Derived principally from analine and its homologues, basic dyes are mainly triphenylmethane dyes.

Sulfur dyes, so called because they comprise sulfur compounds and are used in conjunction with sodium sulfide in the dye bath, are used exclusively on vegetable fibers. They are difficult to treat and their effect upon streams is frequently quite detrimental. Vat dyes are those that acquire their dyeing properties as a result of the reducing action in the dye vat just prior to dyeing. Vat dyes are reduced to a soluble form in the vat by means of a strong reducing agent such as hydrosulfite in conjunction with an alkali. They are generally used with cotton and they may be anthracene, indigo, or carbozol compounds.

The amount, strength, and character of a dye waste from a textile or paper mill depends on the way in which the dye is applied and how often dyes are changed in the operation of each process. When dyes are changed, vats must be dumped and cleaned. At other times, the wastes consist only of the uniform flow of rinses and wash waters (1097).

Dyes in water are detectable to the naked eye at very low concentrations, such as 0.025 mg/l for fluorescein and 0.02 mg/l for magenta (1756).

Despite the fact that dyes are used extensively in industry and despite the fact that numerous references state that dyes are toxic substances, relatively few specific references dealing with the toxicity of dyes were uncovered in this survey. Schaut (632) found that sulfur-black dye wastes, diluted 1:240 with stabilized tap water, had no noticeable effect on minnows during a one-hour exposure. Wright (1162) used Dye 914, a flavine dye, to inhibit or destroy sulfate-reducing bacteria, in a concentration of 4.0 mg/l.

A lagoon that was used to receive and treat wastes from an ink-pigment manufacturing plant in Pennsylvania broke and discharged its entire contents quickly into a small stream. Dyestuffs used in this plant comprised tobias acid, metanitroparatoluene, eosin, methyl violet, erioglaucine, dichlorobenzedine, rhodamine, brilliant green, Victoria blue, paranitroaniline, and Orange II. Many inorganic salts were also used, but the commonly suspected toxic chemicals were not used. The sudden shock load of wastes killed a great number of fish in the creek, but considering the B.O.D. load and the available dilution, it could be shown that deoxygenation alone would account for the fish deaths. Some of the distressed fish were taken from the stream, kept in clean water and later returned to the stream with apparently no ill after-effects. It was suspected, therefore, that the aforementioned wastes were not directly toxic to fish (7).

Clemens and Sneed (2979, 2981) found the 24-, 48-, and 96-hour TL_m values of Malachite green (zinc oxalate of tetramethylpara-aminotriphenylcarbinol) at 25°C all to be 0.14 mg/l toward fingerling channel catfish. With the dye "Luxantholschwarz G," Lodemann (3449) reported that small guppies were harmed at a concentration of 5 mg/l but for carp, the threshold of harmful effect was 75 mg/l. On the other hand, 100 mg/l of eosin, fluorescein, or uranin (dyes widely used as tracers) were found to be non-toxic to trout and roach (3236).

Experiments in Germany prior to 1900 using tench primarily and carp occasionally led to the results shown in the following table (2977):

Dyestuff	*Concentration in mg/l*	*Exposure in days*	*Effects On Fish*
Methylene blue	177.5-217.5	7	lethal
Methylene blue	57.5-157.5	7	lethal
Diamineazobenzene	5.0	0.3	lethal
Bismark brown	8.0	4	lethal
Bismark brown	5.0	4	no effect
Martius yellow	85	4	lethal
Martius yellow	57.5	4	no effect
Indigo blue	471.5	5	no effect
Naphthol black	150	3	no effect
Wool black	150	3	no effect
Diamond fuchsin	125	14	no effect
Metanilic yellow	120	14	no effect
Congo red	110	5	no effect
Azoarchil	100	4	no effect
β-naphtholorange	100	14	no effect

Dyestuff	*Concentration in mg/l*	*Exposure in days*	*Effects On Fish*
Naphthol green	100	3	no effect
Dinitrocresol	80	3	no effect
Dinitroresorcinol	80	6	no effect
Diazotobenzide salicylate	60	3	no effect

Bringmann and Kuhn (2158) tested the toxicity of three dyes to *Daphnia* exposed for two days at 23°C and *Scenedesmus* for four days at 24°C. Threshold effects were noted at the following concentrations:

	Threshold Concentration, mg/l	
Dye	*Daphnia*	*Scenedesmus*
Congo red	4	0.6
Fuchsin	20	1.0
Methylene blue	2	0.1

EDTA

(see Ethylenediaminetetraacetic Acid)

EMCOL-888

(see Chapter X)

EMULSIFIERS

(see Chapter X)

EMULSIN

(see also Amygdalin)

This carbohydrase is an enzyme that specifically hydrolyzes beta glucosides, such as amygdalin. Schaut (362) reports that 17.1 mg/l of this substance in stabilized tap water was not harmful to minnows during 24 hours of exposure, but the dissolved-oxygen content of the water was lowered 41 percent.

ENDOTHAL

(see Chapter IX)

ENDRIN

(see Chapter IX)

EPN

(see Chapter IX)

ETHYL ALCOHOL C_2H_5OH

Industrial ethyl alcohol, produced largely by fermentation of molasses, is widely used in industry, as a starting material for the manufacture of many organic substances, for perfumes, flavors, and drugs; in beverages; for paint and varnish; for fuel; and for many other common purposes. It is a clear, colorless, flammable liquid with a pleasant odor and a burning taste. It is highly miscible with water and many organic liquids. The acute oral toxicity toward rats has been given as 7.4 mg/kg of body weight (3248).

Fish and other aquatic life can apparently withstand high concentrations of ethyl alcohol but, like humans, fish become intoxicated by it. Ellis (313) quotes references to the effect that tench survived a two-hour exposure to 10,000 mg/l in tap water, without injury; but Jones (467) reports that stickleback fish will avoid such a concentration. If forced to remain in tap water containing a 40,000 mg/l of ethyl alcohol, sticklebacks become intoxicated. For gudgeon, a European fresh-water fish, the lethal range is reported (2977) to be 7000 to 9000 mg/l. In contrast, it is interesting to note that only 250 mg/l of ethyl alcohol in distilled water killed goldfish in 6 to 11 hours (313). It is probable that the effect in this instance was attributable to the changes in osmotic pressure when the fish were placed in pure water, i.e., the alcohol may have had no influence.

Toward other aquatic life, ethyl alcohol is similarly toxic only in high concentrations. Anderson (358) showed that the threshold concentration for immobilization of *Daphnia magna* after prolonged exposure in Lake Erie water at 25°C was 18,400 mg/l. A concentration of 198,000 mg/l was required to stimulate the movement of the water beetle (2956). The following threshold concentrations were reported for organisms in Lake Erie water at 20 to 25°C: *Daphnia magna* (young), 7,800 mg/l; *Daphnia magna* (adult), 12,100 mg/l; and *Mesocyclops leuckarti*, 12,000 mg/l (2955).

ETHYLAMINE $C_2H_5NH_2$

This flammable liquid is miscible with water, producing an ammoniacal odor and an alkaline reaction, probably from its dissociation and the formation of ammonium ion. It is used in oil refining, organic synthesis, and the manufacture of synthetic rubber, dyes, and pharmaceuticals (364). The oral LD_{50} for rats has been reported as 400 mg/kg of body weight (2978).

Gillette et al. (1442) found that all creek chub, a fish of average tolerance, in well-aerated water at 15-21°C withstood 30 mg/l of ethylamine for 24 hours but all died at 50 mg/l. The approximate 24-hour TL_m was about 40 mg/l. Shelford reported that 400 to 800 mg/l in tap water killed orange-spotted sunfish in one hour (313, 363). According to Bringmann and Kuhn (2158, 3343) the toxic threshold for *Daphnia* during a two-day exposure at 23°C was 140 mg/l, for *Microregma* it was 40 mg/l, but for *Scenedesmus* at 24°C for 4 days it was only 10 mg/l. A concentration of 1000 mg/l had no apparent effect on *Escherichia coli* at 27°C.

ETHYLENE $CH_2{=}CH_2$

This colorless, flammable gas is prepared commercially from petroleum gases or by dehydration of alcohol, and it occurs naturally in ripening fruit and illuminating gas. It is used to ripen the color of fruits, and in the manufacture of plastics, alcohol, mustard gas, ethylene oxide, and other organics. Shelford (363) reports that orange-spotted sunfish were killed in one hour by a concentration of 22 to 25 mg/l, while Gutsell (463) gives lethal concentrations of 22 to 65 mg/l in an hour or longer.

ETHYLENEDIAMINE $NH_2CH_2CH_2NH_2$

Freely soluble in water, this strong alkaline liquid forms a hydrate in water. By dissociation it reacts to form ammonium ions and to raise the pH. It is used industrially as a solvent and emulsifier. The oral LD_{50} in rats is reported as 1.16 grams/kg of body weight.

According to Gillette et al. (1442) the critical range for creek chub, a fish of average tolerance, exposed for 24 hours in well-aerated water at 15-21°C, was 30 to 60

According to Rohlich and Sarles (759), the halogenation of an aromatic series markedly influences tastes and often a change from sweet to bitter is effected. Benoclor, a trade product consisting of a mixture of the non-emulsifiable forms of mono-, di-, and trichlorobenzene, is used in high-pressure subsurface sprays to control aquatic vegetation. It is reported by Goudey (897) to have a pungent odor but to have no physiological effect on humans at odoriferous concentrations.

Straus (890) reports that more than 500 mg/l of Benoclor in water is required to injure crops. According to Goudey (897), Benoclor is toxic to fish, crayfish, and clams, but concentrations are not given. The toxic dose of Benoclor for sheep is 2666 mg/l, according to Straus (890) but the taste is so bad that animals will avoid the water.

HALOGENATED PHENOLS

1. General. Chlorine, iodine, and bromine have been introduced into the phenol molecule in various manners to produce compounds with toxicities differing markedly from that of phenol. Iodophenols appear to be more toxic than chlorophenols or bromophenols, and of the iodophenols, the para compound is most toxic and the meta the least (613).

2. Cross References. Phenols, Pentachlorophenols, Halogenated Hydrocarbons, Chapter IX.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The chlorination of water supplies containing traces of phenol results in medicinal tastes (see Phenols). The type of chlorophenol formed depends on the concentration of chlorine and phenol, upon temperature, and upon catalytic agents. Rohlich and Sarles (759) quote references to the effect that chlorine in doses up to 0.1 mg/l did not produce odor in water containing up to 0.015 mg/l of phenol. The critical range for chlorophenol tastes was for chlorine dosages from 0.175 to 0.4 mg/l with phenol from 0.002 to 0.017 mg/l. A combination of 0.22 mg/l of chlorine with 0.007 mg/l of phenol caused a strong chlorophenol odor, but heavier dosages of chlorine eliminated the odor.

Several investigators have reported the taste or odor threshold concentrations for various chlorinated phenols. Their results have been summarized and tabulated below:

Compound	*Taste (t) or Odor (d) Threshold Concentration in mg/l*	*Reference*
o-chlorophenol	.00033 (d) at 30°C	2982
	.0001 (t)	459
	.002 (d) at 25°C	3493
	.0025 (d) at 60°C	2982
	.004 (t) at 25°C	3493
	.006 (t)	2059
m-chlorophenol	.100 (d) at 60°C	2982
	.200 (d) at 30°C	2982
	.900 (t)	2059
	1.0 (t)	459
p-chlorophenol	.033 (d) at 30°C	2982
	.1204 (d)	3494
	.143 (d) at 60°C	2982
	.250 (d) at 25°C	3493
	1.0 (t)	459
	1.35 (t)	2059
	>1.0 (t) at 25°C	3493
2, 4-dichlorophenol	.00065 (d) at 30°C	2982
	.002 (d) at 25°C	3493
	.0065 (d) at 60°C	2982
	.008 (t) at 25°C	3493
	.02 (t)	459
2, 5-dichlorophenol	.00045 (d) at 60°C	2982
	.0033 (d) at 30°C	2982
2, 6-dichlorophenol	.002 (t) at 25°C	3493
	.003 (d) at 25°C	3493
2, 4, 5-trichlorophenol	.011 (d) at 30°C	2982
	.333 (d) at 60°C	2982
2, 4, 6-trichlorophenol	.10 (d) at 30°C	2982
	.667 (d) at 60°C	2982
	>1.0 (d) at 25°C	3493
	>1.0 (t) at 25°C	3493
trichlorophenol (undesignated)	1.0 (t)	459
2, 3, 4, 6-tetrachlorophenol	.915 (d) at 30°C	2982
	47.0 (d) at 60°C	2982
pentachlorophenol	.857 (d) at 30°C	2982
	12.0 (d) at 60°C	2982

According to Chamberlin and Griffin (1576) concentrations as low as 0.001 mg/l of mixtures of o-chlorophenol, p-chlorophenol, and trichlorophenol can sometimes be detected in drinking water, and the presence of these compounds may be detrimental to certain industries, such as the manufacture of paper food containers.

b. Industrial Water Supplies. Halogenated phenols produce tastes and odors in many of the food and beverage industries (179, 223, 225). See Chapter V for more details.

c. Stock and Wildlife Watering. The oral LD_{50} for male rats fed 2, 4, 5-trichlorophenol was 2.96 grams per kg of body weight (3490). There was no evidence of adverse effects in rabbits fed 500 mg/kg of body weight or in rats fed 300 mg/kg. McCollister et al. (3490) conclude that this material is not likely to present a health hazard from ingestion.

d. Fish and Other Aquatic Life. The 96-hour TL_m of o-chlorophenol for bluegill fingerlings was found to be 8.4 mg/l. According to Gersdorff and Smith (939), ortho-, meta-, and para-chlorophenols are slightly less toxic to goldfish than is phenol itself, as shown below:

Compound	*Relative Products of Concentration and Killing Time*
phenol	1.00
o-chlorophenol	1.15
m-chlorophenol	1.51
p-chlorophenol	1.89

In concentrations as low as 0.1 mg/l, chlorophenols may impart a taste to the flesh of fish (656), Henderson et al. (2958) report that 2.0 mg/l of o-chlorophenol gave a taste to the flesh of adult bluegills.

At a concentration of 10 mg/l, 95 to 100 percent of the snails (*Australorbis glabratus*) that are the intermediate host of *Schistosoma mansoni* were killed by 2, 4, 6-triiodophenol and its sodium salt, by 2, 4, 6-tribromophenol, by pentabromophenol, and by sodium and copper pentachlorophenates, according to Berry et al. (975).

Applegate et al. (3491) tested several halogenated mononitrophenols against the larvae of sea lamprey and also against three fresh-water fish. Results were as follows:

to 300 lbs of ammonium sulfate per acre; but the liquor must be free of oils and tar, and low in thiocyanates.

GELATIN

(see also Biochemical Oxygen Demand)

This protein, obtained by boiling skin, tendons, ligaments, and bones in water, is used extensively for food products, adhesives, medicines, plastics, and in numerous other ways. Apparently its only detrimental effect in water is its oxygen utilization in decomposition. Schaut (362) reported that 17.1 mg/l in stabilized tap water was not harmful to minnows after 24 hours of exposure, but it lowered the dissolved-oxygen content of the water by 57 percent.

GERMANIUM Ge

This trace mineral, relatively unimportant until recently, is finding increasing use in industry, especially in electronic devices. It is employed in the manufacture of the semi-conductor triode which consists of a small germanium crystal with three electrical contacts. Germanium is also used in metallurgical processes and in electroplating (364, 2121).

Germanium is present in many soils in trace amounts and it may be picked up by certain forms of vegetation. There is no evidence, however, that germanium is an essential element for plant or animal nutrition. It does not accumulate in animal systems. Although it is readily assimilated it is rapidly excreted in the urine (2121, 2129).

Elemental germanium, the oxide, and the sulfide are relatively non-toxic and pharmacologically inert (2129). Food containing 100 mg/kg or water containing 100 mg/l of neutralized germanium oxide caused some inhibition in the growth of rats and a 50 percent mortality in four weeks (3489). Animals that survived beyond four weeks were able to continue such dosages with ill effects.

GIUTHION

(see Chapter IX)

GLYCERIN

(see Glycerol)

GLYCEROL $CH_2OHCHOHCH_2OH$

A syrupy liquid with a sweet warm taste, glycerol (commonly called glycerin) is miscible with water and alcohol. It is used extensively in food production, printing inks, glues and cements, textile printing, antifreeze and shock absorber fluids, dynamite, and over 1500 other processes (364). In natural waters it appears to exert no inimical effect other than an oxygen demand arising from decomposition. Ellis (313) quotes references to the effect that tench survived a 16-hour exposure to 100,000 mg/l of glycerol in tap water at 8°C without apparent harm.

GLYERCOL MONOLAURATE

(see Chapter X)

GOLD Au

Widely distributed in nature, gold occurs in trace quantities in many rocks, in sea water, and in copper, lead, zinc, and other ores. The pure metal is extremely inactive, and insoluble in water. A few gold salts are soluble in water, but owing to the fact that the hydroxide, monochloride, monoxide, and several other combinations are insoluble, gold ions are not likely to be found in natural waters. Gold salts are used in photography, for electrogilding, for painting on porcelain and glass, and for therapeutic purposes. Because it has a short half-life and seeks out certain organs of the body, colloidal radioactive gold has been used for the treatment of internal cancer in humans. Gold salts have also been used in the treatment of arthritis (364, 2129).

According to Jones (2941), the lethal concentration limit of gold ions to the stickleback is 0.40 mg/l.

GOLD TRICHLORIDE, ACID

(see Chloroauric Acid)

GREASE

(see Animal and Vegetable Oils, Oil-Petroleum)

GROUNDWOOD PULP MILL WASTES

Wastes from mechanical or groundwood pulping consist chiefly of finely divided spruce, balsam or poplar that escape in the processes of disintegration. Very little dissolved organic matter occurs in these wastes and hence their B.O.D. is low. According to Westfall (673), however, a finely divided wood, like pine, gives a grinding effluent that is toxic to aquatic organisms (see also Sawdust). This waste blankets stream beds, covers spawning beds, and promotes the growth of undesirable fungi and bacteria in harbors. Pulp that has been pretreated with steam leaves toxic soluble substances in the effluent. Young fish often become tangled in mats of fine wood pulp and even adult gills are affected. (For a contrary opinion, see Sawdust).

GUAIACOL $CH_3OC_6H_4OH$

This white or slightly yellow crystalline substance, a methylcatechol, is obtained from guaiac resin or from hardwood tar. It was formerly used for chronic infections of the respiratory tract but it may cause gastrointestinal irritation (364). It is moderately soluble in water.

According to Hoak (2982, 3403), the odor threshold of guaiacol in hot water (60°C) is 0.0025 mg/l and in warm water (30°C) it is 0.002 mg/l. The killing strength of guaiacol to perch is reported to be 70 to 80 mg/l (365). Toward ciliated protozoa it is given as 1520 mg/l (2904).

GYPSUM

(see Calcium Sulfate)

HALOGENATED HYDROCARBONS

There are numerous halogenated hydrocarbons for which the literature reports detrimental effects to benefical uses of water. Most of these compounds are covered elsewhere in this chapter or in Chapter IX as pesticides.

containing 400 to 1600 mg/l of formaldehyde, finding such solutions to be irritating (467).

Toward lower organisms, Bringmann and Kuhn (2158, 3343) reported the median threshold effect of formaldehyde toward *Daphnia* occurred at 2 mg/l during a two-day exposure at 23°C. Toward *Scenedesmus* held for 4 days at 24°C, it was 0.3 mg/l, toward *Microregma* 5.0 mg/l, and toward *Escherichia coli* at 27°C it was 1.0 mg/l. Gellman (3485) indicated that the toxic concentration of formaldehyde toward aerobic organisms lay between 130 and 175 mg/l while Hermann (2923) found that 740 mg/l inhibited oxygen utilization by 50 percent. It is possible, however, to develop cultures of aerobic organisms that will withstand formaldehyde concentrations of 265 mg/l (660) and 1500 mg/l (3485).

The Mersey River Board in England (2950) has established working standards for sewage and industrial effluents that limit the formaldehyde content to 1.0 mg/l.

FORMIC ACID AND FORMATES HCOOH

(see also Fatty Acids)

Formic acid is a colorless liquid with a pungent odor. A strong reducing agent, it is dangerously caustic to the skin. It is used as a decalcifier, as a reducer in dyeing wool-fast colors, for dehairing and plumping hides, in tanning, in sizes, and in rubber processing. Sodium formate, HCOONa is used in dyeing and printing operations in textile mills. According to Anderson (352), the threshold concentration of sodium formate for immobilization of *Daphnia magna* in Lake Erie water at 25°C, after 48 hours of exposure, was approximately 4700 mg/l. This substance is probably toxic only when present in sufficient concentration to exert unfavorable osmotic pressure.

Concentrations of 20,000 mg/l of formic acid or 40,000 mg/l calcium formate in the drinking water of guinea pigs for 30 days caused no apparent changes in growth, blood, or urine (2980). The oral LD_{50} of formic acid for dogs is given as 4 gm/kg of body weight (3486).

Toward *Daphnia*, the median threshold concentration of formic acid was 120 mg/l for a two day exposure at 23°C; and toward *Scenedesmus* 100 mg/l in 4 days at 24°C (2158). A concentration of 1000 mg/l of formic acid had no effect on *E. coli* (2158) but 550 mg/l inhibited the 5-day BOD of sewage by 50 percent.

FURFURAL C_4H_3OCHO

This colorless oily liquid aldehyde, soluble in water, is used in the manufacture of plastics, as a solvent in petroleum refining, in the manufacture of varnishes, and as an insecticide, fungicide, and germicide (364). The oral LD_{50} for dogs is 2.3 grams/kg of body weight. In the drinking water of albino rats, 10,000 to 50,000 mg/l proved to be lethal in 114 to 548 days (2980). The odor threshold has been reported (2058) as 4 mg/l, above which a musty odor is evident.

Toward the bluegill sunfish, Turnbull et al. (2093) found the 24-hour TL_m to be 32 mg/l at 20°C and the 48-hour TL_m was 16 mg/l. In highly turbid water at 23-24°C, Wallen et al. (2940) reported the 24-hour TL_m as 44 mg/l whereas the 48- and 96-hour TL_m values were both 24 mg/l. According to Ettinger et al. (3487), biochemical degradation of furfural is rapid in rivers seeded with the proper organisms as a result of previous furfural pollution, with up to 25 mg/l disappearing in 5 to 8 days.

GALLIC ACID $C_6H_2(OH)_3COOH$

This light-colored, odorless, crystalline solid is used in the manufacture of pyrogallol and ink dyes, as a photographic developer, in tanning, and in analyses. Ellis (313) quotes references to the effect that gallic acid killed goldfish and minnows at a concentration of 143 mg/l. According to LeClerc et al. (2942, 2943), the minimum lethal dose for minnows at 21°C was 30-35 mg/l in hard water and 15-20 mg/l in distilled water during a six-hour exposure.

GAMLEN CW

(see Chapter X)

GAMMEXANE

(see Chapter IX-Benzene Hexachloride)

GASOLINE

(see Oil, Petroleum)

GAS-PLANT WASTES

(see also Ammonia, Cyanides, Hydrogen Sulfide, Phenol, Benzene, Halogenated Hydrocarbons, Halogenated Phenols)

Gas liquors may be detrimental to receiving waters as a result of (a) high B.O.D. (b) direct toxic action of some of the constituents, and (c) tastes and odors (346). The effects of individual substances that may occur in gas-plant wastes are given elsewhere in this report.

As early as 1917, Shelford (363) reported that illuminating gas, gas liquor, and 31 out of 34 representatives of chief groups of compounds found in gas and gas liquors were very toxic to fishes, in concentrations varying from 1.0 to 1500 mg/l. He noted that fish appear to develop a preference for gas-polluted water and are lured into it, only to be harmed. According to Allen et al. (668), the chlorinated effluents of sewage-treatment plants that contained gas-plant wastes were harmful to fish although the unchlorinated effluents were not. It is possible that cyanogen chloride was formed in such effluents.

Wastes from gas works had the following effects upon fish, as recorded by Knight (540).

Percent of Waste By Volume	*Type of Fish*	*Time of Kill*
5.0	perch	20 minutes
2.0	perch	30 minutes
0.5	perch	12 hours
0.5	stickleback	10 days

Schaut (362) found that minnows could possibly tolerate for short periods a 1:2400 dilution of gas liquors from cyanogen scrubbers, but they lost equilibrium after 15 minutes. At a dilution of 1:240 there was a 50 percent mortality after one hour. At this dilution, the water was black, opaque, and strongly odoriferous.

Silcox (3488) reported that the ammoniacal liquor from gas works can be used directly as a fertilizer in agriculture, with 400 gallons (Imperial) being equivalent

c. Irrigation Water. Concentrations of fluoride likely to be found in natural waters or in polluted streams apparently will have no detrimental effects on plants. Moreover, fluoride added to soil or water has little or no effect on the fluoride content of plants grown in such soil (1049, 1182, 3457). At high concentrations, fluoride has been reported to produce the following effects:

Concentration of Fluoride, mg/l	*Effect*	*Reference*
10	No injury to peach, tomato, and buckwheat plants	3458
100	Peach and buckwheat plants severely injured in 3 days	3458
100–500	Inhibited sprouting of beans	1180
180	Did not injure buckwheat at pH over 5.5	3459
200	Killed peach, tomato, and buckwheat in short time	3458
360	Injurious to peach and buckwheat even at pH 6.5	3459
1000	Stunted growth of large bean plants	1180

The use of fluoride-bearing insecticides appears to cause no harmful concentrations of fluoride in the soil moisture (1182).

d. Stock and Wildlife Watering. The effects of fluorides in drinking water for animals is analogous to those for humans. Table 6-6 lists the reported effects as reported in the survey literature, and indicates that 1.0 mg/l appears to be the threshold value below which no harm results. It is interesting to note that the addition of fluorides to a cow's ration or drinking water had no influence on fluorides in the milk (1181, 1188), and doses of 500 mg/l in the drinking water did not increase the milk fluoride above 0.5 mg/l (1186).

TABLE 6-6

REPORTED EFFECTS OF FLUORIDES IN DRINKING WATER FOR LIVESTOCK

Fluoride Concentration in mg/l	*Dose*	*Animal*	*Remarks*	*Reference*
1.0	--	cattle	harmless	292
1.0	--	sheep	fluoride poisoning	1183
1.4–4.5	--	mice	mottling of teeth	3460
--	0.4 mg per kg	cattle	no mottling	353
--	1 mg per kg	rats	mottled teeth	353
--	1 mg per kg	cattle	mottled teeth	1190, 3462
--	3 mg per kg	cattle	bone damage and death	353
--	5 mg	dogs	gave hypotension	3461
4.0	--	sheep	mottled and pitted teeth	1184
5.0	--	cows	disliked water	3457
5.0	--	sheep	slight dental mottling	1571
6 to 16	--	hogs, etc.	severe mottling	1005
11.78	--	cows	mottled teeth	1178
15	--	mice	affected thyroid and kidney	3460
18	--	cows	slowly increasing fluorosis	1190
20	--	sheep	5 percent reduction in weight	1571
25–100	--	young cattle	teeth lesions	3464
44–61	--	sheep	chronic fluoride poisoning	1184
50	--	hamsters	dental fluorosis in 10 weeks	1185
55	--	cows	disliked such water and drank less	1186
--	60 mg per day	sheep	affected teeth and bones	1187
--	65 mg per day	dogs	no effect on organs	3452
--	120 mg per day	sheep	threshold for general health	1187
--	200 mg per kg	rabbits	lethal dose	353
100	--	cattle	no economic harm	3463

e. Fish and Other Aquatic Life. Fluoride ions appear to have direct toxic properties toward aquatic life, and in addition there seems to be a relationship between the fluorides in water and the condition of the teeth of the fish (1189). The following effects of fluorides on fish have been reported:

Concentration of Fluoride, mg/l	Salt used	Type of fish	Effect	Reference
1.5	--	eggs	slower and poorer hatching	247
2.3–7.3	NaF	trout	TL_m at 18°C. in soft water	3465, 3466
2.6–6.0	NaF	trout	TL_m at 13°C. in soft water	3465, 3466
2.7–4.7	NaF	trout	TL_m	3467
5.9–7.5	NaF	trout	TL_m at 7.5°C. in soft water	3465, 3466
7.7	--	minnows	not harmed in one hour	353
64	KF	--	10-day TL_m	2407
75–91	NaF	carp	TL_m	3467
100	--	goldfish	survived over 4 days	353
120	--	goldfish	killed in 4 days	3468
358	NaF	rainbow trout	toxic in soft water	1756
419	NaF	mosquito-fish	96-hour TL_m in turbid water	2940
678	NaF	*Tinca vulgaris*	lethal dose	3271
1000	--	goldfish	killed in 12 to 29 hours in soft water	353
1000	--	goldfish	killed in 60 to 102 hours in hard water	353

For toxicities toward lower aquatic organisms, see Sodium fluoride.

4. Summary. On the basis of the foregoing information, it appears that the following concentrations of fluoride will not interfere with the specified beneficial uses:

a. Domestic water supply — 0.7 to 1.2 mg/l
b. Industrial water supply — 1.0 mg/l
c. Irrigation water — 10.0 mg/l
d. Stock watering — 1.0 mg/l
e. Aquatic life — 1.5 mg/l

FORMALDEHYDE HCHO

This simple aldehyde is formed by the oxidation of methyl alcohol by air in the presence of metallic silver or copper at high temperatures (300°C). It results also from the incomplete combustion of many organic substances and is found in the atmosphere over cities. It also occurs in some tannery wastes, penicillin wastes, and effluent from the manufacture of plastics and resins. At ordinary temperatures it is a colorless, flammable gas with a pungent suffocating odor, and it is intensely irritating to mucous membranes. It is very soluble in water, and a 37-40 percent solution in water is sold as "formalin". Because of its toxicity to lower forms of life, formaldehyde is used for preserving biological specimens.

The odor of HCHO is reported to be detectable at 50 mg/l (2983) and also at 20 mg/l (3483). The oral LD_{50} for rats is given as 800 mg/kg of body weight (3484).

In a concentration of 10 mg/l, formaldehyde had no apparent effect on rainbow trout in three days but 50 mg/l killed them in one to three days of exposure (659). For killing shiners in 120 hours at 18°C, the minimum lethal concentration was also 50 mg/l (190, 344). In stabilized tap water saturated with oxygen, minnows were harmed by a short exposure to 146 mg/l (362). For rainbow trout, the critical level of formaldehyde was reported (2091) as less than 31.8 mg/l and for young chinook salmon less than 28.2 mg/l. Clemens and Sneed (2979, 2981) investigated the toxicity of formalin (37 percent formaldehyde by weight) toward fingerling channel catfish. They found the 24-hour TL_m to be 32 mg/l as formaldehyde while the 48- and 96-hour TL_m concentrations were 25 mg/l. All fish survived at 18 mg/l as formaldehyde. If they are given a chance to do so, during short-term exposure, fish will avoid solutions

ion in drinking water to aid in the reduction of dental decay, especially among children. A review of such treatment processes is not relevant to this report, but it is significant to note that the presence of about 1.0 mg/l of fluoride ion in natural waters may be more beneficial than detrimental.

There is evidence to support the contention that fluorides in excess of the threshold for mottling of teeth and up to 5 mg/l produce no harmful effects other than mottling (1463, 1564, 1566). Radiologic surveys of 114 persons who had lived for over 15 years at Bartlett, Texas where water had 8 mg/l of fluoride revealed minimal evidence of an increase in density of bones of only 12 percent of those examined, but in no case was there found any interference with the use of bones or joints. Comparisons of mortality rates from nephritis, heart disease, or cancer in high or low fluoride areas has failed to show an association of these diseases with the fluoride content of water (1563, 1564). It has been estimated that daily intakes of about 15-20 mg of fluoride over a period of several years are required to induce chronic fluorosis in an adult man (1567).

The taste of sodium fluoride is salty, but less so than sodium chloride. A solution of sodium fluoride at a concentration of 2.4 mg/l of fluoride can be distinguished from distilled water (1568).

Shay (729, 730) used statistical evidence to show that the incidence of poliomyelitis is lower in districts where the surface waters contain over 1.0 mg/l of fluoride than in areas where the fluoride content is lower. Fellenberg (1163) investigated the correlation between goiter incidence and fluoride in the drinking water, but reached no definite conclusions.

The USPHS Drinking Water Standards (2036) of 1962 set a mandatory limit on fluorides that is based on the annual average of maximum daily air temperatures in accordance with the following table. It is reasoned that children drink more water in warm climates and hence the fluoride content of the water should be lower to prevent excessive total fluoride consumption (1563, 1564, 1565).

Annual Average of Maximum Daily Air Temperatures, °F	*Recommended Control Limits of Fluoride Concentrations, mg/l*		
	Lower	*Optimum*	*Upper*
50.0-53.7	0.9	1.2	1.7
53.8-58.3	0.8	1.1	1.5
58.4-63.8	0.8	1.0	1.3
63.9-70.6	0.7	0.9	1.2
70.7-79.2	0.7	0.8	1.0
79.3-90.5	0.6	0.7	0.8

The WHO International Drinking Water Standards (2328) of 1958 do not set a limit on fluoride concentration, but the WHO European Drinking Water Standards (2329) of 1961 prescribe a recommended limit of 1.5 mg/l.

TABLE 6-5

REPORTED EFFECTS OF FLUORIDES IN DRINKING WATER FOR HUMANS

Concentration of Fluorides, in mg/l	*Reported Effect*	*Reference*
0.2	Mottled teeth in 1 percent of children	1164
0.6	No effects at this concentration, or lower	555
0.7	Mild dental fluorosis in 8.5 percent of children	3451
0.8	No effects at this concentration, or lower	36, 1165
0.8 to 0.9	Mild mottling of teeth	1166
0.8 to 1.5	Threshold for mottling of teeth	219
0.9	Mild mottling of teeth	1165, 1167
0.9	Mottling occurred as a result of high water use	353
0.9	Critical concentration for mottling	555, 1168
1.0	Threshold for mottling of teeth	741
1.0	10 percent of children had mottled teeth	1169, 1164
1.0	90 percent of children had mottled teeth	1170
1.0 to 2.0	Mild to moderate mottling	1165
1.2	No effect at this concentration	353
1.4	No skeletal schlerosis found	353
1.5	Limiting concentration for drinking water	1171
1.7 to 1.8	50 percent of children had mottled teeth	1169, 1164
2.0	Gave mottling and weakening of tooth structure	1172
2.0 to 3.0	Retained in system	555
2.0 to 3.0	Moderate to severe mottling	1165
2.5	75 to 80 percent children had mottled teeth	555, 1169
2.5	No evidence of skeletal fluorosis	3452
3 to 4	Not likely to cause endemic cumulative toxic fluorosis in adults	1173, 1174
3 to 6	Gave severe mottling	1165
3.5 to 6.2	No adverse effect on carpol bones of children	3453
4.0	90 percent of children had mottled teeth	1164
4.0	No disorders other than dental mottling	3454
4.4 to 12	Caused chronic fluorosis and affected skeletal system	1175, 1176
5.0	This concentration had no effect on height, weight or bone	1177
6.0	Threshold for appreciable effect on bones	1168
6.0	100 percent of children had mottled teeth	1164
6.0	Gave pitting and chipping of tooth enamel	1165
8.0	No deleterious bone changes except dental mottling	3456
10	Some cases of skeletal fluorosis	3455
11.8	Gave chronic fluorine intoxication to adults	1178
12	Affects deciduous teeth	1179
13.7	100 percent of children had mottled teeth	353
115	Sub-lethal in drinking water	152
180	Toxic to man in drinking water	555
2000	Lethal dose in drinking water	152

b. Industrial Water Supplies. Excessive fluorides may be harmful in certain industries, particularly those involved in the production of food, beverages, pharmaceutical and medical items, according to Bratton (1569). If wet milling of corn is carried on with water containing one mg/l of fluoride, it is estimated that the concentrated steep water will contain more than 6 mg/l and the corn syrup more than 5 mg/l. Malt syrup made with similar water may contain up to 8 mg/l of fluoride. Weir (1570) points out that fluoride up to 10 mg/l in dough water has no effect on bread, that one mg/l stimulated the yeast fermentation of malt, that 10 mg/l may stimulate or depress yeast fermentation, and that 25 mg/l inhibits yeast activity.

In brewing, fluoride concentrations of 1 to 5 mg/l appear to stimulate yeast metabolism. Continued re-use of yeast in wort containing 10 mg/l of fluoride results in severe deterioration after six fermentations (2349). Concentrations of fluoride permissible in domestic water should have no deleterious effects on brewery processes (2348).

Fluoride concentrations of 1.0 mg/l caused no change in the amount or rate of corrosion of iron, copper, or lead (3482). Fluoride limits have been recommended for some industrial processes, as described in Chapter V and tabulated below:

Use	*Recommended Threshold Values in mg/l*
Brewing	1.0
Carbonated beverages	0.2 to 1.0
Food canning and freezing	1.0
Food equipment washing	1.0
Food processing, general	1.0

year-old girl was caused by ingestion of 1500 mg of $FeCl_2$.

FERROUS OXIDE FeO

Using highly turbid water at 16-23°C and the mosquito-fish (*Gambusia affinis*) as the test organism. Wallen et al. (2940) found the 96-hour TL_m of ferrous oxide to be over 10,000 mg/l.

FERROUS SULFATE $FeSO_4$ and $FeSO_4 \cdot 7H_2O$

1. General. The anhydrous and crystalline forms of this substance are highly soluble in water, and the salts are used in many industrial operations. Sources of pollution by ferrous sulfate include canneries, tanneries, textile mills, mines containing pyrites, and metal-cleaning operations involving the use of pickling liquors. Ferrous sulfate is sometimes used as a coagulant in water and sewage treatment.

2. Cross References. Iron, Distilled Water, Sulfates, and other iron salts.

3. Effects Upon Beneficial Uses.

a. Fish and Other Aquatic Life. The threshold concentration of ferrous sulfate for immobilization of *Daphnia magna* in Lake Erie water was found to be less than 152 mg/l (358).

The following concentrations of ferrous sulfate have been harmful or lethal to fish in the time specified:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Species of Fish*	*Reference*
2.9	distilled	4-24 hours	shiners, suckers, carp	313
6.4	--	24 hours	shiners, suckers, carp	359
100	--	24 hours	minnows, goldfish, trout	359
100	--	4.2-7 days	bass	1035, 1030
		2.5-3.5 days	sunfish	1035, 1030
133	--	24 hours	brook trout	359
315	distilled	3 hours	minnows	313
500	--	1.3-5 days	goldfish	1030
1,000	--	9-23 hours	bass	1030
1,000	--	48 hours	very young carp	1459
1,000	--	5-30 hours	goldfish	1030
		2.5-9 hours	bass	1030
1,000	hard	2-10 hours	goldfish	313
1,390	--	144 minutes	minnows	991
2,721	tap	31-66 minutes	trout, salmon	313
6,950	--	104 minutes	minnows	991
10,000	--	1 week	tench	1459
10,000	--	1 day	other fish	1459
13,900	--	68 minutes	minnows	991

The following concentrations of ferrous sulfate have been reported as not harmful to fish within the time specified:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Species of Fish*	*Reference*
5	--	24 hours	carp, shiners, suckers	359
17.1	--	1 hour	minnows	362
50	--	7 days	bass, bluegills	1459
50	--	24 hours	trout	359
			bass, sunfish	1035, 1030, 359
100	hard	96 hours	goldfish	313
		7 days	goldfish	1035, 1030
100	--	7 days	goldfish	1459
100	--	--	carp, tench	1459
380	--	185 minutes	minnows	353
1,000	--	over 1 week	mature fish	1459

Ferrous sulfate has also been reported to be lethal to fish at the following concentrations of iron:

Concentration of iron, mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
1.28	distilled	24 hours	fish	1459
368	--	2-10 hours	goldfish	1466

On the other had, 37 mg/l of iron has not been harmful to goldfish in 100 hours (1466).

The effects of disposal of as much as 3000 tons per day of acid ferrous sulfate solution at sea have been investigated independently by Arnold and Royce (1466) and Redfield and Walford (1467, 1561). They found no evidence of significant changes or harmful results among the aquatic life in the areas studied.

FERROUS SULFIDE FeS

This black solid is highly insoluble in water. Wallen et al. (2940) reported its 96-hour TL_m toward mosquito-fish in highly turbid water at 20-26°C to be over 10,000 mg/l. Undoubtedly the ferrous sulfide remained in suspension or settled out of suspension, for it would not be expected to go into solution.

FERROUS SULFITE $FeSO_3 \cdot 2\frac{1}{2}H_2O$

Using highly turbid water at 20-21°C, Wallen et al. (2940) found the 24-, 48-, and 96-hour TL_m concentrations toward the mosquito-fish (*Gambusia affinis*) to be 350 mg/l.

FERTILIZER MANUFACTURING PLANT WASTES

Ellis (611) reported that wastes from a fertilizer manufacturing plant in Mississippi constituted no hazard to fish.

FLUORIDES F^-

1. General As the most reactive non-metal, fluorine is never found free in nature but it is a constituent of fluorite or fluorspar, calcium fluoride, in sedimentary rocks and also of cryolite, sodium aluminum fluoride, in igneous rocks. Owing to their origin only in certain types of rocks and only in a few regions, fluorides in high concentrations are not a common constituent of natural surface waters, but they may occur in detrimental concentrations in ground waters (152).

Fluorides are used as insecticides, for disinfecting brewery apparatus, as a flux in the manufacture of steel, for preserving wood and mucilages, for the manufacture of glass and enamels, in chemical industries, for water treatment, and for other minor uses (364). While not normally found in industrial wastes, they may be present in traces, or in higher concentrations resulting from spillage.

2. Cross References. Hydrogen Fluoride and various fluoride salts.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Fluorides in sufficient quantity are toxic to humans, with doses of 250 to 450 mg giving severe symptoms and 4.0 grams causing death (364). The fatal dose has also been reported (1161) as 0.5 gms per kg of body weight and as 2.5 grams (3481).

There are numerous articles describing the effects of fluoride-bearing waters on dental enamel of children and a few papers pertaining to skeletal damage. The effects reported in many of these references, summarized in Table 6-5, lead to the generalization that water containing less than 0.9 to 1.0 mg/l of fluoride will seldom cause mottled enamel in children, and for adults concentrations less than 3 or 4 mg/l are not likely to cause endemic cumulative fluorosis and skeletal effects.

Abundant literature is also available describing the advantages of maintaining 0.8 to 1.5 mg/l of fluoride

FERRIC CHLORIDE $FeCl_3$ and $FeCl_3 \cdot 6H_2O$

1. General. Highly soluble in water, this substance has an acid reaction and imparts a brownish-yellow or orange color. Potential sources of pollution by ferric chloride include mining, ore milling, the chemical and dyeing industries, and water and sewage works. The oral LD_{50} for rats is given (364) as 900 mg/kg of body weight.

2. Cross References. Distilled Water, Iron, Chlorides.

3. Effects Upon Beneficial Uses.

a. Fish and Other Aquatic Life. The following concentrations of ferric chloride have been reported to be harmful or lethal to fish:

Concentration of $FeCl_3$ in mg/l	*Type of Water*	*Time of Exposure*	*Species of Fish*	*Reference*
0.6	tap	--	goldfish	1459
1.2	tap	6 days	stickleback	598
4.35	tap	3 days	goldfish	1459
9	distilled	20 hours	goldfish	313
20	tap	6 hours	goldfish	1459
74	turbid	96-hour TL_m	mosquito-fish	2940
100	soft	1.5 hours	goldfish	598, 313
540	--	86 minutes	minnows	991
2700	--	69 minutes	minnows	991
5400	--	54 minutes	minnows	991

The following concentrations of ferric chloride were reported to be not harmful to fish:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Species of Fish*	*Reference*
1	tap	10 days	stickleback	598
5	--	50 hours	young eels	1459
10	soft	96 hours	goldfish	313
100	hard	96 hours	goldfish	313, 598
270	--	90 minutes	minnows	353

Jones suggested that the toxic effect of $FeCl_3$ might be due principally to the increasing acidity of the solutions as the concentration of ferric chloride was increased (1459). In ferric chloride solution containing 1.0 mg/l of Fe^{+++} (at pH 5), Jones' fish lived for 10 days, as long as the controls (1459).

The threshold concentration of ferric chloride for immobilization of *Daphnia magna* in Lake Erie water was found to be 130 mg/l (358); and in another case less than 18 mg/l, for a 64-hour period (598). For *Polycelis nigra* the threshold for toxicity was 58 mg/l (608).

The following threshold concentrations of ferric chloride toward lower organisms in Lake Erie water at 20-25°C have been reported (2955):

Organism	*Threshold Concentration mg/l for Immobilization*
Daphnia magna, young	18
Cyclops vernalis	116
Diaptomus oregonensis	126

FERRICYANIDES AND FERROCYANIDES

(see Potassium Ferricyanide and Sodium Ferricyanide)

FERRIC OXIDE Fe_2O_3

(see also Iron and other iron salts)

This insoluble substance is used as a polishing powder for glass and metal, in ceramics, and as a pigment in iron paints. It is also a component of iron rust. For ice making, ferric oxide concentrations in excess of 0.14 to 0.20 mg/l are considered to be objectionable (168, 228, 231) and for carbonated beverages the limit is 0.3 mg/l, as Fe_2O_3. Nielson (1159) reports that concentrations of 2.0 mg/l of ferric oxide are sufficient to kill trout, salmon, and roach by blocking their gills, but small crustaceans are more resistant.

FERRIC POTASSIUM SULFATE $FeK(SO_4)_2$

(see also Iron and other iron salts)

Ellis (313) quotes references to the effect that ferric potassium sulfate, used as a mordant for dyeing and printing calico, was not fatal to tench during a one-minute exposure at a concentration of 10,000 mg/l. The maximum concentration of this salt tolerated by young eels for over 25 hours was reported (1459) to be about 14 mg/l.

FERRIC SULFATE $Fe_2(SO_4)_3$

(see also Iron and other iron salts)

This compound, slowly soluble in water, is used in dyeing, in ink manufacture, and in water and sewage treatment. Ellis (313) quotes a reference to the effect that a concentration of 0.716 mg/l in distilled water killed shiners, carp, and suckers in 12 to 24 hours. In a solution of ferric sulfate in distilled water, a concentration of only 0.1 mg/l of iron was reported to have killed certain fish within 24 hours (1459). (see also Distilled Water).

In highly turbid water at 19-23°C, Wallen et al. (2940) found the 24-, 48-, and 96-hour TL_m concentrations for the mosquito-fish *Gambusia affinis*, to be 133 mg/l. In Lake Huron water at 12°C, a concentration of 5.0 mg/l of iron sulfates (valence not given) had no effect on rainbow trout, bluegill sunfish, or sea lamprey during a 24-hour period (2976).

FERROUS CARBONATE $FeCO_3$

(see also Iron and other iron salts)

Insoluble in normal water and soluble only in water containing appreciable carbon dioxide, ferrous carbonate is seldom found in surface waters but occurs in some ground waters (364). According to Klut (945) a concentration of 0.3 mg/l of iron carbonate gives water an inky taste. Thresh (32) says that no potable water should contain more than 0.5 mg/l of iron as carbonate, but this amount cannot possibly have any effect upon health.

FERROUS CHLORIDE $FeCl_2$

(see also Iron and other iron salts)

Ferrous chloride is used in dyeing and printing fabrics, and in metallurgy. It is highly soluble in water (364). The threshold concentration of ferrous chloride for immobilization of *Daphnia magna* in Lake Erie water has been reported to be less than 38 mg/l for a 64-hour period. Concentrations of 1000 mg/l in hard water have killed goldfish within 10 hours; concentrations of 10 mg/l in hard water have not been harmful to goldfish in 96 hours (598). The highest concentration of ferrous chloride tolerated by young eels for more than 50 hours was reported to be about 13 mg/l (1459).

The ingestion of 200 to 800 mg of ferrous chloride daily by dogs induced no noteworthy changes in physiological behavior (353), but an acute poisoning of a 2½-

mg/l, i.e. all fish survived 30 mg/l but all died at 60 mg/l. Bringmann and Kuhn (2158, 3443) reported that the toxic threshold for *Daphnia* exposed for 48 hours at 23°C to ethylenediamine hydrochloride occurred at 8 mg/l, while for *Scenedesmus* the threshold concentration was 20 mg/l. *Echerichia coli* at 27°C showed an adverse response at 200 mg/l, and *Microregma* (a protozoan) at 30 mg/l.

ETHYLENEDIAMINETETRAACETIC ACID (EDTA)

The sodium salt of this acid, a soluble white powder, is used as a chelating agent under trade names such as Versene, Sequestrene, and Komplexon. One gram complexes 201 mg of calcium carbonate. Clemens and Sneed (2981) tested its effect on fingerling channel catfish in University of Oklahoma tap water at 25°C. They found the 24-, 48-, and 96-hour TL_m to be 167, 133, and 129 mg/l respectively. During each period, all fish withstood 100 mg/l and all succumbed at 316 mg/l.

ETHYLENE DICHLORIDE

(see Dichlorethane)

ETHYLMERCURIC BROMIDE

ETHYLMERCURIC PHOSPHATE

ETHYLMERCURIC OXALATE

(see Mercuro-Organic Compounds)

ETHYL PHTHALATE $C_{12}H_{14}O_4$

A colorless, practically odorless, oily liquid with a bitter disagreeable taste, this substance is used in the manufacture of celluloid and as a solvent for cellulose acetate in varnishes and dopes. It is also known as diethyl phthalate and it is nearly insoluble in water (364). Fish exposed in a fish bowl to cellulose acetate material known to contain ethyl phthalate died within a few hours, despite the low solubility of this substance. One drop of ethyl phthalate in 50 ml of water, corresponding to a concentration of about 1.2 mg/l killed a goldfish within 30 minutes (1559).

EXPLOSIVES

(see also Trinitrophenol, TNT, Toluene, Tetryl)

Liquid wastes from the manufacture of munitions and other explosives may contain mixtures of many substances, such as ammonium picrate, picric acid, toluene, mono-nitro-toluene, TNT, nitrobenzene, and orthodichlorobenzene. The principal detrimental effects of such substances appear to be their tastes and odors, although in higher concentrations they are inimical to aquatic life.

During World War II, according to Bandt (616) "bitter-almond" tastes in fish flesh were frequently caused by waste water from factories producing explosives, the main cause being traces of mononitrotoluene, nitrobenzene, and orthodichlorobenzene, which causes tastes when present in concentrations much smaller than those causing damage to the health of fish. Ruchhoft and Norris (655) reported on the concentration of ammonium picrate in wastes from shell-loading plant, pointing out that 0.5 mg/l of this substance can be tasted in water and that 1.0 mg/l is objectionable.

Red-liquor wastes from TNT-manufacturing plants are highly alkaline and contain toluene, sodium salts, and some TNT, but exhibit no B.O.D. Degani (544) shows that the threshold concentration to fish occurs when the increase in total-solids content from red liquor was 87.5 mg/l. A concentration of 1750 mg/l killed minnows in 130 to 500 minutes but did not kill carp or bullheads. At 7000 mg/l, however, minnows were killed in 37 to 120 minutes, carp in 126 minutes, and bullhead in 254 minutes.

The chief objectionable pollutants in wastes from a small-arms ammunition plant were soap solutions, cleansers, lubricants, acid liquors, and rinse waters containing copper and zinc (657).

FATS

(see Animal and Vegetable Oils, Oil-Petroleum)

FATTY ACIDS—GENERAL RCOOH

(see also Acetic Acid, Formic Acid, Oleic Acid)

Fatty acids, so named because two of the higher members of the series—palmitic and stearic acid—are prepared from animal fat, belong to the homologous series that may be represented by the general formula RCOOH, where R indicates a saturated hydrocarbon chain. The series starts with formic acid, HCOOH, and acetic acid, CH_3COOH, and runs through stearic acid, $C_{17}H_{35}COOH$. The higher fatty acids are used extensively in the soap industry and occur in wastes from laundries, bottle-washing processes, chemical plants, and pulp and paper mills. The limiting or threshold concentrations of a few specific fatty acids are given elsewhere in this chapter, but a few references have dealt with the fatty acids as a group.

Gehm (344, 658) summarizes literature to the effect that the sodium salts of fatty acids have a minimum lethal dose, MLD, for fish of 5.0 mg/l. Sodium oleate has a MLD of 5.0 mg/l and sodium linolate has a MLD of 10.0 mg/l. Van Horn et al. (190, 574) report that the sodium salt of the fatty acid fraction of crude soap from sulfate pulp mills had MLD of 5.0 mg/l for killing shiners and minnows in 120 hours at 18°C. For *Daphnia*, however, the MLD was only 1.0 mg/l. With sodium oleate and shiners, the MLD was also 5.0 mg/l in 120 hours at 18°C.

According to Grin (3450), synthetic fatty acids affect the organoleptic properties of water. Concentrations of 3-4 mg/l inhibits nitrification. He recommends that the limiting concentration of all synthetic fatty acids in domestic water supply reservoirs be considered as 0.1 mg/l.

FATTY AMINO COMPOUNDS

(see Chapter X)

FENURON

(see Chapter X)

FERBAM

(see Chapter IX)

Compound	Concentration in mg/l to Kill all larvae of Sea Lamprey	Concentration in mg/l Causing 10 Percent Mortality to		
		Rainbow Trout	Brown Trout	Bluegill Sunfish
2-bromo-4-nitrophenol	5	13	11	--
sodium salt	7	15	--	--
3-bromo-4-nitrophenol	5	11	--	15
5-chloro-2-nitrophenol	3	5	5	--
2, 5-dichloro-4-nitrophenol	3	13	7	--
sodium salt	5	17	--	--
3, 4, 6-trichloro-2-nitrophenol	5	17	15	--
sodium salt	13	23	--	--
3-trifluoromethyl-4-nitrophenol	2	9	7	--
sodium salt	2	7	--	--

e. Shellfish Culture. Water polluted with chlorophenols to the extent of 0.001 mg/l imparted a taste to oysters (3492).

HARDNESS

1. General. The term "Hardness" is applied to the soap-neutralizing power of a water. Any substance that will form an insoluble curd with soap causes hardness; but since iron, manganese, copper, barium, lead, zinc and other trace elements are seldom present in appreciable concentration in natural waters, hardness is attributable principally to calcium and magnesium ions.

Soap will not cleanse, nor lather, until all of the hardness is precipitated as insoluble salts of the fatty acids. This property of soap is used in an accepted test for hardness, whereby a standard soap solution is added to an aliquot portion of water until a persistent lather forms (469). The soap-neutralizing power, or hardness, is then expressed in terms of an equivalent concentration of calcium carbonate. In recent years, however, the "versenate" test for hardness has been perfected and it has largely supplanted the more-cumbersome soap test in standard laboratory procedure.

While hardness is caused only by cations such as calcium and magnesium and consequently is independent of the anions in the solution, the terms "temporary hardness" and "permanent hardness," or the corresponding terms "carbonate hardness" and "noncarbonate hardness," have been used extensively. Where carbonates or bicarbonates are present in concentrations equivalent to or greater than the calcium and magnesium, the scale that will form upon evaporation or heating will consist primarily of calcium carbonate and magnesium hydroxide. Such hardness is termed "carbonate" or, because the scale can be removed with acid, "temporary." Where alkalinity is low, the more-soluble sulfates and chlorides may form scales that cannot readily be dissolved by acid, and such hardness is called "non-carbonate" or "permanent."

Hardness in water may be caused by the natural accumulation of salts from contact with soil and geological formations, or it may enter from direct pollution by industrial wastes such as beam-house liquors from tanneries. Some industrial wastes have an indirect effect upon hardness in ground water by increasing the carbonic-acid content and favoring the solution of calcium and magnesium salts. Return flow from irrigation drainage also increases hardness.

The detrimental effects of hardness, as described hereinafter, include excessive soap consumption in homes and laundries; the formation of scums and curds in homes, laundries, and textile mills; the gallowing of fabrics; the toughening of vegetables cooked in hard water; and the formation of scales in boilers, hot-water heaters, pipes, and utensils.

The references described below deal with literature in which the detrimental effects of calcium and magnesium are grouped and described only as "hardness", without regard to the concentrations of the constituents. For other references, where specific pollutants are given, see the appropriate substance.

2. Cross References. Alkalinity, Bicarbonates, Calcium, Carbonates, Magnesium, and Chapter V.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The literature contains numerous statements relating to the desirable limits for hardness in municipal water supplies. Some of them are condensed in the following paragraph. Hardness over 100 mg/l becomes increasingly inconvenient, for it results in a waste of soap and the incrustation of utensils (330). In a few hard-water areas, a commonly prescribed limiting hardness is 140 mg/l (36). In good water, the total hardness is usually below 250 mg/l (632). The hardness of waters of "good" quality should not exceed 270 mg/l (630). In France, hardness in excess of 300 mg/l is considered suspicious and possibly indicative of pollution (32). In England, it is believed that water with a hardness in excess of 315 mg/l should be softened, primarily to remove harmful magnesium salts; but the use of water with 315 mg/l hardness without preliminary softening has been sanctioned by the government. Hardness above 500 mg/l is considered unsuitable for general domestic purposes (32, 36). In Germany, natural carbonate hardness above 350 mg/l is seldom found, and water with hardness above 448 mg/l is considered to be polluted (839).

The major detrimental effect of hardness is economic. Prior to the amount of synthetic detergents (see Chapter X), many studies showed that the decreased cost of soap alone justified the expense of municipal water softening. Now that syndets are widely used in lieu of soap products, there is a common misconception that hardness is no longer an economic factor. Aultman (34) dispelled that illusion by showing that the annual cost of cleansing agents for a family of five doubles as the hardness increases from zero to 375 mg/l. In a comprehensive economic analysis, De Boer and Larson (3151) found that the savings for soaps, syndets, general household cleansers, scouring compounds, bleaches and other additives were $1.15 per capita per year for each 100 mg/l reduction in hardness (based on 1960 costs).

Since calcium is absorbed and assimilated only in the presence of other essential nutritional factors, such as phosphorus and vitamin D, it is questionable whether calcium in water can be utilized by the body. However, it has been suggested that calcium in water may partially compensate for calcium deficiency in modern foods (330), and from several parts of the world have come reports that the incidence of tooth decay is lower in hard water areas than in soft water areas (629, 868, 921, 938, 1041, 1047). On the other hand, at least one study has found no significant correlation between hardness and caries (36).

Hard waters have had no demonstrable harmful effects upon the health of consumers (32, 36, 653, 947, 1047).

There is no conclusive proof that hardness causes urinary concretions or other diseases of the kidney or bladder (629, 1194, 1195); or biliary calculi, arteriosclerosis, dyspepsia, goiter, diarrhea, constipation, rheumatism, or stomach disorders (623, 1196); but the drinking water of an area may be considered as one of the factors predisposing to formation of calculi (3365). The role of hard water in the formation of urinary concretions is still controversial (1194); Rosenau (629) has cited a reference indicating a higher frequency of calcification of arteries among hard water users; and Murray et al. (1197) have suggested that iodine concentrations that are sufficient in soft waters to prevent goiter may not be adequate in hard waters.

Several investigators (3368, 3369, 3370, 3516, 3517) have found a negative correlation between hardness in the drinking water of an area and death rates from degenerative cardiovascular disease, i.e., softer water was associated with higher death rates. These variations are unexplained on dietary, racial, or social bases. They have been observed in Japan, England, South Africa, the Canary Islands, Australia, and the U.S. Although the correlation appears to exist, its causative factors remained unexplained as of 1961.

Water with a bicarbonate hardness in excess of 90 mg/l is reported to cause formation of a film in tea (1160). A study of the effects of different types of waters on the blanching of vegetables indicated that the greatest loss of ascorbic acid occurred in peas blanched in hard water. The highest calcium contents were found in vegetables blanched in hard waters with high calcium concentrations (220). A study of the effects of hard water used for cooking purposes revealed no changes in weight and composition of feces that could be related to the hardness of the water used for cooking legumes (1198).

Waters with a "temporary" hardness below 57 mg/l are often corrosive to metals (32). Poisonous concentrations of lead in drinking water are generally due to the corrosive action on lead pipes of water with an oxygen concentration below 6 mg/l, pH below 7, and carbonate hardness below 125 mg/l (1073, 1199). Waters with a hardness greater than 125 mg/l form protective coating on lead pipes within a few weeks, thereby limiting the solution of lead (1200); however, hardness does not absolutely prevent plumbosolvency (36, 1201). For further information on the relationship between hardness, carbonate equilibria, and corrosion, see Langelier (692).

b. Industrial Water Supplies. Excessive hardness is undesirable in water for laundries, carbonated beverages, metal finishing, dyeing and textile industries, food processing, paper and pulp, bottle washing, and photography, leather goods, and other industrial processes.

In laundries, if the total hardness of the raw-water supply is more than 286 mg/l, or if the temporary hardness is much more than 214-228 mg/l, water softened by ordinary base-exchange methods contains so much bicarbonate that yellow stains are formed on clothes during drying and finishing (237). In wool-washing, waters with zero hardness remove grease more readily from wool than waters of 57 to 72 mg/l of total hardness; but, owing to the formation in wool of lime soaps which absorb dirt and fatty acids, moderately hard waters yield a cleaner, softer product. The optimum hardness varies with the water and materials (260).

In baking, medium-hard water strengthens gluten, causing it to retain carbon dioxide better, and produces a finer-grained structure. Soft waters soften the gluten, thus producing a sticky dough and soggy bread. Very hard waters significantly retard fermentation (160). In the canning of fruits and vegetables, salts of calcium and magnesium may combine with pectous substances forming insoluble pectides that toughen the product (224). Hard water toughens peas and some varieties of beans (164, 217), when the hardness exceeds 200 mg/l. Hard water does not toughen corn and its use sometimes results in a product with a more desirable color than soft water. Hard water, with hardness below 350 mg/l, does not toughen beets, but may cause a white coating on the surface of the beets (217). Hardness tends to decrease water absorption in the reconstituting of dehydrated fruits and vegetables (3518).

A survey of paper mills indicated that water supplies in use at different factories varied in hardness from zero to 766 mg/l (243) (for recommendations, see the following tabulation).

Distilled water, with no hardness, has a disintegrating effect on Portland cement (1202). Water with a hardness greater than 10 to 20 mg/l will not attack cement if the CO_2 is less than 10 mg/l. If the pH is greater than 6.5, natural waters will attack concrete only if the hardness is less than 50 mg/l. With low hardness (less than 10 mg/l) attack occurs even when or the pH is as high as 7.5 or the CO_2 is negligible (360). When filtered through powdered cement, more lime was removed by hard water than by water free from carbonates (1203). The effect of hard waters on concrete varies with the pH and carbon-dioxide content of the water (1204). Hardness in mixing water had no effect on the "air-entrainment" properties of concrete because the hardness of the aqueous portion of a concrete mixture is so great that it is not appreciably affected by the hardness of the water supply (194).

The levels of hardness tolerable or desirable in water for various industrial purposes are given in Chapter V and summarized in the following tabulation:

RANGE OF RECOMMENDED VALUES FOR HARDNESS IN INDUSTRIAL WATER SUPPLIES

Industry and Process	*Limiting or Recommended Values, in mg/l*
Boiler feed water:	
at 0-150 psi	80
at 150-250 psi	40
at 250-400 psi	12
over 400 psi	2
Brewing	200–300
Carbonated beverages	200–250
Cooling	50
Food canning and freezing:	
general	50–85
legumes	25–75
fruits and vegetables	100–200
peas	200–400
Food equipment washing	10
Food processing, general	10–250
Laundering	0–50

Industry and Process	*Limiting or Recommended Values, in mg/l*
Pulp and paper making:	
groundwood pulp	200
soda pulp	100
kraft pulp, bleached	100
kraft pulp, unbleached	200
fine paper pulp	100
Rayon:	
pulp production	8
cloth manufacture	55
Steel manufacturing	50
Synthetic rubber	50
Tanning:	
beam house	513
tan house	50–135
Textile manufacture	0–50

c. Irrigation. After lime-soda softening, the water at Columbus, Ohio, appeared to have no effect either on the soil reaction or on the development of greenhouse plants (1205). Softened Colorado River water with a high content of sodium produced more bushy and compact begonia, fuschia, and camellia plants than unsoftened water with a high salt content (636). It has been reported that no injury to plants grown in calcareous soils result from watering with hard water; injury caused by applying hard water to acid soils can be prevented by adding a trace of borax (921).

Schroder reports that cyclamen sprinkled with tap water containing excessive hardness as $CaCO_3$ (186 mg/l) deposited a white crust of the carbonate and showed poor growth. Well water with 70 mg/l of carbonate hardness, but with considerably higher non-carbonate hardness, showed no plant injury because the calcium was held in solution as sulfate and bicarbonate (921, 1206). Spurway (1207) maintains that carbonates in hard water may produce alkalinity in greenhouse soils, thereby adversely affecting the growth of plants.

Wilcox stated that hard water is superior to soft water for irrigation (269). For a detailed discussion of the effects of calcium and sodium in irrigation water, see Chapter V.

d. Fish and Other Aquatic Life. The growth of fish in aquaria may be limited by the hardness of the water (1208). The concentration of calcium in vertebrae of fish is not affected by the hardness of the water (1209).

Soft water solutions increase the sensitivity of fish to toxic metals; in hard waters toxic metals may be less dangerous (313, 1111, 1210, 2154, 2936, 2942, 2943, 2944, 2954, 3519). For example, in hard water copper sulfate is less dangerous to fish than in soft water (602). In hard water insoluble copper compounds are precipitated, thereby removing some of the toxicity (600, 900, 1011, 1211). Tarzwell and Henderson (2154) reported that certain beryllium uranyl, zirconium, titanium, vanadium, cadmium, lead, and copper salts were more toxic in soft water than in hard. Dichromates and non metallic compounds such as ammonia, phenol, and naphthenic acid have also been shown (2936, 2954) to be less toxic in hard water than in soft. LeClerc et al. (2942, 2943, 2944) ran numerous fish toxicity tests in distilled water and hard water, finding in most instances that hard water raised the minimum dose causing death to minnows. Sigler and his colleagues (3465, 3467) reported that the effects of fluorides on fish are counteracted by calcium and magnesium.

e. Stock and Wildlife Watering. Experiments with calves and chicks have indicated that those supplied with hard water developed somewhat better than those supplied with distilled water (1047).

The role of hard waters in the development of urinary concretions is still controversial. (see Domestic Water Supplies, above).

HEAVY METALS

1. General. The heavy metals included under this heading comprise copper, lead, zinc, and others of nonspecific designation that may originate in sewage and industrial wastes. All of the references relating specifically to such substances are covered elsewhere in this chapter under the designated metal. This section deals only with references that group heavy metals into one category.

2. Cross References. Each heavy metal, by name.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Goudey (992) has suggested an upper limit of 5.0 mg/l for heavy metals in Class AA and A waters; 10 mg/l for Class B and C waters.

b. Industrial Water Supplies. Aluminum has been damaged by water supplies containing high concentrations of salts of heavy metals (562). Heavy metals are undesirable in water for use in the textile industry (256). The upper limit recommended for heavy metals in water to be used in rayon industries is 0.01 mg/l (405, 550).

c. Irrigation Waters. The presence in water of low concentrations of heavy metals may be beneficial for irrigation (563). An upper limit of 10 mg/l has been suggested by Goudey (992) for heavy metals in ground waters to be classified as safe for irrigation use.

d. Fish and Other Aquatic Life. River Boards in England have specified that heavy metals, individually or in total, shall not exceed 0.5 mg/l (1756) and 1.0 mg/l (2950) in sewage or industrial effluents discharged to the watershed.

HEPTACHLOR

(see Chapter IX)

HEPTANE $CHCH_3(CH_2)_5CH_3$

(see also Hydrocarbons—General)

One of the saturated (paraffin) hydrocarbons, heptane is a volatile, flammable liquid, used as a standard in testing knock of gasoline engines. It may occur in gasoline or other petroleum wastes. It is soluble in water to the extent of 52 mg/l at 15.5°C (911). Hubault (461) reports that the concentrations of n-heptane required to kill white roach in one to four hours at 21°C was 30 mg/l. In contrast, Wallen et al. (2940) tested the effect of heptane on mosquito-fish *(Gambusia affinis)* in highly turbid water at 20-27°C. They found the TL_m values

after 24, 48, and 96 hours all to be 4924 mg/l. Turbidity was not reduced by this concentration of heptane.

HEXACHLOROCYCLOHEXAN

(see Chapter IX—Benzene Hexachloride)

HEXANE $CH_3(CH_2)_4CH_3$

This colorless, volatile liquid is soluble in water to the extent of 138 mg/l at 15.5°C (911). According to North et al. (2108), 10 mg/l of n-hexane showed little or no effect on the photosynthetic activity of the giant kelp *(Macrocystis pyrifera)* during a 96-hour exposure.

HEXAVALENT CHROMIUM

(see Chromium)

HEXADECANOL

(see Cetyl Alcohol)

HUMIC ACID

This collective term is used to describe the acid product resulting from the decomposition of organic matter, particularly dead plants. It is a dark-brown dust-like powder that is slightly soluble in water. Sartorius and Wever (674) state that the presence of humic acid in sufficient quantities to cause color is not dangerous in itself but bacteria live longer in such water and *E. coli* increase in numbers. Humic acid from various sources interfered with chlorination, according to Moiseev (675) but the extent of interference varied with the sources of acid. In some cases, no interference resulted until the concentration of humic acid approached 100 mg/l; in other cases, interference occurred at 0.1 mg/l. Iron humate in a concentration of 0.5 mg/l gives water a marshy, earthy taste (945).

HYAMINE

(see Chapter X)

HYDRAZINE NH_2NH_2

(see also Hydrogen Peroxide, Phenylhydrazine)

Hydrazine is a fuming oily liquid with a penetrating odor, extremely explosive and a violent poison. It is used in rocket fuels. Hydrazine hydrate, $NH_2NH_2.H_2O$, a fuming refractive liquid with a faint characteristic odor, is miscible with water and alcohols. It is used as a reducing agent and as a solvent for inorganic materials; also, to decompose hydrogen peroxide in V-2 rockets.

Southgate (659) reported that waste waters from the testing of rocket motors contained hydrazine hydrate, of which 0.7 mg/l caused fingerling trout to lose equilibrium in less than 24 hours. On the other hand, hydrazine hydrate and hydrazine sulfate, $NH_2NH_2 \cdot H_2SO_4$, had no effect on sea lampreys exposed for 24 hours at a concentration of 5 mg/l (2976).

Corti (146) reports that rainbow trout exposed to 146 mg/l of NH_2NH_2 at pH 8.35 and 13.5°C demonstrated an adverse reaction after 14 to 18 minutes and succumbed completely in 22 to 35 minutes.

The oral LD_{50} for rats and mice is reported as 60 mg/kg (3495, 3496). Confronted with drinking water containing 100 mg/l of hydrazine, white rats restricted their fluid intake and with higher concentrations they were even more reluctant to drink the water. Apparently white rats will not ingest voluntarily sufficient hydrazine in drinking water to bring about pathological changes usually associated with hydrazine intoxication (3496).

For industrial process waters, hydrazine has been used in boiler-feed-water treatment as an oxygen scavenger and bactericide (3497).

HYDROCARBONS (GENERAL)

(see also Oil (Petroleum), Methane, Heptane, Pentane, and other individual hydrocarbons).

Although they may act as bactericides, fungicides, algicides, insecticides, and weed-killers, and although they may be capable of toxic action toward fish and shellfish, many gaseous, liquid, and solid hydrocarbons are capable of being attacked and decomposed by certain bacteria, yeasts, and molds, of which about 100 species have been identified by Zobell (676).

There is a possibility that leaks from natural-gas transmission lines will affect water supplies that are near enough to become polluted, according to Soule (1577). The natural gas tested comprised 94.40 percent methane, 3.37 percent ethane, 0.98 percent propane, 0.36 percent butanes, 0.09 percent pentanes, and 0.19 percent hexanes by volume, the remainder being N_2 and CO_2. It was found by experiment that natural gas will impart to water an odor similar to benzene, the odor being dependent on the volume of gas, the volume of water, and the period of contact.

HYDROCHLORIC ACID

(see Hydrogen Chloride)

HYDROFLUORIC ACID

(see Hydrogen Fluoride)

HYDROGEN CHLORIDE HCl

(see also Acidity, Chlorides, pH)

This strong acid, with innumerable industrial applications, is highly soluble in water, dissociating as hydrogen and chloride ions. Inasmuch as chloride ions are relatively innocuous, the principal effect of HCl lies in changing the hydrogen-ion concentration, except in highly buffered waters. Only when the pH value is depressed to 5.0 or lower will HCl prove lethal to fish (361). Cairns and Scheier (2933, 2934, 2935) show that the 96-hour TL_m at 20°C for the bluegill sunfish occurs when the pH value is lowered to 3.6 by HCl. Corresponding lethal pH values of 4.0 and 4.6 have been reported for goldfish and sticklebacks respectively (2920). At pH 5.0, sticklebacks survived 9 days in water to which HCl had been added, at pH 4.0 only 10.5 hours, and at pH 3.0 only 80 minutes (2941).

It is the resulting pH rather than the concentration of HCl that governs lethality toward aquatic life; hence the discrepancies in the following reports of lethal doses of HCl toward fish.

Concentration of HCl in mg/l	Time of Exposure	Species of Fish	Remarks	Reference
3.6	48 hours	sunfish	lethal in distilled water	313
3.65	24 hours	carp, shiners, suckers	lethal	359
4-6	6 hours	minnows	MLD in distilled water	2942
8.0	24 hours	sunfish	lethal	359
10	24 hours	trout	MLD	359
67.5	--	whitefish, pickerel	toxic threshold	359
80	24 hours	creek chub	lethal	1442
100-110	6 hours	minnows	MLD in hard water	2942
166	4-7 hours	goldfish	killed in hard water	313
200	--	perch, roach	collapsed in distilled water	313
282	96-hour TL_m	mosquito-fish	turbid water	2940
1000	2-5 minutes	trout	overturned in tap water	313

In contrast, Belding (359) reports that 20 mg/l had no effect on minnows and goldfish. According to Ellis (313) in hard water 157 mg/l did not injure goldfish in over 100 hours of exposure. Gillette et al. (1442) indicate that creek chub survived 60 mg/l for 24 hours, but succumbed at 80 mg/l.

The toxic threshold of HCl toward *Daphnia magna* has been reported at 56 to 62 mg/l in soft water and Lake Erie water respectively (313, 358).

It has been reported that HCl can be tasted at a concentration of about 32 mg/l in water (1578) although it is apparent that this threshold is dependent upon the buffering power of the water.

HYDROGEN CYANIDE HCN

1. General. This colorless gas or liquid boils at 26°C and is highly soluble as a gas or miscible with water as a liquid (364). It is used for exterminating rodents and insects in ships and buildings, or for killing insects on trees (677). For this literature review, HCN is particularly significant because it forms in water whenever soluble NaCN or KCN are added thereto. In water HCN dissociates into hydrogen and cyanide ions according to the reaction:

$$HCN \rightleftharpoons H^+ + CN^-$$

the degree of dissociation being governed by the equilibrium equation:

$$\frac{[H^+][CN^-]}{[HCN]} = 7.2 \times 10^{-10}, \text{ at } 25°C$$

or by rearranging

$$\frac{[CN^-]}{[HCN]} = \frac{7.2 \times 10^{-10}}{[H^+]}$$

It is evident from this equilibrium that the ratio of cyanide ion to undissociated HCN is a function of pH. At pH values of 7 or below, less than 1 percent of the cyanide molecules are in the form of cyanide ion; at pH 8, only 6.7 percent; at pH 9, only 42 percent; and at pH 10, 87 percent of cyanide is dissociated (911).

When NaCN or KCN is added to water in dilute concentrations, the salts dissociate immediately but the CN^- ions react with H^+ ions in the water to form HCN with a resulting rise in pH. It is now generally recognized that undissociated HCN is more toxic toward aquatic life than the cyanide ion (1539, 1580, 2109); but Southgate (346) found that there was no appreciable change in toxicity within a pH range of 6.0 and 8.5. For more discussion of this point, see Cyanides.

2. Cross References. Cyanides, Cyanogen Chloride, Nitriles, Ammonium Thiocyanate, Potassium Cyanate, Potassium Cyanide, Potassium Ferricyanide, Potassium Thiocyanate, Sodium Cyanate, Sodium Cyanide, Sodium Ferricyanide, Sodium Thiocyanate.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The average fatal dose for man by ingestion is 50 to 60 mg (364, 1117). A dose of 161 mg was fatal to man in 15 minutes (353). No information on the effect of a daily intake of HCN was uncovered, but for cyanide ions, 2.9 to 4.7 mg per day were reported as not harmful (922), with 37.8 mg or over being fatal. The average fatal dose for HCN ingestion by man has also been given as 50-60 mg (2963, 3498). Carson (1483) states that the average fatal dose of HCN for a man is 0.7 to 3.5 mg per kg of body weight. On this basis, the fatal dose for a man weighing 165 pounds would be about 52 to 260 mg. See Cyanides for a discussion of drinking water standards.

b. Stock and Wildlife Watering. Toxic and lethal doses of HCN have been reported (353) as follows:

Animal	Dose of HCN	Animal	Dose of HCN
Cow	0.39 to 0.92 grams	Horse	0.39 grams
Sheep	0.04 to 0.10 grams	Dog	0.03 to 0.04 grams

For sheep, toxic doses have also been given as 1.05 mg per kg of body weight. Samples of water fatal to cows and ducks contained 103 mg/l of hydrogen cyanide (353, 1118). Rats fed for two years with food containing 100 to 300 mg/kg of HCN showed no signs of cyanide toxicity. There were no hematological or pathological evidences of adverse effects (3499).

c. Fish and Other Aquatic Life. Most of the literature on the effects of HCN and other cyanides expresses toxicity effects in terms of the cyanide ion (see Cyanides). Garrett and Daugherty (1441, 2959, 2960) used the marine pin perch (*Lagodon rhomboides*) as a test animal and found the 24-hour TL_m to be 0.069 mg/l, while all fish died at 0.10 mg/l. They concluded that it would be unsafe to discharge HCN to ocean waters when it resulted in a concentration greater than 0.05 mg/l. According to Czensny (1575) concentrations of 0.10 to 0.15 mg/l of HCN are toxic to trout. For further experimental results, see Cyanides.

HYDROGEN FLUORIDE

(see also Fluorides)

This colorless gas is very soluble in water, dissociating to hydrogen and fluoride ions (see Fluorides). Hydrogen fluoride, per se, is reported to be harmful to fish at 40 mg/l and lethal at 60 mg/l (353).

HYDROGEN ION

(see pH)

HYDROGEN PEROXIDE H_2O_2

(see also Hydrazine)

This colorless, unstable liquid with a bitter taste is miscible with water and is sold commercially as a 3 per-

cent solution for medical use. In the pure state it is used in rocket fuels and has occurred in waste waters from the testing of rocket motors. Southgate (659) showed that over 40 mg/l of hydrogen peroxide was toxic to fingerling trout, but less than 40 mg/l had no harmful effect in 48 hours. Concentrations of 200 mg/l are bactericidal but do not impart a taste to water. Higher dosages cause very unpleasant tastes (1581).

HYDROGEN SULFIDE H_2S

1. General. This flammable, poisonous gas with a characteristic odor of rotten eggs is highly soluble in water, to the extent of about 4000 mg/l at 20°C and one atmosphere. Upon solution it dissociates according to the reactions:

$$H_2S \rightleftharpoons HS^- + H^+$$

and

$$HS^- \rightleftharpoons S^{--} + H^+$$

The dissociation equilibria are such that, at 18°C, the ratios between H_2S, HS^-, and S^{--} are as follows:

$$\frac{[HS^-]}{[H_2S]} = \frac{9.1 \times 10^{-8}}{[H^+]}$$

$$\frac{[S^{--}]}{[HS^-]} = \frac{1.2 \times 10^{-5}}{[H^+]}$$

Thus, at pH 9 about 90 percent of the sulfide is in the form of HS^-, at pH 7 it is about equally divided between HS^- and H_2S, and at pH 5 less than 1 percent is present as HS^-; consequently, H_2S will escape to the air. Only at pH 10 and above is sulfide ion present in appreciable proportions.

The sources of hydrogen sulfide in water include natural processes of decomposition, sewage, and industrial wastes such as those from tanneries, paper mills, textile mills, chemical plants, and gas-manufacturing works.

In the presence of certain sulfur-utilizing bacteria, sulfides and hydrogen sulfide can be oxidized to colloidal sulfur, and these bacteria or their deposits may be considered as corollary pollutants.

2. Cross References. Sulfur, Gas-plant Wastes, pH, Chapter VII-Sulfur Bacteria.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Hydrogen sulfide is present in many municipal supplies as a result of the anaerobic decomposition of underground organic deposits. While generally considered undesirable because of their odors, such waters are sometimes prescribed or recommended for respiratory, metabolic, and skin disorders, especially in Europe where health resorts have been founded upon such waters (e.g. Spa, Bad Wiessee) (653). The minimum concentration of hydrogen sulfide detectable by taste in drinking waters is given as 0.05 mg/l (2059).

b. Industrial Water Supplies. The concentration of hydrogen sulfide in certain industrial waters, especially those used for foods and beverages, is frequently limited. These restrictions are described in Chapter V, from which the following material was condensed.

Industrial Use	*Range of Recommended Threshold Values in mg/l*
Baking	0.2
Boiler feedwater	
0 to 150 psi	5
150 to 250 psi	3
over 250 psi	0
Brewing	0.2
Carbonated beverages	0.0 to 0.2
Confectionary	0.2
Cooling Water	5.0
Food canning and freezing	1.0

In preparing photographic developers, H_2S is particularly bad impurity, according to Crabtree and Mathews (242).

c. Fish and Other Aquatic Life. The following concentrations of hydrogen sulfide have been reported as toxic to fish within the time specified:

Concentration of H_2S in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
0.86	--	24 hours	trout	359, 311
1.0	--	--	minnows, shiners	190, 3500
1.0	--	--	king salmon, cutthroat trout	3501, 3502
1.0	--	24 hours	trout	362, 1046, 658
1.0	--	--	salmon	1582
1.2	15°C	--	silver salmon	3501, 3502
1.38	--	48-hour TLm	fathead minnows	3335
3.3	--	24 hours	carp	359
3.8	--	24 hours	suckers	359, 311
4.3	--	24 hours	goldfish	359, 311
4.9 to 5.3	--	1 hour	sunfish	363, 313
5	hard	200 hours	goldfish	313
5	--	1 hour	fish	463, 311
5-6	--	24 hours	minnows	362
6.25	--	24 hours	carp	359, 311
8-12	--	24 hours	carp	362, 1046
10	hard	96 hours	goldfish	313, 367
10	tap	15 minutes	trout	313
25	--	24 hours	goldfish	359
100	tap	3 hours	tench	313

In contrast, the following concentrations of hydrogen sulfide were survived by the test fish:

Concentration in mg/l	*Time of Exposure*	*Type of Fish*	*Reference*
0.3	--	king salmon	3503
0.5	--	cutthroat trout	3503
0.7	--	silver salmon	3503
4.0	24 hours	minnows	362

According to Haydu et al. (1582) the maximum concentration of H_2S tolerated without injury by the most sensitive fish was about 0.3 mg/l, while Nellist (1466) considers 1.0 mg/l to be the limiting toxic concentration. Murdick (1460) sets the critical concentration at 0.5-1.0 mg/l.

For *Daphnia magna,* the minimum lethal concentration has been given as 1.0 mg/l (190, 313, 574); for mayfly larvae, 1.0 mg/l; and for chironomous larvae 750 mg/l (190, 3500). Hydrogen sulfide is toxic to most algae (1452); however, hundreds of species of plant microorganisms have been found thriving in stagnant ditches containing 0.22 to 0.45 mg/l of H_2S.

d. Shellfish Culture. According to Oliveira (1215), the hydrogen sulfide generated by a deposit on the bottom of a bay was an important factor in causing the death of young oysters.

HYDROQUINONE $C_6H_4(OH)_2$

(see also Benzene Derivatives, Phenol, Pyrocatechol, and Resorcinol)

Known also as p-dihydroxybenzene, this crystalline substance is highly soluble in water. It is used as an anti-

oxidant, as a reducer in photographic developing, and as a chemical reagent in phosphate tests. The oral LD_{50} for rats is given as 320 mg/kg of body weight (364).

According to Sollman (1474), the approximate 48-hour TL_m for goldfish was 0.287 mg/l. In Lake Huron water at 12°C, rainbow trout succumbed to 5 mg/l of hydroquinone in 4 hours, bluegill sunfish in 6 hours, and sea lampreys in 14 hours (2976). Threshold concentrations of hydroquinone have been given by Bandt (2904) as 0.2 mg/l for carp, perch, and stickleback at 16°C in moderately hard water.

For lower organisms in River Havel water, Bringmann and Kuhn (2158, 3343) determined the threshold concentrations for adverse effect to occur as follows:

Organism	*Temperature, °C*	*Threshold Concentration, mg/l*
Daphnia	23	0.6
Microregma	--	2.0
Scenedesmus	24	4.0
E. coli	27	50.0

Bandt (2904) reports the toxic threshold for the water flea, *Gammarus,* to occur at 1.5 mg/l.

HYDROXIDES OH-

(see also Alkalinity, Carbonates, Bicarbonates, pH, and cations that form hydroxides, such as calcium, potassium, and sodium).

The concentration of hydroxyl ions in any solution is indicated by the pH value (see pH). Hydroxides also contribute to the alkalinity of water and markedly influence carbonate equilibria (see Alkalinity, Carbonates, and Bicarbonates). The hydroxyl-ion concentration is given by the relations:

$$[OH^-] = \frac{10^{-14}}{[H^+]} \text{ in mols per liter}$$

$$\text{or Hydroxides} = \frac{17{,}000 \times 10^{-14}}{[H^+]} \text{ in mg/l as } OH^-$$

$$\text{or Hydroxides} = \frac{50{,}000 \times 10^{-14}}{[H^+]} \text{ in mg/l as } CaCO_3$$

Thus, at pH 10, the alkalinity attributable to hydroxide ion is only 5.0 mg/l and at lower pH values the concentration of hydroxyl ions in relations to other forms of alkalinity is insignificant.

While not present in appreciable concentrations in natural streams, hydroxides may be increased materially by the discharge of alkaline industrial wastes such as those from beam-houses in tanneries, soda and sulfate pulping mills, cotton kiering, wool scouring, and finishing in textile mills.

Most of the references dealing with the effects of hydroxides on the beneficial uses of water express concentrations in terms of salts such as KOH, NaOH, and $Ca(OH)_2$. Such literature is covered under the appropriate cation in this report. Only those references in which hydroxide is reported as such are discussed below.

The N.E.W.W.A. Committee on Water Quality Tolerances for Industrial Uses (159) suggests the following limits for the hydroxide-ion concentrations in boiler feed waters:

Pressure, in psi	*Hydroxide, in mg/l*
0-150	50
150-250	40
250-400	30
400 and over	15

In testing the relative toxicities of the various anions of sodium salts toward *Polycelis nigra,* a flatworm, Jones (354) found the toxic threshold of hydroxide to be 13 mg/l. For toxicities of hydroxides to fish and other organisms, see the specific salts.

HYDROXYLAMINE NH_2OH

This hygroscopic solid is very soluble in water with an alkaline reaction, apparently from dissociation and the formation of ammonium ions. Corti (146) tested rainbow trout (*Salmo irideus*) at a concentration of 150 mg/l of hydroxylamine and a temperature of 13.5°C. The fish displayed their first adverse reaction in 5 to 10 seconds, turned over in 4 to 6 minutes, and made their last motion in 9 to 10 minutes.

HYPOCHLORITES

(see Calcium Hypochlorite, Chlorine, Sodium Hypochlorite)

HYPOCHLOROUS ACID

(see Chlorine)

IGEPAL CA

IGEPON T

(see Chapter X)

INDOLE C_8H_7N

This highly odoriferous compound occurs in coal tar, feces, and some plants. Soluble in hot water, it produces a pleasant odor in dilute concentrations; hence it is used in perfumes. In Lake Huron water at 12-13°C, exposure to 5 mg/l of indole for ten hours produced death or obvious distress to trout, but the same concentration for 24 hours had no effect on bluegill sunfish or sea lamprey (1976).

INSECTICIDES

(see Chapter IX)

IODINE I

Elemental iodine and iodides occur only as traces in fresh natural waters, but their concentration in sea water is somewhat higher. There iodine is absorbed by certain seaweeds, the ash of which constitutes a source of sodium iodide and iodine for commercial use. Because they are used only sparingly in industry, e.g. for medicines, germicides, and analytical chemistry, iodine and iodides are not likely to occur in polluted waters in significant concentrations. There is a possibility, however, that radioactive iodine from medical and research institutions may be discharged accidentally (see Chapter VIII).

In contrast with chlorine, elemental iodine is only slightly soluble in water, except in the presence of iodides, and it is not appreciably hydrolyzed in weak acid

solution. Its disinfecting power, therefore, is not attributable to the HOI molecule or to iodide, but rather to molecular iodine or possibly to tri-iodides (1216). Extensive unpublished research on the mechanism of disinfection by iodine and the effects of iodine upon human physiology has been performed by Fair and others at Harvard University. Iodine has been used successfully for the disinfection of water in swimming pools (3504) with residual concentrations of free iodine in the range of 0.2 to 0.6 mg/l.

There is extensive literature dealing with the relationship between iodine deficiencies in water or food and the incidence of goiter, but a thorough review of it is not within the province of this survey. To overcome an iodine deficiency and to minimize goiter in the community, Rochester, New York, added iodine to its water supply from 1923 to 1933; but, owing to economic factors, the availability of a superior vehicle (salt) for the dispersing of iodine, and the adverse effect upon certain individuals sensitive to iodine, the Rochester experiment was abandoned and further mass treatment for iodine deficiency was discouraged (152, 330). The iodine content of blood appears to be independent of the iodine in domestic water supplies (3505, 3506).

It has been reported (1586) that 8 mg/l of iodine destroys all forms of water-borne pathogens, but no adverse affects were noted when personnel in the tropics used drinking water containing NaI at a rate of 12 mg of iodine per man per day for 16 weeks and 19.2 mg per day for the next 10 weeks (1587).

Ellis (313) quotes references to the effect that 28.5 mg/l of iodine killed minnows and goldfish.

With the advent of nuclear testing and the extensive use of radioisotopes in medicine and industrial research, radioiodine (I-131) has become a significant environmental factor. The maximum permissible level of I-131 in drinking water has been given as 0.03 microcurie per liter (3375, 3507, 3508). Algae and other plankton concentrate radioiodine from water by factors in excess of 100,000 within a few days after the iodine becomes available (3509). For further information on concentration factors, see Chapter VIII.

IODOACETIC ACID CH_2ICOOH

This white crystalline substance is highly soluble in water. Hiatt et al. (3350) found that 10 mg/l of iodoacetic acid produced a moderate irritant activity in marine fish.

IPC

(see Chapter IX)

IRON BACTERIA

(see Chapter VII)

IRON Fe

1. General. Metallic iron and its common alloys are of interest in this survey primarily because they are corroded by water in the presence of oxygen. The resulting products of corrosion, in which the iron is in the ionic or molecular state, may in themselves be pollutants of water. In addition to corrosion products, natural waters may be polluted by iron-bearing industrial wastes such as those from pickling operations and by the leaching of soluble iron salts from soil and rocks, e.g. acid-mine drainage and iron-bearing ground water.

Although many of the ferric and ferrous salts such as the chlorides are highly soluble in water, the ferrous ions are readily oxidized in natural surface waters to the ferric condition and form insoluble hydroxides (3510). These precipitates tend to agglomerate, flocculate, and settle or be absorbed on surfaces; hence, the concentration of iron in well-aerated waters is seldom high. In ground water, the pH and Eh may be such that high concentrations of iron remain in solution (3510, 3520).

The following material deals with references to iron or iron ions when no designation of a salt or anion has been made. For literature covering the various ferric and ferrous salts, see the cross references.

2. Cross References. Ferric Chloride, Ferric Oxide, Ferric Potassium Sulfate, Ferric Sulfate, Ferrous Carbonate, Ferrous Chloride, Ferrous Sulfate, Chapter VII-Iron Bacteria.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The 1962 Drinking Water Standards of the USPHS (2036) included a recommended limit of 0.3 mg/l for iron. The 1958 WHO International Standards (2328) contain a "permissible limit" of 0.3 mg/l and an "excessive limit" of 1.0 mg/l but prescribe no maximum allowable limit. The 1961 WHO European Standards set a recommended limit of 0.1 mg/l for iron. Some authorities (1217, 1218) maintain that drinking water should not contain more than 0.1 mg/l of iron.

The WHO and USPHS limits are based not upon physiological considerations, for iron in trace amounts is essential for nutrition. Indeed, larger quantities of iron are taken for therapeutic purposes (555, 1077). The daily nutritional requirement is 1 to 2 mg, and most diets contain 7 to 35 mg per day, with an average of 16. Consequently, drinking water containing iron in unpalatable and unesthetic concentrations, say 1.0 mg/l, would have little effect on the total daily intake.

Instead of physiological reasons, therefore, the limit is based on esthetic and taste considerations. Iron and manganese tend to precipitate as hydroxides and stain laundry and porcelain fixtures. It has also been reported (1160) that ferric iron combines with the tannin in tea to produce a dark violet color.

The taste threshold of iron in water has been given (3511) as 0.1 and 0.2 mg/l of iron from ferrous sulfate and ferrous chloride respectively. It has also been reported (3300) that ferrous iron imparts a taste at 0.1 mg/l and ferric iron at 0.2 mg/l. In contrast, Lockhart et al. (3241) indicated that the taste threshold of ferric sulfate was 10 mg/l of iron. Using a statistically controlled taste panel, Cohen et al. (3301) found that the median taste threshold for ferrous sulfate in spring water occurred at 1.8 mg/l of iron, but for the most sensitive individuals it was 0.12 mg/l. Similarly, for ferrous sulfate in distilled water, the median taste threshold concentration was 3.4 mg/l of iron but the most sensitive individuals tasted 0.04 mg/l.

Iron in domestic water supplies containing organic matter may be present in chelated form and consequently will not precipitate readily (2338, 3510, 3512). Furthermore, it may occur in microbial protoplasm as described under part 3e, following.

b. Industrial Water Supplies. The ranges of recommended threshold values for iron in process and cooling waters are given in Chapter V. Most of this information has been reassembled and tabulated below:

Industrial Use	*Range of Recommended Threshold Values in mg/l*
Baking	0.2
Brewing	0.1 to 1.0
Carbonated beverages	0.1 to 0.2
Cooling water	0.5
Confectionary	0.2
Electroplating	traces
Food canning and freezing	0.2
Food equipment washing	0.2
Food processing, general	0.2
Laundering	0.2-1.0
Oil-well flooding	0.1
Photographic processes	0.1
Pulp and paper making	
Ground wood pulp	0.3
Soda pulp	0.1
Kraft pulp, bleached	0.2
Kraft pulp, unbleached	1.0
Fine paper pulp	0.1
Rayon manufacture	0.0 to 0.05
Sugar making	0.1
Tanning processes	0.1 to 2.0
Textile manufacture	0.1 to 1.0

c. Irrigation. Iron is one of the minor constituents of irrigation water, usually occurring in low concentrations. It is generally of little importance in irrigation practice (268). Chelated iron has been used to combat chlorosis in plants.

d. Stock and Wildlife Watering. Iron is an essential constituent of animal diets (553), but animals are sensitive to changes in iron concentration (945). Cows will not drink enough water if it is high in iron, and consequently, milk production is affected (549).

e. Fish and Other Aquatic Life. Most of the references dealing with this beneficial use are expressed in terms of specific iron salts (see cross references). When iron is added to water in the form of chlorides, sulfates, or nitrates, the salt dissociates but the resulting ferrous or ferric ions combine with hydroxyl ions to form precipitates. Hence, very little of the iron remains in solution; but if the dosage is sufficient and the water is not strongly buffered, the addition of a soluble iron salt may lower the pH of the water to a toxic level. Furthermore, the deposition of iron hydroxides on the gills of fish may cause an irritation and blocking of the respiratory channels. Finally, heavy precipitates of ferric hydroxide may smother fish eggs (346, 2109, 3513).

Knight (540) tested the effects of wastes from nail-making plants on trout, stickleback, and perch. These wastes contained high concentrations of chloride, hydrogen, ferric, and ferrous ions, but the pH values were not specified. Concentrations of 1000 mg/l of these mixed salts killed most fish within a few hours, but hardy stickleback were not killed until five hours exposure to 2500 mg/l. Much of the killing action was attributed to coatings of iron oxide or hydroxide precipitates on the gills. According to Southgate (346), the toxicity of iron and iron salts depends on whether the iron is present in the ferrous or ferric state and whether it is in solution or suspension. The following limiting concentrations have been noted:

Concentration of Iron, in mg/l	*Remarks*	*Reference*
0.2	Threshold concentration for lethality to three types of fish	1023
0.9	Carp will die at this concentration if pH is 5.5 or lower	306
1-2	Death of pike, tench, and trout at pH 5.0-6.7	1459, 1588
1-2	No deaths among dogfish during one week	3415
1-2	Indicative of acid pollution and other conditions unfavorable to fish	247
5	Killed dogfish in 3 hours	3514
10	Caused serious injury or death to rainbow trout in 5 minutes	604
40	Not lethal to fingerling channel catfish in 96 hours when added as ferrous disodium versanate	2981
50	Upper limit for fish life	801

Waters that support good fish fauna in the United States, according to Ellis (310), have the following concentrations of iron:

Concentration of Iron, in mg/l	*Percent of Waters Having This Concentration, or Less*
0.0	5
0.3	50
0.7	95

Crenothrix, Gallionella, and other iron bacteria utilize iron as a source of energy and store it in their microbial protoplasm. They may accumulate in wells, treatment plants, pipelines, and other waterworks structures; or they may pass into the distribution system and cause customer complaints (2700, 2701, 2705). At Wilmington, California, where the residual iron content was only 0.025 mg/l, Wilson (1219) found growths of *Crenothrix.* Trouble with this organism is experienced frequently when the iron exceeds 0.2 mg/l.

Radioactive iron, Fe-59, may be concentrated by certain microorganisms in aquatic and marine environments. For further details on concentration factors for radionuclides, see Chapter VIII. The following concentration factors have been reported for Fe-59:

Concentration Factor	*Organism*	*Reference*
720-1030	flagellate, *Platymonas*	3386
1000	vertebrates, soft parts	2440
1550-4480	alga, *Ochromonas*	3386
4220	diatom, *Navicula conf.*	3386
5000	vertebrates, skeleton	2440
5533	diatom, *Nitzschia*	3386
6000	flagellate, *Chlamydomonas*	3386
7500	flagellate, *Rhodomonas*	3386
10,000	fish	2436
10,000	invertebrates, soft parts	2440
20,000	algae, non-calcareous	2440
100,000	insect larva	2436
100,000	filamentous algae	2436
100,000	invertebrates, skeleton	2440
200,000	phytoplankton	2436

4. Summary. On the basis of the foregoing information, the following concentrations of iron should not be deleterious to the designated beneficial uses:

a. Domestic water supply ..... 0.3 mg/l
b. Industrial water supply ..... 0.1 mg/l

IRON HUMATE

(see Humic Acid)

ISODRIN

(see Chapter IX)

ISOPRENE C_5H_8

This unstable, oxidizable liquid, insoluble in water, is used in the manufacture of synthetic rubber (364). According to Klimkina (3413), it has an odor threshold in water at a concentration of 0.005 mg/l. For warm-blooded animals, the threshold limit in drinking water is 5 mg/l, but deoxygenation of wastes is not inhibited by 30 mg/l.

IVORY SNOW

(see Chapter X)

JAUNDICE

(see Chapter VII—Infectious Hepatitis)

KELTHANE

(see Chapter IX)

KEROSENE

(see Oil, Petroleum)

KRAFT PULP MILL WASTES

1. General. Certain types of wood, primarily coniferous, are pulped in digesters with a strong caustic solution containing sodium hydroxide, sodium sulfate, and sodium sulfide. This procedure is known as the "Kraft" process. When sulfur compounds are not used, as in the pulping of deciduous wood, the digestion is known as the "soda" process. In either process, the concentrated alkaline wastes that are drained and washed from the pulped wood are known as "black liquor". Economy of operation dictates that such black liquors be kept as concentrated as possible and that they be processed for the recovery and re-use of the sodium salts. In well-operated soda or Kraft mills, therefore, the only liquid wastes result from rinse or wash waters that are too dilute to be recovered economically.

Black liquors from Kraft mills, although having a much lower B.O.D. than sulfite waste liquors, have been shown to be much more toxic to aquatic life (465, 683, 684). This toxicity appears to be related to the sulfur compounds, especially the mercaptans, and the resinous and fatty-acid components (685). Kraft-mill black liquors contain mercaptans, dimethyl sulfide, turpentine, methyl alcohol, ammonia, lignin, fatty and resinous acids, formic acid, acetic acid, lactonic acid, and sodium salts of organic and inorganic acids. Most of the organic constituents of black liquors are derived from the cellulose-binding substances in the wood, such as the lignins, pectins, and hemicelluloses.

2. Cross References. Sulfite Waste Liquors, Soaps, Resins, Mercaptans, and other substances noted above.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Kraft-mill wastes discolor water, cause tastes and odors, and raise the pH. A report by the Technical Association of the Pulp and Paper Industries (686) states that a Kraft-mill waste contained 33 mg/l of sulfide and 12 mg/l of mercaptans. To render it odorless, a dilution of 1:50,000 was required; but after chlorination of the waste to a 1.5 mg/l residual, the required dilution was only 1:40. Insofar as domestic water supply is concerned, the elimination of tastes and odors appears to be the controlling factor.

b. Irrigation. Stabilized Kraft-mill effluents have been used directly for spray irrigation of several crops. The yield of crops was increased and there was no damage to the soil resulting from continued application for several years (3522).

c. Fish and Other Aquatic Life. Most of the references dealing with effect of Kraft-mill wastes on fish are expressed in terms of dilution, and inasmuch as the strengths of the wastes are not known the results in the following table are not strictly comparable.

Dilution	*Aquatic Organism*	*Remarks*	*Reference*
1:2	Fish	Killed in 7 minutes	683
1:20.8	Sockeye salmon	Maximum non-lethal concentration	3521
1:200	Fish	Killed	687
1:200	Fish	Killed	311, 688
1:500	Fish	Irritated but not killed	311, 688
1:500 to 1:1000	Fish	Killed in 10 days or less	673
1:1000	Fry and fingerlings	Killed	1225
1:2000	Fish	Killed	689

The Department of Fisheries of the State of Washington (2091) conducted tests with synthesized Kraft-mill effluents and salmon. It was found that older chinook salmon in flowing sea water were least tolerant, requiring a dilution of 60 to 1 to prevent a kill. Silver salmon in flowing fresh water required only 30 to 1. For black liquor, itself, chinook salmon required a dilution of 1093 to 1 in aerated sea water.

In a report of the National Council for Stream Improvement, (690, 3523) the toxicities of various components of Kraft mill waste were compared, as shown in the following table:

	Minimum Lethal Concentrations, mg/l		
Waste Component	*Minnows*	*Daphnia*	*Insect Larvae*
Sodium hydroxide	100	100	125–1000
Sodium sulfate	100	5000	--
Sodium sulfide	3	10	2–1000
Sodium carbonate	250	300	--
Sodium sulfite	--	300	--
Methyl mercaptan	0.5	1	50
Crude sulfate soap	5.0	5–10	50
Resin acid soap	1.0	3	50–100
Hydrogen sulfide	1.0	1	--

In a subsequent report (3501, 3503) the N. C. S. I. listed the minimum lethal concentrations (for 100 percent kill) and the maximum concentrations for no kill of seven components of the Kraft-mill effluents toward king salmon, silver salmon, and cutthroat trout. The results are shown herein under the specific chemicals, viz, hydrogen sulfide, methyl mercaptan, sodium sulfide, sodium sulfhydrate, sodium hydroxide, sodium carbonate, and sodium sulfate.

Extrom and Farner (691) studied the toxicities of effluents from a sulfate mill and the effluents after dilution in the river, using largemouth bass, bluegills, and sunfish. At 50 percent of the concentration of the mill effluent, with 80,000 mg/l of total solids and 3300 mg/l of sodium sulfide, all bass died in 9 days and sunfish in 15 days. Wastes being tested were aerated to keep the dissolved-oxygen content above 4.0 mg/l, so that toxicity was not attributed to oxygen deficiencies. After dilution by the river, such that the concentrations of sodium sulfide were only 0.099 mg/l, none of the fish died.

Jones et al. (3524) showed that chinook salmon tried to avoid concentrations of Kraft-mill effluent, while coho salmon and steelhead trout were less sensitive to this waste.

For the N. C. S. I., Gehm (344, 658, 692) summarized the work of many people to show the effects of sulfate liquors and their components on fish. The critical concentration (minimum lethal dose) for each component is shown by the following table:

Component	*Critical Concentration, mg/l*
Sodium hydroxide	100
Sodium sulfide	3
Methyl mercaptan	0.5
Hydrogen sulfide	1.0
Formaldehyde	50
Crude sulfate soap	5.0
Unsaponifiable fraction of sulfate soap	6.0
Sodium salts of saponifiable fraction of sulfate soap	3.0
Sodium salts of fatty acids	5.0
Sodium salts of resin acids	1.0
Sodium oleates	5.0
Sodium linoleate	10.0
Sodium salts of abietic acid	3.0
Phytosterol	3.0
Sodium thiosulfate	5.0
Sodium sulfate	100
Sodium chloride	2500
Sodium hydrogen sulfide	0.5
Sodium sulfide (as sulfide)	1.2

c. Shellfish Culture. Galtsoff et al. (693) report that the threshold concentration of sulfate-pulp-mill effluents dumped into the York River, Virginia, occurred at a dilution of 1:2000, for oysters. At this dilution, there was a decrease in feeding. The toxic factor in these wastes tends to concentrate in the soap portion, resin acids, and sodium and calcium salts.

KURON

(see Chapter IX)

LACTATES

(see Lactic Acid, Mercury)

LACTIC ACID $CH_3CHOHCOOH$

(see also Dairy Wastes)

This organic acid occurs in sour milk and other sour foods, such as sauerkraut, as the result of bacterial decomposition of the milk sugar, lactose. It also occurs in blood and urine. Industrial uses include numerous processes in food and beverage production and medicine, in dyeing baths, as a mordant in textile printing, in tanneries, and as a solvent. Hence it may occur in wastes from any of these processes.

Ellis (313) reports that 430 mg/l of lactic acid in hard water was not lethal to goldfish in over 100 hours, but 654 mg/l at 18-23°C killed them in 6 to 43 hours. At low dissolved-oxygen contents, lactic acid was even more toxic (311). In soft water, which is generally weakly buffered, the toxic limit appears to be lower. Leclerc et al. (2942, 2943) exposed minnows for 6 hours to water at 20°C containing lactic acid. In hard water the minimum lethal dose was 470-480 mg/l but in distilled water it was only 18-20 mg/l. The effect of these dosages upon pH is not indicated. For *Squalius leuciscus* the lethal level is 1000 mg/l and for *Salmo irideus* it is 400 mg/l, but harmful symptoms develop at 100 mg/l. For trout, 100 mg/l is lethal in about 18 hours (2977).

Doudoroff and Katz (361) claim that lactic acid can be directly lethal to fully developed fish only when the pH value is reduced to 5.0 or lower. This explanation agrees with the antagonistic effect of hardness and alkalinity toward the toxicity of lactic acid.

According to Westfall (3525) the lethality of lactic acid to goldfish is independent of the oxygen level. Inasmuch as no precipitate of mucus was observed on the gill filaments except in very strong solution, he suggested that lactic acid diffuses into the blood and death results from systemic effects of the absorbed acid.

For *Daphnia magna*, Ellis (313) reports a toxic limit of 170 mg/l in 26 to 72 hours in soft water whereas Anderson (358), gives the threshold concentration of 243 mg/l for immobilization in Lake Erie water after prolonged exposure at 25°C.

LACTONITRILE $CH_3CH(OH)CN$

(see also Cyanides, Hydrogen Cyanide)

Among the common organic cyanides, lactonitrile is unique in that it dissociates appreciably in water to release cyanide ion which then combines with hydrogen ion to form the toxic HCN principle. According to Henderson et al. (2958) the cyanide ion released is almost equivalent to the CN content of the lactonitrile. Renn (2968) reported that lactonitrile yielded tolerance values only slightly less than those for potassium cyanide, both expressed as N. Consequently, it is not surprising that several investigators report this compound to be highly toxic.

The critical concentration of lactonitrile for a mixed population of common fish was reported by Renn to be about 0.09 mg/l as N (or 0.457 mg/l as lactonitrile). Garrett (2959, 2960) found the 24-hour TL_m for pin perch to be 0.215 mg/l and all fish were killed at 0.50 mg/l. Daugherty and Garrett (1441) postulated that it would be unsafe for marine fish such as pin perch if the lactonitrile concentration in sea water exceeded 0.10 mg/l.

Henderson et al. (2958) conducted bioassays using three species of common fish in both hard and soft water saturated with oxygen at 25°C. Their results as shown below are expressed as median toxic limits based on commercial lactonitrile.

Kind of Fish	*Type of Water*	*TL_m in mg/l*		
		24-hour	*48-hour*	*96-hour*
Fathead minnows	hard	0.90	0.90	0.90
Fathead minnows	soft	0.90	0.90	0.90
Bluegills	soft	0.90	0.90	0.90
Guppies	soft	1.37	1.37	1.37

Adult bluegills exposed up to four weeks in water containing 0.25 mg/l of lactonitrile did not pick up any organoleptic properties that could be detected by a taste panel (2958).

LANTHANUM La

(see also Chapter VIII—Radioactivity)

This rare-earth metal occurs in nature chiefly as the oxide in several minerals, frequently associated with cerium. It is used as a weighting agent for silk and rayon, in the manufacture of glass and ceramics, and in "flint" lighters (364, 2121). The chloride, hydroxide, and nitrate salts are highly soluble in water; but the carbonate, oxide, and sulfate are insoluble. Hence, lanthanum ions in waste waters are likely to be precipitated and removed by sedimentation or adsorption, as carbonates or sulfates.

Lanthanum is not recognized as a toxic element for man or animals. The LD_{50} values for rats, in terms of lanthanum were reported (3526, 3527) as 4440 mg/kg for lanthanum acetate, 1450 mg/kg for the nitrate, 2450 mg/kg for the sulfate, and over 8500 mg/kg for the oxide.

Using water from the River Havel from which the test organisms were recovered, Bringmann and Kuhn (2158, 3343) studied the threshold effects of lanthanum acetate on four organisms. For *Daphnia* exposed for 48 hours at 23°C, the median threshold effect occurred at 160 mg/l, expressed as lanthanum. For *Scenedesmus* exposed for 4 days at 24°C, the median limit was only 0.15 mg/l of lanthanum; for *E. coli* at 27°C it was 0.4 mg/l, and for a protozoan (*Microregma*) it was 24 mg/l.

LARVICIDES

(see Chapter IX)

LAURYL DIMETHYL BENZYL AMMONIUM CHLORIDE

(see Chapter X)

LEAD Pb

1. General. Some natural waters contain lead in solution, as much as 0.4-0.8 mg/l, where mountain limestone and galena are found. In the U.S.A., lead concentrations in surface and ground waters used for domestic supplies range from traces to 0.04 mg/l averaging about 0.01 mg/l (3528). Lead may also be introduced into water as a constituent of various industrial and mining effluents, or as a result of the action of the water on lead in pipes.

In the examination of waters for various beneficial uses, their action on lead may be of great importance and should always be considered since lead is frequently used for service and domestic pipes and water-storage chambers. The factors that determine the action of plumbo-solvent waters are complex and not yet fully understood. Practically all waters have a slight action on new lead pipes, although in most cases it is only transient and insufficient to be harmful. Normally the internal lead surfaces quickly acquire a protective coating, after which no further lead is dissolved by the water. With certain types of water, however, no such protective covering is developed, or its formation may be so slow that the solution of lead can go on for a long time. The characteristics of water, soft or hard, that appear to be conducive to plumbo-solvency include comparative absence of calcium and magnesium bicarbonates, low pH, high dissolved oxygen, and high nitrate content (36, 1199, 1226).

The following material was gathered from references in which the effects of lead were given without details of the entire compound, if any. Where specific lead salts were mentioned, the data were listed under the appropriate salt. Certain lead salts, such as the acetate and chloride, are readily soluble, but owing to the fact that the carbonate and hydroxide are insoluble and the sulfate is only sparingly soluble, lead will not remain long in natural waters.

2. Cross References. Dissolved Oxygen, pH, Lead Acetate, Lead Arsenate, Lead Chloride, Lead Fluoride, Lead Nitrate, Lead Sulfate, Tetraethyl Lead.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Foreign to the human body, lead is a cumulative poison. It tends to be deposited in bone as a cumulative poison. The intake that can be regarded as safe for everyone cannot be stated definitely, because the sensitivity of individuals to lead differs considerably. Typical symptoms of advanced lead poisoning are constipation, loss of appetite, anemia, abdominal pain, and tenderness, pain, and gradual paralysis in the muscles, especially of the arms. A milder and often undiagnosed form of lead poisoning also occurs in which the only symptoms may be lethargy, moroseness, constipation, flatulence, and occasional abdominal pains (36). Lead poisoning usually results from the cumulative toxic effects of lead after continuous consumption over a long period of time, rather than from occasional small doses. Immunity to lead cannot be acquired, but sensitivity to lead seems to increase (1227). Lead is not among the metals considered essential to the nutrition of animals or human beings (2121).

Lead may enter the body through food, air, and tobacco smoke as well as from water and other beverages. Consequently, the total intake of lead must be considered in setting standards for water. The exact level at which the intake of lead by the human body will exceed the amount excreted has not been established, but it probably lies between 0.3 and 1.0 mg per day (152). The mean daily intake of lead by adults in North America is about 0.33 mg. Of this quantity, 0.01 to 0.03 mg per day are derived from water used for cooking and drinking (3528, 3529). A total intake of lead appreciably in excess of 0.6 mg per day may result in the accumulation of a dangerous quantity of lead during a lifetime. Lead in an amount of 0.1 mg ingested daily over a period of years has been reported to cause lead poisoning (1228, 1229). The daily ingestion of 0.2 mg lead is considered excessive by one authority (1230). On the other hand one reference considered 0.5 mg per day safe for human beings, and a daily dose of 2.0 mg for a one-year period apparently did not affect the health of one adult (353).

Lead poisoning among human beings is reported to have been caused by the drinking of water containing

lead in concentrations varying from 0.042 mg/l to 1.0 mg/l or more (27, 29, 32, 36, 629, 997, 1231, 1232, 1233, 1234, 1235, 1236, 1237, 1238, 1239, 1240, 1241, 1242, 1243, 1244, 1245, 2326, 3530, 3531). On the other hand, concentrations of 0.01 to 0.16 mg/l have been apparently non-poisonous over long periods of time (1077, 1201, 3532). However, there is a feeling that 0.1 mg/l may cause chronic poisoning if the water is used continuously, especially among hypersensitive persons (29).

For many years, the mandatory limit for lead in the USPHS Drinking Water Standards was 0.1 mg/l; but in the 1962 Standards, the limit for lead was lowered to 0.05 mg/l. In the WHO International Standard and WHO European Standards (see Chapter V) the limit for lead has been set at 0.1 mg/l. In the past, the Netherlands and Germany have permitted a temporary lead concentration up to 0.3 mg/l in water that had been in pipes for 24 hours (997, 1229, 3530). Uruguay, on the other hand, has used a standard as low as 0.02 mg/l (1457). Several countries use 0.1 mg/l as a standard.

Owing to the fact that the total intake of lead from food, inhaled atmosphere, and tobacco smoke in industrial urban areas appears to be increasing, with little chance of regulation and diminution, and inasmuch as the concentration of lead in drinking water can be controlled without undue hardship on water purveyors, the USPHS Drinking Water Standard for lead was lowered to 0.05 mg/l in 1962 (2062, 2672). It is significant, however, to note that the U.S. Government has also established a tolerance of lead in food at 7 mg/kg, more than 100 times the limit for drinking water (3533).

b. Industrial Water Supplies. Traces of lead in metal-plating baths will affect the smoothness and brightness of deposits (214).

c. Irrigation. Inorganic lead salts in irrigation water may be toxic to plants and should be investigated further (269, 1014). Klintworth (1493) stated that lead is harmful to plants at all concentrations. On the other hand, the addition of 2.0 mg of lead per kg of dry soil in pot tests increased the nitrogen content of peas (2926). In the culture of oats and potatoes, lead nitrate in concentrations of 1.5 to 25 mg/l had a stimulating effect, but at concentrations over 50 mg/l all plants died in a week's time (2997). See also lead salts.

Lead at a concentration of 51.8 mg/l of nutrient solution was slightly injurious to sugar beets grown in sand culture (1473). Germination of cress and mustard seeds in solution culture was completely inhibited by a 2760 mg/l lead solution, during an exposure period of 18 days. Germination was delayed and growth was retarded by 345-1380 mg/l of lead (1479).

d. Stock and Wildlife Watering. Farm animals are poisoned by lead from various sources, including paint, more frequently than by any other metallic poison (1247). It is not unusual for cattle to be poisoned by lead in the water; the lead need not necessarily be in solution, but may be in suspension, as, for example, oxycarbonate (36). Chronic lead poisoning among animals has been caused by 0.18 mg/l of lead in soft water (1232); and by concentrations under 2.4 mg/l (1238). Chronic changes in the central nervous system of white rats were observed after an ingestion of 0.005 mg of lead per kg of body weight (3534). Most authorities agree that 0.5 mg/l of lead is the maximum safe limit for lead in a potable supply for animals (1238).

Allcroft (1589) reported that calves given as much as 2.0 grams of lead daily have survived for 2 to 3 years. Calves have tolerated daily doses of 8 mg and 6 mg of lead per kg of body weight for, respectively, many months and up to three years. Doses of 0.2-0.4 grams of lead per kg body weight caused death within a few days (1590). When a calf was given drinking water containing lead nitrate at a concentration of 100 mg/l of lead, it survived for only four months. It was suggested that nitrate was partly responsible for the death (1589). Geese have been reported to have contracted lead poisoning from the ingestion of mine tailings (3535).

e. Fish and Other Aquatic Life. The toxic concentration of lead for aerobic bacteria is reported to be 1.0 mg/l; for flagellates and infusoria, 0.5 mg/l. The bacterial decomposition of organic matter is inhibited by 0.1 to 0.5 mg/l of lead (576, 2977, 3324, 3534).

Carpenter and others have studied the effects of small concentrations of heavy metals, particularly lead, upon fish. Such studies indicate that in water containing lead salts, a film of coagulated mucus forms, first over the gills, and then over the whole body of the fish, probably as a result of a reaction between lead and an organic constituent of mucus. The death of the fish is caused by suffocation due to this obstructive layer (346, 991, 1248, 2411). In soft water, lead may be very toxic; in hard water equivalent concentrations of lead are less toxic (311). Calcium in a concentration of 50 mg/l has destroyed the toxic effect of 1.0 mg/l of lead (353).

Wilder found that lobsters died within 20 days when kept in lead-lined tanks; however, in steel-lined and other control tanks they survived for 60 days or longer (1530). Small sticklebacks appeared to be slightly more sensitive to lead than the larger fish (1460).

The Water Pollution Research Board (74) in England conducted extensive experiments to determine the effects of lead salts on rainbow trout in hard and soft waters. In water of the lowest hardness used, 14 mg/l as $CaCO_3$, all of the lead added as lead nitrate remained in solution and produced a curvilinear relationship of lead concentration vs time of survival. A similar curve was obtained for a water with a total hardness of 27 mg/l as $CaCO_3$, although some precipitation of $PbCO_3$ occurred with lead concentrations greater than 8.0 mg/l. In solutions with a total hardness of 53 mg/l as $CaCO_3$ the maximum concentration of lead remaining in solution was approximately 1.6 mg/l, and the median period of survival of the trout exceeded two days even at lead dosages in excess of 20 mg/l. It seems probable, therefore, that precipitated lead carbonate is not effective as a lethal agent. For further evidence that insoluble lead is not highly toxic to fish, see Lead Oxide.

A chart submitted by the Water Pollution Research Board (74) indicates that the median period of survival of rainbow trout in soft water containing dissolved lead, at 18.5°C, was 18 to 24 hours at 1.6 mg/l and only 10 to 12 hours at 4.0 mg/l.

The Water Pollution Research Board has also shown that the toxicity of lead toward rainbow trout increases with a reduction of the dissolved-oxygen concentration of the water (74, 88, 3321, 3322). The following table

shows factors by which the threshold concentration of lead must be multiplied to determine concentrations of equal toxicity at lower dissolved-oxygen tensions:

Dissolved oxygen (percent of saturation)	100	80	60	40
Factor	1.0	0.95	0.85	0.71

The following concentrations of lead have been reported toxic or lethal to fish in the time specified (see also the various lead salts):

Concentration of lead, in mg/l	Type of water	Time of exposure	Species of fish	Reference
0.1	--	--	Fish	353, 573, 1249
0.1	--	--	Sticklebacks	1460, 2920, 2941
0.1–0.2	very soft	--	Sticklebacks	1459
0.2	soft	--	Fish	3319
0.21	--	--	Guppy	2921
0.25	fresh	--	Fish	598
0.33	--	--	Minnows, brown trout and sticklebacks	311, 313, 353, 2920
0.34	1000-3000 mg/l of dissolved solids	48-hour TL_m	Sticklebacks and coho salmon	3536
0.4	distilled	26-48 hours	Minnows	1459
0.41	1000-3000 mg/l of dissolved solids	24-hour TL_m	Coho salmon	3536
0.53	1000-3000 mg/l of dissolved solids	24-hour TL_m	Sticklebacks	3536
0.75	--	--	Minnows	353
1.0	--	long-term	Carp	3327
1.0	--	8.5 days	Sticklebacks	1444, 1460
1.4	soft	18-24 hours	Rainbow trout	74
1.4	tap	48-hour TL_m	Bluegill sunfish	2093
2.0	tap	24-hour TL_m	Bluegill sunfish	2093
2.4E	soft	96-hour TL_m	Fathead minnows	2154, 2125
2.7	distilled	4-16 hours	Minnows	313
2.8	fresh	--	Fish	2931
4.0	soft	10-12 hours	Rainbow trout	74
4.0	--	--	Fish	313
5.5	stream water	--	Trout	313, 598, 609
5.5	distilled	--	Goldfish	313
6.3	tap	24 & 48-hour TL_m	Bluegill sunfish	2093
10.0	--	--	Goldfish	1459, 2920
17	--	--	Goldfish	1459
27	tap	16-183 days	Catfish	1459
40	--	80 hours	Goldfish	1466
63	--	80 hours	Goldfish	1466
75E	hard	96-hour TL_m	Fathead minnows	2154, 2125

E = exploratory test only

The following concentrations of lead have not visibly harmed fish within the time specified:

Concentration of lead, in mg/l	Type of water	Time of exposure	Species of fish	Reference
0.62	--	48 hours	Trout	1459
0.7	soft tap water	3 weeks	Minnows and Sticklebacks	1459
1.0	very soft	indefinitely	Goldfish	1459
4.0	--	--	Plaice eggs	1459

Toward the giant kelp, *Macrocystis pyrifera*, North and Clendenning (2107) found that lead was less toxic than mercury, copper, hexavalent chromium, zinc, and nickel. Lead nitrate produced no deleterious effect on the rate of photosynthesis of kelp in sea water during a four-day exposure at 4.1 mg/l of lead.

The Mersey and Severn River Boards in England have established "working standards" for several streams whereby the total concentration of all heavy metals, including lead, cannot exceed 1.0 mg/l.

4. Summary. In view of the 1962 Drinking Water Standards of the USPHS, setting a mandatory limit of 0.05 mg/l on lead in water delivered to the consumer, and in light of the fact that lead concentrations as low as 0.1 mg/l have been deleterious to fishlife, especially in soft waters, it is evident that the dissolved lead content of surface waters should be restricted to 0.1 mg/l where these two beneficial uses are involved.

LEAD ACETATE $Pb(C_2H_3O_2)_2 \cdot 3H_2O$

1. General. Known also as "sugar of lead", this freely soluble lead salt is used in printing and dyeing operations, in the weighting of silk, and in the manufacture of other lead salts.

2. Cross References. Acetate, Lead, and other lead salts.

3. Effects Upon Beneficial Uses.

a. Irrigation. When lead acetate was tested, 1000 to 10,000 mg/l of lead severely stunted the growth of water hyacinths in water culture; and 1 to 100 mg/l retarded growth (1525). In nutrient solution containing lead acetate, 1500 to 20,000 mg/l of lead inhibited the germination of cress and mustard seeds; however, even after 18 days of exposure to lead, the seeds germinated when they were transferred to water cultures (1479).

b. Fish and Other Aquatic Life. Dawson (1459) found evidence of injury to blood cells of catfish exposed for 16 to 183 days to a 50 mg/l solution of lead acetate (27 mg/l of Pb) in tap water, renewed at 48-hour intervals. Minnows were killed in 26 to 48 hours by 0.4 mg/l of lead in a solution of lead acetate in non-toxic distilled water, renewed at regular intervals during the observation period.

It has been reported that lead acetate concentrations of 10 mg/l in stream water killed trout (313, 598, 609). In distilled water, 5.0 mg/l of lead acetate killed minnows in 4 to 16 hours; and 10 mg/l in distilled water renewed every second day, killed goldfish in 12 days (313). The Washington Department of Fisheries (2931) reported that lead acetate at a concentration of 2.8 mg/l of lead killed fish in fresh water; but Fujiya (3327) maintained that long-term exposure of carp to a much lower concentration of lead acetate (1.0 mg/l as Pb) resulted in a harmful effect to their serum.

LEAD ARSENATE $PbHAsO_4$

1. General. Lead arsenate sprays are used for insect control on crop lands, especially for the gypsy moth and boll weevil; but it is believed that such use has not resulted in the contamination of water supplies derived from treated watershed areas (152). Fortunately, lead arsenate is highly insoluble in water.

2. Cross References. See Lead, Arsenic, other arsenic compounds.

3. Effects on Beneficial Uses.

a. Domestic Water Supplies. Lead arsenate is more toxic to humans than lead carbonate. A total of 100 mg of lead arsenate ingested by two individuals over a period of 10 days caused no apparent harm (353).

b. Stock and Wildlife Watering. In doses of 1.3 to 56.7 grams per day, lead arsenate killed 18 out of 31 chickens, but the survivors showed no symptoms of poisoning. Drinking water containing about 4800 mg/l of lead arsenate caused no harm to 10 birds after a 60-day

period (1013). Toward male rats, the LD_{50} value of lead arsenate has been reported as 1050 mg/kg. The daily consumption by one cow of 6.48 grams of lead arsenate for an unspecified period was not harmful (1014).

c. Fish and Other Aquatic Life. A concentration of 25 mg/l of lead arsenate has killed trout within 24 hours (359), but a concentration of 17.1 mg/l in stabilized tap water did not harm minnows during a one-hour exposure (362).

LEAD CHLORIDE $PbCl_2$

(see also Chlorides, Lead and other lead salts)

This relatively soluble lead salt is used in the manufacture of certain lead paints and as a solder and flux. The following concentrations of lead chloride have been reported as deleterious toward aquatic life:

Concentration of Lead Chloride, mg/l	Type of water	Time of exposure	Type of organism	Reference
0.01 to 1.0	many kinds	10 days	*Daphnia magna*	598
0.33	--	--	fresh-water fish	598
0.58	Lake Erie	--	whitefish fry	2955
1.25	Lake Erie	--	*Daphnia magna*	2955
3.2E	soft	96-hr. TL_m	Fathead minnows	2154, 2125
10.0	distilled	24 hours	*Daphnia magna*	654
>100E	hard	96-hr. TL_m	Fathead minnows	2154, 2125
128	Lake Erie	--	*Cyclops vernalis*	2955

E = exploratory test only

It was also reported (654) that *Daphnia magna* withstood 10 mg/l of lead chloride for 5 hours, but succumbed in 24 hours.

LEAD OXIDE PbO

This insoluble white powder is used extensively in paints. Wallen et al. (2940) tested its toxicity toward the mosquito fish (*Gambusia affinis*) in water having a turbidity from soil suspension of 315 mg/l, a pH range of 7.1 to 7.2, and a temperature range of 18 to 20°C. He found that the 96-hour TL_m was greater than 56,000 mg/l and there was no significant decrease in turbidity as a result of the addition of lead oxide. Apparently insoluble lead is not toxic to fish.

LEAD NITRATE $Pb(NO_3)_2$

(see also Lead, Nitrates)

Used in the manufacture of matches and special explosives, as a mordant in dyeing and printing of textiles, as an oxydizer in the dye industry, and in photography and engraving, this lead salt is highly soluble in water (364).

For tadpoles in tap water, 1.6 mg/l of lead nitrate is deleterious to growth and 3.3 mg/l is lethal (313). Fish appeared to try to avoid, i.e., react negatively, to solutions of lead nitrate varying in strength from 0.33 to 644 mg/l, but reacted positively to a 6640 mg/l solution after a temporary initial negative reaction (1046).

Westfall found that concentration of dissolved oxygen has a significant effect on the response of goldfish to lead nitrate, for example, all the fish tested survived a 2-hour exposure to 5 mg/l of lead nitrate in the presence of 6.2 mg/l of oxygen, but all the fish tested died within 2 hours in the presence of only 1.4 mg/l of dissolved oxygen (1459).

In fresh water, 3 mg/l lead nitrate has been fatal to *Fundulus* within 12 hours (1459). Using lead nitrate, Jones found the lethal concentration limit of lead for sticklebacks to be 0.1 mg/l. At different concentrations of lead, the average survival times of the fish were as follows: one day at 1.0 mg/l, two days at 0.7 mg/l, four days at 0.3 mg/l, and one week at 0.2 mg/l (1460). However, goldfish died only after an 80-hour exposure to 63 mg/l of lead (1466). Minnows died within 48 hours when exposed to 0.4 mg/l of lead in a solution of lead nitrate in non-toxic distilled water (1459).

The following concentrations of lead nitrate have been reported to have killed fish:

Concentration of Lead Nitrate, in mg/l	*Type of water*	*Time of Exposure*	*Type of Fish*	*Reference*
0.16	tap	--	stickleback	2920, 598
0.53	tap	--	minnows, sticklebacks, and brown trout	2920
10	natural	2.5 hours	trout	313, 598
10	tap	24 and 48-hr TL_m	bluegill sunfish	2093
10	hard	4 days	goldfish	353
16	tap	--	goldfish	2920
16.6	--	20 hours	minnows	353
100	hard	80 hours	goldfish	313
165	distilled	--	fish	313
240	highly turbid	96-hr TL_m	mosquito-fish	2940
250	distilled	4-5 days	goldfish	313
250	distilled	2-3 hours	minnows	313
830	--	3 hours	minnows	991
3320	--	2.2 hours	minnows	991
8300	--	1.5 hours	minnows	991
16600	--	1.4 hours	minnows	991, 1248
44000	--	44 minutes	minnows	1248
53000	--	40 minutes	minnows	1248

On the other hand, 10 mg/l of lead nitrate in hard water has not harmed fish during a 96-hour exposure period (313).

Bringmann and Kuhn (2158, 3343) tested the effects of lead nitrate in River Havel water at pH 7.5 and a temperature of 24°C against several organisms during an exposure of 4 days. The threshold concentrations for deleterious action were as follows:

Test Organism	*Threshold Concentration in mg/l of Lead*
Daphnia magna	5.0
Scenedesmus	2.5
Escherichia coli	1.3
Microregma	1.25

Cleland (3328) found that lead nitrate in a concentration of about 200 mg/l in sea water produced abnormalities in the eggs of sea urchins.

LEAD SULFATE $PbSO_4$

(see also Lead, Sulfates)

Sparingly soluble in water, this salt is used as a paint pigment, for weighting fabrics, in lithography, and in galvanic batteries. In distilled water 25 mg/l of lead sulfate killed goldfish in 4 days and killed minnows in 2 to 3 hours (313). Jones (2920) reports the lethal concentration for goldfish as 26 mg/l.

LEAD TETRAETHYL $Pb(C_2H_5)_4$

Used as an additive in motor fuels to prevent knocking, this lead compound might occur in the effluents from re-

fineries. Turnbull et al. (2093) conducted bioassays with this substance in Philadelphia tapwater, using bluegill sunfish (*Lepomis macrochirus*) as the test fish. They found a 24-hour TL_m of 2.0 mg/l as lead and a 48-hour TL_m of 1.4 mg/l. They estimated the safe concentration at 0.20 mg/l.

LIGNASAN

(see Mercuro-Organic Compounds)

LINOLEIC ACID AND LINOLEATES

(see Fatty Acids)

LISSAPOL (NONYLPHENOL ETHYLENE OXIDE CONDENSATE)

(see Chapter X)

LIME

(see Calcium Hydroxide)

LINDANE

(see Chapter IX—Benzene Hexachloride)

LITHIUM Li

(see also Lithium Chloride)

As one of the alkali metals, related to sodium and potassium, lithium is not widely distributed in nature, being found in a few minerals and in certain spring waters. Being very active, the metal does not occur in the elemental state and when purified as such it must be protected from water or oxygen. It is used in metallurgy, in medicinal waters, in some types of glass, and as lithium hydroxide in storage batteries. Hibbard (250) recommends, without references, that lithium in water for drinking and cooking purposes should not exceed 5 mg/l.

Lithium toxicity in citrus has been identified in Santa Barbara County, California. In green-house experiments, 2 and 5 mg/l of lithium sulfate (in air-dried soil) caused the appearance of toxic symptoms in orange seedlings within 6 months. In the field, 1, 2, and 4 mg/l of lithium chloride in the soil have caused symptoms of lithium toxicity.

Various irrigation waters in the area were found to contain 0.045-0.080 mg/l of lithium. Possibly lithium poisoning has resulted from the accumulation in ground of toxic concentrations over a long period of time. Aldrich (1591) and his collaborators are making further studies of the effects of 0.05-0.1 mg/l of lithium in irrigation water.

LITHIUM CARBONATE Li_2CO_3

This light white alkaline powder, quite soluble in water, is used in the production of glazes on ceramic and electrical porcelain (364). It was reported in 1923 that concentrations of 295-516 mg/l of lithium carbonate retarded larval and pupal development of *Drosophila melanogaster* (1592).

LITHIUM CHLORIDE LiCl

(see also Lithium)

A white deliquescent crystalline solid, lithium chloride is highly soluble in water. It is used in pyrotechnics and in the soldering of aluminum. It is also used in the manufacture of mineral waters and it may be found in some natural mineral springs (364).

In a concentration of 3750 mg/l, lithium chloride in distilled water killed goldfish in 22 to 27 hours (313). For mature small fresh-water fish, the lethal concentration in 24 hours of exposure was found to be 2600 mg/l. The data gathered by Powers and by Iwao indicate that lithium chloride at concentrations between 1950 and 3770 mg/l can kill fresh-water fish in about one day, or sooner at warmer temperatures (1459). In contrast with these high concentrations required for lethal effect, one German publication (2977) reports that 100 mg/l of LiCl is toxic but 33 mg/l is harmless to fish.

The threshold concentration for immobilization of *Daphnia magna* in Lake Erie water was found to be less than 7.2 mg/l (598); but in River Havel water at 23°C the threshold of poisonous effect was observed at 16 mg/l during 48 hours contact (2158). With the protozoan *Microregma* as the test organism, food intake was inhibited at 66 mg/l (3343). Toward *Scenedesmus* and *Escherichia coli*, no toxicity was evident at concentrations less than 1000 mg/l (2158). In order to evoke stimulation and movement of the water beetle, *Laccophilus maculosis*, a LiCl concentration of 19,500 mg/l was required (2956).

It has been reported that dilute concentrations of LiCl are deleterious to the eggs of various aquatic organisms, retarding their development and producing monstrosities (1467). King demonstrated that concentrations of 848 mg/l were highly toxic to fly larvae, preventing emergence of offspring and retarding development of both larvae and pupae (1592).

LITHIUM FLUORIDE LiF

This moderately soluble salt is used as a flux for soldering and welding aluminum and in the manufacture of vitreous enamels and glazes (364). The oral LD_{50} for guinea pigs is 200 mg/kg of body weight (3271). The lethal dose in 48 hours for the fish, *Tinca vulgaris*, is reported as 20,000 mg/l (3271).

LITHIUM SULFATE

(see Lithium)

LOROL METASODIUM SULFOBENZOATE

(see Chapter X)

MAGNESIUM Mg

1. General. As one of the most common elements in the crust of the earth, constituting about 2.1 percent of it, magnesium is widely distributed in ores and minerals (364). Because it is very active chemically, it is not found in the elemental state in nature. With the exception of the hydroxide at high pH values, its salts are very soluble; even the carbonate will dissolve to the extent of 100 to 300 mg/l at normal temperatures (364, 911). The solubility of magnesium hydroxide is governed by the equation:

$$[Mg^{++}]\ [OH^-]^2 = 1.2 \times 10^{-11} \text{ at } 18°C$$

Thus, at pH 7, magnesium ions theoretically can be present to the extent of 1200 mols per liter or 28,800

grams per liter, but at pH 10 the maximum possible concentration of magnesium ions would be 28.8 mg/l and at pH 11 only 0.288 mg/l. This phenomenon is useful in treatment processes to remove magnesium from water, but insofar as natural waters are concerned it is described herein merely to show that at common pH values magnesium ion may be present in high concentrations despite the apparently low solubility product.

The industrial and commercial uses of magnesium and its salts are many, as described hereinafter for the salts. Magnesium metal is used as a constituent of light alloys, in other phases of metallurgy, and in the manufacture of electrical and optical apparatus (364). Magnesium ions are of particular importance in water pollution in that they occur in significant concentration in natural waters, and along with calcium form the bulk of the hardness reaction (see Hardness).

The literature decribed herein deals with articles in which magnesium ions were referred to without mention of associated cations. Where salts were tested and described, the material is covered under the appropriate salt.

2. Cross References. Calcium, Hardness, Dissolved Solids, Tastes.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Magnesium is an essential mineral element for human beings, the daily requirement of magnesium is about 0.7 grams (295). Magnesium is considered relatively non-toxic to man and not a public-health hazard because, before toxic concentrations are reached in water, the taste becomes quite unpleasant (633) (see also Tastes). At high concentrations, magnesium salts have a laxative effect, particularly upon new users, although the human body can develop a tolerance to magnesium over a period of time.

The 1946 USPHS Drinking Water Standards recommended a limit of 125 mg/l but there is no limit in the 1962 standards (2036). The 1958 WHO International Standards (2328) have a "permissible limit" of 50 mg/l and an "excessive limit" of 150 mg/l, but no maximum allowable concentration. The 1961 WHO European Standards (2329) have a recommended limit of 125 mg/l, but if the sulfate exceeds 250 mg/l, the magnesium is limited to 30 mg/l.

Limits ranging from 100 to 200 mg/l have also been proposed for domestic water supplies (942, 1059). Hibbard recommended a limiting concentration of magnesium of 10 mg/l in water for drinking and cooking; 5 mg/l for washing; and no magnesium for laundry (250). Baylis suggested limiting concentrations of magnesium ranging from 50 to 150 mg/l for four grades of drinking water (499). In his system for classifying ground waters, Goudey suggested limits from 15 to 125 mg/l, according to domestic and industrial uses (992).

The taste threshold for magnesium (in $MgSO_4$) has been reported (3241) as 100 mg/l and for the average individual it is given as about 500 mg/l (3392).

The negative correlation between hardness in water and cardiovascular disease does not appear to hold for magnesium as it does for calcium (3368, 3369); yet one investigator (3537) reports the favorable use of magnesium sulfate to treat such cases and claims that magnesium, rather than calcium, is the beneficial element in reducing cardiovascular attacks (see Hardness).

b. Industrial Water Supplies. Like calcium, magnesium in small amounts is beneficial in the mash water for pale beer (175, 1074, 3344). Magnesium in water used for preparing developer solutions causes spots on films, if it is precipitated out of solution (242).

The limiting or threshold concentrations of magnesium that have been recommended for various industrial waters are described in Chapter V. The following table is extracted from that chapter:

Process	Recommended Limits, in mg/l for			
	Magnesium	$MgSO_4$	$MgCl_2$	$Mg(HCO_3)_2$
Brewing	30	100-200	100-200	--
Ice manufacture	--	130-300	171-300	50
Soda pulp	12	--	--	--
Sugar making	10	--	--	--
Textile manufacture	5	--	--	--

c. Irrigation. Magnesium is essential to normal plant growth (635); and magnesium and calcium cations in irrigation water tend to keep soil permeable and in good tilth (268, 281, 348, 1252). It has been reported that magnesium in water in concentrations up to 24 mg/l will probably not affect seriously the condition of underground water basins, growth of trees, or condition of the soil (1060). In very high concentrations (3000-5000 mg/l) $MgCl_2$ and $MgSO_4$ have been toxic to the bean plant (3376).

d. Stock and Wildlife Watering. Animals require magnesium salts in their diet. According to Maynard, calves require about 0.6 grams of magnesium per 100 lbs. body weight, or 600 mg/kg in the dry ration. Chicks require about 400 mg/kg of magnesium in the dry ration. The specific needs of other animals are not known (995).

In the body, calcium and magnesium are antagonistic to a certain degree and calcium may alleviate symptoms of magnesium excess. Diets high in magnesium and low in calcium can cause rickets (284, 295). Provided that both calcium and phosphorus in the diet are sufficient, however, the ingestion of a moderate excess of magnesium in the food or water will not markedly disturb calcium retention, although it may increase the calcium requirement (284, 295).

Magnesium salts act as cathartics and diuretics among animals as well as human beings, and high concentrations in drinking water may cause scouring diseases among stock. Water containing less than 5000 mg/l of magnesium compounds is harmless to cattle which have become accustomed to it (292). An interim threshold limit of 500 mg/l has been suggested by Stander (3373). Ingestion of mixtures of sodium salts and magnesium and nitrate ions caused poisoning among ducks (288).

e. Fish and Other Aquatic Life. The relative concentrations of magnesium and calcium in water may be one factor controlling the distribution of certain crustacean fishfood organisms, such as copepods, in streams (1085). Hart et al. cite a report that among U.S. waters supporting a good fish fauna, ordinarily 5 percent have less than 3.5 mg/l of magnesium; 50 percent have less than 7 mg/l; and 95 percent have less than 14 mg/l (310).

Magnesium chloride and nitrate can be toxic to fish in distilled water or tap water at concentrations between 100 and 400 mg/l as magnesium. However, magnesium

chloride, nitrate, and sulfate, at concentrations between 1000 and 3000 mg/l as magnesium have been tolerated for 2-11 days. Some fresh-water fish have been found in very saline lake water containing over 1000 mg/l of magnesium as well as additional sodium and calcium salts (1459).

As magnesium nitrate, 400 mg/l of magnesium is toxic to a flatworm, *Polycelis nigra*, as magnesium chloride (353). A concentration of 300 mg/l of magnesium has been reported to be toxic to stickleback (353, 2941). Magnesium salts in water affect the toxicity of copper toward fish (1076). For further details on fish toxicity, see the specific magnesium salts.

f. Shellfish Culture. Magnesium was found to be more toxic than calcium to mussels but less toxic than sodium and potassium (353).

MAGNESIUM ACETATE $Mg(C_2H_3O_2)_2 \cdot 4H_2O$

(see also Magnesium, Acetates)

This colorless salt is freely soluble in water. Heller found that 15,000 mg/l of magnesium acetate in drinking water permitted satisfactory growth and reproduction among rats (287, 2980).

MAGNESIUM BICARBONATE

(see Magnesium)

MAGNESIUM CHLORIDE $MgCl_2 \cdot 6H_2O$

1. General. Highly soluble in water, magnesium chloride may occur in water either naturally or as a component of waste waters from oil wells, road run-off, and industry. It is used in manufacturing chemicals, artificial leather, cements, fire extinguishers and fireproofing materials, sweeping compounds, and road coverings (364).

2. Cross References. Hardness, Magnesium, Taste, Chapter V.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The taste threshold of magnesium chloride in water has been reported to be 200 to 750 mg/l (621). Steyn et al. recommended that in order to prevent unpleasant salty tastes and possible appetite disturbances, water should not contain more than 168 mg/l of magnesium chloride (284). Thresh states that much smaller quantities of magnesium chloride, even 40 to 50 mg/l, can render a water useless for many domestic purposes because of its hardness (32).

b. Industrial Water Supplies. Magnesium chloride in water may interfere with its suitability for some industrial uses. In boiler waters it is corrosive (855).

The following maximum concentrations have been recommended:

Use	*Concentration in mg/l*	*Reference*
Brewing	200	173
Brewing	100	170
Ice, raw water	171	173

c. Irrigation Use. Irrigation with effluents of high magnesium chloride content did not appreciably affect the yields and composition of hay (1253). At concentrations in excess of 2000 mg/l, $MgCl_2$ inhibited the growth of bean plants (3376) and guayule (3378).

d. Stock and Wildlife Watering. In experimental animals 4.0 grams of magnesium chloride per 100 grams of diet causes diarrhea, loss of appetite and even death (295). It has been reported that daily oral administration of 20 grams of magnesium chloride to pigs; 60 grams to sheep; and 400 grams to horses had no detrimental effects. A dose of 1.5 grams has been toxic to ducks (284).

Magnesium chloride in water had no effect on tooth decay among rats (1069). Water containing 10,000 to 15,000 mg/l of magnesium chloride interfered with the growth of rats (287). Water containing 10,000 mg/l of magnesium chloride and 5000 mg/l of calcium chloride was not harmful to mature rats, although it did interfere with lactation (287). Water containing 5000 mg/l of magnesium chloride and 20,000 mg/l of sodium chloride inhibited rat growth (284). Concentrations of $MgCl_2$ up to 9500 mg/l in the drinking water had no effect on the food or water intake of male rats (2398).

e. Fish and Other Aquatic Life. Garrey found that magnesium chloride added to water decreased the toxicity of calcium and potassium chlorides toward fresh-water fish, and that calcium chloride decreased the toxicity of magnesium chloride (307).

The following concentrations of magnesium chloride have been reported to have killed fresh-water fish:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
476	distilled	4-6 days	minnows	313
5000	--	96.5 hours	golden shiners	645
6757	distilled	3-21 days	goldfish	313
8132	--	--	carp	3538
10000	--	4.6 hours	shiners	645
15000	--	0.8 hours	shiners	645
16500	turbid	96-hour TL_m	mosquito fish	2940
20000	--	0.5 hours	shiners	645
23800	--	24 hours	*Orizias*	1459

The highest concentration of $MgCl_2$ tolerated by young eels for more than 50 hours was reported to be about 9500 mg/l (1459). Some fish-food organisms, such as *Daphnia* and other cladocera, are less tolerant of magnesium chloride and have been immobilized or killed within two days by concentrations of magnesium chloride from 740 to 3500 mg/l (598).

f. Shellfish Culture. In 1890, Dean reported that the most favorable salt content for oyster growth is about 30,800 mg/l of chlorides, of which magnesium and potassium chlorides constitute about 20 percent (314).

MAGNESIUM FLUORIDE MgF_2

This colorless salt is soluble in water only to the extent of 87 mg/l at 18°C. It finds commercial application in the ceramics and glass industries. The oral LD_{50} for guinea pigs is 1000 mg/kg of body weight (364). To kill tench, a concentration of 10,000 mg/l was required (3271).

MAGNESIUM NITRATE $Mg(NO_3)_2 \cdot 6H_2O$

(see also Magnesium, Nitrates)

This freely soluble compound is used in pyrotechnics. The taste threshold of magnesium nitrate in water is reported to be 500 to 800 mg/l (621). The following

concentrations of magnesium nitrate have been reported to kill fish:

Concentration in mg/l	*Time of Exposure*	*Type of Fish*	*Reference*
300	long-time	stickleback	1460
400	--	stickleback	2920
500	4 days	stickleback	1460
1500	2 days	stickleback	1460
1820	14-16 hours	stickleback	598
2000	one day	stickleback	1460
12500	--	goldfish	313

MAGNESIUM OXIDE MgO

(see also Magnesium)

Known in the dry state as "magnesia", this oxide combines with water to form magnesium hydroxide, which is sparingly soluble at high pH values. It is used medicinally as an antacid and laxative, in doses of 0.25 to 3.0 grams. One authority (1254) reports that drinking water should contain some magnesium and calcium oxides; the most satisfactory ratio of calcium oxide to magnesium oxide is said to be 7:1. In the soft-drink industry, magnesium oxide in the wash water gradually "clouds" the bottles, causing unsightliness (180).

MAGNESIUM SILICOFLUORIDE $MgSiF_6 \cdot 6H_2O$

This highly soluble salt is used for mothproofing fabrics. The oral LD_{50} in guinea pigs is given as 200 mg/kg of body weight (364). A concentration of 50 mg/l is reported to kill tench (3271).

MAGNESIUM SULFATE $MgSO_4 \cdot 7H_2O$

1. General. Known also as Epsom salt, this compound is freely soluble in water. It occurs in natural deposits and soils, thereby contributing to the concentration in natural waters. It is used in weighting cotton and silk, in dyeing and printing calico, in tanning processes, and in fertilizers, explosives, and matches (364).

2. Cross References. Dissolved Solids, Magnesium, Sulfates.

3. Effects on Beneficial Uses.

a. Domestic Water Supplies. The taste threshold of magnesium sulfate is 400 to 600 mg/l (621, 3241). A dose of 30 grams of magnesium sulfate is toxic and 120 grams fatal for man (284).

Magnesium sulfate in excessive concentrations in drinking water may have purgative effects (623). The most sensitive individuals are affected at about 400 mg/l and the average person at about 1000 mg/l (3392). Waters containing 1200 mg/l of magnesium sulfate and 500 mg/l of sodium sulfate have caused diarrhea in humans. Ordinarily, according to Taylor (36) waters containing half this quantity would be regarded as unsuitable for domestic use.

Dosages of 1 to 2 grams of magnesium sulfate have a purgative effect; therefore, in drinking-water standards magnesium sulfate should be limited to 1000 to 2000 mg/l. Concentrations below this limit are physiologically harmless (621).

b. Industrial Water Supplies. The following concentrations of magnesium sulfate have been recommended for industrial waters:

Process	*Concentration, mg/l* Optimum	Maximum	Reference
Brewing, pale ales, I	60-90	---	170
pale ales, II	60-120	---	170
mild ales	60	---	170
stout	60	---	170
Brewing	100	---	170
Brewing, light or dark	---	200	173
Ice, raw water	---	130	173

c. Irrigation. See Calcium, Hardness, and Chapter V-Irrigation.

d. Stock and Wildlife Watering. High concentrations of magnesium sulfate in the drinking water of rats and other small animals have retarded growth, caused emaciation, rough coat, diarrhea, and increased mortality among the young (284, 287, 640). Concentrations from 10,000 to 25,000 mg/l have been harmful to rats. A combination of 5000 mg/l of magnesium sulfate and 20,000 mg/l of sodium chloride has inhibited the growth of rats (640) (see also Dissolved Solids). On the other hand, 5000 mg/l in drinking water has not been harmful to rats (287). Livestock will tolerate 2050 mg/l of magnesium sulfate without laxative effects (2394). In drinking water, 12,000 mg/l had no effect on the water and food consumption of male rats (2398).

e. Fish and Other Aquatic Life. The following concentrations of magnesium sulfate have been reported to have killed fish:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
15,500	turbid	96-hour TL_m	mosquito-fish	2940
20,900-28,400	cistern	14 days	perch	644
24,500-27,500	well	78 days	perch	644

The maximum concentration of magnesium sulfate tolerated by young eels for over 25 hours was reported to be about 12,000 mg/l (1459).

MALATHION

(see Chapter IX)

MALEIC ANHYDRIDE $C_4H_2O_3$

This solid dissolves readily in water, forming maleic acid, HOOCHC = CHCOOH. It is used in the manufacture of alkyd-type resins, dye intermediates, and pharmaceuticals (364). Wallen et al. (2940) exposed mosquito-fish (*Gambusia affinis*) to maleic anhydride in turbid water at 20-23°C. They found the 24- and 48-hour TL_m values to be 240 mg/l and the 96-hour TL_m was 230 mg/l. The pH value was lowered from 8.0 to 5.8 and the 128 mg/l of turbidity was coagulated and removed by this compound. Using bluegill sunfish (*Lepomis macrochirus*) in Philadelphia tap water at 20°C, Turnbull et al. (2093) found the 24-hour TL_m to be 150 mg/l and the 48-hour TL_m to be 138 mg/l. They estimated a safe concentration to be 35 mg/l.

MANGANESE Mn

1. General. Manganese metal is not found pure in nature, but its ores are very common and widely distributed. The metal or its salts are used extensively in steel alloys, for dry-cell batteries, in glass and ceramics, in the manufacture of paints and varnishes, in inks and dyes, in matches and fireworks, and in agriculture to

enrich manganese-deficient soils (2121). Like iron, it occurs in the divalent and trivalent form. The chlorides, nitrates, and sulfates are highly soluble in water; but the oxides, carbonates, and hydroxides are only sparingly soluble. For this reason, manganic or manganous ions are seldom present in natural surface waters in concentrations above 1.0 mg/l. In ground water subject to reducing conditions, manganese can be leached from the soil and occur in high concentrations. Manganese frequently accompanies iron in such ground waters and in the literature the two are often linked together.

2. Cross References. Iron, Manganese Salts, Potassium Permanganate, Turbidity, Tastes.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The 1962 Drinking Water Standards of the USPHS (2036) set a recommended limit for manganese of 0.05 mg/l. The 1958 WHO International Standards (2328) prescribe a "permissible limit" of 0.1 mg/l and an "excessive limit" of 0.5 mg/l, but no maximum allowable limit is given. The 1961 WHO European Standards have a recommended limit of 0.1 mg/l.

These limits have been established on the basis of esthetic and economic considerations rather than physiological hazards. Manganese is essential for the nutrition of both plants and animals (2121, 2129). Diets deficient in manganese result in impaired or abnormal growth, symptoms of central nervous system disturbance, anemia, and possibly interference with reproductive functions (2121, 2129). The daily intake from a normal human diet is about 10 mg (2129). It is absorbed very slightly and deposits mainly in the liver and kidneys (2129).

In concentrations not causing unpleasant tastes, manganese is regarded by most investigators to be of no toxicological significance in drinking water (633, 1077). However, some cases of manganese poisoning have been reported in the literature. A small outbreak of an encephalitis-like disease, with early symptoms of lethargy and edema, was traced to manganese in the drinking water in a village outside of Tokyo; three persons died as a result of poisoning by well water contaminated by manganese derived from dry-cell batteries buried nearby (36, 1225). Excess manganese in the drinking water is also believed to be the cause of a rare disease endemic in Manchukuo. That manganese may be toxic is also indicated by the reports that 0.5 to 6.0 grams of manganese per kilogram of body weight administered daily to rabbits had stunted growth and interfered with bone development (921).

Despite the possible toxic effects of manganese under unusual circumstances, it cannot be considered a physiological hazard because the normal dietary intake is far higher than the amount that would be tolerated esthetically in drinking water.

Manganese is undesirable in domestic water supplies because it causes unpleasant tastes, deposits on food during cooking, stains and discolors laundry and plumbing fixtures, and fosters the growth of some micro-organisms in reservoirs, filters, and distribution systems (1593, 3539, 3540, 3541, 3542) (see Fish and Other Aquatic Life, below).

It has been reported by one observer that manganese salts impart a metallic taste to water at concentrations above 0.5 mg/l (945); and by another reference at above 20 mg/l (759). Cohen et al. (3301) found the taste threshold for manganous ion in spring water to occur at about 180 mg/l for the median of a large panel, but at 32 mg/l for the most sensitive members. In distilled water the taste thresholds were much lower, about 35 mg/l for the median and about 0.9 mg/l for the most sensitive panel members (3301). Manganese in excess of 0.15 mg/l has also been reported to cause turbidity in water (1594).

For domestic water supplies a maximum concentration of manganese, or of iron and manganese together, as low as 0.017 mg/l has been recommended (1256). Concentrations as low as 0.1 mg/l are reported to cause laundry trouble (219, 284); concentrations of 0.2 to 0.4 mg/l are likely to cause complaints (36); and, in general, limiting concentrations from 0.02 to 0.5 mg/l have been recommended (499, 555, 628, 1257, 3541).

b. Industrial Water Supplies. Excessive manganese is undesirable in water for use in many industries, including textiles (255, 256, 257); dyeing (261); food processing, distilling, and brewing (240, 224, 284); ice (234); paper (212, 879); and many others (see Chapter V). The following tabulation summarizes the recommendations as to maximum permissible concentrations of manganese in industrial waters:

	Maximum Permissible Concentration		
Industrial Use	*Manganese in mg/l*	*Iron + Manganese in mg/l*	*Reference*
Air conditioning	0.5	0.5	162
	0.5	--	152
Baking	0.2	0.2	162, 152
Brewing, light and dark	0.1	0.1	162, 152
Canning	0.2	0.2	162, 152
Carbonated beverages	0.2	0.2	162, 152, 184
	--	0.1	179
Confectionary	0.2	0.2	162, 152
Cooling water	0.2	0.2	152
	0.5	0.5	162
Dyeing	0	0	36
Food processing	0.2	0.2	162, 152
Ice	0.2	0.2	162, 152, 234
Milk industry	0.03-0.1	--	2344
Paper and pulp	0	0	36
Groundwood	0.5	1.0	162, 152
	0.1	--	244
Kraft pulp	0.1	0.2	162, 152
Soda and sulfate	0.05	0.1	162, 152
	0.05	--	245
Highgrade paper	0.05	0.1	162, 152
Fine paper	0.05	0.1	350
Kraft paper			
bleached	0.1	--	351
unbleached	0.5	--	351
Photography	0	0	36
Plastics (clear)	0.02	0.02	162, 152
Rayon and viscose			
Pulp production	0.03	0.05	162, 152
Manufacture	0	0	162, 152
	0.02	--	550, 405
Tanning	0.2	0.2	162, 152
Textiles, general	0.25	0.25	162, 152
	---	0.1	852
	0.1	--	256
dyeing	0.25	0.25	162, 152
wool scouring	1.0	1.0	162, 152
bandages	0.2	0.2	162, 152

c. Irrigation. Manganese is essential for plant growth, apparently as an enzyme activator (3543). It is especially abundant in the reproductive parts of plants, seeds being highest while woody sections contain the least manganese (3544). Nuts contain the highest concentrations (22.7 mg/kg) and sea foods the lowest (0.25 mg/kg). Tea diffuses enough so that the normal liquid has 1 to 7 mg/l (2121). Manganese has been used to enrich soil, yet in some concentrations it may be phytotoxic (219, 277, 563).

Manganese in the nutrient solutions has been reported to be toxic to many plants, as grown in solution cultures. The sensitivity and response of the plants to the presence of manganese varies both with the species of plant and the composition of the nutrient solution. Symptoms of manganese injury have been intensified in the presence of molybdenum, vanadium (1595), or nitrate (1596). Symptoms of manganese injury have been diminished in the presence of cobalt (1499), iron, molybdenum, aluminum, phosphorus deficiency (1458), ammonium or ammonium nitrate (1596). The following concentrations of manganese have been reported to be harmful to plants in solution culture:

Concentration of Manganese in mg/l	*Type of Plant*	*Reference*
0.5	Various plants	1597
1-10	Various legumes	1597
3.5	Various plants	1597
5	Orange and mandarin seedlings	1524
5-10	Tomatoes	1499
10-25	Soybean, flax	1595
25-100	Flax	1458
50	Flax	1596
62.5	Various plants	1597
150-500	Oats	1462

It has also been reported that 0.25 mg/l of manganese has permitted good growth of tomatoes, and that up to 5.0 mg/l of manganese has reduced the severity of cobalt poisoning in tomatoes (1499). In the presence of ammonium or of ammonium nitrate, 50 mg/l of manganese was not harmful to flax, although this concentration was harmful in the presence of nitrate without ammonium (1596). Manganese sulfate, at a concentration of 100 mg/l as manganese caused no apparent injury to oat plants (1462).

d. Stock and Wildlife Watering. A deficiency of manganese in animals produces ovarian disfunction, testicular degeneration, poor lactation, lack of growth, bone abnormalties, and symptoms of central nervous disturbance (2121). Cattle are reported to have received dosages of 50 to 600 mg/kg in the diet for 20 to 45 days without serious effects. Birds have received single oral dosages of up to 600 mg/kg without adverse effects, but the continuous excess of manganese in fodder was suspected as an etiological factor in the occurrence of infectious anemia in horses. Manganese appears to oxidize vitamin B in the horse body, producing avitaminosis (1049).

The metabolism of manganese is closely related to that of calcium, phosphorus, iron, copper, and possibly other minerals, and the proper balance must be maintained. The manganese requirement for chicks has been reported to be 30-50 mg/kg (dry ration); for hens, 40-50 mg/kg. However, 1000 mg/kg in the dry ration was not toxic (1551).

e. Fish and Other Aquatic Life. The toxicity of manganese toward fish is dependent upon many factors. Jones (2941) gives the lethal concentration for the stickleback as 40 mg/l; however, the toxic action is slow and manganese does not appear to precipitate the gill secretions. According to Oshima (3545) and Iwao (3546) the toxicities of manganous chloride and manganous sulfate are slight, being about 2400 and 1240 mg/l of manganese respectively. Manganese appears to be somewhat antagonistic to the toxic action of nickel toward fish (1468).

The following concentrations of manganese have been tolerated by fish under the stated conditions:

Concentration in mg/l	*Time of Exposure*	*Type of Fish*	*Reference*
1	--	river crayfish	2977
15	7 days	tench, carp, trout	2151
40*	4 days	fingerling catfish	2981
50**	3 days	stickleback	1459
2700	50 hours	eels	1459

* from manganese disodium versenate
**from manganese sulfate

Manganese and iron in concentrations above 0.1 mg/l stimulate the growth of certain organisms, such as *Crenothrix, Gallionella,* and other related forms in reservoirs, filters, and distribution systems (152, 921, 945, 1258). The addition of as little as 0.0005 mg/l of manganese resulted in increased growth and multiplication of various microbiota in sea water (1259). Guseva (584, 1260), on the other hand, found that concentrations of manganese above 0.005 mg/l had a toxic effect on some algae.

The threshold concentration of manganese for the flatworm *Polycelis nigra* has been reported to be 700 mg/l as manganese chloride and 660 mg/l as manganese nitrate (608). Crustacea, worms, and insect larvae were not harmed by 15 mg/l of manganese during a 7-day exposure (2151).

The permanganates are much more toxic to fish than the manganous salts. Permanganates killed fish in 8 to 18 hours at concentrations of 2.2 to 4.1 mg/l of manganese (3545, 3546). However, permanganates are not stable for long in water.

4. Summary. On the basis of the literature surveyed, it appears that the following concentrations of manganese will not be deleterious to the stated beneficial uses:

a. Domestic water supply	0.05 mg/l
b. Industrial water supply	0.05 mg/l
c. Irrigation	0.50 mg/l
d. Stock watering	10.0 mg/l
e. Fish and aquatic life	1.0 mg/l

MANGANESE CHLORIDE $MnCl_2$ and $MnCl_3$

(see also Manganese, Chlorides)

This highly soluble salt, occurring generally in the manganous form, is used in dyeing operations, in disinfecting, in linseed oil driers, and in electric batteries (364). In fresh water, 12 mg/l has been reported as fatal to minnows (*Fundulus*) within six days (1459), but other fish have been found to be much more tolerant of $MnCl_2$. For the small fresh-water fish (*Orizias*), the 24-hour lethal concentration was about 7850 mg/l (1459) and for other fish 5500 mg/l (3545, 3546). The highest concen-

tration tolerated by young eels for 50 hours was 6300 mg/l (1459). The first toxic effects of $MnCl_2$ for fish were observed at 330 mg/l but the lethal concentration did not occur until 800 mg/l (2977).

Toward lower organisms there is similarly a wide variation in reported toxicity. For immobilization of *Daphnia magna* in Lake Erie water, the threshold concentration was found (598) to be 50 mg/l of $MnCl_2$. In River Havel water at 23°C, the threshold effect of $MnCl_3$ occurred at 50 mg/l of manganese (2158). For the flatworm, *Polycelis nigra,* the threshold concentration of $MnCl_2$ was reported to be 700 mg/l (608).

MANGANESE DIFLUORIDE MnF_2

(see also Manganese, Fluorides)

This highly soluble manganous salt is reported to be lethal to tench in 48 hours at a concentration of 500 mg/l (3271).

MANGANESE NITRATE $Mn(NO_3)_2 \cdot 4H_2O$

This manganous salt is very soluble in water. For sticklebacks in tap water, the minimum lethal concentration of manganese nitrate has been reported to be 40 mg/l as manganese (598, 1460). The average survival times of the fish in different concentrations was as follows: one week at 50 mg/l, four days at 100 mg/l, two days at 150 mg/l and only one day at 300 mg/l, all measured as managanese (1460). For the flatworm, *Polycelis nigra,* the threshold concentration has been reported to be 660 mg/l as manganese nitrate (608).

MANGANESE SULFATE $MnSO_4 \cdot H_2O$

This pale-pink manganous salt, highly soluble in water, is used in dyeing, porcelain glazing, varnishes, and specialized fertilizers (364). In culture solution, 100 mg/l as manganese caused no apparent injury to oat plants, 150-200 mg/l caused chlorosis, and 500 mg/l produced injury (1462).

Toward fish, the toxicity of manganous sulfate is slight. In tap water, 50 mg/l as manganese did not kill sticklebacks within three days (1459). Young eels tolerated 1500 mg/l as manganese sulfate for more than 25 hours. The first influence of this salt toward fish is reported to occur at 500 mg/l as Mn, and at 1000 mg/l as Mn the salt is lethal (2977). Japanese investigators (3545, 3546) report the toxicity of this salt at 3400 mg/l.

MANOXOL OT

(see Chapter X)

MASONITE MANUFACTURING WASTES

Ellis (611) investigated wastes from a Masonite plant in Mississippi, containing chemical compounds, fibers, pigments, and an unidentified substance with a high B.O.D. that was toxic to fish in one to three days at 1:100,000 dilution. Loose fibers menaced fish for 12 miles below the plant.

MERCAPTANS, GENERAL

(see also Methanethiol)

Mercaptans (RSH) are the sulfur analogs of the alcohols (ROH) and phenols (R'OH). They are generally odoriferous and can be detected in very small concentrations. They occur in coal tar and in the wastes from Kraft-process pulp mills.

The threshold concentration for taste and odor of mercaptans from Kraft mill wastes has been reported at less than 0.02 mg/l (686). The untreated waste from the mill, containing 12 mg/l of mercaptans, required a dilution of 1:50,000 to render it odorless, i.e., down to a concentration of 0.00024 mg/l; but after chlorination to a residual of 1.5 mg/l, the required dilution was only 1:40, i.e. down to a concentration of 0.3 mg/l.

Gersdorff (695) shows that phenyl mercaptan, C_6H_5SH (thiophenol), a liquid with a repulsive, penetrating, garlic-odor, and tolyl mercaptan $CH_3C_6H_4SH$ (thiocresol) have a similar toxic effect on goldfish, but the toxic action differs from that of phenol. Metatolyl mercaptan is about four times as toxic, o-tolyl mercaptan about five times, and p-tolyl mercaptan about 8.5 times as toxic as phenol (see Phenols). The relative toxicities of m-o-, p-tolyl mercaptans are in the ratios 1 to 1.19 to 2.19, a relationship nearly the same as that found for the corresponding cresols. The replacement of the oxygenation of the cresol molecule by sulfur appears to cause a fourfold increase in the toxicity of the compound to goldfish (695).

MERCURIC ACETATE

(see Mercuro-Organic Compounds)

MERCURIC CHLORIDE $HgCl_2$

1. General. This salt is soluble in water at 20°C to the extent of 61,000 mg/l (911). It is used in embalming, disinfecting, preserving, printing of fabrics, tanning, electroplating, manufacturing ink, and numerous other processes (364). It may occur in wastes from any of these industries, or in lead mining and chemical wastes (313).

2. Cross References. Mercury, Other Mercury Salts, Mercuro-Organic Compounds, Chlorides, and Chapter V —Fish and Other Aquatic and Marine Life.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The ingestion of 1.0 to 2.0 grams of mercuric chloride is frequently fatal to human beings.

b. Stock and Wildlife Watering. The lethal dose for dogs has been reported as 10 to 15 mg per kg of body weight (353). The LD_{50} value of mercuric chloride for rats was given as 37 mg/kg while that for mercurous chloride (Calomel) was 210 mg/kg (3009, 3067).

c. Fish and Other Aquatic Life. From a study of the relation between concentration of the salt and period of survival, it appears that mercuric chloride is infinitely toxic to fish, i.e. that infinitesimal traces of the compound will be toxic if exposure continues long enough (3547). The following concentrations of mercuric ion from chloride have been shown to injure or kill fish in the time indicated:

Concentration of Mercury, in mg/l	*Time of Exposure*	*Species of Fish*	*Reference*
0.008	--	sticklebacks	1460, 2941
0.01	--	sticklebacks	2962, 2920
0.01	80-92 days	minnows	1459
0.011 *	--	sticklebacks	598
0.02	--	guppies	2921

Concentration of Mercury, in mg/l	*Time of Exposure*	*Species of Fish*	*Reference*
0.02	19-47 days	minnows	1459
0.02	7 days	sticklebacks	1460
0.027	50 hours	young eels	1459
0.05	1 day	minnows	1459
0.1	8 hours	minnows	1459
0.2	4 days	sticklebacks	1459
3.2	--	minnows	362
4.0 to 30	--	fish	468
7.4	15 minutes	sticklebacks	1459
7.4	106 minutes	sticklebacks	1264
9.2 to 37	24 hours	trout	359
10.0	42 minutes	minnows	313
12.6	15 minutes	minnows	362
30	31 minutes	fish	467
370	54 minutes	trout	313
1000	20.5 minutes	minnows	991
5000	12 minutes	minnows	991

*Threshold value for deterimental effect

In varying concentrations, sodium chloride exhibits first a synergistic effect, then an antagonistic effect toward the toxicity of mercuric chloride. The presence of 1,000 mg/l of NaCl decreased the survival time of fish in a solution of 10 mg/l of mercuric chloride from 106 minutes to 54 minutes, but concentrations of 15,000 mg/l of NaCl prolonged the survival time to as much as 190 minutes (1264, 1265). According to Jones (467), fish show no special ability to detect or avoid toxic concentrations of mercuric chloride.

Anderson (598) reports that the threshold concentration for immobilization of *Daphnia magna* in Lake Erie water at 25°C was found to be less than 0.006 mg/l in 64 hours. For *Polycelis nigra*, a flat worm, the threshold of toxicity in 48 hours was 0.2 mg/l of mercury, or 0.027 mg/l of mercuric chloride, according to Jones (608). The effect of mercuric chloride on *Marinogammarus marinus*, an amphipod, was measured by Hunter (1266) who found that 0.1 mg/l of mercury killed in 510 minutes and 1.0 mg/l in 390 minutes. Low concentrations of copper increase appreciably the toxicity to mercury solutions.

Toward the larvae of bivalves, Woelke (2989) reported mercuric chloride to be lethal at a concentration of 0.027 mg/l (0.02 mg/l as Hg). Bringmann and Kuhn (2158, 3343), using River Havel water, determined the concentrations of mercury added as mercuric chloride that produced threshold effects upon four organisms as follows:

Organism	*Threshold Concentration of Hg in mg/l*
Microregma	0.15
Escherichia coli	0.2
Scenedesmus	0.03
Daphnia magna	0.03

In a concentration of 3.0 mg/l, mercuric chloride killed 100 percent of water snails (*Australorbis glabratus*), while at a dosage of 1.0 mg/l only 30 percent were killed (exposure time not specified) (3548). Other inorganic mercury salts were approximately equal in effectiveness.

Mercuric chloride at a concentration of 0.61 mg/l causes a 50 percent decrease in the 5-day utilization of oxygen by synthetic sewage (2923) and at a concentration of 2.0 mg/l there is complete bacteriostasis (3549).

Clendenning and North (2106, 2107) found that 0.05 mg/l of mercury, added as mercuric chloride, caused a 50-percent inactivation of photosynthesis of the giant kelp (*Macrocystis pyrifera*) during a four-day exposure, while 0.1 mg/l caused a 15-percent decrease in photosynthesis in one day and complete inactivation in four days. Mercury was more toxic than copper, hexavalent chromium, zinc, nickel, and lead.

MERCURIC CYANIDE $Hg(CN)_2$

Highly soluble in water, mercuric cyanide has been used as a diuretic, as a topical antiseptic, and as a disinfectant for surgical instruments (364). Bringmann and Kuhn (2158, 3343) tested its effectiveness in water from the River Havel toward four organisms. Concentrations of mercury added as Hg $(CN)_2$ required to cause a median threshold effect were reported as follows:

Organism	*Threshold Concentration of Hg in mg/l*
Daphnia magna	0.02
Scenedesmus	0.15
Escherichia coli	0.20
Microregma	0.16

MERCURIC NITRATE $Hg(NO_3)_2 \cdot H_2O$

This soluble salt is used in the manufacture of explosive caps, felt, and the treatment of skin diseases. In very soft water, it killed sticklebacks in one week at a concentration of 0.02 mg/l as Hg (2977). Toward guppies the LD_{50} value was also reported as 0.02 mg/l as Hg (2921). A concentration of 3.0 mg/l gave a 90-percent mortality among water snails, while 1.0 mg/l showed a 30 percent reduction (3548). Klock and Pearson (2314) reported the 48-hour TL_m toward the stickleback (*Gasterosteus aculeatus*) as 1.7 mg/l. The concentrations producing the first apparent significant response for three organisms were as follows:

Test Species	*Concentration of $Hg(NO_3)_2$ in mg/l*
Isopod (*Neospheroma oregonensis*)	0.015
Fish (*Gasterosteus aculeatus*)	0.015
Polychaete (*Mercierella enigmatica*)	1.00

MERCURO-ORGANIC COMPOUNDS

Organic mercury compounds are used in herbicides, fungicides, and medical treatment of animals and humans. They have been used extensively to control slimes in paper mills and consequently they may be present in discharged white waters. Phenylmercuric acetate is used extensively as a herbicide for the control of crabgrass.

Van Horn (974) tested the survival time of fish in organic mercury compounds and other proprietary substances used for slime control in paper mills. He found that the critical concentrations, i.e., the concentrations that will sooner or later be fatal to fish, were as follows:

Trade Name	*Concentration in mg/l for*	
	Minnows	*Shiners*
Santobrite	0.3 to 0.4	0.2
Merfenel	0.02	0.02 to 0.06
Lignasan	1.0	0.8
Nalco 23	1.4	1.4
Nalco 21	--	0.4

Van Horn and Balch (3550) investigated the toxicity of slime control agents to minnows. The minimum lethal concentration was 0.15 mg/l for pyridylmercuric acetate and 0.04 mg/l for pyridylmercuric chloride.

Ellis (1267) found that in water at a pH value of 7.7 to 7.8 containing 0.5 mg/l of phenylmercuric lactate, all fish died in 16 hours or less. The lowest concentration causing death was about 0.10 mg/l. In terms of the content of mercury, phenylmercuric lactate is more than twice as toxic as mercuric chloride.

Phenylmercuric acetate (Scutl) in concentrations of 0.02 mg/l was fatal to young salmon in less than five days, according to Vallin (357), and the maximum safe concentration was found to be 0.01 mg/l. For mercuric acetate, the safe concentration was 0.02 to 0.05 mg/l. *Daphnia pulex* was found to be more sensitive than fish to phenylmercuric acetate, the safe concentration lying between 0.005 and 0.01 mg/l.

Pyridylmercuric acetate in very small concentrations (about 0.14 mg/l) was found to be beneficial to the health of fish, probably by suppressing the micro-organisms in the water (356). According to Van Horn and Katz (1268), 96 percent of Lake Emerald shiners survived a concentration of 0.15 mg/l but higher concentrations are toxic to fish.

Pyridylmercuric acetate successfully controlled the growth of a fluorescent species of *Pseudomonas*, which had infected yearling blueback salmon, when the fish were immersed for one hour in a solution containing 10 mg/l without harming the fish (1598). This same concentration, however, has been found toxic to other species, being more toxic to rainbow trout fingerlings than to brown or brook trout. Toxicity was manifested by losses of appetite, and it increased with temperature (1599).

A concentration of 5 mg/l for one hour was not toxic to rainbow trout fingerlings less than three inches in length, but it was toxic to larger fingerlings of the same species (1467). A concentration of 2 mg/l applied for one hour to yearling trout resulted in the death of 13 percent of the fish. Two weeks later a repeated treatment caused death of 10 percent of the fish. No fish died after a third treatment when the fish were not fed for 24 hours (1600).

Clemens and Sneed (2981) investigated the toxicity toward fingerling channel catfish of numerous compounds used in fish management and in agriculture. The results for organic mercury compounds in tap water are summarized below:

	Temperature	TL_m in mg/l			
Chemical	°C	*24 hrs.*	*48 hrs.*	*72 hrs.*	*96 hrs.*
Phenylmercuric acetate	19	4.1	3.4*	3.3	3.3
Pyridylmercuric acetate	24	3.8	--	0.49	--
Ceresan M (a)	19	1.8	1.8	1.6	1.6
Lignasan (b)	19	2.0**	2.2	1.7	1.3
Tag 10% solution (c)	20	1.5	0.78	0.60	0.58

* At 45 hours
** At 28 hours
(a) Ethyl mercury p-toluene sulfonanilide, 7.7 percent (total mercury as metallic, 3.2 percent)
(b) Ethylmercuric phosphate, 6.25 percent
(c) Phenylmercuric acetate, 10 percent

Bond and Nolan (3548) found that 13 organic compounds of mercury were 90 to 100 percent fatal to snails (*Australorbis glabratus*) at concentrations of 1.0 mg/l and several were highly toxic at 0.3 mg/l. Toward phytoplankton, the minimum lethal concentration of ethylmercuric bromide, phenylmercuric chloride, and ethylmercuric oxalate was found to be about 0.3 mg/l. Zooplankton were killed by this concentration in 22 minutes (3552). Lignasan (ethylmercuric phosphate, 6.25 percent) is an effective algicide at 1.0 mg/l (3551).

MERCURY Hg

1. General. Although elemental mercury occurs as a free metal in some parts of the world, it is rather inert chemically and insoluble in water; hence, it is not likely to occur as a water pollutant. It is used in scientific and electrical instruments, in dentistry, in power generation, in solders, and in the manufacture of lamps (364, 2121).

Mercuric salts occur in nature chiefly as the sulfide HgS, known as cinnabar, but numerous synthetic organic and inorganic salts of mercury are used commercially and industrially, e.g. as medicinal products, disinfectants, detonators, pigments, and photoengraving. Many of the mercuric and mercurous salts are highly soluble in water.

Most of the references dealing with mercury express concentrations in terms of specific mercuric salts; hence they are covered under that heading. The data in this section relates only to the mercuric compounds for which the anion was not specified.

2. Cross References. Mercuric Salts, Mercuro-Organic Compounds.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The drinking water standards of the USPHS and the WHO do not include limits for mercury but for over a decade the maximum permissible concentration of mercury or mercuric ions in drinking water in the USSR has been set at 0.005 mg/l (460, 3107).

Mercury and mercuric salts are considered to be highly toxic to humans (353, 364, 2121). They are readily absorbed by way of the gastro-intestinal tract, and fatal doses for man vary from 3 to 30 grams (2121). According to Smith (922), adults may safely drink water containing about 4 to 12 mg of Hg per day and a fatal dose of such water would be about 75 to 300 mg per day.

b. Fish and Other Aquatic Life. Mercuric ions are considered to be highly toxic to aquatic life. For freshwater fish, concentrations of 0.004 to 0.02 mg/l of Hg have been reported harmful (2409). For the stickleback (*Gasterosteus aculeatus*) the lethal concentration limit has been found to be 0.008 mg/l (353, 1460, 2941). Mercury salts, such as the unstable compounds mercuric sulfate and nitrate, have killed minnows at a concentration of 0.01 mg/l as mercury, after 80-92 days. At concentrations of 0.05 and 0.1 mg/l as mercury, fish were killed in 6 to 12 days (1459). For further details, see the mercuric salts.

In contrast, Schweiger (2151) reported that 0.2 mg/l of mercury was not harmful to one- and two-year-old tench, carp, rainbow trout, and char, nor to fish-food organisms such as crustacea, worms, and insect larvae.

For phytoplankton, the minimum lethal concentration of mercury salts has been reported (3552) to range from 0.9 to 60 mg/l of Hg. The toxic effects of mercuric salts are accentuated by the presence of trace amounts of copper (3313).

The Severn and Mersey River Boards in England have adopted working standards that limit the total concen-

tration of heavy metals, including mercury, to 1.0 mg/l (1756, 2950).

METAL-PLATING WASTES

(see also Chromates, Cyanides, Zinc, Iron, Acidity)

Pickling liquors from metal-plating works contain principally sulfuric acid, chromates, and iron. Schaut (362) found that a 1:240 dilution of such wastes gave a yellow color and an opaqueness to water, with an iron content of 470 mg/l and an acidity of 30 mg/l. At this dilution, minnows were harmed in 15 minutes and had a 50-percent mortality in one hour. For a dilution of 1:2400 the water was murky yellow in appearance but minnows were normal after one hour of exposure.

METAPHOSPHATES

(see Phosphates and Phosphoric Acids)

METASYSTOX

(see Chapter IX)

METHANE CH_4

(see also Hydrocarbons, General)

A gaseous saturated (paraffin) hydrocarbon, methane is odorless and flammable. It is a product of anaerobic decomposition of organic matter in marshes, mines, and sludge-digestion tanks. Wastes from natural-gas and petroleum plants may contain methane dissolved in water. According to Shelford (363), methane does not appear to be toxic or harmful to sunfish and Schaut (362) reported that a saturated solution (about 65 mg/l) had no effect on minnows after two hours of exposure.

METHANETHIOL CH_3SH

Known also as methyl mercaptan, this flammable gas with an odor of rotten cabbage occurs in coal tar and wastes from pulp mills. At a concentration of 1.0 mg/l in lake water it has been reported to kill white bass in 105 minutes, smallmouth blue bass and blue gills in 6 to 8 hours and rock bass in 11 hours (311, 313, 688, 696). In Kraft-pulp-mill wastes the minimum lethal concentration for shiners in 120 hours at 18°C was given as 0.5 mg/l (190, 344, 658). For minnows, the minimum lethal concentration was also reported as 0.5 mg/l (3523).

Haydu et al. (3501, 3502, 3503) reported on the minimum lethal concentration (defined as the minimum concentration giving complete kills) and the critical concentration (defined as the maximum concentration that caused no mortality) for three species of fish as follows:

Condition	*King Salmon*	*Silver Salmon*	*Cutthroat Trout*
Temperature of test, °C	15.5-19.5	12-18	9-15
Minimum concentration for complete kill mg/l	0.9	1.75	1.2
Maximum concentration for no kill, mg/l	0.5	0.7	0.9

For *Daphnia magna* and may-fly larvae, the minimum lethal concentration is given as 1.0 mg/l (190, 574) but for the larvae of *Chironomus*, the minimum lethal dose is only 50 mg/l. The toxicity of methanethiol to chironomous larvae was reported as follows (690):

Concentration in mg/l	*Survival in percent*
5 to 20	100
50	50
100	25
200	0

METHOXYCHLOR

(see Chapter IX)

METHYL ALCOHOL CH_3OH

This colorless, clear, flammable liquid is obtained from the destructive distillation of wood or by hydrogenation of carbon monoxide. It is used extensively in chemical syntheses, as a fuel, as a solvent, and in the commercial manufacture of many products. The acute oral toxicity to rats has been reported (3248) as 9.1 mg/kg of body weight.

For human consumption it is very toxic. Small amounts will lead to blindness and 10 ml has caused death (364). Aquatic life, however, is much more tolerant to this substance. Trout withstand 10,000 mg/l in tap water for two hours without apparent injury; but in distilled water with 250 mg/l goldfish died in 11 to 15 hours (313). The same effect was noted with ethyl alcohol. For fingerling trout in natural waters, up to 8100 mg/l of methyl alcohol had no harmful effects in 24 hours.

Anderson (358) reports that in Lake Erie water the threshold concentration for immobilization of *Daphnia magna* after prolonged exposure at 25°C was 32,000 mg/l. To evoke a reaction (stimulation of movement) in a group of water beetles *(Laccophilus)* a methyl alcohol concentration of 115,000 mg/l was required (2956).

METHYLAMINE CH_3NH_2

Occurring in nature as a degradation product of proteinaceous matter, methylamine is a gas at ordinary temperatures and pressures. It is highly soluble in water. Industrially, it is used in tanning and in organic synthesis (364).

According to Gillette et al. (1442) the critical range for creek chub, a fish of average tolerance, exposed for 24 hours in well-aerated water at 15-21°C, was 10 to 30 mg/l, i.e., all fish survived 10 mg/l but all died at 30 mg/l. Corti (146) tested rainbow trout *(Salmo irideus)* at a methylamine concentration of 141 mg/l. The resulting pH was 10.2 and the temperature 13.0-13.5°C. The fish showed their first reaction in about two minutes, turned over in 5 to 8 minutes, and made their last movement in 19 to 23 minutes. Bringmann and Kuhn (2158, 3343) found that the toxic threshold for methylamine hydrochloride occurred at a concentration of 4 mg/l for *Scenedesmus* and 50 mg/l for *Microregma* during a 4-day exposure at 24°C. For *Daphnia* at 23°C for 2 days, the threshold was 480 mg/l, while for *E. coli* at 27°C concentrations up to 1000 mg/l had no deleterious effect.

METHYL CHLORIDE CH_3Cl

This colorless gas with an ethereal odor is used as a refrigerant. It is soluble in water to the extent of 4000 mg/l at 20°C or about 9000 mg/l. Yant et al. (3554) investigated the possibility of poisoning by methyl chloride pollution of water fed to dogs. The taste of water

saturated with methyl chloride at 20°C is sharp, sickening, and sweetish when first taken into the mouth, followed almost immediately by a burning sensation. The water was frequently refused by the dogs, but they had no other water supply. Ingestion of water with an average methyl chloride content of 5950 mg/l caused no apparent symptoms of poisoning or changes in the hemoglobin or blood cells (3554).

METHYL CYANIDE

(see Acetonitrile)

METHYLCYCLOHEXANE

(see Cyclohexane)

METHYL DODECYL BENZYL TRIMETHYL AMMONIUM CHLORIDE

(see Chapter X)

METHYL ETHYL KETONE $CH_3COC_2H_5$

Known also as 2-butanone, this flammable liquid is highly soluble in water. It is widely used as a solvent in industry and for the manufacture of smokeless powder and synthetic resins (364). The oral LD_{50} dose for rats is given as 3.98 grams/kg of body weight (3244).

Turnbull et al. (2093) exposed bluegill sunfish *(Lepomis macrochirus)* to methyl ethyl ketone in Philadelphia tap water at 20°C. They found the 24-and 48-hour TL_m values both to be 5,640 mg/l, but the fish were visibly affected at 3,380 mg/l. Using highly turbid water and the mosquito-fish *(Gambusia affinis)* as the test organism, Wallen et al. (2940) found the 24-, 48-, and 96-hour TL_m values all to be 5,600 mg/l. This ketone had no clarifying effect on the turbidity.

METHYL MERCAPTAN

(see Methanethiol)

METHYLNAPHTHOQUINONE $C_{11}H_8O_2$

(see also Naphthoquinone and Dichloronaphthoquinone)

This bright-yellow crystalline solid with a faint acrid odor, also known as Menadione and by other trade names, is almost insoluble in water, but soluble in organic liquids. It is used in vitamin K therapy. Fitzgerald et al. (1542) report that it was toxic to fish in 24-48 hours at concentrations of 0.3-0.6 mg/l, or about the same concentration necessary to kill blue-green algae.

METHYL PARATHION

(see Chapter IX)

METHYL SALICYLATE $HOC_6H_4COOCH_3$

This oily liquid, known also as oil of wintergreen, is used in medicine topically as a counter-irritant. It is also used in perfumery and for flavoring (364). At 30°C it is soluble in water to the extent of 740 mg/l. According to Helbig (3494) the threshold odor of methyl salicylate in water is 0.035 mg/l.

MOLLUSCACIDES

(see Chapter IX)

MOLYBDENUM Mo

1. General. This metal and its salts are not considered to be significant in water pollution at the present time. Although the element occurs in some minerals such as molybdenite, MoS_2, and wulfenite, $PbMoO_4$, it is not widely distributed in nature. The metal and its salts are used principally for metallurgy and for electrical and electronic apparatus. It is also used in the glass and ceramic industries, for the production of pigments, and as a constituent of fertilizers for leguminous crops.

2. Cross References. Ammonium Molybdate, Copper, Selenium.

3. Effects Upon Beneficial Uses.

a. Irrigation. Molybdenum in very low concentrations has been found to be essential for healthy growth of a number of plants (1493, 1498, 1601, 1602, 1603, 3555, 3556), and for certain microbiological systems, where it apparently plays a role in the enzymatic catalysis of nitrate reduction (3557). At higher concentrations it has been injurious to the growth of many kinds of plants in solution or sand culture. The following concentrations of molybdenum have been reported to be injurious to plants:

Concentration of Molybdenum in mg/l	*Plant*	*Effect*	*Reference*
0.5-100	Flax	Abnormal growth	1458
5.0	Clover, lettuce	Toxic effect	1601
10-20	Soybean, flax	Toxic effect	1595
25-35	Cotton	Slight effect	1604
50	Oats	Slight effect	1462
100	Oats	Chlorosis	1462
200	Oats	Stunted growth	1462

It has also been reported that 40 mg/l of molybdate (MoO_4^{--}) in nutrient solution was slightly injurious to sugar beets in sand culture (1473).

On the other hand, 0.1 mg/l of molybdenum has caused no injury to soybean and flax plants (1595). Molybdenum in small amounts reduced the symptoms of manganese, cobalt, and nickel poisoning in flax (1458), and nitrate injury in tomatoes (1596). There is some evidence that 1.0 mg/l of molybdenum in the water applied to cotton plants will reduce chlorosis (3556). The application of molybdenum to soil has overcome necrosis in hops (3555). Peas and beans grown with nitrate as nitrogen source appear to require molybdenum for completion of their life cycle, inasmuch as molybdenum is essential in the nitrogen-fixing mechanism (2121). Recovery of molybdenum deficient plants was effected by the application of 100 mg/l of molybdenum in molybdate solution (1603).

d. Stock and Wildlife Watering. Molybdenum appears to be an essential trace mineral for rats (1605), 2121). The toxic dose for rats has been reported to be 0.4 mg of molybdenum as molybdate (1498). Browning (2121), however, reported the oral LD_{50} for rats to be 125 mg/kg for molybdenum trioxide, 101 mg/kg for calcium molybdate and 333 mg/kg for ammonium molybdate. Comparison of the effects of various molybdenum compounds showed that 500 mg of molybdenite (MoS_2) ingested daily was non-toxic, but animals receiving molybdenum trioxide, calcium molybdate, and ammonium molybdate showed anorexia, listlessness, and loss of weight. In the drinking water, 5 mg/l of molybdenum

increased the mortality rate among rats fed a ration containing 11 mg/l of selenium (1481).

Upon addition to the gross ration 0.1 percent molybdenum (as sodium molybdate, $Na_2MoO_4 \cdot 2H_2O$) has produced toxic symptoms among rabbits (1606). When 1.2 g/kg of ammonium molybdate was given orally to rats and guinea pigs anorexic, colic, trembling, incoordinated movements, and dyspnea resulted (2121). In the diet of chicks, 300 mg/l (on a dry basis) of molybdenum has resulted in a reduced rate of growth of the chicks during a 4-week observation period (1493), and 200 mg/l depressed growth at eight weeks (3311), although copper appears to reduce the toxicity of molybdenum.

The principal detriment of molybdenum, like that of selenium, arises from the fact that it is picked up by plants and concentrated in foliage in pastures. Water-soluble molybdenum compounds in herbage cause cattle to scour severely (1269, 1270, 3557, 3558). Soil from pastures in which cattle had scoured contained 20 to 100 mg/l of molybdenum while non-injurious fields contained less than 5 mg/l. The herbage content of molybdenum varied with species on the same soil (1049).

Barley hay containing 89 mg/l of molybdenum (dry weight) caused symptoms similar to selenium poisoning in livestock, and molybdenum has also been suggested as a cause of bovine hematuria in parts of Australia. Studies are being conducted in Australia to determine the significance of molybdenum (1049). In an area of Florida where molybdenum poisoning among cattle has been common, the molybdenum content of the herbage has been as high as 26 mg/l (dry basis), while the molybdenum content of the water has ranged from 0 to 85 mg/l (1607). The toxic symptoms of molybdenum poisoning appear to be reduced by copper (3311, 3558) and molybdenum appears to affect copper metabolism (1271). Many studies have been undertaken to clarify the metabolic relationships between molybdenum and copper, as well as other minerals (1498, 1575, 1607, 1608, 1609, 1610, 2121, 3311). Copper glycinate has been injected into cattle to overcome the toxic effects of molybdenum (3558).

e. Fish and Other Aquatic Life. Tarzwell and Henderson (2154), in an exploratory test with fathead minnow, found the 96-hour TL_m for molybdic anhydride (MoO_3) to be 70 mg/l in soft water (pH = 7.4, total alkalinity = 18, hardness = 20) and 370 mg/l in hard water (pH = 8.2, total alkalinity = 360, hardness = 400). Molybdenum appears to be essential for the growth of *Scenedesmus* (1602) and indeed the threshold concentration for deleterious effect upon this alga occurs at 54 mg/l (2158). *Escherichia coli* and *Daphnia* tolerate concentrations of 1000 mg/l without perceptible injury (2158). Several species of algae concentrate molybdenum from water by factor of 2 to 15 (2442).

MONOBUTYL BIPHENYL SODIUM MONOSULFONATE

MONOBUTYL PHENYLPHENOL SODIUM MONOSULFONATE

MONOBUTYL PHENYL SODIUM MONOSULFONATE

(see Chapter X)

MONOCHLORO PHENOL

(see Halogenated Phenols)

MONONITRO BENZENE

(see Nitrobenzene)

MONONITRO PHENOL

(see Nitrophenol)

MONONITRO TOLUENE

(see Nitrotoluene)

MUSTARD GAS $ClCH_2CH_2SCH_2CH_2Cl$

(see also Chemical Warfare Agents)

This oily liquid, a deadly vesicant with a weak, sweet, agreeable odor, is sparingly soluble in water (about 480 mg/l). Direct contact of eyes with the unhydrolyzed liquid may cause conjunctivitis or blindness, and serious effects are noted with the respiratory tract and skin. Ingestion of contaminated material may cause nausea and vomiting (364). When hydrolyzed in water, however, mustard gas becomes almost innocuous (697) and non-toxic to man (466). In rabbits, gastro-intestinal inflammation is caused by water containing 500 mg/l of mustard gas (i.e. some unhydrolyzed liquid) and at 650 mg/l the mortality is 33 percent. The skin of man has about the same threshold sensitivity as the gut of a rabbit (697).

In a mustard-gas concentration of 5 mg/l in tap water, guppies become sluggish, and at a concentration of 20 mg/l, guppies died within 15 minutes (698).

NACCONOL NRSF

NALCO 21

(see Chapter X)

NALOAM

(see Chapter IX)

NAPHTHA

(see Petroleum Benzin)

NAPHTHALENE $C_{10}H_8$

The most abundant single constituent of coal tar, naphthalene is a white solid with the odor of moth balls. It is soluble in water at 20°C to the extent of about 30 mg/l. The use of naphthalene in organic syntheses and dye manufacture is extensive, and hence it may occur in wastes from refineries, coal-tar plants, textile mills, and chemical industries.

Shelford (363) and Gutsell (463) report that 4 or 5 mg/l of naphthalene kills sunfish in one hour, but Demyanenko (431) and Ellis (313) set the lethal concentration at 10 mg/l. During an exposure of one hour, 17.1 mg/l did not kill minnows but it caused them to stop eating. A concentration of 4.3 mg/l had no effect (362). For perch, the killing strength has been given as 40 mg/l (365, 699) and as 20 mg/l (2920).

Le Clerc (2942) exposed minnows for 6 hours to naphthalene in distilled water and hard water. The minimum

lethal dose in distilled water at 19°C was 11-13 mg/l and in hard water at 16°C it was 15-18 mg/l. Using highly turbid water and the mosquito-fish *(Gambusia affinis)*, Wallen et al. (2940) found the TL_m values at 22-24°C to be 220 mg/l at 24 hours, 165 mg/l at 48 hours, and 150 mg/l at 96 hours. The naphthalene did not clarify the turbid water. In aerated seawater, the critical level for fingerling silver salmon during a 72-hour exposure was reported (2091) to be between 1.8 and 3.2 mg/l.

In a dilution of 1:2400, commercial naphtha solution made minnows stop eating after 15 minutes of exposure; but the effect was temporary because the naphtha evaporated quickly (362). A concentration of 1.0 mg/l influenced the taste of the flesh of bleak (2904).

NAPHTHALIC ACID $C_{10}H_6(COOH)_2$

Ellis (313) quotes references to the effect that naphthalic acid and other naphthalene derivatives from refinery wastes showed the following lethalities in tap water:

Species	*Lethal Concentration in mg/l*	*Killing Time*
percoid fish	3	6 to 12 hours
pickerel	5	36 to 48 hours
minnows	5	72 hours
carp	20	26 to 36 hours
goldfish	20	8 to 16 hours

NAPHTHOL $C_{10}H_7OH$

This hydroxynaphthalene is used in the manufacture of dyes, intermediates, perfumes, medicinals, and antioxidants for synthetic rubber. The α-naphthol is slightly soluble in water and has an oral LD_{50} in rabbits of 9000 mg/kg of body weight. The β-naphthol is soluble to the extent of about 1000 mg/l. Its oral LD_{50} for rabbits is given as 3800 mg/kg of body weight.

According to Nesmeyanova (3402) the odor threshold of naphthol in water occurs at 7 mg/l; but in chlorinated water at 0.5 mg/l Hoak (2982, 3403) gives the odor thresholds of α-naphthol as 0.455 mg/l at 60°C and 0.500 mg/l at 30°C. For β-naphthol the corresponding thresholds occur at 5.0 and 3.33 mg/l.

Bandt (2904) reports the following effects of naphthols upon fish and the taste of fish flesh:

Effect Upon Fish	*Concentration in mg/l*	
	α-Naphthol	*β-Naphthol*
Thresholds of toxicity for:		
bleak	2	2
bream	3	2
carp	4	2
Threshold of taste in flesh:		
bleak	0.5	-
bleak and carp	-	1

NAPHTHENIC ACID

(see Cyclohexanecarboxylic Acid)

NAPHTHOQUINONE $C_{10}H_6O_2$

(see also Methylnaphthoquinone and Dichloronaphthoquinone)

These oxygenated products of naphthalene occur as 1,2-naphthoquinone and 1,4-naphthoquinone. The former is highly soluble in water, but the latter only sparingly so (364).

In Lake Huron water at 12°C, 1,2-naphthoquinone at a concentration of 5 mg/l killed rainbow trout, bluegill sunfish, and sea lampreys in 2, 3, and 4 hours respectively while a concentration of 1.0 mg/l killed them in 3, 4, and 12 hours respectively. A concentration of 0.1 mg/l had no effect in 24 hours.

According to Fitzgerald et al. (1542), 1,4-naphthoquinone was toxic to fish in 24-48 hours at a concentration of 0.3 to 0.6 mg/l. This concentration was also toxic to blue-green algae.

NASCENT OXYGEN

(see Ozone and Dissolved Oxygen)

NATURAL GAS WELL WASTES

(see also Hydrocarbons, Specific Electrical Conductance, Mercaptans)

Ellis (611) reports that waste waters from natural-gas wells are detrimental to aquatic life owing to (a) osmotic action of brine water, inasmuch as a specific conductance of 4000×10^{-6} mhos at 25°C is approximately the upper limit tolerable by fish, (b) toxic action of calcium and magnesium salts, and (c) toxic action of hydrocarbons, mercaptans, and other organic compounds.

NEOTRAN

(see Chapter IX)

NICKEL Ni

1. General. Elemental nickel seldom occurs in nature, but nickel compounds are found in many ores and minerals. As a pure metal it is not a problem in water pollution because it is not affected by, or soluble in, water. Many nickel salts, however, are highly soluble in water and since they are used in metal-plating works they may be discharged to surface or ground waters.

Although metallic nickel does not merit serious consideration as a water pollutant, nickel ions may be detrimental to beneficial uses. Where concentrations mentioned in the literature are expressed as nickel, they are summarized below; but when nickel salts are described, the results are discussed under the appropriate salt.

2. Cross References. See also Nickel Acetate, Nickel Chloride, Nickel Nitrate, Nickel Sulfate, and Nickel Ammonium Sulfate.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The USPHS Drinking Water Standards (2036) do not place any limit on nickel but Kirkor (460) reports that in Russia the maximum permissible concentration is 1.0 mg/l. According to Sawyer and Brown (561) the existence of 0.09 mg/l of copper, 0.08 mg/l of cobalt and 0.03 mg/l of nickel in public water supplies containing 80 to 90 mg/l of total solids, 4.4 mg/l of chlorides and with a pH of 3.8 to 4.5 caused pitting of aluminum in the kettles. Without these traces, no pitting occurred.

No data on the toxicity of nickel to man were revealed, but the toxicity is believed to be very low (353). Systemic poisoning of human beings by nickel or nickel salts is almost unknown (2121).

b. Irrigation. According to literature cited by the Chilean Nitrate Educational Bureau (921), experiments

performed in 1899 upon horse beans and corn showed that the effect of nickel salts upon the growth of plants was very injurious no matter how small the quantity. This contention is shared by Wilcox (3559).

Nickel is extremely toxic to citrus plants. It is found in many soils in California, generally in insoluble form, but excessive acidification of such soil may render soluble, causing severe injury or death of plants (1445).

Nickel at a concentration of 0.5 mg/l was toxic to flax grown in water culture, but the symptoms were somewhat reduced in the presence of molybdenum (1453). Concentrations of 15.9-29.4 mg/l of nickel, in nutrient solution were injurious to sugar beets, tomatoes, potatoes, oats, and kale grown in sand culture (1473).

c. Stock and Wildlife Watering. An average daily dose of 2 mg of nickel did not harm rats but for dogs doses of 10 to 20 mg per kg of body weight proved lethal (353). Administered in the drinking water, 5 mg/l of nickel caused an increase in mortality among rats fed a ration containing 11 mg/l of selenium (1481). Nickel in the water supply of amphibia, reptiles, birds, and mammals hinders the growth of the liver (3560).

d. Fish and Other Aquatic Life. Nickel appears to be less toxic to fish and river crabs than copper, zinc, brass, and iron (575). For sticklebacks, however, the lethal limit has been reported as 0.8 mg/l of nickel (2941) (see also Nickel Salts). Kaemmerer and Ericksen found that nickel was more toxic to fish than iron and manganese (1468). They also found fish living in water polluted with mine effluent and containing 13-18 mg/l of nickel (1459).

The Mersey and Severn River Boards in England have established standards for industrial wastes that limit the concentration of nickel alone, or in combination with other heavy metals, to 1.0 mg/l (1756, 2950).

Nickel combines readily with cyanide to form a nickel-cyanide complex that is relatively stable. It can be present in water at concentrations greater than 100 mg/l as cyanide without harm to fish life if the water is moderately alkaline. In acid waters, however, the complex breaks down and releases HCN (2109, 2154, 2157, 2410, 2412, 2973, 3561, 3562, 3563)

NICKEL ACETATE $Ni(C_2H_3O_2)_2$

This green crystalline salt of nickel is highly soluble in water. Cats and dogs, fed daily with 4 to 12 mg of nickel acetate per kg of body weight were unaffected in 200 days (353).

NICKEL AMMONIUM SULFATE $Ni(NH_4)_2(SO_4)_2 \cdot 6H_2O$

This bluish-green crystalline salt, highly soluble in water, is used in electroplating baths. The threshold of deleterious effect toward *Daphnia magna,* according to Bringmann and Kuhn (2158, 3343) is 6 mg/l of nickel. Toward *Scenedesmus* the threshold of toxicity is 0.09 mg/l, toward *E. coli* 0.1 mg/l, and toward *Microregma* 0.07 mg/l as nickel.

A nickel concentration of about 27 mg/l from nickel ammonium sulfate caused a 50 percent reduction in the oxygen utilization from synthetic sewage (2949).

NICKEL CHLORIDE $NiCl_2 \cdot 6H_2O$

Occurring generally as the green crystalline solid, $NiCl_2 \cdot 6H_2O$, this salt is highly soluble in water and is used for nickel plating and the manufacture of sympathetic ink (364).

Cats and dogs that received 4 to 12 mg of $NiCl_2$ per kg of body weight per day for 200 days were unaffected (353).

The tolerance of fish toward $NiCl_2$ varies widely, depending on synergism, species, pH, and other factors. In soft water, goldfish survived a concentration of 100 mg/l of $NiCl_2 \cdot 6H_2O$ for 19 to 50 hours and 10 mg/l for 200 to 210 hours. Jones (2920) reports the lethal concentration of $NiCl_2 \cdot 6H_2O$ for goldfish to be 10 mg/l. In tap water, 8.1 mg/l $NiCl_2 \cdot 6H_2O$ killed the top minnow *Fundulus* in a few hours but the same species survived 259 mg/l of $NiCl_2 \cdot 6H_2O$ in salt water (313, 598, 1459). Toward the guppy, the median lethal concentration of $NiCl_2 \cdot 6H_2O$ was reported as 4.8 mg/l (2921). It has also been reported (1466) that nickel chloride at a concentration of 4.5 mg/l as nickel killed goldfish in 200 hours. Exploratory tests by Tarzwell and Henderson indicated that the 96-hour TL_m for fathead minnows was 4.0 mg/l in soft water and 24 mg/l in hard water, expressed as $NiCl_2 \cdot 6H_2O$.

Using water of the River Havel from which test organisms were taken, Bringmann and Kuhn (2158) found the threshold of toxic effects toward *Daphnia magna* to be 6.0 mg/l as nickel. Anderson (598) had previously determined the threshold concentration for immobilization of this organism in Lake Erie water at 25°C to be less than 0.7 mg/l in 64 hours of exposure. In similar studies, Bringmann and Kuhn (2158, 3343) set the threshold concentration for *Scenedesmus* at 1.5 mg/l, for *Escherichia coli* at 0.1 mg/l and for *Microregma* at 0.05 mg/l as nickel.

The biochemical oxidation of sewage was inhibited 50 percent by 15 mg/l of nickel added as nickel chloride (2922), by 9.4 mg/l of N: (2949) and by 12 to 16 mg/l of nickel (3411).

NICKEL NITRATE $Ni(NO_3)_2$

This salt of nickel occurs generally as the green deliquescent crystal $Ni(NO_3)_2 \cdot 6H_2O$ which is highly soluble in water. It is used for nickel plating and the manufacture of brown ceramic colors (364). According to Anderson (598), the threshold concentration for stickleback in tap water was 2.44 mg/l of $Ni(NO_3)_2$. Jones (608) gives the threshold concentration of nickel nitrate for toxicity to a flatworm *(Polycelis nigra)* as 45 mg/l measured as nickel.

The lethal concentration limit of nickel nitrate for sticklebacks was reported to be 0.8 mg/l of nickel. At higher concentrations, the survival times were as follows: one week at 1.0 mg/l, four days at 1.5 mg/l, and only one day at 10 mg/l, as nickel (1460). Jones (2920) indicates that the lethal concentration of nickel nitrate toward sticklebacks at 15-18°C is 1.0 mg/l as nickel.

A nickel concentration of about 13 mg/l from nickel nitrate caused a 50 percent reduction in the oxygen utilization from synthetic sewage (2949).

NICKEL SULFATE $NiSO_4 \cdot 6H_2O$

Occurring generally as a green crystalline salt, $NiSO_4 \cdot 6H_2O$, this compound is highly soluble in water. It has a sweet astringent taste and it is used in nickel plating, dyeing, and printing of fabrics (364). Applied in a culture solution it was slightly toxic to oat plants at a concentration of 1.0 mg/l as nickel. At 2.5-5.0 mg/l as nickel it was severely toxic and markedly reduced growth (1462).

The maximum concentration of nickel sulfate tolerated by young eels for an average of 31 hours was found to be about 16 mg/l. In a tap water solution of nickel sulfate, 50 mg/l (as nickel) killed sticklebacks in 49-60 hours. A rainbow trout was not affected by one mg/l of nickel in a 51-hour period of exposure (1459).

In water culture, 2.5 mg/l of NiO from nickel sulfate caused plants to wilt or die (2977).

North and Clendenning (2107) measured the effects of many heavy metals on the rate of photosynthesis by the giant kelp, *Macrocystis pyrifera*. They found that zinc sulfate showed no appreciable effect at 1.31 mg/l as zinc but gave a 50 percent reduction in photosynthesis in 4 days at 13.1 mg/l.

A nickel concentration of 3.6 mg/l from nickel sulfate caused a 50 percent reduction in the oxygen utilization from synthetic sewage (2949).

NICOTINE

(see Chapter IX)

NIGROSINE

(see also Dyes, Aniline, Weed Killers)

Nigrosine is an aniline dye that has been used to control weeds in fish-hatchery ponds. It tends to destroy the coarse weeds and to favor the growth of waterbloom. Apparently it is absorbed by plants and used up within a short period of time (700). At concentrations of 10 to 25 pounds per acre, used to control weeds, nigrosine does not harm fish or benthal organisms. *Daphnia magna* successfully survived 7.2 mg/l (about 20 lbs per acre feet of water) for five days. In a dirt-bottomed pond, 10 lbs of nigrosine applied weekly for four weeks caused benthos to increase, but zooplankton decreased.

NITRAMINE

(see Tetryl)

NITRATES NO_3^-

1. General. Nitrates are the end product of the aerobic stabilization of organic nitrogen, and as such they occur in polluted waters that have undergone self-purification or aerobic treatment processes. Nitrates also occur in percolating ground waters as a result of excessive application of fertilizer (1032) or leachings from cesspools. In a few instances, nitrates may be added to a stream or ground water by natural degradation or directly by inorganic industrial wastes, but such sources are relatively insignificant. Wastes from chemical fertilizer-producing plants (apart from fertilizers in the field) are an increasingly important source of nitrate pollution.

In spite of their many sources, nitrates are seldom abundant in natural surface waters, for they serve as an essential fertilizer for all types of plants, from phytoplankton to trees. Photosynthetic action is constantly utilizing nitrates and converting them to organic nitrogen in plant cells. In deep ground waters, however, this action is not possible and, consequently, it is in such waters that excessive and deleterious concentration of nitrates are often found.

2. Cross References. Ammonia, Albuminoid Ammonia, Nitric Acid, Nitrites, and Nitrogen.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Until 1962, the USPHS Drinking Water Standards (2036) did not have a requirement for nitrates. At that time, however, a recommended limit of 45 mg/l as nitrates was established. The WHO European Drinking Water Standards (2329) have a recommended limit of 50 mg/l as nitrates. No limit has been set for the WHO International Standards (2328). These limits were established because of the relationship between high nitrates (or nitrites) in water and infant methemoglobinemia. No cases of such poisoning have been evident in the U.S. when the water consistently contained less than 45 mg/l of nitrate as NO_3 or 10 mg/l as nitrogen.

Infant methemoglobinemia, a disease characterized by certain specific blood changes and cyanosis, may be caused by high nitrate concentrations in the water used for preparing feeding formulae. Since the disease often does not occur even where the water is very high in nitrate content, however, it seems likely that not all infants are susceptible to nitrate poisoning, but that some are predisposed to it by physiological conditions such as low gastric acidity or other unknown factors. Many well waters containing over 500 mg/l of nitrate nitrogen have never been linked with reported cases. Most cases, however, have been associated with the use of water containing 50 mg/l or more of nitrate nitrogen. While it is still impossible to state precise concentration limits, it has been widely recommended that water containing more than 10 mg/l of nitrate nitrogen should not be used for infants. The references dealing with nitrates and methemoglobinemia are far too numerous to discuss in detail in this report; however, the following were reviewed: (36, 152, 353, 630, 944, 1273-1283 incl., 1611-1617 incl., 2028, 3530, 3564, 3565, 3566, 3567, 3568, 3569, 3570, 3571, 3572, 3573).

Nitrates are rated among the poisonous ingredients of mineralized waters, with potassium nitrate being more poisonous than sodium nitrate (284). Excess nitrates cause irritation of the mucous linings of the gastrointestinal tract and bladder, with symptoms of diarrhea and diuresis. Drinking one liter of water containing 500 mg/l of nitrate can cause such symptoms (629).

Studies of nitrate-bearing water, both artificial and authentic, seeded with coliform bacteria indicated that when the nitrate-nitrogen concentration was 100 mg/l or higher, the standard presumptive tests for the presence of coliforms gave unreliable and ambiguous results (1618, 1619).

b. Industrial Water Supplies. Nitrates are injurious to the dyeing of wool and silk fabrics (261). Nitrates are

harmful to fermentation processes and cause disagreeable tastes in beer (165, 167, 240, 1094, 2347). For brewing, the nitrate concentration of water should be less than 30 mg/l (170), or less than 15-20 mg/l (3574). In the brewing industry, according to Bonnet (2344), nitrates are partially reduced to nitrites during fermentation; hence they are poisonous toward yeast. They also give a bad taste to the beer when present in large amounts. Nitrates and free nitric acid at concentrations of 15 to 30 mg/l render water harmful for various industrial purposes (855). On the other hand, two mg/l of nitrate in some water protects metal in boilers from intercrystalline cracking (1284).

c. Irrigation. Excess nitrate tends to reduce soil permeability. Nitrates may sometimes accumulate to toxic concentrations in the soil solution, but their effect is usually osmotic (246). In general, nitrates are desirable for their fertilizing value.

d. Stock and Wildlife Watering. Outbreaks of nitrate poisoning (methemoglobinemia) have been reported among cattle following their consumption of hay containing 30,000 to 70,000 mg/l of potassium nitrate (1049), although fodder containing 20,000 mg/kg was without effect. Campbell et al. (3575) report methemoglobinemia among cattle that received water containing 2790 mg/l of nitrate.

Nitrates in the drinking water of rats and livestock have been harmful in lower concentrations than mixtures of chlorides and sulfates of the alkaline metals (286, 641). Where "duck disease" occurs, the soil contains a high concentration of nitrates. Oral feeding to ducks of mixtures of salts with nitrates has resulted in duck poisoning (1285).

Nitrate in water and food consistently inhibited dental decay among a group of experimental rats (1069).

Special attention should be paid to the concentration of nitrates in stock waters, especially when the total salt concentration exceeds 570 to 1000 mg/l (284).

Although the drinking water administered to two cows over a 10-day period contained 114 mg/l of nitrate nitrogen, no positive test for nitrates could be demonstrated in the milk (1277).

e. Fish and Other Aquatic Life. High nitrate concentrations in effluents, and water stimulate the growth of plankton and aquatic weeds (380, 584, 1286, 1287, 1288). By increasing plankton growth and the development of fish food organisms, nitrates indirectly foster increased fish production (1288).

Hart et al. report references to the effect that among United States waters supporting a good fish life, ordinarily 5 percent have less than 0.2 mg/l of nitrates; 50 percent have less than 0.9 mg/l; and 95 percent have less than 4.2 mg/l (310).

The toxic threshold concentration of nitrate, as sodium nitrate, for the flatworm *Polycelis nigra* is reported to be 2670 mg/l (354).

NITRIC ACID HNO_3

1. General. One of the most-widely used acids in industrial processes, nitric acid is a clear, colorless liquid that is very hygroscopic and miscible with water. It dissociates completely into hydrogen ions and nitrate ions with a resulting tendency to lower the pH value. Its principal deleterious effect, therefore, is its acid reaction. For further information about the effects of acids, see Acidity and pH; and for further data pertaining to the nitrate ions, see Nitrates.

2. Cross References. See also Nitrates, Acidity, pH.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Rudolf has reported that free nitric acid and nitrates in concentrations of 15 to 30 mg/l render water harmful or dangerous for domestic, drinking, and industrial purposes (855).

b. Industrial Water Supplies. Nitric and nitrous acids in high concentrations are injurious to the dyeing of wool and silk fabrics; they not only color the fabrics yellow, but may also react with some of the dyes (261).

c. Fish and Other Aquatic Life. The threshold concentration of nitric acid for the immobilization of *Daphnia magna* in Lake Erie water at 25°C is reported to be 107 mg/l (358).

Doudoroff and Katz (361) cite references to the effect that nitric, as well as other strong acids, are directly lethal to fully developed fish only when they reduce the pH to 5.0 or less. The lethal threshold concentration of nitric acid for stickleback is reported to be 0.01 mg/l of hydrogen ion (353), i.e. a pH value of 5.0. The following concentrations of nitric acid have been cited as toxic or lethal to fish:

Concentration in mg/l	*Resulting pH*	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
1.6	--	--	--	Trout	353
1.56-20	--	--	--	Fish	359
15.6	--	--	24 hours	Trout	359
72	6.2	Turbid	96-hr TL_m	Mosquito-fish	2940
98.5 (threshold)	--	--	--	Pickerel	353
113 (threshold)	--	--	--	Whitefish	353
200	--	--	--	Minnows, goldfish	359
750	3.4	Hard	½-1 hr.	Goldfish	313, 353
1000	--	Tap	½ hr.	Trout	313
1000	4.4	--	7 hrs.	Minnows	313

In contrast, the following concentrations of nitric acid have not been harmful to fish within the time specified:

Concentration in mg/l	*Resulting pH*	*Time of Exposure*	*Type of Fish*	*Reference*
5.75	5.2	--	Minnows	313
5.75	--	--	Shiners	359
5.75-20	--	--	Fish	359
20	--	--	Carp, goldfish, suckers	359
200	--	>100 hrs.	Goldfish	313
200	4.9	>4 days	Goldfish	35

NITRILES

(see Acetonitrile, Acrylonitrile, Adiponitrile, Benzonitrile, Cyanides, Lactonitrile, Oxydipropionitrile).

NITRITES NO_2^-

1. General. In water, nitrites are generally formed by the action of bacteria upon ammonia and organic nitrogen. Owing to the fact that they are quickly oxidized to nitrates, they are seldom present in surface waters in significant concentrations. In conjunction with ammonia and nitrate, nitrites in water are often indicative of pollution. However, the presence of nitrite does not always signify pollution (155).

2. Cross References. See also Nitrates, Nitrogen, Ammonia.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Nitrites are poisonous compounds, but in the minute amounts ordinarily found in drinking waters they can scarcely have a pharmacological effect (629, 630). Old Swiss standards for drinking and mineral waters required the complete absence of nitrites (944). A generally accepted limit for nitrites in domestic water supplies is 2 mg/l (170), however, another reference states that the ammonia and nitrite content of drinking water should not exceed 0.1 mg/l (855). In southern Bavaria, 70 poisonings including 18 deaths have been attributed to the use of nitrites in the foodstuff industry (3576).

b. Industrial Water Supplies. Nitrite is undesirable in waters for use in brewing (1094, 2344), and in the dyeing of wool and silk fabrics (261). Nitrite-containing waters are objectionable in brewing because (a) they show the presence of organic matter in decomposition, (b) the preservation of beer is poor, (c) nitrites are poisons for the yeast, and (d) they are not suitable for malting as they cause an invasion of the malt by molds and bacteria.

c. Irrigation. Nitrite usually occurs in very low concentrations in irrigation water and is of little significance (268).

d. Fish and Other Aquatic Life. Nitrite and nitrate stimulate the growth of plankton in reservoirs (1287). For further details, see Nitrogen and Nitrates. Tests with minnows showed that a concentration of 50 mg/l of sodium nitrite could be fatal in 14 days, while 10,000 mg/l destroyed the minnows in 1.5 hours (3577). The snail hosts of schistosomes *Planorbis glabratus* and *Bulinus contortus* were killed in 24 hours by 10,000 mg/l of sodium nitrite or potassium nitrite (3578).

NITROANILINE $C_6H_4NH_2NO_2$

(see also Dyes, Analine)

This compound exists with the NO_2 group in the meta-, ortho-, or para-position. They are all used as dyestuff intermediates. Meta- and paranitroaniline are moderately soluble in water but the ortho-form is only slightly soluble. Bringmann and Kuhn (2158) found the threshold of harmful effect toward *Daphnia* occurred at 24 mg/l and toward *Scenedesmus* at 20 mg/l. A concentration of 100 mg/l had no effect on *E. coli* at 27°C.

NITROBENZENE $C_6H_5NO_2$

(see also Dinitrobenzene and Trinitrobenzene)

Moderately soluble in water, this oily liquid with an almond odor is used in the manufacture of analine, soaps, and shoe polishes. The oral LD_{50} for rabbits is given as 700 mg/kg of body weight (364). The approximate concentration in water that results in a faint odor has been reported as 0.03 mg/l (1756).

Hiatt et al. (3350) indicate that 10 mg/l of nitrobenzene in sea water produced a slight irritant activity in marine fish. According to LeClerc (2942), the minimum lethal dose for minnows during a 6-hour exposure in distilled water at 22°C was 20-24 mg/l, and in hard water 90-100 mg/l.

Toward lower aquatic life, Bringmann and Kuhn (2158, 3343) found the thresholds of toxicity to be 28 mg/l for *Daphnia*, 30 mg/l for *Microregma*, 40 mg/l for *Scenedesmus*, but 600 mg/l for *Escherichia coli*. Herman (2923) reported that 630 mg/l caused a 50 percent decrease in oxygen utilization in the BOD test.

NITROCHLOROBENZENE $C_6H_4ClNO_2$

This yellow crystalline compound is insoluble in water in the o- and p-isomers. It is used in dye chemistry (364). Orthonitro-chlorobenzene has been detected in river waters and has been observed to persist for many weeks (3102, 3356).

NITROGEN N

(see also Albuminoid Ammonia, Ammonia, Cyanides, Nitrates, Nitriles, Nitrites, Nitrogen Gas, Organic nitrogen)

Nitrogen occurs abundantly in nature, constituting 78.06 percent by volume of the atmosphere. It is also an essential constituent of protein in all living organisms, and it is present in many mineral deposits as nitrates (364). In organic matter it undergoes changes of decomposition from complex proteins through amino acids to ammonia, nitrites, and nitrates; and also changes of synthesis from nitrates into plant and animal forms. This so-called nitrogen cycle in nature is dependent upon bacterial action for decomposition and upon photosynthesis for reconstitution of organic matter. In natural or polluted waters, nitrogen may be present in many forms, but the ones that are measured by the common tests include ammonia, albuminoid ammonia, organic nitrogen, nitrites, and nitrates (469). Results of investigations that are expressed in terms of a particular form of nitrogen are presented elsewhere in this chapter under the appropriate term. The references described below deal with the term "nitrogen" without reference to the form. In some instances total nitrogen is involved.

In water suitable for most beneficial uses, the total concentration of nitrogen compounds should be less than 10 mg/l according to an anonymous German reference (632). This same limit has been recommended for critical low flows in the Miami River from Dayton to its mouth (929). For irrigation purposes, however, nitrogen in any form is seldom deleterious; in fact, it is usually beneficial, for nitrogen compounds form the basis of most organic and chemical fertilizers (1289, 1290, 1291, 1292).

Toward algae, crustacea, and other fish-food organisms, the total concentration of nitrogen is not as important as the form in which it exists. Organic nitrogen, amino acids, and ammonia may inhibit biological growth whereas nitrates stimulate phytoplankton (1293) (see also Ammonia). Some green plants apparently do not need to depend upon complete nitrification but can utilize nitrogen from ammonia or nitrites (1294). Extensive studies have been made of the effects of nitrogen and phosphorus in three Wisconsin lakes upon the nuisances from odors caused by algal "blooms" (375, 377, 1295). According to Sawyer (374), the critical concentration of nitrogen, below which algal growths were not troublesome, was 0.30 mg/l provided that phosphorus was kept below 0.015 mg/l. Fish production is highest in ponds

and streams containing the most organic nitrogen (1296).

Sawyer et al. (1450) reported that for some algae, the optimum nitrogen: phosphorus ratio appeared to be about 30 to 1; for other algae, ratios of 15-18:1 were evident. The presence of 0.01 mg/l of phosphorus and 0.30 mg/l of inorganic nitrogen in ponds or lakes at the time of the spring overturn would probably foster the production of nuisance blooms. Gerloff and Skoog (2209, 2210, 2211) suggest that in many instances nitrogen, rather than phosphorus, may be the limiting element in growth of algae. Imhoff (2170) and Mueller (3579, 3580) point out that enormous growth of plants in streams and lakes does not occur if the nitrate as N is kept below 0.3 mg/l and the total nitrogen as N is below 0.6 mg/l.

NITROGEN GAS

Natural waters in equilibrium with air contain dissolved nitrogen gas as well as dissolved oxygen in accordance with Henry's Law. Although nitrogen is considerably less soluble than oxygen, it constitutes about 78 percent by volume of normal air, and hence water in equilibrium with air at 20°C will contain about 15.6 mg/l of dissolved nitrogen gas (911). Unlike oxygen, however, dissolved nitrogen is inert and it is not consumed in biological stabilization; consequently, the dissolved-nitrogen-gas content of surface waters is not likely to fluctuate other than in accordance with temperature changes.

No literature was uncovered to describe the effects of dissolved nitrogen gas on beneficial uses of water other than for fish and aquatic life. Under normal conditions of saturation, no detrimental effects toward fish are anticipated, and extreme supersaturation or undersaturation is not likely to occur in surface waters. When nitrogen gas occurs in excess of saturation, gas bubbles may form on rough and warm surfaces, producing gas-bubble disease in fishes (3553). It has been reported (3581) that supersaturation of nitrogen above 130 percent may be fatal to fish.

NITROGEN TRICHLORIDE NCl_3

(see also Chlorine and Chloramines)

Known also as agene, this compound may be produced in waters treated by breakpoint chlorination, if ammonia or nitrogenous organic compounds are present and if the pH is below 5.0. It may cause unpleasant odors in treated waters (1620, 1621).

NITROPHENOL $C_6H_4OHNO_2$

Metanitrophenol is highly soluble in cold water, p-nitrophenol moderately so, and o-nitrophenol only sparingly soluble. The ortho isomer is used in chemical manufacturing (364). According to Le Clerc et al. (1560, 2942) the minimum lethal doses toward minnows during a 6-hour exposure at 22°C in distilled or hard water were as follows:

Isomer	Minimum Lethal Dose in mg/l: In Distilled Water	In Hard Water
o-nitrophenol	14-18	125-130
m-nitrophenol	9-10	20-22
p-nitrophenol	4-6	30-33

Lammering and Burbank (3582) used bluegill sunfish and standard bioassay procedures at 20°C to determine the effects of o-nitrophenol. They found the 24-hour TL_m to be 66.9 mg/l and the 48-hour TL_m to be 46.3 to 51.6 mg/l.

Toward lower organisms in River Havel water, Bringmann and Kuhn (2158, 3343) found the following thresholds of toxic effect:

Organism	Temp.°C	Concentration of Nitrophenol in mg/l: Ortho-	meta-	para-
Daphnia	23	60	24	14
Scenedesmus	24	36	28	72
Microregma	—	40	20	20
Escherichia coli	27	>1000	300	100

Applegate et al. (3491) tested the effects of several halogenated nitrophenols toward the larvae of sea lamprey and the adult form of three fresh water fishes. Results were as follows:

Compound	Concentration in mg/l to kill all sea lamprey larvae	Concentration in mg/l to cause 10 percent fish kill: Rainbow Trout	Brown Trout	Bluegill Sunfish
2-bromo-4-nitrophenol	5	13	11	--
3-bromo-4-nitrophenol	5	11	--	15
5-chloro-2-nitrophenol	3	5	5	--
2, 5-dichloro-4-nitrophenol	3	13	--	--
3, 4, 6-trichloro-2-nitrophenol	5	17	15	--
3-trifluoromethyl-4-nitrophenol	2	9	7	--

NITROTOLUENE $NO_2C_6H_4CH_3$

(see also Trinitrotoluene)

Metanitrotoluene is moderately soluble in water but o- and p-nitrotoluene are almost insoluble. These compounds are used in the manufacture of dyes and other chemicals (364). According to Le Clerc (2942) the minimum concentrations producing death to minnows in 6 hours at 23°C in distilled water or at 21-22°C in hard water were as follows:

Isomer	Minimum Lethal Concentration, mg/l: in distilled water	in hard water
o-nitrotoluene	18-20	35-40
m-nitrotoluene	14-18	25-30
p-nitrotoluene	20-22	45-50

NONYLPHENOL ETHYLENE OXIDE CONCENTRATE (Lissapol)

NOPALCO

NOPCO 2272-R

NOPCOGEN 14-L

(see Chapter X)

OCTADECENYL DIMETHYL ETHYL AMMONIUM BROMIDE

(see Chapter X)

OCTOPHENOL

(see Chapter X)

OCTYL ALCOHOLS $C_8H_{17}OH$

Octyl alcohol is a generic term that includes a number of isomeric alcohols of which 2-ethyl hexanol and n-octyl

alcohol are industrially the most common. The octyl alcohols are colorless liquids, generally with a penetrating aromatic odor. They are used in the manufacture of perfumes and esters, in mercerizing textiles, and as a solvent for dyes, resins, and oils. Ellis (313) reports that among 23 octyl alcohols, the least toxic produced paralysis in goldfish in four hours at 200 mg/l. Primary octyl alcohol produced paralysis in 15 minutes at a concentration of 67 mg/l. A concentration of 5 mg/l had no effect on the sea lamprey during a 24-hour exposure in Lake Huron water at 12°C (2976).

N-OCTYL SODIUM SULFATE

(see Chapter X)

ODOR

(see also Tastes)

Disagreeable odors and tastes in water are associated with the presence of any of a great variety of objectionable substances, particularly living microscopic organisms or decaying vegetation, including weeds, bacteria, fungi, actinomycetes, and algae; decaying organic matter; sewage; and industrial-waste products. Problems of odor and taste are very complex because the senses of smell and taste are intimately related and their responses are often difficult to differentiate clearly. In addition, it is frequently difficult, if not impossible, to identify the specific cause of an odor or taste, for many substances can cause what appears to be the same effect, or because mixtures of substances may be involved.

It would be impractical to attempt to make a complete list of all the types of industry whose waste products are potentially odoriferous in water supplies, but among them are the following: pulp and paper; explosives; petroleum, gasoline, and rubber; wood distillation; coke and coal tar; gas; tanneries, meat-packing and glue; chemicals and dyes; and milk products, canneries, beet-sugar, distillation and other food products. Among the chemicals responsible for tastes and odors are halogens, sulfides, ammonia, turpentine, phenols and cresols, picrates, various hydrocarbons and unsaturated organic compounds, mercaptans, tar and tar oils, detergents, pesticides, and innumerable others, many of unknown identity. For odor and taste threshold concentrations of specific substances, see each potential pollutant in this chapter and also Chapters VII, IX, and X. Odor thresholds of many compounds have been tabulated by Hoak (2982, 3403), Nesmeyanova (3402), Baker (2058), and Filicky et al. (2040).

Odorous substances in water must be vaporizable in order to be smelled. While such substances may often also be tasted, the sense of smell is generally much more acute than that of taste (759) and in water-works practice the separation of odor and taste is rarely made. Standard Methods for the Examination of Water and Wastewater (469) does not prescribe a procedure for taste determination. For a discussion of the complexities of the odor-taste differentiation problem, see the paper by Rohlich and Sarles (759).

Odors can be caused by volatile substances in concentrations too small to be detected by ordinary analytical techniques; therefore, reliance must be placed upon the sense of smell, in spite of its subjectivity and lack of precision (3584). Methods devised for determining the presence of odor in either cold or hot water are described in Standard Methods (469). Results are reported in terms of "threshold odor numbers" (TON) calculated from the amount of sample in the most diluted portion giving perceptible odor. The threshold odor number equals the volume of the dilution divided by the volume of the sample in the dilution. The 1962 Drinking Water Standards of the USPHS (2036) indicate that the threshold odor number should not exceed 3. In the literature on odor and taste in water are also found many references to the "threshold concentrations" of odor and taste-producing substances, i.e. the lowest concentrations, in mg/l giving perceptible odor and/or taste. The variety of odors possible is almost numberless, and although many descriptive terms have been assembled (16, 469, 759) they are still considered to be inadequate.

Knowledge about the sources of odors in water was enhanced considerably by the development at the R. A. Taft Sanitary Engineering Center of the carbon-filter technique for separating and identifying organic components in dilute concentrations (1622, 1623, 2983, 3004, 3102, 3354, 3355, 3356, 3357, 3358, 3583, 3585, 3586, 3587, 3588, 3589, 3590, 3591). Studies of numerous surface waters indicate that hydrocarbons are a major source of taste and odors. Among the hydrocarbons, the neutral fractions have the highest threshold odor numbers. For more detail, see Carbon Chloroform Extract and Oils, Petroleum, or the original references.

Odor and taste problems may be aggravated by the impounding of water (962, 2314) or by the sedimentation of suspended solids, resulting in the trapping of decaying matter on lake or stream bottoms (780). In addition, chlorination of waters polluted by even very low concentrations of TNT wastes, algae and protozoa, detergents, or phenolic or cresolic compounds may result in the production of foul odors and tastes. On the other hand, chlorination may reduce the odors and tastes caused by some wastes, such as Kraft mill wastes, sulfides, mercaptans, and turpentine (686). Treatment methods for combating odors and taste vary in effectiveness with the substances involved and the composition of the water.

Waters free from odor and taste nuisance are desirable for drinking and domestic use, industry, irrigation, fish production and fishing, shellfish culture, and recreation (345, 964, 965). Odors and tastes are particularly undesirable in waters for drinking and domestic purposes, beverages, dairying, distilling, brewing, and other food-processing industries (173).

Offensive odors and tastes may be imparted to fish and shellfish from polluted waters by algae, petroleum products, turpentine, resins and resin acids, vegetable and mineral oils, insecticides, wastes from synthetic rubber and explosives factories, petroleum refineries, phenols, and cresols. Phenol in concentrations of 15 mg/l (431) to 25 mg/l (935) and cresols in concentrations of 10 mg/l have rendered fish unpalatable.

Many state and interstate water-pollution-control agencies have included references to odors and taste in their standards, but such requirements are qualitative

and not quantitative. For details and exact statements, see Chapter III and its appendices.

OIL, PETROLEUM

1. General. A comprehensive report on "Oily Substances and Their Effects on the Beneficial Uses of Water" was published in 1956 by the California State Water Pollution Control Board (3596) as Publication No. 16. Much of the following material appears in that reference, but for a thorough analysis of oils in relation to beneficial uses the reader should consult the full publication.

Potential contributors to oil pollution are all agencies engaged in production, transportation, handling, and use of oil, such as ships, oil wells, oil-loading points, refineries, railroads, civic dumps, salvage dumps, garages, and industries (981). Garland (982) lists the principal sources of oil pollution of seas and harbors as (a) the discharge of oil-contaminated ballast and bilge, (b) cleaning and flushing of oil tanks at sea, and (c) accidents to oil lines in fueling. The average oil content of such wastes is 2 to 3 percent and the average ship contains 1000 to 2000 tons of such material.

The British Minister of Transport appointed a committee to consider and report on practical measures to prevent oil pollution of seas (1624). The report indicated that fouling of seas, hundreds of miles offshore, damages flora and fauna of the coast line and open ocean. There is evidence that the oils causing the worst pollution are very stable. There may be very slow oxidation, so slow as almost to defy detection, but the oils keep floating almost indefinitely. In experimental tanks, oil on water floated for nearly 18 months until the water had evaporated. Experiments on the behavior and drift of oil discharged on the sea indicated that 15 tons of fuel oil covered an area of 8 square miles six days after discharge and drifted about 20 miles in an eight-day period. Wind and tide influence the drift.

Sources of pollution include unburned fuel oil escaping through funnels of ships as well as crude oil residues, leakage, breakage, and the discharge from ballast tanks. Damage caused by oil may involve (a) spoiling of beaches and coastal resorts, (b) destruction and injury of sea birds, (c) fouling of boats, fishing gear, and quays, (d) the risk of fire in harbors and other enclosed areas, and (e) damage to marine flora and fauna. The disappearance of eel-grass is believed by some to be a result of such pollution. Large areas of eel-grass provided special habitat for fish and food for waterfowl; the long fronds were effective as breakwaters. Where large areas of eel-grass have disappeared there has been a great decrease in molluscs and crustaceans, wild fowl have moved elsewhere, and coastline erosion has often followed (1624).

The Oil Pollution Act of 1924 of the U. S. Congress forbids the discharge or dumping of oil by any means into navigable coastal waters in which the tide ebbs and flows (614). Oil may also reach inland surface waters from industrial processes such as machining operations (983) and from garages and filling stations.

Oil films may interfere with gas exchange, coat bodies of birds and fish, impart a taste to fish flesh, exert a direct toxic action on some organisms as a result of water-soluble components, and interfere with fish-food organisms and the natural food cycle (614, 1624, 3535, 3592, 3593, 3594, 3595). Oil from surface films becomes adsorbed on clay particles, settles to the bottom, and there remains a source of pollution, for it may be stirred up to float again or may leach toxic principles (614). Experiments in England (2001, 2518) have shown that films of oil less than 10^{-4} cm thick do not affect the rate of solution of oxygen.

The American Petroleum Institute (984) investigated the spreading of oil on water surfaces and presented the following tabulation of thicknesses and descriptions.

Gallons of Oil Per Square Mile of Surface	*Approximate Film Thickness, Inches*	*Appearance*
25	1.5×10^{-6}	Barely visible under most favorable light conditions
50	3.0×10^{-6}	Visible as a silvery sheen on surface of water
100	6.0×10^{-6}	First trace of color may be observed
200	12.0×10^{-6}	Bright bands of color are visible
666	40.0×10^{-6}	Colors begin to turn dull
1332	80.0×10^{-6}	Colors are much darker

Films up to 3.0×10^{-6} inches do not persist for more than five hours on agitated water surfaces. A slug of oil at sea required 40 to 100 hours to thin out to 40×10^{-6} inches but thereafter disappeared entirely in less than 24 hours. The API reports that oil may be discharged uniformly at a rate of 10 gallons per hour per square mile without becoming visible, but a rate of 28 gallons per hour per square mile would result in a continuous iridescent film (984) (see also Hydrocarbons).

In well waters, as little as 10^{-6} mg/l of oil has been detected by a fluorescence test (3597).

2. Cross References. Animal and Vegetable Oils, Hydrocarbons, Tars, Tastes and Odors, Chapter IX.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supply. The potential deleterious effects of oily substances upon domestic water supplies may be grouped into the following categories: (a) hazards to the health of consumers, (b) the production of tastes and odors, (c) the presence of turbidity, films, or iridescence and (d) the increased difficulty of water treatment. The first two categories are discussed in detail in the following sections of the report. The third category is one involving esthetic problems that are unlikely to arise inasmuch as the taste and odor of oily wastes become objectionable at much lower concentrations of oil than those required to produce turbidity, iridescence, or films (3596).

The fourth category is an outgrowth of the second and third. Floating or emulsified oil in raw-water supplies will complicate the coagulation, flocculation, and sedimentation processes at a treatment plant. Oil-coated floc may not settle properly. If free or emulsified oil reaches sand filters or ion-exchange beds it will coat the grains, decrease the effectiveness of filtration, and interfere with backwashing. Taste- and odor-producing compounds will require the greater use of activated carbon or heavy

chlorination. Again, however, the taste-and-odor factor will control the threshold or limiting concentration of oily material acceptable in a domestic water supply (3596).

An exhaustive search of literature to determine the toxic concentrations of the components of refinery waste waters toward humans revealed a paucity of toxicological data on the ingestion of such substances by humans or by test animals (2980). From the approximate order of magnitude based on data from the fields of occupational health and industrial hygiene, it was concluded that any tolerable health concentrations for oily substances far exceed the limits of taste and odor. It appears, therefore, that hazards to human health will not arise from drinking oil-polluted waters, for they will become esthetically objectionable at concentrations far below the chronic toxicity level (2980).

The principal detriment of petroleum oils toward domestic water supplies lies in their tastes and odors. This effect has been reported as the threshold odor concentration (TOC) in mg/l and as the threshold odor number (TON), the dilution ratio at which odor is just detectable (see Tastes and Odors). Kirkor (460) reports the TOC of refined petroleum in water to be 1.0 to 2.0 mg/l, and for raw petroleum 0.1 to 0.5 mg/l. According to Halstrick (952) mineral oil imparts a taste to water at a concentration of 5.0 mg/l and an odor at 2.5 mg/l. The odor of gasoline is more easily detected than that of crude oil, but the latter is tasted more quickly. Aging causes petroleum odors to become musty, probably as a result of biochemical decomposition. Cohen et al. (2984) give the TOC of deodorized kerosene as 0.082 mg/l. Melpolder et al. (3589) report the TOC of commercial gasoline in water as 0.005 mg/l, with 0.010 mg/l giving a strong odor.

By far the most comprehensive work in relation to the odors of oily substances has been that of Ruchhoft, Middleton, Ettinger, Braus, and Rosen at the R. A. Taft Sanitary Engineering Center in Cincinnati (459, 1469, 2983, 3353, 3354, 3358, 3585, 3586, 3588, 3591). These investigators developed the carbon-filter techniques to remove minute concentrations of odor-producing substances from surface waters and liquid wastes.The materials adsorbed on the carbon filters are eluted with an organic solvent and then separated into acidic, basic, amphoteric, phenolic, neutral, and water-soluble groups. The neutral group consists primarily of the aromatic and aliphatic hydrocarbons, and closely related compounds that do not form salts with acids or bases. The acidic group comprises a mixture of organic acids including fatty acids; and the basic group is derived largely from nitrogen bases such as the pyridines.

Numerous tests of effluents from refineries and of river water and lake water known to be polluted by oily substances by Ruchhoft and his colleagues have shown that the neutral fractions are the most odoriferous, with TOC values as low as 0.003 mg/l.

Other investigators have reported the odor of oily substances in terms of the threshold odor number (TON), or the dilution ratio at which odor is just detectable. Gibbons (953) tabulated the dilutions required to reach the threshold odor for various petroleum products as follows:

Petroleum Product	*Threshold Odor Number (Dilution Ratio)*	
	Hot (65°C)	*Cold (20°C)*
Gasoline	8,000,000	5,000,000
No. 1 heating oil	4,000,000	1,500,000
No. 2 heating oil	12,000,000	3,000,000
No. 4 heating oil	10,000,000	2,000,000
Bunker C fuel oil	7,600,000	2,000,000
20 W lubricating oil	80,000	40,000

Mueller (1739) notes that some persons can detect gasoline in water at a TON of 1,100,000, most people notice it at 500,000, and it is quite evident at 100,000. Gibbons (953) recorded a TON of 5,000,000 for gasoline while Halstrick (952) gave a TON value of 400,000 for petroleum oil. German authors (1739, 3598, 3599, 3600, 3601) report on the pollution of ground water and its effect on the odor of drinking-water supplies. TON values as high as 5,000,000 were indicated. In one of these cases (1739), the gasoline contained lead, but in only one sample of ground water was the lead concentration as great as 0.5 mg/l and in other samples it was 0.1 mg/l or less. No danger of lead poisoning was present.

Coincident with the appearance of gasoline in wells, nitrates could no longer be determined, and only traces of nitrites were found. As the gasoline odor diminished, nitrates gradually increased to their normal value. Gasoline in the soil appears to be oxidized first by atmospheric oxygen and then by means of nitrates with the aid of catalytic or biological processes. When the groundwater table is lowered, the lighter gasoline were absorbed by the soil, but they became evident again when the water table rose.

b. Industrial Water Supplies. In steam production, the presence of oil in boiler feed water may cause foaming, priming, overheating of tubes resulting in blistering or failure, and poor transmission of heat from the metal to the water. As a result, the American Boiler Manufacturers Association in its standard guarantee on steam purity specifies that "the total quantity of oil or grease, or substance which is extractable either by sulfuric ether or by chloroform, shall not exceed 7 ppm in the boiler water when the sample being tested is acidified to 1 percent hydrochloric acid, or 7 ppm in the feed water when the sample being tested is first concentrated at low temperature and pressure to the same ppm total solids as the boiler water."

Powell (212) recommends that "No oil should be present in boiler feedwater. Serious damage has resulted from only a few parts per million of oil in feedwater fed to high-pressure boilers. When oil is present in the feedwater, it forms deposits on the boiler tube surfaces, preventing proper heat transfer, thus causing overheating and final failure of the metal."

Cooling waters may be used once and discarded, but the trend is toward water conservation by recirculation through cooling towers. In this process, evaporation will cause impurities in the water to become more concentrated. Moreover, aeration over huge surfaces favors biological action so that cooling waters tend to become slimy, especially when organic pollutants and nutrient materials are present. Oily substances in cooling water may possibly retard corrosion of metal in some instances, but their disadvantages in cooling waters outweigh this possible advantage. Powell (212) reports that "waters

contaminated by oil and grease are highly objectionable when used as cooling water for heat exchangers, surface condensers, and similar types of equipment."

Oil in water used for hydraulic transport or buoyancy may coat the product being transported (e.g. fruit or vegetables in a cannery) or the containing vessels or flumes. Biological slimes may result from presence of such oily substances in flowing waters.

For many processing and production operations, the major deleterious effect of oily substances is the possibility of taste and odor in the manufactured product. This is especially true for brewing, carbonated beverages, bakeries, food canning and freezing, ice manufacture, sugar production, and food-equipment washing. While the literature dealing with food and beverage processes does not specify numerical concentrations for oil, the recommended threshold or limiting values for oil, taste and odor, or organic matter are given as "none," "low," "infinitesimal," or "barely noticeable."

Oil is detrimental in water used for paper making in that it may adhere to the stock, form slick spots on the paper, or promote the growth of undesirable organisms. The Portland Cement Association (191) states that water used for making concrete should be free of acids, alkalies, and oil.

c. Irrigation Waters. Aromatic solvents of petroleum origin have been used to kill weeds in irrigation ditches. According to the United States Bureau of Reclamation (890), a concentration of 300 mg/l in the stream for 30 minutes will kill weeds without harming crops that are subsequently irrigated, owing to volatilization and dilution. Roberts (986) claims that oil films do not prevent the growth of fresh-water plants. It has been reported (667), however, that pollution by oil from ships is a possible contributing factor to the disappearance of eel grass from European and American coastal waters.

The phytotoxic mode of action of oils is described by McKee (3596) based on the work of van Overbeak and his colleagues. Instances are also cited where crude petroleum, added directly to the soil, appeared to improve rather than interfere with crop production. Heavy applications (4-5 percent in the soil) inhibited the rate of growth of some plants, presumably by interfering with the availability of soil moisture.

From the foregoing information it appears that the pollution of irrigation waters by oil is not likely to become a serious problem.

d. Stock and Wildlife Watering. Literature dealing with the effects of oily wastes in water on livestock and wildlife is both scant and inconclusive. In 1935, Ferguson and his committee (3602) stated that some cattle, sheep, and hogs develop a liking for crude oil and suffer adverse physiological effects from ingestion of oil-polluted waters. The adverse effects may have been due to laxative properties or possibly to toxicity. Whatever the cause, the animals failed to thrive and have been reported to die as a result.

Bruns et al. (2873 describe tests with guinea pigs to determine the possible ill effects of an emulsion containing 400 and 800 mg/l of aromatic hydrocarbons (mostly xylene). The animals consumed considerably less treated water than untreated, and they almost refused to drink the 800 mg/l water until the third day when they became very thirsty. After three days of confinement on treated water, none of the 32 animals showed ill effects. It is believed that the strong odor and disagreeable taste of water heavily polluted with hydrocarbons make it unlikely that animals will drink such water. Where an occasional heavy dose occurs, animals will postpone drinking until the water clears up or an alternate source is available.

The oral LD_{50} of kerosene for rabbits has been given (3603) as 28,350 mg/kg of body weight.

Floating oil may smear birds so that they cannot fly (615, 981, 3592). The wrecking of the oil tanker Frank H. Buck off San Francisco in March 1937 proved disastrous to many species of aquatic birds along the coast (987), but sea gulls escaped.

Unwin (988) describes effluent standards set up by the Ohio Department of Health for Mills Creek, including a limit of 30 mg/l for emulsified oils, because the creek is used for grazing cattle.

e. Fish and Other Aquatic Life. Oily substances may possibly be harmful to fresh-water aquatic life in the following manners:

1. Free oil and emulsions may act on the epithelial surfaces of fish, i.e. they adhere to the gills and interfere with respiration. Within limits, however, fish have a defensive mechanism to combat such action. They can secrete a mucous film to wash away irritants. If the concentration of oil is too heavy, oil will accumulate on the gills and cause asphyxia (311).

2. Free oil and emulsions may coat and destroy algae and other plankton, thereby removing a source of fish food. The coated organisms may agglomerate with suspended solids and settle to the bottom of the stream.

3. Settleable oily substances may coat the bottom, destroy benthal organisms, and interfere with spawning areas.

4. Soluble and emulsified material, ingested by fish, taint the flavor of the flesh.

5. Organic materials may deoxygenate the waters sufficiently to kill fish.

6. Heavy coatings of free oil on the surface may interfere with the natural processes of reaeration and photosynthesis. Very light coatings would not be detrimental in this respect, however, for wave action and other turbulence would maintain adequate reaeration.

7. Water-soluble principles may exert a direct toxic action on fish or fishfood organisms. Such toxicity may be acute or chronic. Acute toxicity will produce death or debility in 96 hours or less. Chronic toxicity exerts a long-time effect, through an accumulative action or through subtle changes in the ecology. By its very nature, chronic toxicity is difficult to detect and generally even more difficult to prove. The material presented below deals primarily with acute toxicity attributable to soluble principles (3596).

Certain petroleum products appear to have no soluble poisonous substances, but when emulsified by agitation with water, such oils prove deadly to fish (463). Chipman and Galtsoff (615) report that crude oil in concentrations as weak as 0.4 ml per liter (i.e., about 0.3 mg/l) is extremely toxic to fresh-water fish. Kerosene applied as an insecticide at a rate of 25 gallons per acre had no effect on fresh-water fish (612). The lethal limit of gasoline

for rainbow trout has been reported as 100 mg/l (2977). After 15 minutes exposure at 50 mg/l, rainbow trout show irritation and after 60 minutes they are tired. The toxic threshold is about 40 mg/l (2977). Agitated solutions of automobile gasoline and jet aviation fuel were found to be lethal to fingerling salmon at concentrations of 100 and 500 mg/l respectively (2218).

Turnbull et al. (2093) tested the toxicity of commercial cutting oils toward bluegill sunfish at 20°C in tap water. They found 24-hour TL_m values of 5540 and 14,500 mg/l for No. 1 and No. 2 soluble cutting oils respectively. For a commercial dissolved and floating kerosene, the 24-hour TL_m was 2990 mg/l.

Albersmeyer and Erichsen (3404) found that carbolated oil and light oil, both dephenolated, imparted a taste to fish flesh more pronounced than that caused by naphthalene and methyl naphthalene. They concluded that the hydrocarbons were more responsible for tastes in fish flesh than were the phenolic compounds.

Oils are toxic to mosquito larvae and also to the microorganisms on which the larvae feed (877). Crude oil, diesel oil, and Navy-grade special fuel oil in sea water are toxic to the hydrozoan *Tubulareacrocea,* to barnacles, and to the embryos of toadfish (615). Shaw and Timmons (3604) note that the concentration of emulsified aromatic hydrocarbons used to destroy weeds in irrigation ditches (300 mg/l) was sufficient to eliminate crayfish. Other fish were also killed.

The biochemical oxidation of hydrocarbons can be accelerated by the use of seeding material. With 28 mg/l of motor oil at the start of a test, 52 percent disappeared in 6 days with the use of a seed developed from a refinery cooling-tower effluent. With 70 mg/l initially present, 72 percent destruction occurred in 17 days with seed developed from oil-saturated soil. Both seeds gave approximately 50 percent reduction in 6 days (1625).

Petroleum oils are used extensively as solvents or vehicles for pesticides. While the oil may not in itself be toxic, it frequently increases the toxicity of the pesticide. For further details, see Chapter IX.

Toward the giant kelp, *Macrocystis pyrifera,* North and Clendenning (2106, 2866, 2867) found that 100 ml/l of boiler fuel oil and diesel fuel oil was injurious in 12 to 48 hours and concentrations of emulsified fuel oils above 10 ml/l were deemed to be deleterious.

f. Shellfish Culture. The heavy mortality of oysters in 1932-33 in certain areas of Louisiana was believed to be due to oil-well wastes, according to McConnell (319) and Gowanloch (967). In laboratory tests at that time, sea water that had been in contact with oil caused a 90 percent mortality of oysters in three months as compared with 17 percent for unpolluted sea water. The oil itself was not in contact with the oysters but the sea water extracted toxic substances from the oil. Sedentary animals such as clams may be destroyed by direct contact with oil (615).

According to Speer (317) surface films of floating oil can kill free-swimming larvae in a few hours and heavier portions are deposited on the bottom where oysters are killed by direct contact. Oil destroys plankton, especially diatoms, diminishes aeration at surface, destroys spawning grounds, and gives offensive tastes to oysters. Galtsoff (989) reports that oil imparts a taste to oyster flesh that makes the meat unsalable. Also, oysters in oil-polluted water show a decreased glycogen content.

g. Swimming and Other Recreational Uses. Oil slicks are visible when the concentration reaches 50 gallons of oil per square mile (984) and higher concentrations may coat bathers. Mixtures of brine and oil cause skin irritation to bathers (985) and the fire hazard to a water front is increased by oil pollution.

OLEIC ACID AND OLEATES

(see Fatty Acids)

OLEYLPOLYOXYETHYLENE GLYCOL ETHER

(see Chapter X)

ORGANIC NITROGEN

(see Nitrogen)

ORGANIC PHOSPHATES

(see Chapter IX)

OMAZENE

(see Chapter IX)

ORTHOPHOSPHATES

(see Phosphates and Phosphoric Acids)

OSMOTIC PRESSURE

(see Dissolved Solids, Specific Electrical Conductance, Distilled Water)

OVATRAN

(see Chapter IX)

OXALATES $^{-}OOC—COO^{-}$

(see also Oxalic Acid)

The salts of oxalic acid are likely to occur in the same industrial wastes that contain oxalic acid. Inasmuch as many oxalates dissociate readily and inasmuch as oxalic acid is a weak acid, the net effect is an increase in pH. Consequently, the result of adding oxalates to water is just the opposite of adding oxalic acid, insofar as pH is involved.

Jones (354) tested the threshold of toxicity of sodium oxalate to *Polycelis nigra,* a flatworm, and found that at 15-18°C the threshold occurred when the concentration of oxalate ion was 970 mg/l. In Lake Erie water at 25°C, however, Anderson (352) reports that 214 mg/l of sodium oxalate immobilized *Daphnia magna* in 48 hours. Toxicity appeared to have been due to factors other than osmotic pressure.

Using turbid water at 19-23°C and mosquito-fish (*Gambusia affinis*) as the test animal, Wallen et al. (2940) found the 24-, 48-, and 96-hour TL_m values for sodium oxalate to be 1350 mg/l. This compound had very little effect on the turbidity.

OXALIC ACID HOOC—COOH

(see also Oxalates)

This simple organic acid occurs widely in plants and vegetables, for it is one of the products of metabolism

of many molds. As a dihydrate, the crystalline solid sublimes readily into fumes that irritate the respiratory tract (364). It is highly soluble in water and in this form it is used extensively in industrial processes, such as those of the textile, leather, ceramic, paper, and rubber industries.

According to Doudoroff and Katz (361) oxalic acid cannot be toxic to fully developed fish without lowering the pH to 5.0, i.e. toxicity is not attributed to the undissociated acid. Consequently, the pH value is a good index of serious pollution by oxalic acid. Furthermore, a given dose of oxalic acid would be less likely to cause harm in a highly buffered water than in a relatively pure water.

Ellis (313) reported that 200 mg/l of oxalic acid in hard water was not lethal to goldfish after 100 hours, but 1000 mg/l in hard water killed goldfish in 30 minutes. German authorities (2977) report that 100 mg/l is lethal for fingerlings of trout in 20 to 50 minutes. For other fish the threshold of toxicity lies at about 20 mg/l.

In Lake Erie water, according to Anderson (358), the threshold concentration for immobilization of *Daphnia magna* after prolonged exposure at 25°C was 95 mg/l. Herman (2923) indicates that 43 mg/l of oxalic acid inhibits the 5-day BOD of sewage by 50 percent.

OXYDIPROPIONITRILE $(CH_3CH_2CN)_2O$

(see also Cyanides, Hydrogen Cyanide)

Henderson et al. (2958) conducted bioassays using three common species of fish in both hard and soft water saturated with oxygen at 25°C. Commercial oxydipropionitrile was used as the test compound. Apparently it does not dissociate measurably in water for little if any of the toxic principle, HCN, is formed. The following median toxic limits were reported:

Kind of Fish	*Type of Water*	*TL_m in mg/l*		
		24-hour	*48-hour*	*96-hour*
Fathead minnows	Hard	5700	5700	3900
Fathead minnows	Soft	4650	3850	3600
Bluegills	Soft	4300	4200	4200
Guppies	Soft	7350	7350	4450

Adult bluegills exposed up to four weeks in water containing 100 mg/l of oxydipropionitrile did not pick up any organoleptic properties that were apparent to a taste panel (2958). For rats, the oral LD_{50} dose of this compound was reported (2978) as 2830 mg/kg.

OXYGEN

(see Dissolved Oxygen)

OXYGEN CONSUMED

1. General. Like acidity, alkalinity, and BOD, this term is applied not to a specific pollutant but to a measure of the strength or character of many substances. The full name of the test, oxygen consumed from permanganate, refers to the amount of oxygen used by the sample when digested for 30 minutes in a boiling bath with a definite strength of acid or alkaline permanganate. Although potassium permanganate oxidizes the carbon but not the nitrogen in organic matter, the test does not indicate the total amount of carbonaceous organic matter, for the carbon in nitrogenous organic matter (e.g., proteins) is not so readily oxidized as that in carbonaceous organic matter (e.g., sugars). Furthermore, the test does not differentiate directly the carbon present in unstable organic matter from that in fairly stable organic matter (469, 3605). Thus, it measures primarily the readily oxidizable carbonaceous organic matter. Formerly popular and routine for analyses of water and sewage, it is used less frequently now, having been replaced by the more significant test for BOD. According to Phelps (330), "The test is no longer regarded as having any significant value as an index of pollution in the sanitary water analysis". It is of value, however, in estimating the strength of certain industrial wastes in conjunction with, or in lieu of, the BOD test. Although many of the references cited below are outdated, they are presented for what they are worth; but the reader should weigh them against the significance of the oxygen-consumed test.

2. Cross References. BOD.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. As described by Taylor (36), if the oxygen absorbed from NaOCl is greater than the oxygen absorbed from permanganate, animal pollution is indicated, but if it is less, then organic pollution is probably of vegetable origin. Beger (630) considers a water to be of good quality when it contains, among other things, not more than 12 mg/l of organic matter expressed as oxygen consumed from permanganate.

b. Industrial Water Supplies. The Progress Report of the N.E.W.W.A. Committee on Water Quality Tolerances for Industrial Uses (159) suggested the following limits for the oxygen-consumed test for boiler feed waters.

Pressure in psi	*Oxygen consumed, mg/l*
0-150	15
150-250	10
250-400	4
400 and over	3

For ice making and refrigeration a threshold concentration of 3.0 mg/l has been recommended (229) and polluted water with a potassium-permanganate demand of more than 8.0 mg/l is reported to impart a bad odor to textiles washed in it (259). Water having an oxygen-consumed value greater than 3.0 mg/l caused trouble when used in making synthetic rubbers at a plant on the Kanawha River in West Virginia (1213). The limit recommended (184) for carbonated beverage is 1.5 mg/l. Schwalbe (552) proposed a tentative specification for the quality of water used in pulp and paper manufacturing. For higher-grade products he proposed a standard of 10 mg/l for oxygen consumed from permanganate and for lower-grade papers a permissible tolerance of 20 mg/l. In discussing water for brewing, Laufer (170) indicates that the generally accepted limit for oxygen consumed is 0.5 to 2.0 mg/l with a maximum of 5.0 mg/l.

OZONE AND NASCENT OXYGEN O_3 and O

Although it is the most reactive form of oxygen and deteriorates rapidly into oxygen and nascent oxygen according to the reaction,

$$O_3 \rightarrow O_2 + O \text{ (nascent)}$$

ozone is found in the atmosphere in varying amounts (about 0.05 mg/l by volume at sea level), inasmuch as

it is produced continuously in the outer layers of the atmosphere by the action of solar ultra-violet radiation on the oxygen of the air (364). It can also be produced in the laboratory or commercially by passing dry air between plate electrodes charged by an alternating current. The commercial product is used as a disinfectant for water and air, and for bleaching paper pulp, textiles, waxes, and oils. Its occurrence in wastes from these processes is extremely unlikely, however, for ozone is the strongest oxidizing agent known and consequently is converted rapidly to oxygen and oxidized products.

Owing to its powerful oxidizing action, ozone has been used as a bactericide and cysticide. Kessel et al. (1297) report that the time required to destroy cysts of *Entamoeba hystolytica* and bacteria was less for solutions containing 0.3 mg/l of ozone than in solutions of 0.5 to 1.0 mg/l of chlorine. According to Chang (742), ozone concentrations of 0.1 to 0.3 mg/l killed these cysts in a few minutes.

Toward fish, ozone and nascent oxygen have been shown to be extremely deleterious. Ellis (313) reports that minnows and shiners may be killed by 0.033 mg/l of nascent oxygen and fish are irritated by ozone in concentrations less than 0.01 mg/l (311, 361, 2920).

PALLADIUM CHLORIDE $PdCl_2$

This dark-brown crystalline salt, occurring as $PdCl_2 \cdot 2H_2O$, is highly soluble in water. It is used in photography, toning solutions, indelible ink, and galvano-coating of precision parts (364). The lethal concentration of $PdCl_2$ for three mature small freshwater fish (*Orizias*) exposed for 24 hours to only 20 ml of test solution, was reported to be about 7 mg/l (1459).

PAPER MILL WASTES

(see Kraft Pulp-Mill Wastes, Groundwood Pulp-Mill Wastes, Paper Mill White Waters, Sulfite Waste Liquors)

PAPER MILL WHITE WATERS

The so-called "white waters" from paper mills, which may be highly colored at times, consist primarily of the water that is drained or drawn from the paper stuff on the paper machines. Such water contains fine fibers that escape the machine or occur in clean-up wastes, sizing materials, alum, clay, other weighting substances, dyes, and bactericidal or slime-controlling agents. The fine cellulose fibers may cause difficulty in a stream by blanketing the bottom and killing important fish-food organisms (684, 701). Schaut (362) reported that a 1:240 dilution of a white waste in stabilized tap water gave a white turbidity and caused minnows to stop feeding. At a 1:2400 dilution, there was no noticeable effect on minnows.

PARADICHLOROBENZENE

(see Dichlorobenzene)

PARA-OXON

(see Chapter IX)

PARATHION

(see Chapter IX)

PARIS GREEN (COPPER ACETOARSENITE)

(see Chapter IX)

PENETRANTS

(see Chapter X)

PENTABORANE

(see Boron)

PENTABROMOPHENOL

(see Halogenated Phenols, Chapter IX)

PENTACHLOROPHENOL AND PENTACHLOROPHENATES

(see Chapter IX)

PENTANE C_5H_{12}

(see also Hydrocarbons, General)

This flammable liquid, one of the saturated (paraffin) hydrocarbons, occurs in natural petroleum and as a constituent of petroleum benzin. It is soluble in water to the extent of 360 mg/l at 16°C. The concentration required to kill fish (white roach) at 20.2°C is given by Hubault (461) at more than 60 mg/l.

PENTENE C_5H_{10}

Shelford (363) found that amylene (1-pentene) which occurs in some gas-manufacturing wastes and coal tar, killed sunfish in one hour when the concentration of this colorless liquid in tap water was 655 mg/l.

PEPTONE

Peptone is the product of the pancreatic digestion of meat, the dried powder containing about 15 percent nitrogen and 4 percent ash. It is highly soluble in water. According to Schaut (362), 17.1 mg/l of peptone in stabilized tap water produced no noticeable effect on minnows after 24 hours of exposure, but the dissolved-oxygen content of the water decreased 58 percent.

PERTHANE

(see Chapter IX)

PETROLEUM

(see Oil, Petroleum)

PETROLEUM BENZIN

(see also Pentane, Hexane)

Known also as petroleum ether, benzin, or naphtha, petroleum benzin consists chiefly of hydrocarbons of the methane series, principally pentanes and hexanes. It is clear, nonflourescent, colorless, highly flammable liquid with a characteristic odor, used as a solvent for fats and waxes, as a detergent, in paints and varnishes, as fuel, and as an insecticide.

pH

1. General. The symbol "pH" is used to designate the logarithm (base 10) of the reciprocal of the hydrogen-ion concentration. Thus, if $[H^+] = 10^{-5}$ mols per

liter, then pH = 5. Inasmuch as an excessive concentration of hydrogen ions may adversely affect water for one or more beneficial uses, pH is a measure of a potential pollutant.

Not only is the hydrogen ion a potential pollutant in itself; it is also related intimately to the concentration of many other substances, particularly the weakly dissociated acids and bases. A few of the common equilibria in which hydrogen ions are involved are shown below:

Reaction	Reaction
$H_2O \rightleftharpoons H^+ + OH^-$	$H_2S \rightleftharpoons H^+ + HS^-$
$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$	$HS^- \rightleftharpoons H^+ + S^{--}$
$HCO_3^- \rightleftharpoons H^+ + CO_3^{--}$	$HOCl \rightleftharpoons H^+ + OCl^-$
$H_3BO_3 \rightleftharpoons H^+ + H_2BO_3^-$	$H_3PO_4 \rightleftharpoons H^+ + H_2PO_4^-$
$H_2BO_3^- \rightleftharpoons H^+ + HBO_3^-$	$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{--}$

The foregoing reversible reactions are but a few of the ones involving hydrogen ions in natural waters, but they illustrate that the concentration of hydrogen ions, i.e., the pH, controls the degree of dissociation of many substances; and since the undissociated compounds are frequently more toxic than the ionic forms, pH may be a highly significant factor in determining limiting or threshold concentrations (361). For an example, see Ammonia.

Conversely, the concentration of weakly dissociated acids and bases markedly affects the pH value and the ease with which it can be altered. For this reason, pH should not be confused with acidity and alkalinity. The presence of carbonates, phosphates, borates, and similar ions give water a buffering power so that the addition of an acid or base is less likely to be deleterious.

Owing to their origin in municipal water supplies, most domestic sewages are neutral or slightly alkaline, with a strong buffering action. Many industrial wastes, on the other hand, are so strongly alkaline or acid that they have a marked effect upon the pH of receiving waters. Among the acid wastes may be included tan liquors, acid dyes, coal-mine drainage, sulphite waste liquors, pickling liquors, and some brewery wastes; and among the strongly alkaline wastes are beam-house effluents, kier liquors, wool-scouring wastes, soda- and sulfate-pulp rinse waters, laundry wastes, and bottle wash waters.

Most of the state and interstate agencies that have established stream standards or effluent standards have included limits for the pH values. For details of stream standards for specific states and agencies, see Chapter III and its appendices. Where effluent standards have been promulgated, they are generally expressed in terms of the resulting pH of the stream. The standards for the discharge of industrial wastes to public water courses in Russia (460) state that the wastes shall not lower the pH below 6.5 nor increase it above 8.5.

2. Cross References. Acidity, Alkalinity, Ammonia, Bicarbonates, Carbonates, Chlorine, Hydrogen Sulfide, Hydroxides, Phosphates, Protozoa.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The hydrogen-ion concentration of a raw-water source for domestic water is important in that it affects taste, corrosivity, efficiency of chlorination, treatment processes such as coagulation, and industrial applications. The United States Public Health Service Drinking Water Standard of 1962 (2036) set no limits to pH in domestic waters.

Butterfield et al. (493) have shown that the killing power of chlorine diminishes with increasing pH values and, consequently, it is advantageous to apply chlorine to a water with a pH value of 7 or less. On the other hand, Langelier (692) has demonstrated that high pH values favor corrosion control. Natural waters do not dissolve lead if their pH value is above 8.0, according to Holl (1073). Water acquires a "sour" taste at pH 3.9 or below, except in highly buffered solutions, according to Rohlich and Sarles (759). The optimum dosage of coagulating chemicals is influenced by the pH as well as by the buffering action of the water (387, 711). In a lighter vein it is interesting to note that during Prohibition days waterworks operators received many complaints from customers who preferred water with a pH below 7.0 for home brewing (164).

b. Industrial Water Supplies. Recommended threshold values of pH for various industrial process waters are given in Chapter V, from which the following table has been abstracted:

Industrial Process	*Recommended pH Values*	
	Minimum	*Range*
Boiler feed water:		
0 to 150 psi	8.0	--
150 to 250 psi	8.4	--
250 to 400 psi	9.0	--
400 psi and over	9.6	--
Brewing	--	6.5 to 7.0
Confectionary	7.0	--
Food canning and freezing	7.5	--
Laundering	--	6.0 to 6.8
Oil-well flooding	7.0	--
Rayon manufacturing	--	7.8 to 8.3
Steel making	--	6.8 to 7.0
Tannery operations	--	6.0 to 8.0

In addition to the foregoing tabulations, the following observations are of interest. Collins (164) states that distilled water is not satisfactory for making hard candies because it is likely to have a pH of 6.1 to 6.5 which favors inversion of sucrose and makes the candy sticky. According to Behrman (1017), the optimum pH range for waters used in carbonated beverages is 2.7 to 3.5. Medberry (404) sights problems of foaming of carbonated beverages when the pH was above 8.0, but none when the pH was below 7.0. For higher-grade pulp and paper, Schwalbe (552) proposed that the pH of the water supply be 7.0 and for lower-grade products 6.7 to 7.3.

Low pH values increase the corrosion action of water toward concrete (see Chapter V). Antill (1298) states that caution should be observed for corrosion of concrete if the water has a pH below 7, and Tremper (1299) reports that the degree of attack is inversely proportional to the pH value in waters ranging from pH 6.0 to 7.0. Attack is negligible at pH values greater than 7.0. According to Ley (694), ground water with a pH less than 6.0 is potentially dangerous to concrete through corrosive properties. The corrosion of concrete is not a function of

pH alone but varies with the content of hardness and alkalinity constituents, with free carbon dioxide being particularly detrimental (see Carbon Dioxide). Low pH values favor the formation of carbonic acid from bicarbonates (360, 1204).

c. Irrigation. The optimum pH for irrigation water depends on the type of crops to be grown and the physical and chemical properties of the soil. In the western United States, where alkaline soils predominate, waters with low pH values are desirable. Wilcox (269) reports that waters with pH values over 9.0 are unsuitable for irrigation use. In eastern acid soils where liming is practiced, waters of moderately high pH are not detrimental for most crops. However, Stevens (1300) noted that a cranberry crop in Wisconsin improved when the fields were flooded with waters of pH 5.3 and 6.8 in contrast with the normally alkaline water used on such fields. In the other direction, the yield of corn grown in culture solution at pH 4.5 was about half of that grown at pH 6.0 (1447).

d. Fish and Other Aquatic Life. Ellis (313 reports that the pH values of most inland waters containing fish range between 6.7 and 8.6, with extremes of 6.3 and 9.0; but Cole (311) states that fish are euryionic and can live in a wide pH range, with limits as broad as 4.7 to 8.7. Of United States waters that support a good fish fauna, only 5 percent have a pH less than 6.7; 50 percent have a pH less than 7.6; and in 95 percent the pH is less than 8.3 (310).

The permissible range of pH for fish depends upon many other factors such as temperature, dissolved oxygen, prior acclimatization, and the content of various anions and cations. The pH value determines the degree of dissociation of weak acids and bases, some of which may be more toxic in the molecular than ionic form (361). For example, Jones (1046) showed that the toxicity of sodium sulfide to trout tends to increase with acidity as the pH changes from 9 to 6, i.e., as sulfide and HS^- ion are converted to H_2S (see Hydrogen Sulfide). The tolerance of fish to low concentrations of dissolved oxygen varies markedly with pH (1301, 3650, 3701). The toxicity of nickel cyanide increased over 1000-fold as the pH of the solution was decreased from 8.0 to 6.5 (1632) (see also Cyanide). The toxicity of sodium sulfide to fish increased as the pH decreased; 8.0 mg/l of sodium sulfide was lethal at pH 8.2, but at pH 5.2, as little as 0.55 mg/l was lethal (1468). Stiemke and Eckenfelder (270) presented an empirical formula to relate lethality, pH, and total acidity of common acids for predicting survival of fish (see Acidity). Fish that can tolerate pH values as low as 4.8 will die at pH 5.5 if the water simultaneously contains 0.9 mg/l of iron (306, 1023).

The Aquatic Life Advisory Committee of the Ohio River Valley Water Sanitation Commission (2408) concluded that direct lethal effects of pH are not produced within a range of 5 to 9.5, but from the standpoint of productivity it is best to maintain the pH in the range of about 6.5 to 8.2.

Some of the minimum and maximum pH values, and recommended ranges in pH, as reported in the literature, are given in the following tabulation for fish.

Limiting pH Values			
Minimum	*Maximum*	*Remarks*	*References*
3.3	10.7	Trout survived without adverse effects	1467
3.6	---	Tench survived two weeks but carp died	1302
3.6	10.5	96-hour TL_m range for bluegill sunfish	2934
3.8	10.0	Fish eggs could be hatched, but abnormal young were produced of extreme pH's	1468
4.0	10.0	The most resistant fish can tolerate such extreme pH values indefinitely after acclimatization	1460
4.0	10.1	Limits for the most resistant species	361
4.0	10.4	Limits for bluegill sunfish with HCl and NaOH	3606
4.1	8.5	Range tolerated by speckled trout in nature	1467
4.1	9.5	Range tolerated by trout	862
4.3	---	Carp died in 5 days	1303
4.4	8.7	Toxic limits for trout	3609
4.5	8–9	Trout eggs and larvae develop normally	1636
4.6	9.5	Toxic limits for perch	3609
4.6	---	Tench died in two days	2977
4.8	---	Lower limit for trout	1304
4.8	---	Illness and early death for carp	2977
4.8	9.2	Toxic limits for fish	1305
5.0	---	Toxic limit for sticklebacks	2941
5.0	9.0	Tolerable range for most fish	361, 2409, 3609
---	8.7	Upper limit for good fishing water	717
5.4	---	Lower limit for carp and tench	1306
---	9.2	Upper limit for trout and perch	2977
5.5	---	Lower limit for general fish protection	1307
5.4	11.4	Fish avoided waters beyond these limits	1046
6.0	11.0	Fish did not avoid waters in this range	1046
6.0	7.2	Optimum range for fish eggs	1468
6.5	8.4	Range tolerated by most freshwater fish	1466
For other aquatic organisms, ranges were reported as follows:			
1.0	---	Mosquito larvae destroyed at this value	1308
2.5	---	Tolerated by *Chlamydomonas, Fragilaria, Asterionella, Aphanizomenon*	2168
3.3	4.7	Mosquito larvae thrived in this range	1309
4.0	---	Toxic for *Paramecium, Volvox, Asplanchna*	2977
7.5	8.4	Good range for plankton production	1021
---	8.5	Algae are destroyed above this value	

Wiebe (3607) investigated the effects of rapid changes of pH on fish. A majority of the species tested tolerated extensive and rapid changes in pH, e.g. from 7.2 to 9.6 and from 8.1 to 6.0.

When the pH falls below 5.0, specialized flora and fauna develop (3608) and some species have been observed at pH values as low as 2.9

e. Shellfish Culture. According to Loosanoff and Tommers (323), oysters live in brackish waters of which the pH is usually above 7.0. At pH values of 6.5 or lower, the rate of pumping and the time shells remained open were decreased 90 percent. With respect to high pH values, Gaarder (324) found that oyster larvae are injured

at pH 9.0 and die at pH 9.1 in a few hours. For crabs, the upper limit of pH is 10.2 (2977).

Loosanoff and Tommers found that oysters pumped normally at pH 7.75. At pH values of 6.75-7.00 they pumped vigorously for a few hours, but then fell behind the controls. At pH 6.5 the rate of pumping was markedly reduced; and at pH 4.14 the pumping rate was off 90 percent (1448).

f. Swimming and Other Recreational Uses. A decrease in the pH of swimming pool water from 8 to 7 resulted in an increase in the number of cases of eye irritation among swimmers in a controlled pool (1104).

PHAGES

(see Chapter VII—Bacteriophages)

PHENANTHRENE $C_{14}H_{10}$

(see also Coal-Tar Wastes)

This solid, highly insoluble in water, is found in some coal-tar manufacturing wastes. Shelford (363) reports that concentrations of 1.02 to 2.0 mg/l killed sunfish in one hour, while Gutsell (463) gives 4.0 to 5.0 mg/l as the lethal concentration for fish during one hour exposure. In Lake Huron water at 12°C, 5.0 mg/l killed rainbow trout and bluegill sunfish in 24 hours but had no effect on the sea lamprey (2976).

PHENOL C_6H_5OH

1. General. This colorless, crystalline substance with a characteristic odor is commercially obtained from coal tar and is widely used as a disinfectant, for the manufacture of synthetic resins, medical, and industrial compounds, and as a reagent for chemical analyses. It is highly soluble in water (one gram per 15 ml) and in alcohol, benzene and other organic solvents (364). Phenolic wastes arise from the distillation of wood, from gas works, coke ovens, oil refineries, chemical plants, sheep dips, and from human and animal refuse (923).

Despite the fact that it is used as a bactericide in strong concentrations, weak phenol solutions are decomposed by bacterial and biological action in streams (924). Mischonsniky (925) reports that, in the presence of earth and aquatic plants, phenol is decomposed at a rate of 3.0 to 5.0 mg/l per day with an accompanying decrease in dissolved oxygen. In the absence of earth and plants, decomposition is only 2.0 mg/l per day.

In a concentration of 1.0 mg/l, phenol has been found to be biologically dissimilated at 20°C in 1 to 7 days under aerobic conditions (1538). At 4°C, complete dissimilation required 5-19 days. Under anaerobic conditions, dissimilation occurs but at a slower rate (1538).

In tests conducted by the San Bernardino County Flood Control District (926) wastes containing 24 mg/l of phenols were leached through columns of soil 50 inches long and 12 inches in diameter. The concentration of phenol in the effluent rose gradually from 0.31 mg/l to 22 mg/l with 23 gallons of flow. Then distilled water was added and the concentration of phenol in the effluent decreased gradually to 3.6 mg/l with 37 gallons. The lagooning of water containing 3.0 mg/l of phenol reduced the phenols to 0.28 mg/l in seven days and to 0.02 mg/l in 14 days. Sayre and Stringfield (927) report that phenols from the Ohio River have not affected the wells at Parkersburg, W. Virginia, although the wells are only 150 feet from the river.

State and interstate agencies that have promulgated stream standards frequently limit phenol concentrations in the waters. For details, see Chapter III.

2. Cross References. Halogenated Phenols, Cresols, Nitrophenols, Pentachlorophenol, Chapter III.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The ingestion of concentrated solutions of phenol will result in severe pain, renal irritation, shock and possibly death. A total dose of 1.5 grams may be fatal (364). It is not likely, however, that harmful concentrations of phenol will be consumed in drinking water because such concentrations are much higher than taste considerations would allow. The 1962 USPHS Drinking Water Standards (2036) limit the concentration of phenolic compounds to 0.001 mg/l, because of tastes resulting from the action of chlorine on such waters. The 1958 WHO International Standards (2328) set a permissible limit of 0.001 mg/l and an excessive limit of 0.002 mg/l. The 1961 WHO European Standards (2329) have a recommended limit of 0.001 mg/l. Bean (1745) recommends that the concentration of phenolic substances in drinking water be limited to 0.0005 mg/l.

Other authorities have reported that the threshold concentrations for taste or odor are as follows:

Threshold Concentration in mg/l	*Reference*
A. For Phenol Alone	
0.010	130
0.020-0.030	460, 929
0.10	459, 871
0.25-4.0	2983
0.7-1.0	3610, 3611
5.0 (at 60°C)	2982, 3403
7.13	3494
10.0 (at 30°C)	2982, 3403
25	3402
60	2059
B. For Phenol in Chlorinated Water	
0.00001-0.001	3610
0.0005-0.001	3402
0.001	459, 499, 687
0.002	871, 930, 946
0.002-0.010	931
0.005	932
0.005-0.020	460, 3611

Kinney (3584) points out that the method of analysis for phenol measures not phenol alone but a whole series of organic compounds that are called "phenolics"; yet it does not measure all phenol-like compounds. Phenolics are found not only in discharges from coke plants and refineries but also in mountain streams from decaying vegetation, in the urine of rabbits and pregnant mares, and in countless other sources (3612). Strong medicinal odors may occur when phenol concentration is 0.002 mg/l, yet at other times when the phenol concentration was over 0.050 mg/l there were no tastes or odors (3584).

b. Industrial Water Supplies. Phenol is deleterious in many of the food and beverage industries (see Chapter V) and may cause obnoxious tastes and odors (179, 223). Kohman (225) reports that pears packed in syrups made from chlorinated river waters had "iodoform" tastes. On the other side of the ledger, however, Pickett (934)

reports that refineries discharge wastes containing up to 60 mg/l of phenol into Dominguez Channel in Los Angeles County without harmful effects and that a beneficial action occurs in that the phenol retards development of objectionable marine growths in the harbor. Similar discharges elsewhere might be highly objectionable.

c. Irrigation Waters. Phenol in irrigation water is not considered to be deleterious to crops. In Germany, a municipal sewage containing 40-50 mg/l of phenol from a gas plant was used for irrigation without any damaging effect on the crops (2977).

d. Stock and Wildlife Watering. According to Heller and Pursell (941) rats that drank water containing phenol from 15 to 1000 mg/l showed no deleterious physiological effects. In concentrations up to 5,000 mg/l, phenol did not interfere with digestion, adsorption, or other metabolic functions. Above 7,000 mg/l, growth was stunted and many young died at birth. The authors conclude that appreciable concentrations of phenol are not toxic to animals.

e. Fish and Other Aquatic Life. Phenolic compounds may affect fish in two ways: first, by a direct toxic action and second, by imparting a taste to the fish flesh. There are numerous references concerning the effects of phenolic compounds on aquatic life, especially fish. The reported lethal concentrations vary widely not only because of the common variables such as species, temperature, time of contact, dissolved oxygen and mineral quality of water but also because of synergistic and antagonistic effects of other substances in the water. Some of the tests have been run on pure phenols, but others were conducted with phenolic wastes for which the phenol equivalent is given. Many phenolic compounds are more toxic than pure phenol and consequently mixed phenolic compounds may have a lower lethal limit than phenol itself. For this reason, the Aquatic Life Advisory Committee of ORSANCO (2109) recommends that any criterion for toxicity to fish be set for phenolic compounds as a group rather than for phenol itself. Indeed, much of the toxicity of a phenolic waste may be due to non-phenolic substances such as cyanide.

The following concentrations of phenol have been reported as lethal or damaging to fish:

Concentration in mg/l	*Time of Exposure*	*Type of Water and Temperature,°C*	*Type of Fish*	*Reference*
0.079	30 minutes	River	Minnows	936
0.28	---	River	Mixed fish	3613
0.4-0.6	8.5 hours	---	Fish	313
0.5-1.0	---	---	Trout	3405
0.71	1 hour	---	Sunfish	311
1-5	---	---	Tench	3405
3-5	---	---	Fish	3614
4.3	12 hours	River	Trout	3615
5.0	10 hours	Lake	Trout	2976
5.0	24 hours	---	Trout	359
5.0	---	---	Perch	827
5.0	24-hour TL_m	18°	Salmonide embryos	3404
5-10	---	---	Fish	311
5-10	---	---	Trout	311
5-20	1 hour	Aerated	Sunfish	311
6.0	---	---	Perch	3405
6.0	---	---	Rainbow trout	2920
6.2	24 hours	---	Brook trout	359
7.5-12.5	60 minutes	12°	Yearling trout	3245
9	---	---	Perch	365
9-10	---	---	Trout	1637
9.5	156 minutes	---	Brook trout	3616
10	156 minutes	---	Dobule	3616
10	72 hours	Hard	Goldfish	313
10	---	---	Fish	247
10	---	---	Tench	311
10-15	---	---	Fish	935
10-20	---	Non-aerated	Fish	937
11.5-20	96-hour TL_m	Standard, 20°	Bluegill sunfish	2933
12	---	---	Perch	1637
12	156 minutes	---	Perch	3616
13.5	96-hour TL_m	Soft, 18°	Bluegill sunfish	2936
14.2	24 hours	---	Minnows	359
14.5	24-hour TL_m	18°	Tench	3404
15.5	48-hour TL_m	---	Stickleback	2985
15.5	48-hour TL_m	Synthetic, 15°	Stickleback	3617
16-20	---	---	Fish	313
16.7	96-hour TL_m	Tap	Fingerling catfish	2981
17	---	---	Minnows	1637
17	156 minutes	---	Minnows	3616
17	20 minutes	---	Perch	3405
18-20	6 hours	Hard, 17°	Minnows	2942
19	48-hour TL_m	Aerated, 20°	Bluegill sunfish	2093
19.3	96-hour TL_m	20°	Bluegill sunfish	3618
20	110 minutes	21°	Roach	910
20	---	---	Fish	431
20	1 hour	---	Sunfish	311
20	---	17°	Minnows	2920
20	16.2 minutes	---	Minnows	3405
20-25	---	Aerated	Fish	937
20.2	96-hour TL_m	Hard, 18°	Bluegill sunfish	2936
22.2	48-hour TL_m	Synthetic, 20°	Bluegill sunfish	3582
23	10 minutes	---	Perch	3405
24	96-hour TL_m	Soft, 30°	Bluegill sunfish	2936
24-28	6 hours	Distilled, 19°	Minnows	2942
24.9	24-hour TL_m	18°	Carp	3404
28.5	96-hour TL_m	Hard, 30°	Bluegill sunfish	2936
28.9	48 hours	Tap	Goldfish	1474
30	150 minutes	9.5°	Roach	910
40	30.7 minutes	---	Eels	3405
40	48-hour TL_m	---	Fathead minnows	3335
51	90-140 minutes	Distilled	Goldfish	313
56	96-hour TL_m	Turbid, 23-26°	Mosquito-fish	2940
70-75	1 hour	Tap	Sunfish	363
100 or less	1 hour	---	Fish	463
100	---	18-23°	Goldfish	2920
333	24 hours	---	Goldfish	359
400	15 minutes	---	Trout	614
1600	Brief	Tap	Trout, perch	1638
1900	Brief	River	Roach	1467

The following concentrations of phenol have been reported not to harm fish in the indicated time of exposure:

Concentration in mg/l	*Time of Exposure*	*Type of Fish*	*Reference*
0.002	---	Mixed	3613
0.079	---	Goldfish	936
0.10	---	Fish	938
0.5	---	Fish	935
1.0	100 hours	Goldfish	313
1.3	---	Trout	3619
2.7	---	Bluegills	1639
3.0	48 hours	Stickleback	2985
5.0	24 hours	Bluegills and lamprey	2976
9.0	156 minutes	Brook trout	3616
10	6 hours	Carp	936
10	---	Fish	925
15	---	Fish	313, 431
17.1	24 hours	Minnows	362
20	90 minutes	Eels	3405
19,000	Brief	Carp	1467, 1638

On the basis of the foregoing tabulations it is evident that there is a wide spectrum of toxic levels and considerable overlap between the lethal or damaging concentrations and those that have not harmed fish in the designated times. Much of the reliable information relating to pure phenol under carefully standardized conditions indicates that the 24-, 48-, and 96-hour TL_m concentrations are in the general range of 10-20 mg/l at 20°C. The threshold concentration, however, may be considerably lower.

The Halsbands (3619) attempted to determine the "threshold of disturbance" of phenol toward trout. They maintained that lethal action toward fish and fish-food organisms should not be the criterion but rather any

disturbance that has a pathological effect on the animal. For trout, they found that the threshold of disturbance lies at 1.3 mg/l. Phenol appears to act as a nerve poisoning, causing too much blood to get to the gills and to the heart cavity of the fish (3620). Threshold responses were also noted for stickleback at 3.0 mg/l (2985) and 0.6 mg/l (3617). Kalabina (3613) found an abundant and diversified fish fauna in a river containing 0.02 mg/l of phenolic compounds, but found no fish in parts of the river where phenolic compounds were 0.28 mg/l. On the basis of the foregoing figures, 3.0, 1.3, 0.6, and 0.28 mg/l as threshold concentrations, it is apparent that somewhere in the vicinity of 1.0 mg/l represents a threshold effect for fish.

The toxicity of phenol toward fish increases as the dissolved-oxygen concentration is diminished (3321, 3322), as the temperature is raised (3405), and as the hardness is lessened (2904, 2036). The effect of combinations of different phenolic compounds is additive (610, 3405).

The second major effect of phenolic substances on fish relates to the odor imparted to flesh. Mixed phenolic substances appear to be especially troublesome in this respect (2109). Chlorophenols produce a bad taste in fish flesh even at concentrations far below lethal or toxic doses (2904). Mineral oils also give a bad taste to fish meat by way of the fish-food organisms living in oil-containing muds (2904). The relationship between true phenols and taste in fish flesh is far from clear. In experiments carried out with fish exposed for 36 hours at concentrations below the TL_m, no connection between the phenol uptake of the fish and the impairment of taste could be found. It was concluded that the phenols occurring in waste waters of phenol-working industries could not be considered as the intrinsic cause of so-called phenolic tastes in fish. On the other hand, there was in intense impairment of taste from carbolated oil and light oil, both dephenolated. It was concluded that taste was due to non-phenolic materials accompanying the phenols in waste water (3404).

Tests carried out on the sperm of carp and the eggs of this fish and the stickleback indicate that the propagation products of fish are not damaged by phenol concentrations tolerated by fish (3404).

Over a concentration range of 4 to 400 mg/l of phenol, minnows appear to have little or no power of discrimination between phenol and water; hence they will swim into the solution and become intoxicated (1535). While it is possible that a slight degree of pollution may do no harm if the effluent is highly diluted immediately to a maximum degree, the possibility does exist that if phenolic pollution mixes only slowly with the stream and forms pools where solution of comparably high concentrations may be found, fish may venture into these regions and be harmed. Solutions of phenol cause a fish to lose its sense of balance and capability of coordinated movement. The time the solutions take to produce this effect is much shorter than the survival time (1535).

Phenols appear to be less toxic toward fish-food organism and other lower aquatic life than towards fish. The following concentrations of phenol have been reported to be damaging or deleterious toward lower organisms:

Concentration in mg/l	*Organism*	*Effect*	*Reference*
1	*Platymonas*	Limits photosynthesis	3621
3	*Mytilias edulis*	Threshold effect	3617
5	*Daphnia* (young)	Threshold effect	2955
8-10	*Daphnia magna*	Killed	313
14	*Daphnia* (adult)	Threshold effect	2955
15	Tubeworm	Threshold effect	3617
15	Isopod	Threshold effect	3617
16	*Daphnia*	Threshold effect	2158
25	*Gammaridae*	Lethal	935
30	*Microregma*	Threshold effect	3343
40	*Scenedesmus*	Threshold effect	2158
40-50	*Culex* larvae	Threshold effect	3404
50	Isopod	Threshold effect	2985
50-60	*Daphnia*	Threshold effect	3404
94	*Daphnia magna*	Immobilization	358
94-100	Snail	Toxic action	2936
100-120	*Pionocypris*	Threshold effect	3404
108	*Diaptomus oregonensis*	Threshold effect	2955
122	*Cyclops vernalis*	Threshold effect	2955
150-180	*Cyclops* and *Sayomia* larvae	Threshold effect	3404
200	*Tubifex*	Toxic action	2889
200	Crustacea	Toxic effect	2904
200	Molluscs	Toxic effect	2904
251-261	*Navicula*	Toxic effect	2936
800-1000	*Culex* pupae	Threshold effect	3404
1000	Protozoa	Toxic action	2904
1000	Rotifers	Toxic action	2904
1600	Bacteria	Toxic action	2923

At a concentration of 4.3 mg/l, phenol was not damaging to *Gammerus pulex, Tubifex,* or Chironomid larvae in 12 hours (3615).

Toward algae, phenol is less toxic than cresol, but more so than catechol. The toxic effect of phenol toward algae is essentially attributable to its undissociated molecule and to the tendency to form compounds with the albumen of the protoplasm (940).

Bringmann and Kuhn (2158, 3343) tested the effect of several phenolic compounds toward four species of lower aquatic life in River Havel water. The concentrations showing threshold effects are shown below:

Phenolic Compound	*Threshold Concentration in mg/l for*			
	Daphnia (a crustacean) (23°C)	*Scenedesmus (an alga)* (24°C)	*Microregma (a protozoan)* —	*E. coli (a bacterium)* (27°C)
Phenol	16	40	30	>1000
p-Aminophenol	0.6	6	2	8-10
p-Butylphenol	8	10	10	>100
o-Cresol	16	40	50	600
m-Cresol	28	40	20	600
p-Cresol	12	6	10	>1000
o-Nitrophenol	60	36	40	>1000
m-Nitrophenol	24	28	20	300
p-Nitrophenol	14	72	20	100
2, 4-Dinitrophenol	6	40	—	>100
Dinitro-o-cresol	8	36	30	100
o-Xylenol	16	40	10	500
m-Xylenol	24	40	70	>100
p-Xylenol	10	40	50	>100
Resorcinol	0.8	60	40	>1000
Hydroquinone	0.6	4	2	50
Pyrocatechol	4	6	6	90
Pyrogallo	18	8	50	30

As this table indicates, many of the phenolic compounds are more toxic than pure phenol toward these lower organisms.

Clendenning and North (2106, 2866, 2870) tested the effect of phenol on the giant kelp, *Macrocystis pyrifera.* They found that 10 mg/l caused a 50-percent inactiva-

tion of photosynthesis in bottom kelp fronds during a 4-day exposure, but 1.0 mg/l had no apparent effect.

The Mersey River Board in England (2950) has recommended that sewage and industrial wastes discharged to rivers should not contain more than 1.0 mg/l of phenolic compounds.

4. Summary. On the basis of the foregoing information, it appears that the following concentrations of phenol will not interfere with the designated beneficial uses:

a. Domestic water supply	0.001 mg/l
b. Irrigation	50.0 mg/l
c. Stock watering	1000.0 mg/l
d. Fish and aquatic life	0.2 mg/l

PHENYL ETHER $C_6H_5OC_6H_5$

Used in perfuming soaps and for organic synthesis, this liquid is only slightly soluble in water (364, 911). According to Middleton et al. (2983, 3356) it is detectable in water by odor or taste at a concentration as low as 0.013 mg/l.

PHENYLHYDRAZINE $C_6H_5NHNH_2$

(see also Hydrazine)

This combination of benezene and hydrazine is only sparingly soluble in water. It is used in the manufacture of dyes and as a reagent for sugars, aldehydes, and ketones (364). The oral lethal dose for rats is reported as 550 mg/kg of body weight (364). In a concentration of 108 mg/l, phenylhydrazine retards fermentation and at 1080 mg/l decomposition processes are almost completely stopped (2977).

PHENYL MERCAPTAN

(see Mercaptans, General)

PHENYLMERCURIC ACETATE

PHENYLMERCURIC LACTATE

(see Mercuro-Organic Compounds)

PHOSPHATES

1. General. In addition to the phosphates formed from ortho-, meta-, and pyro-phosphoric acids, anhydrous salts may be produced in which the hydrogen of the acid is replaced only partially by univalent or bivalent metals. Regardless of the condition of the anhydrous salt, the water solution of this salt will contain primarily $H_2PO_4^-$ or HPO_4^{--}, depending on the pH of the solution (see Phosphoric acid). The sodium, potassium, and ammonium phosphates are soluble, but of the calcium salts only monocalcium phosphate is soluble. Most of the numerous magnesium phosphates are only slightly soluble (911).

Phosphates may occur in surface or ground waters as a result of leaching from minerals or ores in natural processes of degradation or from agricultural drainage, as one of the stabilized products of decomposition of organic matter, as a result of industrial wastes, as a constituent of cooling waters that have underground phosphate treatment and as a major element of municipal sewage as a result of utilization of syndets. In surface waters, however, phosphates are seldom found in significant concentrations because they are utilized by plants and converted into cell structures by photosynthetic action. Higher concentrations are likely to occur in ground waters that receive leachings from excessive application of fertilizer, from cesspools, or from the recharge of cooling waters.

2. Cross References. Phosphorus, Phosphoric Acid, Sodium and Potassium Salts, Mercuro-Organic Compounds, Chapter VIII—Radioactivity, Chapter IX—Pesticides, Chapter X—Surface Active Agents.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Polyphosphates have been used in municipal water works and especially in boiler-feed-water treatment to prevent scale formation and to inhibit corrosion. There are numerous references dealing with such treatment, the preferred compounds, the optimum doses, and the results; but a review of such treatment is beyond the scope of this report. The subject is relevant to this survey, however, in that such treated waters may be discharged to streams or underground basins. In the concentrations encountered in water treatment, these polyphosphates do not appear to have any physiological significance. They do tend, however, to encourage aftergrowths of organisms in treated water by serving as a nutrient (152, 1311).

According to Konrich and Haase (1312), excessive phosphate is undesirable in water used for preparing food, as it has a buffering action on the acids of the stomach.

Jones (1313) discusses the physiological effects of hexametaphosphates and points out that in the acid condition of the stomach, they are rapidly changed to H_3PO_4 (see Phosphoric acid). In sufficient concentration, they can cause vomiting and diarrhea. Safe doses for oral ingestion were given as follows:

Substance	*Safe Dose, in Grams, for*	
	Rabbit	*Man*
Sodium orthophosphate	15	450
Sodium pyrophosphate	3	90
Sodium metaphosphate	4	120

For rats, amounts up to one-tenth of the total diet, or 200 mg/kg had no ill effects (1314). Manz (3626) reported an incident where sodium polyphosphates added to culinary water in a concentration of 450 mg/l produced diarrhea but no permanent illness. He considered that 50 mg/l should not be exceeded.

In raw-water sources, polyphosphates are detrimental in that they interfere with coagulation, flocculation, and the lime-soda treatment of water (204). Sodium tripolyphosphate and tetrasodium pyrophosphate are the principal builder compounds of synthetic detergents. As such they are appearing increasingly in polluted water and in raw water sources for domestic supply. Several papers (2050, 3622, 3623, 3624, 3625) have reported that phosphate concentrations of 0.5 to 1.5 mg/l interfere markedly with coagulation. Cohen et al. (2049), report that concentrations of sodium tripolyphosphate as low as 0.025 mg/l have an adverse effect on synthetic turbid water being coagulated with 10 mg/l of ferric sulfate. A concentration of 0.4 mg/l virtually prevented any removal of turbidity.

Campbell et al. (2059) reported the taste threshold of trisodium phosphate in water to be 225 mg/l, while Lockhart et al. (3241) found a taste threshold in distilled water at 180 mg/l.

b. Industrial Water Supplies. Owing to their weekly dissociated acid reaction and their inhibitory effects upon scale formation, phosphates are seldom frowned upon in industrial-process or cooling waters. Where they encourage biological aftergrowths, however, they are detrimental (1311). In brewing water, according to LeClerc (166), primary phosphates are converted into secondary phosphates, making the wort more alkaline and deleteriously affecting the aroma and the resistance of the beer to bacterial actions.

c. Irrigation. Phosphates are one of the minor constituents of irrigation waters and are of little importance in irrigation practice (268). Where they do occur, they are likely to be beneficial in increasing the fertility of the soil moisture (905, 1291, 1315). Blueberry plants grown in nutrient water of low phosphate content (1 to 5 mg/l of P) showed signs of phosphorus deficiency and were only three-fourths as large as plants grown with more P. On the other hand, plants that received 60 mg/l of phosphorus showed signs of incipient iron chlorosis. A high P content in the nutrient solution appears to lower the available inorganic iron and thereby produces chlorosis in the blueberry plant (3627).

d. Fish and Other Aquatic Life. The discharge of excessive amounts of phosphates to streams or lakes may result in an overabundant growth of algae with concomitant odors and detriment to fish (373, 374, 1910) (see Phosphorus). In themselves, however, phosphates seldom exhibit toxic effects upon fish and other aquatic life (1316, 1317, 1318) and may be beneficial to fish culture by increasing algae and zooplankton (367).

A mixture of sodium tripolyphosphate and sodium pyrophosphate was not toxic to fingerling rainbow trout during a 24-hour exposure to a concentration of 545 mg/l expressed as phosphate, but a concentration of 1090 mg/l produced death to all fish. Exotic phosphate compounds may be considerably more toxic, owing undoubtedly to the cation employed. For example, Surber (898, 3628) found that tributylphosphate was toxic to bluegill sunfish at concentrations exceeding 15 mg/l. Also, a substituted fluorophosphate was toxic to carp at 20 to 40 mg/kg in fish bait (2529). Diethyl phosphate is toxic toward *Scenedesmus* at concentrations exceeding 250 mg/l, to the protozoan *Microregma* at 100 mg/l in 24 hours, and to *Daphnia* at 25 mg/l in 48 hours (2977). Dimethyl phosphate, on the other hand, is more toxic to *Scenedesmus* (toxic at 12.5 mg/l in 4 days) and less so to *Microregma* (withstanding 500 mg/l for 24 hours) and *Daphnia* (withstanding 125 mg/l for 48 hours) (2977).

Organic phosphates are used extensively in pesticides, and as such exhibit selective toxicity to many forms of aquatic life. For details, see Chapter IX—Pesticides.

According to Eldridge (2826), phosphate is the major factor controlling the growth of *Sphaerotilus*, but extremely small amounts (0.01 mg/l) of phosphate are required to implement the growth of *Sphaerotilus*. Since the Columbia Basin Irrigation Project was initiated, the average phosphates in the Columbia River have doubled, owing perhaps to the use of fertilizers.

PHOSPHINE PH_3

(see also Phosphorus)

This colorless gas with a garlic-like odor is analogous chemically to ammonia, but unlike ammonia it does not form a base with water. It is less soluble, less basic, and more toxic than ammonia (1224). At 17°C it is soluble to the extent of 260 cc per liter, or about 397 mg/l (911). As a gas, it is extremely toxic to man, with the maximum allowable concentration in the atmosphere being given as 0.05 mg/l (364).

Doudoroff and Katz (361) cite a reference to the effect that a concentration of 3.6 mg/l of phosphine in hard water was lethal to rainbow trout.

PHOSPHORIC ACIDS HPO_3, $H_4P_2O_7$, H_3PO_4

(see also Phosphates, Phosphorus, Phosphorus Salts, Chapter VIII, Chapter IX—Pesticides, Chapter X—Surface Active Agents)

With a valence of +5, phosphorus forms three types of phosphoric acids, viz HPO_3, metaphosphoric acid; $H_4P_2O_7$, pyrophosphoric acid; and H_3PO_4, orthophosphoric acid. In water solution, the meta and pyro forms tend to change to the more stable ortho condition. Orthophosphoric acid, hereinafter referred to as phosphoric acid, is of interest in this survey in that in water it dissociates in three steps, to $H_2PO_4^-$, HPO_4^{--}, and PO_4^{---} with corresponding release of hydrogen ions. At 18°C, the constants for these dissociations are 1.1×10^{-2}, 7.5×10^{-8}, and 4.8×10^{-13}, respectively (911). The degree of dissociation, therefore, is a function of pH and the proportion of phosphate ions in each of the four conditions is computed to be as follows:

pH Value	Approximate Percentage of Phosphate Ions at 18°C in Form of			
	H_3PO_4	$H_2PO_4^-$	HPO_4^{--}	PO_4^{---}
1	90.0	9.9	---	---
2	47.6	52.4	---	---
3	8.3	91.7	---	---
4	0.89	99.1	0.01	---
5	0.08	99.2	0.72	---
6	---	93.0	7.0	---
7	---	57.2	42.8	---
8	---	11.8	88.2	---
9	---	1.3	98.64	0.04
10	---	0.12	99.40	0.48
11	---	---	95.42	4.58
12	---	---	67.6	32.4
13	---	---	17.1	83.9

Between pH 2 and 7, therefore, most of the phosphate ions in water will exist as $H_2PO_4^-$ ions, and between pH 7 and 12, most will be in the form of HPO_4^{--} ions. This concept of dissociation is important for an understanding of what occurs when soluble phosphates are added to water. Also it illustrates the effect of phosphates in adding to the buffering power of water.

Phosphoric acid, per se, is used as an acidulant in beverages, in dental cements, in process engraving, and in several other industrial applications (364), but its occurrence in the partially dissociated states in polluted waters is more likely to result from the use of soluble

phosphates in industry, in cooling waters, and in synthetic detergents (see Phosphates).

Doudoroff and Katz (361) report that phosphoric acid can be directly lethal to fully developed fish in most natural waters only when the pH is reduced to 5.0 or lower. For stickleback, the lethal limit of phosphoric acid has been given as 0.01 mg/l of hydrogen ion, i.e., at pH 5 (353). In distilled water, to which phosphoric acid had been added, *Gasterosteus aculeatus* (a stickleback) survived eight hours when the pH was reduced to 4.0, but only 15 to 57 minutes when the pH was 2.2 to 3.4 (353).

Using highly turbid water and the mosquito-fish (*Gambusia affinis*) as the test organism, Wallen et al. (2940) found the 24-, 48-, and 96-hour TL_m concentrations at 22-24°C to be 138 mg/l.

PHOSPHORUS P

(see also Phosphates, Phosphoric Acid, Phosphine, Chapter VIII—Radioactivity, Chapter IX—Pesticides, and Chapter X—Surface Active Agents).

This active element does not occur free in nature, but is found in the form of phosphates in several minerals and it is a constituent of fertile soils, plants, and the protoplasm, nervous tissue and bones of animal life. It is an essential nutrient for plant and animal growth, and like nitrogen it passes through cycles of decomposition and photosynthesis (1319). It combines directly with oxygen, sulfur, hydrogen, the halides, and many metals (1224). For references dealing with the combined forms of phosphorus, see the specific cross reference. The literature cited below is that in which phosphorus has been mentioned without reference to its particular combined form or with the understanding that it pertains to total phosphorus in all forms.

Newcombe (781) discusses the loss of phosphorus as a result of silt pollution, pointing out that the Mississippi River annually removes about 62,000 tons of soluble phosphorus plus $7{,}500 \times 10^6$ cubic feet of suspended matter containing 0.15 percent phosphorus in the combined form. Obukhov (1320) states that more than 0.2 mg/l of phosphorus in ground or surface waters indicates that some phosphorus of sewage origin is present.

Extensive studies have been made of the effects of nitrogen and phosphorus in three Wisconsin lakes upon the nuisances from odors caused by algal "blooms" (375, 377, 1295). According to Sawyer (374), excessive growths of algae developed in these lakes when the average concentration of inorganic phosphorus was over 0.01 mg/l. Gardiner (1321) points out that in lakes and tarns where food supplies are scanty, the addition of nitrogen and phosphorus fertilizers has increased the amount of food available to trout and hastened the rate of growth of trout. The total fish productivity of Minnesota lakes was shown to rise linearly from 100 to 400 lbs per acre as the phosphorus was increased from 0.2 to 1.4 mg/l (3629). It appears, also, that the rate of decomposition of organic matter in fresh water depends on the concentration of phosphorus in the water (432).

Nitrogen and phosphorus concentrations of water appear to be most important factors regulating the biological productivity of a group of 17 Wisconsin lakes. Nitrogen-phosphorus ratios of 30:1, and of 15-18:1 have been reported as optimal for the growth of algal blooms. Sawyer (1450) and Curry and Wilson (3630) suggest a concentration of 0.01 mg/l of inorganic phosphorus as a maximum value permissible without the danger of supporting undesirable growths. It is pointed out that detergents are an increasingly important source of phosphorus pollution.

Total phosphorus determinations were made in regions where a heavy *Gymnodinium* "red tide" occurred off the Florida coast in November, 1952. It was found that although the phosphorus content was high in areas of definite bloom, nevertheless certain other areas minus bloom had much higher phosphorus values, as much as 10 times normal (1640). On the other hand, Ketchum (2727) reported that the total phosphorus in "red-tide" waters was 10 to 20 times that of adjacent unaffected waters.

Isom (3631) studied the toxicity of elemental phosphorus to the bluegill sunfish at 26°C, using yellow phosphorus, in distilled water adjusted to pH 7. When colloidal phosphorus was removed by filtration through a membrane filter, all fish lived for more than 6 days, but when the colloidal material was added, the fish died within 24 hours. He concluded that the TL_m of colloidal P_4 is not greater than 0.105 mg/l at 48 hours nor less than 0.025 mg/l at 163 hours.

Studies of radioactivity have revealed that phosphorus-32 is often the most abundantly concentrated radioisotope in aquatic environments. On the Columbia River below Hanford, for example, the concentration of P-32 in the caddis-fly larvae of the genus *Hydropsyche* was 370,000 times that of the water and in shiners the concentration factor was 165,000 (2535). Waterfowl that have fed from the Columbia River are reported (3702) to have concentrated phosphorus-32 by a factor of 75,000. The freshwater alga *Spirogyra* was found to concentrate P-32 by a factor of 850,000 (3273). For further details about concentration factors, see Chapter VIII—Radioactivity.

PHOSDRIN

(see Chapter IX)

PHYGON

(see Chapter IX, Dichloronaphthoquinone)

PHYTOSTEROL

Phytosterols are complex alicylic alcohols that occur in plants, in contrast to other alcohols, the cholesterols, which occur in animals. Phytosterol is present in some wastes from Kraft process pulping mills. The minimum lethal concentration to kill shiners in 120 hours at 18°C has been reported to be 3.0 mg/l (190, 344, 3632).

PICOLINE $C_5NH_4CH_3$

(see also Pyridine)

Found in coal tar, bone oil, the urine of horses, and some industrial wastes, the alpha, beta, and gamma isomers of methyl pyridine are all freely soluble in water. A colorless liquid with a strong unpleasant odor, alpha-picoline (2-methylpyridine) is used as a solvent and as

an intermediate in the dye industry. Beta-picoline (3-methylpyridine) has the same uses and also is employed in the manufacture of insecticides, waterproofing agents, and nicotinic acid. Gamma-picoline (4-methyl-pyridine) is used similarly (364). The oral LD_{50} for rats has been given (2978) as 1.29 mg/kg of body weight. The minimum concentration of picolines in water that can be detected by taste or odor is reported (2983) to be 0.5 to 1.0 mg/l.

In Lake Huron water at 12-13°C, a concentration of 5.0 mg/l of alpha-picoline had no effect on trout, bluegill sunfish or sea lamprey during a 24-hour exposure (2976). Picolines are subject to biochemical degradation in surface waters, especially when the proper seed organisms are present (1641, 3633).

PICRIC ACID AND PICRATES

(see Trinitrophenol)

PICROTOXIN $C_{30}H_{34}O_{13}$

This substance is the active constituent of fish berries that are used by East Indians to stupefy fish. It is also the bitter principle isolated from cockle. Intensely bitter, it is used as a respiratory stimulant for humans, in doses of 1.0 to 3.0 mg. Heavier doses may cause nausea, paralysis, and death.

According to Schaut (362), in stabilized tap water 4.0 mg/l killed 25 per cent of minnows exposed for 24 hours and 7.0 mg/l gave a 50-percent mortality.

PLATINIC CHLORIDE

(see Chloroplatinic Acid)

POLYETHYLENE COMPOUNDS, POLYOXYETHYLENE COMPOUNDS, AND RELATED SUBSTANCES

(see Chapter X)

POTASSIUM K

1. General. One of the more common elements, potassium constitutes 2.4 percent of the crust of the earth and occurs in many minerals (364). It is one of the most active metals and reacts vigorously with oxygen and water. For that reason it is not found free in nature but only in the ionized or molecular form. Potassium resembles sodium in many of its properties, and potassium salts can be substituted for sodium salts in many industrial applications. The sodium salts, however, are generally less expensive and hence more frequently used. For fertilizers, some varieties of glass, and for a few other purposes, however, potassium salts are indispensable (1224). Owing to the fact that the common salts of potassium, even the carbonate and hydroxide, are extremely soluble, they are not readily separated from water by natural processes other than evaporation.

The references described below are those that deal with the general subject of potassium or refer to potassium ions without mentioning the salt. For other references dealing with specific salts, see the appropriate heading hereinafter.

2. Cross References. Potassium Salts, Sodium and Sodium Salts, Dissolved Solids, Chapter V, Chapter VII (Protozoa).

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Potassium is an essential nutritional element, but in excessive quantities it acts as a cathartic. A dose of 1 to 2 grams of potassium is cathartic, and 1000 to 2000 mg/l is regarded as the extreme limit of potassium permissible in drinking water (621). According to Lockhart et al. (3241), the taste threshold for potassium from KCl was 340 mg/l and from potassium acetate it was 680 mg/l.

b. Industrial Water Supplies. Foaming may be caused in boilers by more than 50 mg/l of potassium plus sodium in the water (1214). As shown in Chapter V—Industrial Water Supply, potassium ion or potassium salts are not listed among the substances for which limiting or threshold concentrations are given.

c. Irrigation. Potassium in low concentrations is essential for plant nutrition (3634), but for good plant development it must be maintained in proper balance with other mineral requirements, such as phosphorus (269, 1322). In irrigation water, potassium and sodium act alike upon the soil, although potassium is considered less harmful than sodium (268, 348, 3634) (see also Chapter V—Agricultural Water Supply). Potassium in saline irrigation waters appears to prevent the intake of sodium chloride in excess amounts by barley seedlings (1323).

Results of the most recent studies of soil samples at the United States Salinity Laboratory indicate that exchangeable potassium has only a slight or no effect on the physical properties of soil. There is also some evidence that high concentrations of potassium in nutrient or soil solutions may be toxic to some plants; however no data are given (1642).

d. Stock and Wildlife Watering. Potassium is essential for animal nutrition (295, 995, 1049). The effects of excesses are not clearly understood (284) (see also Dissolved Solids).

e. Fish and Other Aquatic Life. The toxicity of potassium to fish is reduced by calcium, and, to a lesser degree, by sodium. Potassium is more toxic to fish and shellfish than calcium, magnesium, or sodium (306). The toxic threshold concentration of potassium for the flatworm *Polycelis nigra* has been reported to be 350 mg/l, as either potassium nitrate or chloride (608). For stickleback, the lethal concentration limit of potassium has been reported to be 50 mg/l (353, 2941).

Several investigators found, independently, that potassium could be toxic to fish in soft or distilled waters at concentrations of 50-200 mg/l when potassium chloride and nitrate were tested. It has also been reported that the threshold concentration of potassium for fish in different kinds of water is about 400 mg/l when potassium chloride, nitrate, or sulfate is used. Young eels could tolerate for more than two days 3900 mg/l of potassium in potassium chloride solution, but survived for only 15-27 hours 1950 mg/l of potassium when the nitrate or sulfate was used (1459).

It has been observed that potassium stimulates plankton growth (1295). The content of potassium in lakes in Germany, Austria, and Scandinavian countries is reported to vary from 0.4 to 1.5 mg/l in oligotrophic and mesotrophic lakes; it runs as high as 5-6 mg/l in very eutrophic lakes (1643, 3635).

For lower organisms, the toxicity level has been reported (2977) as follows: for *Gammaridae,* 200 mg/l of K; for *Chironomidae,* 700 mg/l; and for *Trichoptera* larvae, 1000 mg/l of potassium.

POTASSIUM ALUM

(see Aluminum Potassium Sulfate)

POTASSIUM BICARBONATE $KHCO_3$

(see also Alkalinity, Bicarbonates)

This highly soluble salt is used in baking powders and effervescent antacid salts (364). A concentration of 2000 mg/l of potassium bicarbonate has killed minnows and goldfish (313). It has also been reported (2980) that 50,000 mg/l in the drinking water of sheep daily for 62 days caused a moderate adverse effect on appetite and growth.

POTASSIUM BIFLUORIDE KHF_2

This highly soluble salt is used as a flux for silver solders, for frosting glass, and for treating coal to prevent slag formation (364). Simonin (3271) reports that a concentration of 100 mg/l is lethal to tench.

POTASSIUM CARBONATE K_2CO_3

(see also Carbonates, Alkalinity)

Many industrial processes, such as tanning, soap manufacture, utilize this soluble salt. Potassium carbonate is a valuable plant fertilizer (905), but in excess concentrations is highly toxic to plants (264, 268, 635).

POTASSIUM CHLORATE $KClO_3$

This highly soluble salt is used in matches, fireworks, and explosives as an oxygen donor. It is also used in printing and dyeing textiles (364). According to a German reference (2977), 2500 to 3000 mg/l is the threshold of toxicity for perch and bleak in tap water; and 1250 mg/l is the threshold value for pike in distilled water. A concentration of 1000 mg/l kills algae and fungi in 24 hours (2977).

POTASSIUM CHLORIDE KCl

1. General. Occurring in water as a natural ionized substance or as a component of brines from oil wells and other industrial wastes, potassium chloride is highly soluble.

2. Cross References. Chlorides, Hardness, Potassium, Tastes, Dissolved Solids, Distilled Water.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The taste threshold concentration of potassium chloride in water is reported to be 350 to 600 mg/l (621) and 650 mg/l (3241). Adverse effects on the nervous system and kidneys have been reported (2980) at dosages of 80-100 mg of K per kg of body weight.

b. Stock and Wildlife Watering. Most of the information available concerning the effects of potassium chloride in stock water deals with effects of mixtures of salts (287) (see Dissolved Solids, Chlorides). No adverse effects on sheep were observed when 116 grams of KCl per head per day were administered in the drinking water (1644). Chicks fed water containing 2500 mg/l of KCl for 10 days had a 33-percent mortality (2980).

c. Fish and Other Aquatic Life. Potassium chloride appears to be the most toxic to fish of the chlorides of sodium, calcium, magnesium and potassium. While calcium and sodium chlorides tend to annul the effect of potassium chloride, potassium chloride has no antagonistic effect upon the toxicity of the others (307).

The threshold concentration of potassium chloride for immobilization of *Daphnia magna* is reported to be 373 to 432 mg/l (358, 598). Concentrations up to 1250 or 1860 mg/l in various types of water have killed *Daphnia* and other related crustacean organisms within a few hours (598). In Lake Erie water at 20-25°C the following threshold concentrations were observed for aquatic invertebrates (2955):

Organism	*Threshold Concentration, mg/l for Immobilization*
Daphnia magna, young	430
Leptodora kindtii	127
Cyclops vernalis	640
Mesocyclops leuckarti	566
Diaptomus oregonensis	134

The following concentrations of potassium chloride have been reported to have killed fish:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
74.6	Distilled	4.5-15 hours	Goldfish	313
373	Distilled	12-29 hours	Minnows	313
751	Lake Erie	----	Pickerel	353, 2955
920	Turbid	96-hour TL_m	Mosquito-fish	2940
1360	Well	3 days	Some perch	644
2010	----	96-hour TL_m	Bluegills	2936, 3381
2300	----	24 hours	Small freshwater fish	1459
5500	Synthetic	24-hour TL_m	Bluegills	1459
10368	Lake Erie	----	Whitefish fry	353, 2955
12060	----	24-hour TL_m	Pickerel	353

Some perch, on the other hand, have survived exposure for three days at a concentration of 1360 mg/l in well water (644). The highest concentration tolerated by young eels for 50 hours was 7500 mg/l (1459). At a concentration of 5800 mg/l, KCl stimulated 50 percent of the water beetles (*Laccophilus maculosis*) to greater movement (2956). For the snail *Physa heterostropha* the 96-hour TL_m has been reported (2936) as 940 mg/l.

POTASSIUM CHROMATE

(see Chromium)

POTASSIUM CUPRIC CYANIDE $K_3Cu(CN)_6$

Using water from the River Havel, from which the test organisms had been recovered, Bringmann and Kuhn (2158) determined the median concentration of potassium cupric cyanide causing a threshold effect on the following organisms:

Organism	*Temperature °C*	*Time of Exposure*	*Concentration in mg/l as $Cu(CN)_6$*
Daphnia	23	2 days	0.4
Scenedesmus	24	4 days	1.2
Escherichia coli	27	1–2 days	12.0

POTASSIUM CYANATE KOCN

(see also Cyanides, Hydrogen Cyanide)

Cyanates are formed when cyanides in waste waters are treated by alkaline chlorination. According to Resnick et al. (2974), cyanates are stable in aerobic water at pH 7 and 20 for at least 10 days. Eventially, cyanates

undergo hydrolysis to yield ammonium carbonate. In anaerobic solutions, cyanates are reduced not to cyanide but to a mixture of ammonia and formic acid.

Minnows exposed for 24 hours to potassium cyanate in stabilized tap water showed no effects at a concentration of 17.1 mg/l according to Schaut (362) or 264 mg/l according to Dobson (1115). The toxic threshold to fish is 125 to 250 mg/l as reported in references quoted by Eldridge (1114).

Using water of the River Havel from which the test organisms had been recovered, Bringmann and Kuhn (2158, 3343) found concentrations producing median threshold effects to be as follows:

Organism	*Temperature °C*	*Exposure Time*	*Concentration as CNO, mg/l*
Scenedesmus	24	4 days	520
Daphnia	23	2 days	23
Escherichia coli	27	1–2 days	10
Microregma	--	2 days	21

POTASSIUM CYANIDE KCN

(see also Cyanides, Hydrogen Cyanides, and other cyanide salts)

This highly soluble salt is used in a similar fashion to, but is largely replaced by, sodium cyanide (364). Upon solution in water, the cyanide ions unite with hydrogen ions to form weakly dissociated hydrogen cyanide, thereby tending to raise the pH of the solution. For further explanation of the toxic mechanism of cyanides, see Hydrogen Cyanide.

Herbert et al. (1580, 1645, 3636) ran tests on yearling trout with KCN at 17.5°C with dissolved oxygen near saturation. The pH of the water varied from 7.4 to 8.0. In this range, about 95 percent of the HCN produced by hydrolysis of the added KCN will be undissociated (see HCN).

The mean survival times of the rainbow trout to varying concentrations of cyanide were as follows:

Concentration of Cyanide in mg/l	*Mean Survival Time, in minutes*	*Concentration of Cyanide in mg/l*	*Mean Survival Time, in minutes*
2.00	2.7	0.14	90
0.30	8.8	0.10	2520
0.25	12	0.09	1620
0.20	12	0.08	3600
0.18	25	0.07	4400
0.16	72		

Toxic concentrations of KCN toward fish have been reported as follows:

Concentration of KCN in mg/l	*Time of Exposure*	*Type of Fish*	*Remarks*	*Reference*
0.009	----	Brook trout	Reduced ability to swim	2966
0.09	48 hours	Brook trout	TL_m	2966
0.1	----	Marine fish	Irritating	3350
0.1-0.3	3-4 days	Goldfish	Lethal in hard water	313
0.12	96 hours	Bluegill sunfish	TL_m, with low DO	2954, 2957
0.14	1 hour	Trout	Helpless at 5°C	1113
0.175	73 hours	Rainbow trout	Succumbed	2920
0.22	24 hours	Blacknose dace	TL_m	2965
0.25-0.35	6 hours	Minnows	Lethal in hard water	2944
0.25-1.0	----	Fish	Lethal in unaerated water	1114
0.27	10-120 min.	Trout	Overturned	313
0.45-0.57	96 hours	Bluegill sunfish	TL_m, normal DO	2933, 2935
0.6-1.0	6 hours	Minnows	Lethal in distilled water	2944
0.7	24 & 48 hrs.	Bluegill sunfish	TL_m	2093
0.78	43-118 hrs.	Goldfish	Lethal in distilled water	313
1.6	24, 48 & 96 hrs.	Mosquito fish	TL_m in turbid water	2940
5.0	1 hour	Trout, sunfish	Lethal in lake water	2976
5.0	9 hours	Sea lamprey	Lethal in lake water	2976
15.0		Tadpoles	Lethal	313

The toxicity of KCN toward fish is more severe at low tensions of dissolved oxygen (2954, 2957). This effect appears to be most marked in the lower concentrations of KCN (2964).

Toward other aquatic and marine organisms KCN does not appear to be generally as severely toxic as toward fish. A concentration of 1.0 mg/l caused fresh-water mussels to close and lose their ability to attach to fish (313). The 96-hour TL_m for the pulmonate snail (*Physa heterostropha*) at 18°C was reported by Cairns and Scheier (2957) to 1.08 mg/l in synthetic dilution water with normal dissolved oxygen and only 0.48 mg/l with periodic low dissolved oxygen. Toward bivalve larvae, however, KCN is lethal at 0.014 mg/l.

Using water of the River Havel from which the test organisms had been recovered, Bringmann and Kuhn (2158, 3343) found median threshold effects of KCN to occur as follows:

Organism	*Temperature °C*	*Exposure Time*	*Concentration as CN in mg/l*
Scenedesmus	24	4 days	0.16
Daphnia	23	2 days	0.8
E. coli	27	1–2 days	0.4–0.8
Microregma	--	2 days	0.04

In contrast, concentrations of 65 mg/l of KCN are reported (313) to have killed *Daphnia magna*. Some animal plankton were killed by 40 mg/l of KCN and all by 200 mg/l (1117). Oxygen utilization by synthetic sewage was diminished 50 percent in 5 days by 15 mg/l of KCN (2923). Growth of the amoeba, *Pelomyxa carolinensis* was inhibited by 0.0065 mg/l of KCN (10^{-7} molar) but 10^{-9} molar concentration increased growth.

POTASSIUM DICHROMATE

(see Chromium)

POTASSIUM FERRICYANIDE $K_3Fe(CN)_6$

(see also Cyanides, Hydrogen Cyanide, and Potassium Ferrocyanide)

This compound differs from the ferrocyanide in that the iron is trivalent. Potassium ferricyanide is slowly but highly soluble in water, and an aqueous solution decomposes gradually, especially in sunlight (364). The compound is used chiefly for blueprints, but also in textile processes, as an etching liquid, and in electroplating work (364).

According to Schaut (362), minnows exposed to 17.1 mg/l of potassium ferricyanide for one hour in stabilized tap water showed no harm, and Ellis (313) quotes references to the effect that 2000 mg/l of this compound had no lethal action on minnows and goldfish. It is important to recognize, however, that the ferricyanide and ferrocyanide ions decompose slowly with the release of cyanide ion and the formation of HCN, which is much more toxic than the complex ions. Burdick and Lipschuetz (1324) recognized this phenomenon and demonstrated that concentrations of ferricyanide as low as 2.0 mg/l in the presence of sunlight became toxic to fish life (2412, 2961, 2973, 3409). In hot, bright summer weather, this limit might be even lower, according to the authors (see Potassium Ferrocyanide for details of their tests). A toxic limit as low as 0.5 mg/l of ferricyanide has been reported (353). Hiatt et al. (3350) found that 1.0 mg/l

of potassium ferricyanide produced a slight irritant activity in marine fish.

Using water from the River Havel from which the test organisms had been recovered, Bringmann and Kuhn (2158) found the median threshold effect of potassium ferricyanide to occur as follows:

Organism	*Temperature °C*	*Time of Exposure*	*Threshold Concentration in mg/l as Fe(CN)₆*
Scenedesmus	24	4 days	0.25
Daphnia	23	2 days	2.5
E. coli	27	1–2 days	over 1000

POTASSIUM FERROCYANIDE $K_4Fe(CN)_6$

(see also Cyanides, Hydrogen Cyanide, Potassium Ferricyanide)

This compound, in which the iron is in the reduced or ferrous condition, is readily soluble in water, but the complex ions decompose slowly to release cyanide ion. The industrial uses of this substance are similar to those of potassium ferricyanide (364).

Schaut (362) reports that minnows exposed for 70 days to 17.1 mg/l of potassium ferrocyanide in stabilized tap water showed no harmful effects, and Ellis (313) quotes references to show that 2000 mg/l were not lethal to minnows and goldfish. Trout survived a one-hour exposure to 8,723 mg/l in tap water without symptoms (313).

Burdick and Lipschuetz (1324) noted that fish were killed in a New York stream by the discharge of an industrial waste containing ferro- and ferricyanides when the concentration of the diluted compounds was far less than the generally accepted lethal doses (as noted above). The authors reported that these compounds decompose under the action of sunlight, especially in the presence of dissolved oxygen, to release cyanogen, HCN, and cyanides. Part of the HCN is lost to the atmosphere. In diffuse light, 4000 mg/l of potassium ferrocyanide in solution showed a cyanide content of 0.3 to 0.6 mg/l and killed all fish in 48 hours. This toxic effect is not evident in solutions of 2000 mg/l in diffuse light or in the dark. In direct sunlight, however, the following effects upon fish were noted:

Concentration of Potassium Ferrocyanide in mg/l	*Resulting Concentration of Cyanide, mg/l*	*Effects*
1	0.05 to 0.16	No harm in 5 hours
2	0.36 to 0.48	Fish died in 0.5 to 1.5 hrs.
3	0.72	Fish died in 25 minutes
5	0.50 to 0.84	Fish died in variable times

Using water of the River Havel from which the test organisms had been recovered, Bringmann and Kuhn (2158) found that the median threshold effect of potassium ferrocyanide occurred as follows:

Test Organism	*Temperature °C*	*Time of Exposure*	*Threshold Concentration in mg/l as Fe(CN)₆*
Scenedesmus	24	4 days	0.2
Escherichia coli	27	1-2 days	over 1000

POTASSIUM FLUORIDE KF

Highly soluble in water, this salt is used as an insecticide, for frosting glass, as a flux in soldering, and to prevent unwanted fermentations (364). The 24-hour oral LD_{50} for rats has been reported as 245 mg/kg of body weight (3637). For tench, a concentration of 1500 mg/l has been reported as lethal (3271).

POTASSIUM HYDROXIDE KOH

(see also Alkalinity, Hydroxides)

Sources of potassium hydroxide pollution include wastes from soap works; wood industries; dyeing, bleaching and mercerizing of cotton; paint and varnish removers; electro-plating, photoengraving, and lithography; detergents; and printing inks. The salt is highly soluble and hence occurs in water solution as potassium and hydroxide ions.

A dose of 2.6 grams of potassium hydroxide may cause death in man (353). The taste threshold of potassium hydroxide in water is reported to be 1 to 50 mg/l (621).

The following concentrations of potassium hydroxide are reported to have killed fish:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
28.6	---	24 hours	Minnows	359
50	---	24 hours	Trout	359, 353
56	---	24 hours	Bluegills	359
56	Distilled	4½ hours	Bluegills	313
80-85	Turbid	24 & 96-hour TL_m	Mosquito-fish	2940
140	---	24 hours	Goldfish	359

The following concentrations of potassium hydroxide were not harmful to fish within the period specified:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
28	---	24 hours	Bluegills	359
28	Distilled	10 days	Bluegills	313, 359

POTASSIUM IODIDE KI

(see also Iodine)

This soluble salt is used in photographic emulsions, in animal and poultry feeds to the extent of 10 to 30 mg/l, in table salt, and in analytical chemistry (364). Like iodine, it may cause iodism in sensitive individuals in doses over 0.3 to 1.3 grams.

The taste threshold of potassium iodide in chlorinated water is reported to be 0.005 mg/l (459). A concentration of potassium iodide of 10,000 mg/l in the drinking water of rats permitted fair growth, but no reproduction (287). The oral LD_{50} in mice has been reported as 1982-2068 mg/kg of body weight (3638).

Using water from the River Havel, Bringmann and Kuhn (2158) found the toxic threshold of KI to be 7.5 mg/l for *Daphnia*. For *E. coli* and *Scenedesmus*, however, a concentration of 1000 mg/l had no effect.

POTASSIUM NITRATE (Saltpeter) KNO_3

1. General. This primary source of nitrates is used extensively in industry, for fireworks, fluxes, matches, gunpowder, freezing mixtures, pickling meats, treating tobacco, and for tempering steel (364). It is highly soluble in water.

2. Cross References. Nitrates, Potassium, Dissolved Solids, Chapter V.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Potassium nitrate can be a diuretic for man and animals; large doses cause vomiting, diarrhea, muscular weakness, collapse and death (284, 623). The medicinal dose of potassium nitrate is 0.3 grams (629). A dose of 5 grams of potassium nitrate is toxic to man; and 8 to 39 grams, fatal (284).

b. Stock and Wildlife Watering. A dose of 50 grams of potassium nitrate is toxic to horses; a dose of 240 grams is fatal to cows; a dose of 30 grams is fatal to sheep and swine; and 6 grams are fatal to dogs (284, 3108).

c. Fish and Other Aquatic Life. The following concentrations of potassium nitrate have killed fish:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
127	Natural	--	Stickleback	598
162	Turbid	96-hour TL_m	Mosquito-fish	2940
181	--	One week	Stickleback	1460
258	--	4 days	Stickleback	1460
421	Turbid	24-hour TL_m	Mosquito-fish	2940
517	--	2 days	Stickleback	1460
1203	Tap	15 days	Bluegills	313, 598
1290	--	1 day	Stickleback	1460
3000	Aerated	96-hour TL_m	Bluegills	3381
5050	--	15.7 hours	Young eels	1459

The highest concentration of KNO_3 tolerated by young eels for more than 50 hours was reported (1459) to be about 1000 mg/l.

POTASSIUM PERMANGANATE $KMnO_4$

(see also Manganese)

Potassium permanganate is a powerful oxidizing agent and it is sometimes used as a bactericide (1325). It is frequently used to disinfect fish tanks and to treat certain fish infections, such as fungus growths (313). In concentrations of 0.2 to 0.5 mg/l it has been effective as an algicide in reservoirs (587).

The threshold concentration of potassium permanganate for the immobilization of *Daphnia magna* has been reported to be 0.63 mg/l (358). In river water, 5 mg/l has killed various minute crustaceans, including fish-food organisms (313). The following concentrations of potassium permanganate have been toxic or lethal to fish:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
3.0	--	--	Bluegills	3639
3.2	Tap	24-hour TL_m	Fingerling catfish	2981
4.0	--	--	Largemouth bass	3639
5.0	--	--	Fathead minnows	3639
5.2	U.S.mn.	24-hour TL_m	Bluegill sunfish	2093
5.4	U.S.mn.	48-hour TL_m	Bluegill sunfish	2093
6.25	--	24 hours	Trout	359
10	Hard	12-18 hours	Goldfish	313, 2920
11.8	--	8 hours	Young eels	1459
12	Turbid	24 & 96-hour TL_m	Mosquito-fish	2940
22–62	--	30 minutes	Fish	1326

Young eels tolerated 8 mg/l for 50 hours (1459). Concentrations of 2.0 to 2.5 mg/l of $KMnO_4$ have been used in aquaria and in the field to neutralize the toxicity of 0.05 mg/l of rotenone (3639), without harmful effects.

POTASSIUM PHOSPHATE, TRIBASIC K_3PO_4

This deliquescent crystalline solid is highly soluble in water. Using mosquito-fish *(Gambusia affinis)* in highly turbid water at 20-30°C, Wallen et al. (2940) found the 24-, 48-, and 96-hour TL_m concentrations to be 750 mg/l. This salt reduced the turbidity to less than 25 mg/l.

POTASSIUM SILICOFLUORIDE K_2SiF_6

Used in the manufacture of opalescent glass, porcelain enamels, and insecticides, this solid is only sparingly soluble in water (364). The lethal dose to kill tench has been reported (3271) as 50 mg/l.

POTASSIUM SULFATE K_2SO_4

(see also Sulfates)

This soluble salt is used in fertilizers and for the manufacture of potassium alum and other potassium salts. A dose of 45 grams has been reported as fatal for man (364). Potassium sulfate in a concentration of 869 mg/l has killed bluegills and stickleback in four days (313, 598). The 96-hour TL_m for bluegill sunfish in aerated water was reported by Trama (3381) as 3550 mg/l. The highest concentration of K_2SO_4 tolerated by young eels for more than 50 hours was reported to be about 1300 mg/l (1459).

POTASSIUM SULFIDE K_2S

(see also Sulfides, Hydrogen Sulfide)

In waters used for mixing developer solutions, even very low concentrations of potassium or sodium sulfides can cause a chemical fog (242).

POTASSIUM THIOCYANATE KSCN

(see also Cyanides, Hydrogen Cyanide, Sodium Thiocyanate)

Readily soluble in water, this compound has been used in printing and dyeing of textiles, as an intensifier in photography, and in the manufacture of artificial mustard oil (364). It has a high chlorine demand and can interfere with water purification by decreasing the bactericidal effects of chlorination (362). Schaut (362) reports that fish were killed and minnows harmed by exposure for three days to 171.4 mg/l of KSCN. When chlorinated to a 0.02 mg/l residual, however, as little as 6.0 mg/l of KSCN was toxic to fish, owing to the breakdown of the SCN^- molecule to cyanide.

A concentration of 97 mg/l of KSCN killed *Paramecium aureleaneum* in less than 24 hours but did not affect the minnow, *Gambusia holbrooki*, in 10 days of contact. A concentration of 194 mg/l killed some minnows after two days of exposure and 3880 mg/l killed all in less than two hours (353). The region of harmfulness to fish has also been reported as 1700 mg/l.

Using water of the River Havel, from which the test organisms had been recovered, Bringmann and Kuhn (2158) reported the following median threshold limits for KSCN:

Test Organism	*Temperature °C*	*Time of Exposure*	*Threshold Concentration in mg/l as SCN*
Daphnia	23	2 days	19
Scenedesmus	24	4 days	120
E. coli	27	1-2 days	over 1000

POTASSIUM XANTHOGENATE $C_2H_5OCS_2K$

This substance is very soluble in water, giving a strongly alkaline reaction. It is used in analytical chemistry, in oil flotation, and to free soil from insect pests. According to Ellis (313, 609), goldfish were killed by a concentration of 10 mg/l in hard water in two to four days, and 0.1 mg/l was fatal in five days. Goldfish were also killed by 80 mg/l in 36 hours or less and by 315 mg/l in five to eight hours. Ellis also reports that this compound decomposes rapidly and is not fatal in dilute solutions of 1:100,000 unless constantly renewed. If renewed daily, 1:10,000,000 dilution was toxic to fish. A concentration of 4.0 mg/l stopped the heartbeat of frogs, turtles, and fish immediately, 5.0 mg/l killed crustacea in five days or less, without renewal of the solution, and if renewed daily 0.01 mg/l was detrimental to plankton.

PRESSURE

While it may seem to be remote to consider pressure as a potential pollutant, momentary changes in this physical property may be extremely detrimental to one beneficial use of water, namely, the maintenance of fish and other aquatic life. Sudden changes in pressure, therefore, may be classed as a potential pollutant of the physical type.

Underwater charges of violent explosives, such as may be used in explorations for submarine oil, may be very destructive to fish. Large areas can be more or less depopulated by repeated blasts, though repopulation occurs within a few months (1646).

Fish are resistant to very large and relatively rapid changes in positive pressure, but are very susceptible to negative pressures (i.e., pressures less than atmospheric pressure), and cannot tolerate the almost instantaneous changes induced by high explosives. The tolerance of fish to explosives varies with the nature of the charge and resultant pressure changes. Dynamite and hercomite explosions are very destructive to fish; even when jetted, charges as small as 1.0 to 1.25 lbs. often killed fish; lethal-threshold peak pressures from dynamite explosions varied from 40 to 70 psi.

Black powder explosions are less harmful; charges as high as 45-90 lbs. have not caused excessive loss of fish. Peak pressures of 124-160 psi from black powder explosions did not kill caged fish (1646).

PROPIONIC ACID CH_3CH_2COOH

This colorless liquid with a pungent odor is miscible with water. It is employed in the production of propionate mold inhibitors, and in the manufacture of ester solvents, fruit flavors, and perfume (364). According to Doudoroff and Katz (361), propionic acid can be toxic to fish without lowering the pH to 5.0. Its toxicity is attributed primarily to the undissociated acid in solution; hence pH in itself is not a reliable index of dangerous pollution.

PROPYL ALCOHOL C_3H_7OH

Normal propyl alcohol is a liquid miscible with water. It is used industrially as a solvent. The oral LD_{50} for rats has been reported as 1.87 grams/kg of body weight (2978) and as 3.3 grams/kg (3248). For gudgeon (a European fresh-water fish) the lethal range of n-propyl alcohol is 200 to 500 mg/l; and for isopropyl alcohol the range is 900 to 1100 mg/l (2977). A concentration of 192,000 mg/l was required to evoke a stimulation to movement among 50 percent of a group of water beetles (*Laccophilus*), according to Hodgson (2956).

PULP AND PAPER MILL WASTES

(see Kraft Pulp Mill Wastes, Groundwood Pulp Mill Wastes, Paper Mill White Waters, Sulfite Waste Liquors)

PYRETHRUM (ALLETHRIN)

(see Chapter IX)

PYRIDINE C_5NH_5

(see also Coal Tar and Picoline)

Pyridine is a flammable, colorless liquid with a disagreeable odor and a sharp taste. It occurs in coal-tar and gas-plant wastes and may be washed from recently burned-over areas. It is miscible with water and can be used as a solvent for many organic and mineral substances.

In a concentration of 2.5 mg/l, pyridine produces a characteristic odor in water. At 1.0 mg/l it inhibits biochemical oxidation, but not at 0.5 mg/l. Albino rats fed pyridine doses of 0.125 mg/kg of body weight developed pathological effects. A maximum permissible concentration of 0.25 mg/l of pyridine in water has been suggested (3640).

According to Negus (633), pyridine is a by-product of the ammonium-sulfate treatment of waters when the ammonia is of coke origin; but inasmuch as solutions as high as 10 percent have been used therapeutically for respiratory diseases, any concentrations found in drinking water should be safe for human consumption.

Toward fish and other aquatic life the following effects of pyridine have been reported:

Concentration in mg/l	*Type of Organism*	*Effect*	*Reference*
15	Fish	Toxic action on nervous system	311, 753,
40	*Daphnia*	Threshold effect	2158
100	*Alburnus* (fish)	Threshold toxicity	2977
160	Bleak	Threshold toxicity	2904
180	Bream	Threshold toxicity	2904
200	Carp	Threshold toxicity	2904
200	Perch	No damaging effect	3405
200	*E. coli*	Threshold effect	2158
200	*Microregma*	Threshold effect	3343
400	Yearling trout	Toxic limit	3245
<1000	Perch	Lethal concentration	2920
1000	Fish	Threshold effect	313, 363, 431
1300	Mosquito-fish	96-hour TL_m in in turbid water	2940
1480-1580	Orange-spotted sunfish	Killed in one hour in tap water	363
1830	Goldfish	Killed in 10-30 hours in distilled water	363

Pyridine is subject to rapid biochemical degradation in surface waters when the proper seed organisms are

present (1641, 3633). At a concentration of 5.0 mg/l, pyridine influenced the taste of bleak and carp (2904).

PYRIDYLMERCURIC ACETATE

PYRIDYLMERCURIC CHLORIDE

(see Mercuro-Organic Compounds)

PYROCATECHOL $C_6H_4(OH)_2$

(see also Phenols, Guaiacol, and Resorcinol)

Highly soluble in water, this solid phenolic-type compound is used in photography and for dyeing fur. To distinguish it from resorcinol, it is known also as 1, 2-benzenediol. The oral LD_{50} for rats is reported as 3890 mg/kg of body weight (364).

According to Sollman (1474) the approximate 48-hour TL_m for goldfish is 14 mg/l. For perch, the lethal concentration has been reported as 5 to 10 mg/l (2977). Another authority (3405) gives the lower toxic limit for perch at 12 mg/l, while 27.5 mg/l was toxic in 20 minutes. In Lake Huron water at 12°C, the sea lamprey was not affected during a 24-hour exposure to 5.0 mg/l (2976). For minnows, 20 mg/l was toxic in 51.9 minutes (3405). Bandt (2904) reports the thresholds of toxic effect at 10 mg/l for bleak and 15 mg/l for carp. The taste of the flesh of carp is influenced by 2.5 mg/l of pyrocatechol.

Toward lower aquatic life, Bringmann and Kuhn (2158) found that the toxic threshold for *Daphnia* occurred at 4 mg/l during a 48-hour exposure at 23°C. For the diatom *Scenedesmus*, the threshold was 6 mg/l during a 96-hour exposure at 24°C. The beginning of a poisoning effect toward *Escherichia coli* at 27°C occurred at 90 mg/l. Toward protozoa, the toxic effect of pyrocatechol occurs at about 860 mg/l (2904).

PYROGALLOL $C_6H_3(OH)_3$

(see also Benzene Derivatives)

This white, odorless, crystalline substance is highly soluble in water. It is used in the dyeing of woolens and furs, for staining leather, and as a developer in photography (364). The oral LD_{50} for dogs is 25 mg/kg of body weight.

Toward goldfish in tap water at room temperature, Sollman (1474) found the approximate 48-hour TL_m for pyrogallol to be 18 mg/l.

The threshold concentration for toxic action has been reported at 20 mg/l for bleak and 50 mg/l for carp (2904). The taste of the flesh of bleak and carp is influenced at 20-30 mg/l (2904). For the sea lamprey in Lake Huron water at 12°C, 5 mg/l caused no deleterious effect in 24 hours (2976).

Bringmann and Kuhn (2158, 3343) reported the threshold of toxicity toward *Daphnia* held for 2 days at 23°C to be 18 mg/l; toward *Scenedesmus* during 4 days at 24°C to be 8 mg/l; toward *Microregma* 50 mg/l; and toward *E. coli* for 1-2 days at 27°C to be 30 mg/l. For protozoa, a toxic effect has also been reported at 375 mg/l (2904).

PYROPHOSPHATES

(see Phosphates and Phosphoric Acids)

QUATERNARY AMMONIUM COMPOUNDS

(see Chapter X)

QUINACRINE HYDROCHLORIDE

$C_{23}H_{30}ClN_3O \cdot 2HCl \cdot 2H_2O$

Known commonly as atabrine, this soluble crystalline solid is used in the prevention and treatment of malaria. The oral LD_{50} for roosters is reported as 714 mg/kg of body weight (364). Using fingerling channel catfish, Clemens and Sneed (2981) found this compound to be highly toxic. At 25°C, the 24-, 48-, and 120-hour TL_m concentrations were 0.93, 0.89, and 0.80 mg/l respectively. At 120 hours, all fish survived 0.5 mg/l but all died at 1.3 mg/l.

QUINALDINE $C_{10}H_9N$

(see also Quinoline)

This colorless, oily liquid, insoluble in water, is known also as 2-methylquinoline. The oral LD_{50} in rats is given as 1230 mg/kg of body weight (364). Toward fingerling trout at 12-13°C, a concentration of 5.0 mg/l was toxic in 60 minutes (3245).

QUINHYDRONE $C_{12}H_{10}O_4$

Slightly soluble in cold water, this green crystalline substance is used as an electrode in pH determinations. The oral LD_{50} for rats is given as 225 mg/kg of body weight (364). In Lake Huron water at 12-13°C, rainbow trout, bluegill sunfish, and sea lamprey were killed by the following concentrations at the designated times (2976):

	Time in Hours Until Death at		
Type of Fish	*5.0 mg/l*	*1.0 mg/l*	*0.1 mg/l*
Rainbow trout	1	14	No effect
Bluegill sunfish	3	4	14
Sea lamprey	9	14	No effect

In River Havel water, Bringmann and Kuhn (2158, 3343) found the threshold of toxic effect to occur at 0.4 mg/l for *Daphnia;* 0.7 mg/l for *Microregma,* a protozoan; 4.0 mg/l for the alga *Scenedesmus,* and 5 mg/l for *Escherichia coli.*

QUININE $C_{20}H_{24}N_2O_2$

In the form of quinine dihydrochloride, this alkaloid of cinchona bark is used in drugs, especially to suppress malarial attacks. It has been reported to impart a taste to water at a concentration of 0.5 mg/l (759) and at 4.0 mg/l (1578).

Hora and Nair (758) reported that pollution of water by the effluent from a quinine factory had very little injurious effect on fish. According to Clemens and Sneed (2981), the 24-, 48-, and 96-hour TL_m concentrations for quinine sulfate toward fingerling channel catfish at 23°C were 42.0, 38.6, and 34.0 mg/l respectively.

QUINIZARIN $C_{14}H_8O_4$

This orange crystalline substance is highly soluble in water. In Lake Huron water at 12-13°C, a concentration of 5.0 mg/l had no effect on rainbow trout, bluegill sunfish, or sea lamprey during a 24-hour exposure (2976).

QUINOLINE C_9H_7N

(see also Coal Tar)

Quinoline occurs in coal tar in small amounts. It is an extremely hygroscopic liquid, absorbing up to 22 percent water, but it is difficultly soluble in cold water. The commercial applications of quinoline include the manufacture of dyes and drugs, and use as a solvent for resins and terpenes. It may occur in coal-tar and gas-plant wastes.

Toward fish and other aquatic life, the following effects of quinoline have been reported:

Concentration in mg/l	*Type of Organism*	*Effect*	*Reference*
5.0	Rainbow trout	Lethal in 14 hours at 13°C	2976
5.0	Bluegill sunfish	Lethal in 4 hours at 13°C	2976
5.0	Sea lamprey	Caused illness in 4 hours at 13°C	2976
7.5	Fish	Lethal	2961, 2977
7.5–10	Yearling trout	Lethal in 60 minutes at 13°C	3245
10	Bleak, bream, carp	Toxic threshold	2904
<30	Perch	Lethal	2920
50	Perch, sunfish	Lethal	313, 363, 365, 463
50	*Microregma*	Toxic threshold	3343
52	*Daphnia*	Toxic threshold at 23°C	2158
140	*Scenedesmus*	Toxic threshold at 24°C	2158
600	*E. Coli*	Toxic threshold at 27°C	2158
750	Ciliates	Toxic	2904

According to Cole (311) quinoline paralyzes the respiratory muscles. It is reported (2904) to influence the taste of the flesh of carp at concentrations of 0.5 to 1.0 mg/l.

QUINONE $C_6H_4O_2$

With a penetrating chlorine-like odor, quinone is only slightly soluble in water. It is used in photography, dye manufacture, tanning, and as an oxidizing agent. The oral LD_{50} in rats is given as 130 mg/kg of body weight (364).

According to Hiatt et al. (3350), a concentration of 1.0 mg/l caused a slight irritant activity in marine fish, while 10 mg/l caused moderate activity from irritation. The lethal concentration for perch has been reported as 5 to 10 mg/l (2977). For p-quinone the threshold concentration for toxic effect has been reported (2904) as 0.1 mg/l for bleak, 0.2 mg/l for bream and 1.0 mg/l for carp and tench. It begins to influence the taste of flesh of tench and carp at 0.5 mg/l.

Quinone compounds are selectively toxic toward blue algae at 1.0 mg/l but not toward green algae or most higher plants (2977). In River Havel water, Bringmann and Kuhn (2158, 3343) found that the thresholds of toxicity occurred at 0.4 mg/l for *Daphnia* (23°C), 2.0 mg/l for *Microregma*, 6 mg/l for *Scenedesmus* (24°C) and 50-60 mg/l for *E. coli* (27°C). Toxic effects have also been reported for ciliates and rotatoria at 1.0 mg/l (2904).

RADIUM Ra

(see also Chapter VIII)

A product of the distintegration of uranium, natural radium has long been used in physical research, radiotherapy, and the manufacture of luminous paints. With the increasing availability of other radioisotopes, however, the need for and use of radium has decreased. Radium undergoes spontaneous disintegration with the formation of radon, one gram of radium producing about 10^{-4} ml of radon per day (364). This property is utilized in measuring radium in natural waters (3641, 3642).

Hursh (3641) measured the radium content of the raw water and tap water of 42 sources of supply, serving approximately one-fifth of the population of the U.S. The radium in raw water ranged from 0.002 to 6.54×10^{-9} mg/l with a median of 0.049×10^{-9} mg/l, whereas in tap water the range was 0.00 to 5.79×10^{-9} mg/l with a median of 0.028×10^{-9} mg/l. In general, ground-water sources contained a higher concentration of radium than surface water supplies. Hursh also measured the radium content of 25 deceased persons at Rochester, N.Y. where the radium content of tap water is close to the median of the 42 other sources of supply. The average whole-body content of radium was found to be 120×10^{-9} mg, approximately three orders of magnitude lower than the level judged safe for workers in the radium industry. Hursh (3641) concluded that the accumulation of naturally occurring radium from water and food does not constitute a health hazard for the population as a whole.

Lucas (3643) reported that the Ra-226 content of certain midwest water supplies ranged from less than 0.03 to 37×10^{-9} mg/l. Surface supplies and wells in glacial deposits or limestone formations were with rare exception found to contain less than 1.0×10^{-9} mg/l whereas wells penetrating deep sandstones had Ra-226 contents of 1.0 to 37×10^{-9} mg/l. A population of 1,000,000 was identified as consuming water in excess of 1.0×10^{-9} mg/l of Ra-226. Smith et al. (3642) measured the natural radioactivity of ground-water supplies in Maine and New Hampshire, finding the average Ra-226 activity of drilled and dug wells in Maine to be 66 and 73 pc/l respectively and in New Hampshire 6.9 and 4.1 pc/l respectively. Since one gram of radium emanates one curie, these activities correspond to 4.1 to 73×10^{-9} mg/l of radium, or somewhat higher than the results of Hursh (3641) or Lucas (3643). A tabulation of natural radioactivities of natural waters, presented by Smith et al. (3642) shows a variation from as low as 0.01×10^{-9} mg/l in the River Thames to as high as 7.1×10^{-4} mg/l for spring waters in Japan. The average for all river waters was given as 0.07×10^{-9} mg/l and for all sea waters as 0.08×10^{-9} mg/l.

The 1962 Drinking Water Standards of the U.S.P.H.S. (2036) limit the radioactivity from Ra-226 to 3 picocuries/l, corresponding to 3×10^{-9} mg/l of radium; but the WHO International and European Drinking Water Standards (2328, 2329) both limit total alpha emitters, including Ra-226, to 1.0 pc/l, or 1.0×10^{-9} mg/l. On the basis of the foregoing resume of the Ra-226 content of water supplies in the U.S., it is apparent that many of them do not meet the U.S. standards.

Tsivoglou et al. (3644, 3645, 3646, 3647, 3648) have reported on problems of radioactive waste disposal to surface waters, especially along the Animas River which receives wastes from uranium processing. Uranium is the parent of a series of 14 radioactive isotopes that occur in nature. Inasmuch as the uranium only is desired, all of the daughter elements are discarded as wastes. Radium is the most hazardous radioelement in these wastes. The bulk of the radium, perhaps 98 to 99 percent, is generally retained undissolved in tailings ponds; but the remaining dissolved portion can constitute a significant stream pollution problem, affecting domestic and industrial water supply, irrigation, stock watering, and aquatic life. Indeed, a portion of the undissolved radium also escapes to the stream and constitutes a hazard as it builds up in bottom muds.

REOVIRUSES

(see Chapter VII—Enteroviruses)

RESINS

The term "resin" is applied here to the mixture of various solid or semi-solid organic substances derived from the residue of wood pulping or the distillation of turpentine. Ellis (611), investigating a plant in Mississippi using pine for the production of turpentine, pine oil, and resin, found in the wastes some turpentine, pinene ($C_{10}H_{16}$), and turpene. In a dilution of 1:1000, this waste was rapidly lethal to fish, and few survived a 1:10,000 dilution. Lethality was attributed to certain organic acids and turpene derivatives.

Waste liquors from a synthetic resin plant had a strong odor of phenol and formaldehyde, according to Schaut (362) and produced a medicinal taste, when chlorinated, at a dilution of 1:25,000,000. At a dilution of 1:2400 in stabilized tap water, minnows became distressed in five minutes.

Resin acids, which occur in Kraft mill wastes, are toxic to fish in concentrations reported at 1.0 mg/l to 5.0 mg/l by several references (311, 344, 658, 766, 1687, 3649). The sodium salts of such resin acids are toxic to fish at concentrations of about 1.0 mg/l (190, 344, 658, 3500, 3523, 3553, 3632). *Daphnia magna* is slightly more resistant, succumbing at about 3.0 mg/l of the sodium salts of resin acids while for *Chironomous* larvae the minimum lethal concentration is about 50 mg/l (190, 574, 690, 3500, 3523, 3553).

RESORCINOL $C_6H_4(OH)_2$

(see also Pyrocatechol)

This crystalline solid is distinguished structurally from pyrocatechol in that the hydroxyl groups are in the 1, 3 positions. It is known also as 1, 3-benzenediol. Highly soluble in water, it is used in explosives, dyes, resins, and tanning operations (364). It is also used as an antiseptic agent, especially for skin diseases.

Nesmeyanova (3402) reported that the odor threshold for resorcinol was 40 mg/l in unchlorinated water, but no lower with chlorination. The threshold of toxic action toward fish was given (2904) as 35 mg/l for bleak and carp. A concentration of 30 mg/l gave the first indication of taste in the flesh of carp (2904).

According to Bringmann and Kuhn (2158) the threshold of toxic effect toward *Daphnia* occurred at 0.8 mg/l during a 48-hour exposure at 23°C; but toward the diatom *Scenedesmus* the toxic threshold was 60 mg/l at 24°C for 96 hours. Resorcinol at concentrations up to 1000 mg/l had no effect on *E. coli* at 27°C. For the protozoan *Microregma,* the threshold of toxic effect occurred at 40 mg/l (3343).

RICIN

This white powder is a highly toxic albumin that is obtained from castor seed. According to Schaut (362) 17.1 mg/l in stabilized tap water produced a 75 percent mortality among minnows in 20 hours of exposure. At 6.0 mg/l, the mortality was 50 percent after 48 hours. Chlorination appeared to have destroyed the toxicity.

ROAD DRAINAGE

(see Tars)

ROCCAL

(See Chapter X)

ROTENONE

(see Chapter IX)

RUBBER PLANT WASTES

In waste water from synthetic-rubber plants in Germany, according to Bandt (616), the substances that impart taste to the flesh of fish are the higher, partially unsaturated aldehydes and hydrocarbons. Styrol, in spite of its low solubility, imparts taste to fish. Rubber waste products are attacked and decomposed by a wide variety of bacteria and fungi, with a resulting B.O.D. and the production of CO_2 (666). Waste liquors from rubber-reclaiming plants at a dilution of 1:2400 in stabilized tap water, gave a dirty yellow color and a distinct odor, according to Schaut (362). At this dilution, minnows would not eat but they recovered their appetite when transferred to clear water. At a dilution of 1:2400 they were normal.

RUBIDIUM Rb

Occurring widely in nature in small concentrations, this alkali metal is highly reactive; hence it is found only in the combined or ionized state and never as the pure metal. Rubidium salts are very soluble in water. They occur in some mineral springs. They are used in making zeolite catalysts and also in photoelectric cells (364, 2121). Rubidium is fairly abundant in food elements, especially beans and barley, and it occurs in concentrations of 20-40 mg/kg in animal tissues (2121). There is no evidence that it serves any essential metabolic function, but it can be substituted for potassium in some physiological processes (2121). High concentrations of rubidium in a low-potassium diet for animals causes irritation of muscular nerves. There is no evidence of rubidium toxicity toward human beings (2121).

Using water from the River Havel, Bringmann and Kuhn (2158, 3343) found that the toxic threshold of rubidium, added as rubidium chloride, was 14 mg/l for the alga *Scenedesmus* and the protozoan *Microregma.*

Toward *Daphnia* and *Escherichia coli* concentrations up to 1000 mg/l had no apparent effect.

RUTHENIUM Ru

This gray, lustrous, hard metal occurs in the ores from which platinum, nickel, and copper are extracted. It also occurs in the combined form in the minerals osmiridium and laurite. Some of its salts are soluble in water. Until the advent of nuclear fission, little attention was paid to ruthenium. It was used in platinum and palladium alloys as a hardener, and for colors in ceramics (364, 2121). There is no evidence that ruthenium is highly toxic, nor does it appear to be essential for animal or plant nutrition.

As fission products of uranium, ruthenium-103 and ruthenium-106 occur in fallout from nuclear explosions. Morgan (3386) reports the following concentration factors for ruthenium-103.

Organism	*Concentration Factor*
Ochromonas (a flagellate)	4000-6500
Navicula (diatom)	7900

Since ruthenium-106 does not become adsorbed on rocks or soil, it may be expected to travel great distances in ground water (1976).

SALICYLIC ACID AND SALICYLATES $C_6H_4OHCOOH$

Salicylic acid is a crystalline substance, soluble in water to the extent of about 2000 mg/l. It is used in the manufacture of salicylates, acetylsalicylic acid (aspirin), and dyes. It is also used as a food preservative, but such use is forbidden in some countries (364).

Ruchhoft (459, 946) quotes references to the effect that, in chlorinated water, the minimum concentrations of salicylates usually giving tastes are as follows:

Substance	*Concentration in mg/l*
Salicylic acid	0.01
Sodium salicylate	0.10
Ammonium-salicylate	0.10
Salicylaldehyde	less than 0.001

In Lake Erie water at 25°C, the threshold concentration of sodium salicylate for immobilization of *Daphnia magna* with 48 hours of exposure was 1450 mg/l, according to Anderson (352). Toxicity was attributed to factors other than osmotic pressure. Hermann (2923) reported that 110 mg/l inhibited the 5-day BOD of synthetic sewage by 50 percent.

SALINITY

(see Dissolved Solids, Specific Electrical Conductance)

SALT WORKS WASTES

Ellis (1327) studied the pollutional effects of various wastes from salt works on fish and *Daphnia magna*. The wastes included bittern liquor (the supernatant fluid after natural brine has lost most of its sodium chloride), bromine-still waste and neutralized bromine-still wastes, and a concentrated solution of calcium chloride. In dilutions of 1:50, all of these effluents were dangerous to goldfish and *Daphnia magna*. The bittern liquor was harmless when diluted 1:100 and well-neutralized bromine still waste was not injurious in dilutions of 1:500. If not properly neutralized, however, it was dangerous up to dilutions of 1:1000. Fresh bromine-still waste was harmful up to 1:10,000 dilution.

SANTOBRITE

(see Chapter IX, Pentachlorophenol and Pentachlorophenates; also Chapter X)

SANTOMERSE

(see Chapter X)

SAPONINS $C_{32}H_{54}O_{18}$

Saponins are glycosides that are used in medicine and industry as foam producers. They are found in nature in many plants, and in horse-chestnut seed (777), and they occur in wastes from potato-starch and sugar-beet factories. In addition to foaming, they produce an aromatic woody odor in water in a concentration of 17.1 mg/l, and when such water is chlorinated the taste becomes medicinal (362).

The toxic limit to most fish for beet saponin or acid saponin appears to be about 5.0 mg/l (311, 458, 778). A concentration of 17.1 mg/l, however, did not affect minnows during 24 hours of exposure (362). Ellis (313) found that 10 mg/l in hard water produced marked distress in goldfish in five hours and 100 mg/l killed them in 7 to 24 hours.

SAPROPHYTIC BACTERIA

(see Chapter VII)

SARIN

(see Chapter IX)

SAWDUST

There appears to be a difference of opinion among the authors of the references dealing with sawdust in water. Ellis (779) reports that extract leached from one gram of pine sawdust in 24 hours killed fish when diluted with 20,000 parts of fresh stream water. Extracts from old sawdust piles after a slow, drizzly rain were also highly toxic to fish. Knight (540) on the other hand, claims that trout lived for two weeks in water heavily polluted with sawdust, with no trace of injury. He states, however, that sawdust in millstreams can interfere with the breeding of fish, especially those that require sandy or gravelly bottoms. Also, sawdust may destroy insect life, reduce fish-food organisms, and harm the development of young fish.

Ward (560) avers that it is incorrect to assume that pulp and sawdust from lumber and paper mills kill fish by clogging their gills, inasmuch as wastes are now known to be dangerous because of toxic substances or oxygen depletion. Healthy fish are not suffocated by solid particles and their gills become choked with debris only when the fish are weakened by other causes and are already dying.

SCHISTOSOMES

(see Chapter VII)

SCHRADAN

(see Chapter IX)

SECONDARY SODIUM ALKYL SULFATE (Teepol)

(see Chapter X)

SELENIOUS ACID H_2SeO_3

(see also Selenium)

When selenium dioxide, SeO_2, is dissolved in water, selenious acid is formed. At normal pH values, i.e. 5 to 8, this acid is only partially dissociated in water, with most of the molecules occurring as $HSeO_3^-$ (911). In stabilized tap water, minnows were not harmed during a one-hour exposure to 17.1 mg/l of selenious acid (362).

SELENIUM Se

1. General. Analogous to sulfur in many of its chemical combinations, selenium is used in its elemental form and as several salts in a variety of industrial applications, such as pigmentation in paints, dyes, and glass production; as a component of rectifiers, semiconductors, photo-electric cells, and other electrical apparatus; as a supplement to sulfur in the rubber industry; as a component of alloys; and for insecticide sprays (364, 2121, 2129). Selenium occurs in some soils as basic ferric selenite, as calcium selenate, as elemental selenium, and in organic compounds derived from decayed plant tissue. In some areas of South Dakota and Wyoming, soils may contain up to 30 mg/kg of selenium (2121). Selenium may be expected in trace quantities in the municipal sewage from industrial communities. Although selenium is found in some natural waters, food appears to be a more likely source for human ingestion (1049, 1328, 1329).

2. Cross References. Arsenic, Selenious Acid, Sodium Selenite.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Proof of human injury by selenium is scanty and definite symptoms of selenium poisoning have not been identified; but it is widely believed that selenium is highly toxic to man (353, 997, 1329, 1330). It has been stated that the symptoms of selenium poisoning are similar to those of arsenic poisoning (555).

In areas where selenium poisoning of livestock is common, the toxic effects appear to be associated with the consumption of high concentrations of selenium in food rather than in the water (152, 1331). In food stuffs 4.0 mg/kg is considered an extreme tolerance limit; and 3 mg/kg is regarded as a safer limit (353).

Mild chronic selenium poisoning has been observed in humans living in areas where the soil and produce are rich in selenium. In addition, there have been cases of selenosis at industrial establishments that use or produce selenium compounds. Hydrogen selenide (H_2Se) in the air in concentrations as low as 0.2 parts per million by volume produces definite toxic symptoms (2129).

Selenium in trace amounts appears to be essential for the nutrition of animals, including man, although very little is known about the mechanism of its action (3217). Arsenic and selenium are apparently antagonistic in their toxicity, tending to counteract each other. Selenium salts are rapidly and efficiently absorbed from the gastro-intestinal tract and excreted largely through the urine. Retention is highest in the liver and kidney (2121, 2129).

Surveys have shown that dental caries rates of permanent teeth were significantly higher in seleniferous areas than in non-seliniferous areas (2253). There is also a tendency for increased malocclusion and gingivitis in seleniferous areas (2253).

The U.S. Food and Drug Administration placed a "zero tolerance" on selenium and seleniferous materials with respect to permissible residue on fruits and other edible crops (3651).

The USPHS Drinking Water Standards (2036) have restricted selenium to 0.05 mg/l on a mandatory basis for many years (See Table 5-1). In 1962, however, the new standards lowered the mandatory limit to 0.01 mg/l. The WHO International (2328) and European (2329) Drinking Water Standards prescribe a mandatory limit of 0.05 mg/l. These strict standards were undoubtedly set because of the similarity between arsenic and selenium poisoning, the dental effect, and the known toxicity to livestock, as described below.

b. Irrigation. In general, the soil in parts of the world where selenium poisoning occurs naturally contains 1 to 6 mg/kg of selenium in the top eight inches. However, plants vary in their ability to absorb selenium; the final selenium concentration in the plant will be determined by many factors, including the species and age of the plant, season of the year, and the concentration of soluble selenium compounds in the root zone (1049).

Plants can absorb relatively large amounts of selenium from irrigation water or soil without apparent injury to themselves. Certain weeds, such as *Astragulus*, may contain as much as 4500 mg/kg of selenium (1049). Other plants grown on seleniferous soil have been found to contain as much as, or more than, 1610 mg/kg of selenium (dry weight) (1003, 1328). Byers believes that any vegetation containing 5.0 mg/kg of selenium is potentially dangerous for livestock; and that any soil containing more than 0.5 mg/kg is also hazardous (1329). Russell has pointed out that fodder containing as little as one mg/kg of selenium has caused poisoning, and states that 4.0 mg/kg is the tolerance limit of livestock (1049).

Cereals and grains concentrate selenium from the soil, the amount depending on the chemical condition of the selenium, the type of crop, and the sulfate content of the soil. High sulfates diminish the uptake of selenium. Up to 63 mg/kg of selenium have been found in wheat and up to 17.8 mg/kg in onions (2121).

Plants have been injured by selenium in the soil; wheat plants have been severely damaged by 30 mg/kg of sodium selenate in the soil (1328). However, injury to plants can be reduced or prevented by sulfate ion, when it is present in a concentration about 12 times as high as that of selenium (1328). Klintworth (1493) believes that selenium is harmful to plants at all concentrations. Walker and Rigdon (1688) have prepared an extensive bibliography of papers on selenium toxicity to plants and animals.

The USDA Regional Salinity Laboratory at Riverside, California, has not established limits on selenium, but

Dr. Beath of the Research Chemistry Department, University of Wyoming, suggests the following tentative limits of selenium in water for irrigation (3652):

Irrigation Class	*Selenium in mg/l*	*Remarks*
1-low	0.00-0.10	No plant toxicity anticipated
2-medium	0.11-0.20	Usable, but with possible long-term accumulations under particular conditions should be watched
3-high	0.21-0.50	Doubtful-probable toxic accumulation in plants except under especially favorable conditions
4-very high	over 0.50	Non-usable under any conditions

c. Stock and Wildlife Watering. Selenium poisoning ("alkali disease" or "blind staggers") occurs frequently among livestock in the Great Plains regions of the United States and Canada, and also in Mexico. It can be produced in laboratory rats, as well as livestock, by feeding abnormal amounts of inorganic selenium compounds or seliniferous feed. In general, the soil in affected areas contains 1 to 6 mg/kg of selenium in the top eight inches, but higher values have also been found. Not all such soils give rise to toxic vegetation, partly because of differences in the solubility of the selenium compounds present and their availability to plants. Where poisoning is prevalent, samples of vegetation have been found to contain as little as one mg/kg of selenium. About four mg/kg appears to be the tolerance limit for livestock. Acute poisoning is caused by ingesting large amounts.

Selenium poisoning occurs naturally among cattle, sheep, horses, pigs, and even poultry, in both chronic and acute forms. It is characterized by loss of hair from mane and tail and soreness of the feet, as well as by deformity, loss of condition, and emaciation. Among poultry, the eggs give rise to abnormal or weak chicks (1049, 1328).

Impairment of vision, weakness of limbs, and respiratory death have resulted from livestock feeding on plants containing 100 to 1000 mg/kg of selenium. At lower concentrations in vegetation, up to 24 mg/kg, livestock is subject to "alkali disease" involving lack of vitality, loss of hair, sterility, lameness, and possibly death from anemia and malnutrition (2129).

Rats show toxic effects from diets containing 3 to 40 mg/kg of selenium. A dose of 4 grams of powdered selenium introduced into a dog's stomach appeared to be harmless; but 4 mg of soluble selenate per kilogram of body weight was quickly fatal (353).

The lethal dose of selenium salts for cattle is believed to be 2 mg per animal pound (1003). As sodium selenite administered by mouth, the minimum lethal dose of selenium for horses is about 1.5 mg per kilogram of body weight; for cows and calves, 4.5 to 5.0 mg per kg; and for pigs, 6 to 8 mg per kg. Chronic poisoning of cattle has been caused by as little as 1 to 4 mg/kg of selenium in fodder (1049). Milk from cows in poisoning areas contains 0.3 to 1.2 mg/l of selenium (1049).

In water, 0.4 to 0.5 mg/l of selenium is believed to be non-toxic to cattle. Such water may contribute to selenium poisoning, but the selenium content of the feed is a more critical factor (997, 1003).

The toxicity of 9 to 18 mg/kg of selenium in the dry ration of rats, pigs, and dogs has been counteracted by various arsenic compounds administered in the drinking water (see also Arsenic) (921, 1005).

Selenium in the dry ration at a concentration of 0, 2, or 4 mg/kg did not affect the health of poultry or the hatchability of eggs. Chicks fed 5-8 mg/kg of selenium showed no harmful effects. A concentration of 5 mg/kg (dry ration) should be considered the toxic threshold for poultry (1551).

Kohlmeyer and Moxan found that 5 mg/kg of selenium (dry ration) did not affect the hatchability of turkey eggs, but 9 mg/kg caused abnormalities in young turkeys (1551).

An increase in mortality among rats fed a ration containing 11 mg/kg of Se has been caused by the presence in drinking water at concentrations of 5 mg/l of various chemicals. The presence of 5 mg/l of soluble tungsten in the drinking water reduced the mortality slightly. For a comprehensive presentation of the selenium problem, particularly in feeds, the reader is referred to the paper by Moxan and Rhian (1481).

d. Fish and Other Aquatic Life. Added as sodium selenite, 2.0 mg/l of selenium has been toxic to goldfish in eight days, and lethal in 18 to 46 days (353). Minute concentrations of selenium appear not to be harmful to fish during an exposure period of several days; however, constant exposure to traces of selenium has caused disturbances of appetite and equilibrium, pathological changes, and even deaths of fish after several weeks. Concentrations considered safe for human beings over a period of weeks have been toxic to fish (770, 3203).

Barnhart (3653) describes a reservoir in Colorado that has been stocked repeatedly with a variety of game fishes, but the fish die in a few weeks. Bottom deposits in the reservoir contain high concentrations of selenium. It is believed that selenium is passed up through the food chain to the fish which accumulate this element in the liver in lethal concentrations.

Using water from the River Havel, from which the test organisms had been recovered, Bringmann and Kuhn (2158, 3343) studied the threshold effect of selenium added as sodium selenite. For *Daphnia* the median threshold effect for a 48-hour exposure at 23°C occurred at 2.5 mg/l of selenium; for *Scenedesmus* exposed at 24°C for 4 days, the median threshold level was also 2.5 mg/l as selenium; but for *Escherichia coli* at 27°C it was 90 mg/l of selenium, and for a protozoan (*Microregma*) it was 183 mg/l.

SESONE

(see Chapter IX)

SETTLEABLE SOLIDS

(see also Suspended Solids, Silt)

Settleable solids may consist of sewage and industrial sludges, including many kinds of suspended solids that tend to settle out slowly on the stream bottom, thereby smothering bottom organisms, covering and destroying spawning beds, blanketing bacteria, fungi, and decomposing organic wastes, and in general trapping and maintaining offensive and deleterious stream conditions. Deposits of solids interfere with recreation, navigation, fish and shellfish production, and destroy esthetic values

of water. They may decompose to produce putrefactive odors and they may exude products of decomposition to overlying waters (611, 664, 673, 684, 780, 781, 782, 783, 2407, 3654, 3655).

Ellis has recommended that the stream bottom should not be blanketed to a depth of more than one-quarter of an inch by sedimentary deposits in order to prevent destruction of bottom fauna and shellfish (347).

According to many authorities, settleable solids should be removed from sewage and effluents before discharge into streams or ocean, or on land for irrigation purposes (784, 785, 786, 787, 788). Several alternative recommendations or practices have been mentioned in the literature, and most standards or criteria for streams include a provision that wastes discharged thereto should not form sludge banks or deposits. In this connection it is significant to recognize that dissolved or colloidal solids may be synthesized by bacteria in a stream to form suspended and settleable sludge consisting primarily of bacterial and biological slimes. Sulfite waste liquors, for example, contain little or no settleable solids but they can result in sizeable sludge deposits as a result of biological action in the stream (7).

Specific limiting or threshold concentrations of settleable solids permitted in natural waters are not to be found in the literature. Instead, it is generally required that settleable solids be removed from sewage or industrial wastes.

SEVIN

(see Chapter IX)

SILICA AND SILICATES SiO_2, SiO_3^{--}

1. General. The element silicon is not found free in nature but it occurs as silica in sand or quartz and as silicates in feldspar, kaolinite, and other minerals. Silicon dioxide, or silica, is insoluble in water or acids, except hydrofluoric, but it may occur in natural waters as finely divided or colloidal suspended matter, in concentrations of one to 40 mg/l (32, 2342). Silicon is used in metallurgy and silica is widely employed in industry for making glass, silicates, ceramics, abrasives, enamels, petroleum products, etc. (364). Silicates have been used in water treatment as coagulants and corrosion inhibitors while sodium silicofluoride has been used as a fluoridating agent.

2. Cross Reference. Turbidity, Suspended Solids, Silt.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Sodium silicates have been used as coagulants for the removal of turbidity and iron (1332) and dosages of 4.0 to 8.0 mg/l have been used to form protective coatings on pipes to inhibit corrosion of iron (206, 1333, 1334, 3305, 3656) or lead (1335). In concentrations found in natural or treated waters, silica or silicates appear to have caused no adverse physiological effects (36). According to Raux (178), silica in a concentration greater than 50 mg/l may cause difficulties arising from turbidity. When municipal water is used for boiler feed water, however, the presence of silicates may be detrimental (see below).

b. Industrial Water Supplies. A summary of the recommended limits of silica in water for various industrial processes, as discussed in Chapter V, is presented below:

Industrial Use	*Recommended Limit in mg/l*
Brewing	50
Boiler feed water, 0-150 psi	40
150-250 psi	20
250-400 psi	5
400 psi and over	1
Pulp and paper mills:	
Groundwood pulp	50
Soda and sulfate pulp	20
Kraft pulp, bleached	50
Kraft pulp, unbleached	100
Fine paper	20
Rayon pulp production	25

Silica appears to be particularly undesirable in boiler feed waters (212, 1336, 1337, 1338) because it passes over in the steam and deposits in the tubes of heaters and on steam-turbine blades. Silica appears to have a volatility or solubility in steam (3657), for as much as 0.4 mg/l of silica per ml of steam has been found leaving the boilers, according to Schwartz (1339), who found that silica in boiler feed water gave the following concentrations in condensate:

Silica in Feedwater, mg/l	*Silica in Condensate, mg/l*
25	0.43
100	0.07 to 0.90
200	1.8

For the maximum concentration of silica in boiler feed water, Reichelt (1689) recommended 0.5 mg/l while Stassart (1690) preferred a limit of 0.3 mg/l for high-pressure boilers. Imhoff (1340) suggested that the silica content of steam be kept below 0.1 mg/l to control turbine-blade deposits at operating pressures of 350 psi.

c. Irrigation. Silica, per se, is generally considered to be of little importance in irrigation practice (268). Limits for several constituents suggested for irrigation water by the U. S. Department of Agriculture include silica at 10 to 50 mg/l.

d. Fish and Other Aquatic Life. An abundance of silica in water, along with other necessary nutrients, favors the growth of diatoms (36, 1287) (see also Silt, Turbidity, and Suspended Solids). Indeed, blooms of diatoms synthesize silica into the tests of the organisms and thereby lower the content in the water. The diatom *Asterionella,* and possibly other algae, require silica in concentrations above 0.5 mg/l for growth in ponds, and cannot utilize silica when present at lower concentrations (1453). For factors by which silicon (radioactive or otherwise) is concentrated from water into biological forms, see Chapter VIII.

SILICON

(see Silica and Silicates)

SILT

1. General. By the term "silt" is meant loose sedimentary material, rock particles, mud, or clay that settle readily and may be transported by bed-load movement. Silt results from erosion, logging, mining, dredging, or irrigation operations, steel mills, gravel washing, the

precipitation of certain industrial dusts, such as those from limestone saw-mills, and also from operations in cotton and wool-cleansing plants (779, 2194, 2879, 3655, 3658, 3659). Fine silt may stay in suspension and be classified as "suspended solids", while the larger particles settle to the bottom of the water course. Like other suspended solids in streams, silt may have far-reaching effects on the nature and biota of the stream by reducing the amount of light transmitted by the water, by altering the rate of temperature change in water strata, by adsorbing organic matter and other substances that create unfavorable conditions on the bottom, and by blanketing the stream bottom (2183). Although a moderate amount of silt may have a beneficial effect on aquatic life by increasing the amount of mineral nutrients available (789), excessive quantities have been reported to affect deleteriously all of the desirable uses of water (346, 780, 781, 790, 2879, 2895, 3654, 3660, 3661).

2. Cross References. Suspended Solids, Settleable Solids, Turbidity.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Silt and mud are undesirable in domestic and industrial water supplies, and the cost of removing mud from water may exceed all other costs in making water suitable for use (780).

b. Irrigation. Mud constituents of water can fertilize the soil and may also be valuable for closing up cracks in the earth channels of water. However, grit and sand may injure crops (791). Silt from fertile land is beneficial, but silt from the subsoil of eroded areas deposited on the surface of productive soils can markedly decrease their productivity (246). Silt consisting of clay-like matter can result in the packing of sediment on soil to form an impervious blanket, keeping air and water out of the soil (792). Over a three-year period of observation, silty water did not affect the yields of corn as compared with the yields from plots irrigated with clear water; however, oat fields were reduced when sediment was deposited in sufficient quantities before germination to prevent the emergence of the seedlings; and heavy silting killed bean plants grown in furrows, but not those grown on ridges (793).

c. Aquatic Life. Silt in water reduces light penetration, alters the rate of temperature change in different water levels, and carries organic wastes to the bottom where processes of decay may continue to operate over a long period, leading to oxygen depletion, changes in pH, and other unfavorable conditions. Erosion and silting hinder fish production, smother food organisms, destroy spawning grounds, and fill pools (308, 611, 781, 790, 794, 795, 796, 797). Smith has reported that silting has reduced the bottom fauna of the Rogue River by 25 to 50 percent (798). According to Ellis, hard particles cause abrasive injuries to the delicate external organs, such as gills, spiracles, and fins, of miscellaneous aquatic animals, and fine particles tend to coat and destroy fish eggs (347, 799, 800). Ellis has recommended, therefore, that suspensoids of a hardness of one or more should be so finely divided as to pass through a 1000-mesh screen, and that the stream bottom should not be blanketed to more than one-quarter of an inch (see also Turbidity) (313, 347, 801).

Salmon and trout can withstand a heavy load of silt for several weeks; nevertheless, by limiting successful reproduction and the food supply, silting is harmful to fish (800). Ward (560, 882) agrees that silt deposits may smother spawning beds and nests, but believes that natural and mining silts are not always inimical to fish life; they may possibly be beneficial because of the food organisms and particles they carry and because muddy waters afford protection to fish against predatory animals and anglers. Ward cites experiments in which young salmon were maintained for three to four weeks without harm in circulating water containing a suspension of mud in a concentration above 1000 mg/l. Griffin (802) has reported experiments of three to four weeks' duration in which trout and salmon fingerlings fed and grew apparently well in muddy water carrying a constant silt load of 300 to 750 mg/l, and for short intervals daily during stirring, a load as high as 2300 to 6500 mg/l (For comparison, the Willamette River in heavy flood, when it appeared muddy, carried a silt concentration of only 42 mg/l).

Ziebell (2687) and Wagner (3662) measured the productivity in terms of aquatic insect populations upstream and downstream from a gravel dragline operation. He found a productivity decline of 85 percent as a result of the gravel operation. Turbidities were increased from zero to 91 mg/l and suspended solids from 2 mg/l upstream to 103 mg/l downstream. Tarzwell (2407) cites an instance where bottom samples from a silted area averaged only 36 organisms per sq ft whereas in a clean stream bottom the average was 249 per sq ft.

d. Shellfish Culture. Most fresh-water mussels have been unable to maintain themselves in either sand or gravel bottoms when a layer of silt one-quarter to one inch deep was allowed to accumulate on the surface (790). Silt deposits can smother both mussels (331, 795, 798) and oysters (332, 790). Silt in suspension chokes the respiratory and intestinal passages of young shellfish (314, 332, 803). Silt concentrations from 1000 to 4000 mg/l have depressed the pumping and feeding rate of oysters as much as 94 percent; and in heavy suspensions oysters have been stopped feeding entirely (804). On the other hand, some species of oysters thrive in silt-laden water (315, 804), and silt from dredging operations in the Susquehanna River did not cause oyster deaths (795).

As little as 100 mg/l of silt or other turbidity-producing substance seriously reduces the pumping rate of oysters to as low as 40 percent; 1000 mg/l caused a drop to 20 percent of the normal rate; and 3000-4000 mg/l resulted in a reduction to 4 percent. Silt, kaolin, and chalk all gave comparable results. When the oysters were returned to clear water, however, they resumed their normal pumping (1448).

e. Recreation and Navigation. Silt and mud destroy esthetic values and produce deposits in harbors that cause significant economic losses (780, 781, 1033).

SILVER Ag

1. General. In nature, silver is found in the elemental state and combined in ores such as argentite, Ag_2S, horn silver, AgCl, proustite, Ag_3AsS_3, and pyrargyrite, Ag_3SbS_3. From these ores, silver ions may be leached into

ground waters and surface waters, but since many silver salts such as the chloride, sulfide, phosphate, and arsenate are insoluble, silver ions cannot be expected to occur in significant concentration in natural waters. Nine municipal water supplies in the U.S. have shown the presence of 0.05 mg/l of silver ion and at Denver the concentration is reported as 0.2 mg/l (152). Silver nitrate is highly soluble and silver sulfate moderately so (1224).

Silver metal is used in jewelry and silverware, in alloys, for electroplating, and in the processing of food and beverages. Silver nitrate is used in photography, ink manufacture, electroplating, coloring porcelain, and as an antiseptic (364, 2121). From such sources, traces of silver can be expected to reach natural waters.

2. Cross References. Nitrates.

3. Effects on Beneficial Uses.

a. Domestic Water Supplies. Silver oxide has been used for water purification at Warsaw, Poland, and other cities. There is abundant literature on the oligodynamic action of silver and its bactericidal power, but a review of such literature is beyond the scope of this report. Dosages of 0.000001 to 0.5 mg/l of silver have been reported as sufficient to sterilize water (577, 1341, 1342, 1343, 3408, 3663, 3664, 3665). At such dosage rates, silver is not an irritant, has no toxic action toward humans, and does not interfere with the taste (1344). The fatal dose of silver nitrate for humans has been reported as 10 grams (2129).

Silver occurs in soil humus up to 5 mg/kg, in grain and cereals up to 0.9 mg/kg, and in mushrooms up to several hundred mg/kg (2121). Silver nitrate solution has been used as an antiseptic, especially in the upper respiratory system.

Prior to 1962, no restriction was placed on silver in the USPHS Drinking Water Standards (2036), nor does any limitation presently appear in the WHO International or European standards (2328, 2329). The 1962 edition of the USPHS Standards (2036) sets a mandatory limit of 0.05 mg/l of silver. Presumably, this restriction was set not because of the danger of silver in natural sources of water supply but to prevent its intentional addition to water, in excessive amounts, for disinfection. The limit was based on cosmetic considerations rather than public-health effects.

The chronic administration of silver salts, not uncommon in the early part of the 20th century, led to a bluish permanent darkening of the skin called "argyria". With prolonged administration of silver salts, a small proportion is absorbed and deposited in the skin where it is reduced by light to metallic silver (2129). This impregnation of the tissues with silver causes no recognizable disturbance to health (3704), but is obviously undesirable from an esthetic viewpoint. Although the total dosage of ingested silver required to produce argyria is not known, the intravenous injection of 1 gram will cause this effect. Based on the USPHS limit of 0.05 mg/l of silver and two liters per day of water consumption, it would take over 27 years to ingest one gram of silver, a large part of which would pass through the intestinal tract without assimilation (2121). This calculation ignores the daily intake of silver from food, which might be appreciable for mushroom lovers.

b. Fish and Other Aquatic Life. Using silver nitrate, Marsh and Robertson (1459) found that 0.04 mg/l killed some (but not all) test salmon fry in tap water within 48 hours; 0.44 mg/l of silver nitrate proved decidedly toxic. A concentration of 0.033 mg/l of silver nitrate did not prove fatal in 48 hours. The highest concentration of silver nitrate tolerated by young eels for more than 24 hours was reported to be about 0.2 mg/l. Toward guppies, the LD_{50} value was found to be 0.0043 mg/l, as silver (2921).

Jones (1460) found that the lethal concentration limit of silver applied as silver nitrate, for sticklebacks at 15-18°C was 0.003 mg/l. In different concentrations of silver the average survival times of the fish were as follows: one week at 0.004 mg/l, four days at 0.01 mg/l and only one day at 0.1 mg/l, as silver. Anderson (598) reported that the toxic threshold of silver nitrate for sticklebacks was 0.0048 mg/l.

The level of toxicity of silver toward lower organisms has been reported by several observers. Using water from the River Havel from which the test organisms were recovered, Bringmann and Kuhn (2158, 3343) studied the threshold effects of silver, added as silver nitrate, on various species during a 4-day exposure, at 23-27°C. For *Daphnia* and for *Microregma* the median threshold effect occurred at 0.03 mg/l of silver, for *E. coli* at 0.04 mg/l, and for *Scenedesmus* at 0.05 mg/l. Jones (608) tested the toxicity of silver nitrate toward *Polycelis nigra*, a flatworm, and found the threshold concentration to be 0.15 mg/l of silver. Toward *Daphnia magna* in Lake Erie water at 25°C, the threshold for immobilization was reported (598) to be 0.0051 mg/l of silver nitrate. Hodgson (2956) studied the stimulating effects of several substances on the water beetle, *Laccophilus maculosus*. He found that 580 mg/l of silver nitrate stimulated 50 percent of the beetles into movement, but no lethal dose was reported.

The Severn and Mersey River Boards in England (2950) have set working standards of 1.0 mg/l on the total of all heavy metals, including silver.

SILVEX

(see Chapter IX)

SIMAZINE

(see Chapter IX)

SINOX

(see Chapter IX)

SKATOLE C_9H_9N

This constituent of coal tar, feces, and some plants is soluble in hot water, giving a fecal odor (364). The odor is detectable at a concentration of 0.0012 mg/l (1756).

SLAKED LIME

(see Calcium Hydroxide)

SOAPS

(see also Chapter X)

Soaps are salts of fatty acids, usually made by the saponification of a vegetable oil with sodium hydroxide.

They may be formed also by resinous acids in the Kraft process at paper mills.

Toward fish, crude sulfate soap from Kraft mill wastes had a minimum lethal concentration of 5.0 mg/l toward minnows and shiners, according to research by the National Council for Stream Improvement (190, 344, 574, 658, 690, 3500, 3523). LeClerc and Devlaminck (3666) report that for minnows the minimum lethal concentration of sodium palmitate and sodium oleate was 10-12 mg/l in distilled water and 900-1000 mg/l in hard water. For sodium stearate it was 10-12 mg/l in distilled water and 250-300 mg/l in hard water. The effect of water hardness was confirmed by Henderson et al. (1910) who found that the 96-hour TL_m values for three packaged soaps ranged from 29 to 42 mg/l in soft water but averaged 1470 mg/l in hard water. Soaps form insoluble complexes or precipitates in hard water which are apparently non-toxic to fish. The pure compound, sodium stearate, was somewhat less toxic than the packaged soap products (1910).

Cole (311) claims that dairy wastes contain soaps that are toxic to fish at 600 mg/l and Schaut (362) reports that soapy waste waters at a dilution of 1:240 had no effect on minnows. Turnbull et al. (2093) reported that the 48-hour TL_m of truck-washing soap solution toward bluegill sunfish at 20°C occurred at dilutions of 1:357 to 1:416.

Toward other aquatic organisms, crude sulfate soap from Kraft mills had a TL_m of 50 mg/l for *Chironomus* larvae, 10 mg/l for mayfly larvae, and 5.0 mg/l for *Daphnia magna* (190, 574, 690, 3500, 3523). Soaps in concentrations of 1000 to 2500 mg/l were lethal to mosquito pupae by reducing the surface tension of the water so that the pupae were unable to maintain their position and suffocated (805). Solutions containing 5,000 to 10,000 mg/l were directly toxic to the pupae. In another instance (756) soap in a concentration of 2000 mg/l completely destroyed mosquito larvae and pupae.

SODA PULP MILL WASTE

(see Kraft Pulp Mill Wastes)

SODIUM Na

1. General. This very active metal does not occur free in nature, but sodium compounds constitute 2.83 percent of the crust of the earth (364). Owing to the fact that most sodium salts are extremely soluble in water, any sodium that is leached from soil or discharged to streams by industrial wastes will remain in solution. Some sodium may be removed by ion-exchange phenomena in the passage of ground waters through certain soils. For other effects of sodium on soils, see Chapter V—Agricultural Supply.

Sodium is the cation of many salts used in industry and as such is one of the most common ions in process wastes. The information presented below pertains to literature in which the sodium ion, per se, was mentioned without reference to the sodium salt. Where concentrations were given in terms of the salt, the literature has been abstracted under the appropriate sodium compound.

2. Cross References. Sodium Salts, Potassium, Dissolved Solids, Chapter IV, Chapter V.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Sodium in drinking water may be harmful to persons suffering from cardiac, renal, and circulatory diseases (1242, 3667), and as much as 200 mg of sodium from drinking water may be injurious (152). Although it has been reported (627) that drinking water of good quality may contain up to 115 mg/l of sodium, Hibbard (250) recommends a limit of 10 mg/l as desirable. According to Lockhart et al. (3241) the taste threshold of sodium in distilled water varies with the sodium salt used, as follows:

Salt Tested	*Taste Threshold of Sodium in mg/l*
Sodium acetate	140
Sodium carbonate	34
Sodium bicarbonate	290
Sodium chloride	135
Trisodium phosphate	75

b. Industrial Water Supplies. More than 50 mg/l of sodium plus potassium in boiler waters may cause foaming (1214). Other limitations for sodium, as given in Chapter V, are expressed in terms of the sodium salts (see Sodium Chloride, Carbonate, and Sulfate).

c. Irrigation. Sodium is required in very limited amounts for most plant growth (3634). An exception is the desert plant, halogeton, which apparently needs sodium for optimum growth (3668). In high concentrations sodium is not only toxic to plants but deleterious to soil conditions (268, 635, 1322) (see also Chapter V—Irrigation). Sodium carbonate and bicarbonate are more harmful than other sodium salts to plants (273). On a weight basis, chlorides are generally more toxic to plants than sulfates, but at equal osmotic pressures, sodium sulfate appeared to be more injurious to flax than sodium chloride (1093).

High concentrations of sodium (e.g. 106-212 mg/l) in water used for sprinkler irrigation can, under certain conditions and with a few plants, cause deleterious accumulations of sodium in the foliage (3669, 3670). Sufficient sodium has been absorbed by citrus leaves from a single sprinkling with water containing 69 to 190 mg/l of sodium to cause serious leaf burn and defoliation (3670).

Sodium-saturated soils are greasy to feel. Sodium soil colloids swell, closing the pores of the soil, reducing soil permeability to water and air, and increasing the pH of the soil solution to dangerous levels. The deterioration of soil quality because of sodium in the irrigation water is a steadily cumulative process, with impaired drainage resulting in even more highly concentrated soil solutions (280).

Kelley reported that an average concentration of 82 mg/l of sodium in water from the Colorado River was not expected to affect seriously either the growth of citrus trees or soil conditions in the South Coastal Basin of California (1060). For an instance where sodium figured prominently in a court case, see *Barakis vs American Cyanamid Co.* in Chapter IV.

d. Stock and Wildlife Watering. Excess concentrations of various sodium salts in the drinking water are deleterious to various animals. Well waters containing 7000 mg/l of sodium were toxic to chicks (1551). A threshold limit of 2000 mg/l of sodium for livestock has been suggested by Stander (3373). For further details,

see Sodium Salts, Dissolved Solids, Chapter V. Sodium bicarbonate is less injurious than sodium carbonate (640).

e. Fish and Other Aquatic Life. Of the United States waters supporting a good fish fauna, ordinarily the concentration of sodium plus potassium is less than 6 mg/l in about 5 percent; less than 10 mg/l in about 50 percent; and less than 85 mg/l in about 95 percent (310).

The toxicity threshold of sodium for *Polycelis nigra* is 4370 mg/l from sodium chloride, and 1000 mg/l from sodium nitrate (353, 608). A sodium concentration of 500 mg/l is lethal to stickleback (353). On the other hand, moderate amounts of sodium in water may reduce the toxicity of potassium and ammonium salts toward fish (306).

The possible accumulation of radioactive sodium (Na-24) in aquatic life, fish, or oysters is not considered to be hazardous owing to the short half life (about 15 hours) of radiosodium (2442).

Several investigators have reported independently that 500-1000 mg/l of sodium was toxic to fish in distilled and soft waters when sodium chloride or nitrate was tested 1450, 2941). However, Gueylard reported as harmless to sticklebacks concentrations up to 4720 mg/l of sodium in distilled water and up to 7870 mg/l in tap water when sodium chloride was added (1459).

Freeman and Fowler (1691), using a standardized double-distilled water, studied the toxicity of five inorganic sodium salts to *Daphnia magna* at 23°C. The 100-hour toxicity thresholds were determined for each salt alone, in combinations of two, and in combinations of three. The 100-hour toxicity threshold was defined as the concentration that will immobilize 50 percent of the organisms in 100 hours. Rather than list their results separately under the specific sodium salts involved, all of their data are presented under this heading in order that the magnitudes can be compared. For each of the five salts tested singly, the 100-hour toxicity thresholds and the resulting pH values are shown in the following tabulation:

Compound Tested	*100-Hour Toxicity Threshold in mg/l*	*pH at Threshold*
Sodium bisulfite	102	7.3
Sodium carbonate	524	9.5
Sodium chromate	0.42	7.8
Sodium silicate	247	9.1
Sodium sulfate	4547	7.7

When these salts were tested in combinations of two, the results were as follows:

Compounds Tested	*100-Hour Toxicity Thresholds in mg/l*	*pH at Threshold*
Sodium bisulfite Sodium carbonate	85 436	9.2
Sodium bisulfite Sodium chromate	70 0.286	7.3
Sodium bisulfite Sodium silicate	177 427	7.5
Sodium bisulfite Sodium sulfate	82 3642	7.5
Sodium carbonate Sodium chromate	408 0.33	9.3
Sodium carbonate Sodium silicate	180 85	9.3
Sodium carbonate Sodium sulfate	221 1918	9.2
Sodium chromate Sodium silicate	0.159 93	8.7
Sodium chromate Sodium sulfate	0.276 2984	7.9
Sodium silicate Sodium sulfate	158 2899	9.0

In combinations of three, the toxicities of the sodium salts were as follows:

Compounds Tested	*100-Hour Toxicity Thresholds in mg/l*	*pH at Threshold*
Sodium bisulfite Sodium carbonate Sodium chromate	87 440 0.35	9.3
Sodium bisulfite Sodium carbonate Sodium silicate	38 194 92	8.8
Sodium bisulfite Sodium carbonate Sodium sulfate	58 295 2562	9.0
Sodium bisulfite Sodium chromate Sodium silicate	144 0.861 506	6.9
Sodium bisulfite Sodium chromate Sodium sulfate	75 0.306 3312	6.9
Sodium bisulfite Sodium silicate Sodium sulfate	52 126 2308	8.5
Sodium carbonate Sodium chromate Sodium silicate	182 0.146 86	9.1
Sodium carbonate Sodium chromate Sodium sulfate	240 0.192 2079	9.3
Sodium carbonate Sodium silicate Sodium sulfate	155 73 1343	8.8
Sodium chromate Sodium silicate Sodium sulfate	0.201 119 2180	8.5

From the foregoing tabulations it is noted that the toxicity of sodium salts depends largely on the anion involved, the chromate being exceedingly toxic and the sulfate least so. Moreover, there appears to be no correlation between the toxicity displayed and the pH value, but since *Daphnia magna* is known to live within the pH range 6.2-9.5, which embraces all of the foregoing results, pH was apparently not the sole cause of mortality. It is also noted that the thresholds for specific compounds are lower when tested in combinations of two or three with the exception of sodium bisulfite with sodium silicate, or with sodium silicate and sodium chromate. This latter variation may be attributable to the possible oxidizing action of the chromate on the bisulfite (1691).

SODIUM ACETATE $NaC_2H_3O_2 \cdot 3H_2O$

Highly soluble in water, this compound is used as a mordant in textile dyeing, as a buffering agent in chemical production, in photography, and in medicine as a diuretic, expectorant, or alkalizer (364). According to Lockhart et al. (3241), the taste threshold of the anhydrous salt is 500 mg/l, or for the crystalline form 830 mg/l. Hodgson (2956) studied the stimulating effects of

sodium acetate on the water beetle (*Laccophilus maculosis*) and found that a concentration of 32,600 mg/l was required to stimulate the movement of 50 percent of the beetles.

SODIUM ACID PYROPHOSPHATE $Na_2H_2P_2O_7$

Soluble in water with an acid reaction, this white fused substance is used chiefly in baking powder (364). A concentration of 400 mg/l in sea water caused a decrease in irritability of oysters, but 500 mg/l caused oyster deaths within 44 hours (1448, 1467).

SODIUM ALKYL ARYL SULFONATE

SODIUM ALKYL BENZENE SULFONATE

SODIUM ALKYL SULFATE

(see Chapter X)

SODIUM ALUMINATE $NaAlO_2$

This highly soluble salt is used in water treatment, for sizing in paper mills, and for printing in textile plants (364). Wallen et al. (2940) tested its toxicity toward mosquito-fish (*Gambusia affinis*) in turbid water at 17-19°C. They found 24-, 48-, and 96-hour TL_m values of 199, 126, and 111 mg/l respectively. This salt coagulated the water and reduced turbidity.

SODIUM ANTHRAQUINONE-α-SULFONATE

SODIUM ARYL ALKYL POLYETHER SULFONATE

(see Chapter X)

SODIUM ARSENATE Na_2HAsO_4

(see also Arsenic, Arsenic Trioxide, Sodium Arsenite)

This colorless, odorless salt, readily soluble in water, is used in dyeing and printing fabrics and in various fungicides and insecticides.

It is not highly toxic to fish or other aquatic life. According to Jones (2120) the lethal concentration of sodium arsenate toward minnows at 16-20°C is 234 mg/l, as arsenic. It has also been reported (617) that minnows survived for 16 hours in a solution of sodium arsenite equivalent to 250 mg/l of arsenic.

Toward lower forms of aquatic life, the toxicity of sodium arsenate is variable. The threshold concentration for immobilization of *Daphnia magna* in Lake Erie water has varied from 18 to 31 mg/l as sodium arsenate, or 4.3 to 7.5 mg/l as arsenic (352, 353, 358). The toxic threshold for the flatworm *Polycelis nigra*, is reported to be 670 mg/l as AsO_4, or 361 mg/l as arsenic (354).

SODIUM ARSENITE $NaAsO_2$

(see also Arsenic, Arsenic Trioxide, Sodium Arsenite)

Freely soluble in water, sodium arsenite is used in dyeing, in arsenical soap making, in termite extermination, and in weed-killers. As a commercial preparation it is often a mixture of several sodium and arsenic compounds. Therefore, references to sodium arsenite solutions are often reported in terms of arsenic concentration or as arsenic trioxide equivalents.

Sodium arsenite has been used extensively as a herbicide for the control of mixed submerged vegetation in static water. Commercial sodium arsenite contains varying amounts of other arsenic compounds and impurities; hence it is labeled in terms of equivalent arsenic trioxide (As_2O_3). For the control of submerged vegetation in ponds and lakes, applications of 2 to 5 mg/l as arsenic trioxide (1.5 to 3.8 mg/l as As) have been found effective. Although these concentrations are generally considered to be safe for fish and other aquatic animals, it is advisable to spray only a part of the pond or lake at one time so that fish may avoid the sprayed area. Fish are reported to be sensitive to sub-lethal doses of sodium arsenite and will generally swim away into fresh water (890, 2861, 3671). Commercial sodium arsenite in concentrations up to 10 mg/l (arsenic content not stated) have been used for weed control in Wisconsin lakes without harm to the fish population (1006).

The following concentrations of sodium arsenite, expressed as arsenic, have been reported as lethal, toxic, or otherwise deleterious to aquatic organisms:

Concentration, as Arsenic, mg/l	*Time of Exposure*	*Organism*	*Reference*
1.0-2.0	--	Fish	1692
1.4-2.3	--	Fish food organisms	574, 1007
1.9-3.0	--	Midges, mayflies, amphipods	3009
4.6	48 hours	*Daphnia*	2158
5.0	10 days	Pink salmon	2091
5.0	48 hours	*Microregma* (protozoan)	3343
5.2	--	*Daphnia magna*	352
8.4	48-hour TL_m	Chum salmon fry	2900
15	48 & 72-hour TL_m	Fingerling channel catfish	2981
17.8	--	Minnow	2920
20	36 hours	Rainbow trout, minnows	3005
20	36 hours	Minnows	617
27	72-hour TL_m	Minnows	3672
27.6	24-hour TL_m	Fingerling channel catfish	2981
29	48-hour TL_m	Minnows	3672
35-46	48 hours	*Scenedesmus*	2158
45	24-hour TL_m	Minnows	3672
290	48 hours	*Escherichia coli*	2158

The literature on weed control contains statements that applications of sodium arsenite up to 10 mg/l as As_2O_3 (7.6 mg/l as As) are not harmful to fish, but the foregoing table would indicate that the threshold level is somewhat lower than expected. Furthermore, fish-food organisms are susceptible at concentrations as low as 1.0 mg/l.

On the other hand, the following concentrations of sodium arsenite, expressed as arsenic, have been reported to have no harmful effects on the organism noted:

Concentration, as Arsenic, mg/l	*Time of Exposure*	*Organism*	*Reference*
1.3	---	Fish or fish-food organism	1007
1.3-1.5	---	Fish-food organisms	1007, 1008
1.4-2.9	---	Fish	416
2.9	---	Chironomus larvae	574
5.0	24 hours	Trout, bluegills, sea lamprey	2976
8-16	---	Damselflies, dragonflies, sowbugs, water mites	3009
15	96 hours	Minnows	3672

Lawrence (3673, 3674) reports that two applications of 4 mg/l of sodium arsenite applied one month apart in experimental ponds reduced the number of bottom organisms an average of 34 percent and reduced bluegill production an average of 42 percent as compared with control ponds. Two applications of 8 mg/l of sodium arsenite applied one month apart reduced the number of bottom organisms an average of 45 percent and bluegills 65 percent as compared with control ponds. An application of 4 mg/l killed all microcrustacea and greatly reduced the population of rotifers.

SODIUM AZIDE NaN_3

Highly soluble in water, this crystalline solid is used in chemical manufacturing and in medicine as a hypotensive agent (364). Using water of the River Havel, Bringmann and Kuhn (2158, 3343) determined the thresholds of toxicity to be 0.3 mg/l for *Daphnia*, 3 mg/l for *Microregma* (a protozoan), 4 mg/l for *Scenedesmus* (an alga), and 19 mg/l for *Escherichia coli*. Toward trout, bluegills, and sea lamprey in Lake Huron water at 12°C, 5 mg/l of sodium azide had no apparent effect during a 24-hour exposure (2976).

SODIUM BENZENE SULFONATE

(see Chapter X)

SODIUM BENZOATE C_6H_5COONa

(see also Benzoic Acid)

This highly soluble salt is used as a preservative in food products with concentrations up to 1000 mg/kg being permitted. The oral LD_{50} for rats is given as 4100 mg/kg of body weight (364).

In Lake Erie water at 25°C, the threshold concentration of sodium benzoate for immobilization of *Daphnia magna* with 48 hours of exposure was less than 650 mg/l, and Anderson (352) estimated the true threshold concentration to be about 585 mg/l. Toxicity was due to factors other than osmotic pressure.

SODIUM BICARBONATE $NaHCO_3$

1. General. This freely soluble salt dissociates completely in water into sodium and bicarbonate ions. The concentration of bicarbonate ions, in turn, depends upon the resulting pH value (see Bicarbonates, Carbonates). The bicarbonate acts as a powerful buffering agent. Sodium bicarbonate is used in the manufacture of many sodium salts, as a source of carbon dioxide, in fire extinguishers, in effervescent salts and baking powder, in cleaning compounds, and in many industrial processes.

Goudey (992) suggested classification of ground waters and included the following recommendations as to sodium bicarbonate limits: Class AA (domestic), 0 mg/l; Class A (irrigation), 0 to 50 mg/l; Class B (irrigation), 50 to 100 mg/l; and Class C (irrigation and industry), more than 100 mg/l.

2. Cross References. Sodium Carbonate, Bicarbonates, Chapter V.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The taste threshold of sodium bicarbonate in water is reported (3241) to be 1060 mg/l.

b. Industrial Water Supplies. It has been recommended (173) that the sodium bicarbonate concentration of raw water to be used for making ice not exceed 35 mg/l.

c. Irrigation. Water containing sodium carbonate or bicarbonate as the predominant salts may be more harmful to plants than water with other sodium salts (273). Sodium bicarbonate, although it is an important buffer in water, may be directly or indirectly harmful to plants. It may be toxic, it tends to increase soil alkalinity, and in addition, by precipitating calcium carbonate out of solution, it reduces calcium concentration of the soil and soil permeability (264, 268). A concentration of 1500 to 2000 mg/l of sodium bicarbonate in the water has caused a 50-percent reduction in the dry weight of tomatoes grown in liquid culture (1345). In irrigation water 4000 mg/l has rendered soils alkaline and impaired their fertility (921).

d. Stock and Wildlife Watering. The following concentrations of sodium bicarbonate in drinking water have been reported as injurious to animals by causing thirst, diarrhea, and impaired growth:

Concentration in mg/l	*Animal*	*Reference*
6000	Chicks	287, 284, 2980
15000	Rats	287, 284
20000	Rats	287, 284

Concentrations from 12,000 to 24,000 mg/l have caused deaths among chicks (284).

e. Fish and Other Aquatic Life. The toxic threshold concentration of sodium bicarbonate for *Polycelis nigra*, a flatworm, was found to be 7140 mg/l (354). The threshold concentration of sodium bicarbonate for immobilization of *Daphnia magna* in Lake Erie water was reported to be 2350 mg/l (352) and 4200 mg/l (358). Cairns et al. (2933, 2934, 2935) found the 96-hour TL_m values for bluegill sunfish in standard dilution water at 20°C to be 8250 to 9000 mg/l depending on the size of the fish. Using highly turbid water at 20-22°C and the mosquito-fish as the test organism, Wallen et al. (2940) found the 96-hour TL_m to be 7550 mg/l. The 24-hour TL_m was 7700 mg/l.

SODIUM BIFLUORIDE $NaF \cdot HF$

(see also Sodium Fluoride)

This highly soluble powder produces an acid reaction in solution; hence it is used as a "sour" in laundrying (364). According to Simonin (3271) the fish *Tinca vulgaris* is killed by 100 mg/l of this compound.

SODIUM BISULFATE $NaHSO_4$

(see also Sulfates, Sulfuric Acid)

Known also as sodium acid sulfate, this compound is freely soluble in water and dissociates into Na^+ and HSO_4^- ions. The concentration of the bisulfate ion in solution depends upon the resulting pH value and above pH4 almost all bisulfate will be converted to sulfate ion.

For immobilization of *Daphnia magna*, the threshold concentration of sodium bisulfate in Lake Erie water was found to be less than 145 mg/l (352). Later work by Fairchild (2946) showed that the threshold toxicity value is dependent on dissolved oxygen. For *Daphnia*

exposed 100 hours at 23°C, the threshold concentrations were as follows:

Dissolved Oxygen, mg/l	*Threshold Level of Toxicity, mg/l*
6.5	153
8.9	145
1.55	106

SODIUM BISULFITE $NaHSO_3$

Used as a disinfectant and bleach in textile, laundering, paper, and fermentation industries, this compound is freely soluble in water, dissociating into Na^+ and HSO_3^- ions. The concentration of the undissociated bisulfite ion depends upon the resulting pH value; furthermore, it is readily oxidized to sulfate (364).

The toxicity threshold (100-hours at 23°C) to *Daphnia magna* has been reported at 102 mg/l (1691), but in the presence of other sodium salts, this threshold may be somewhat lower (see Sodium). Fairchild (2946) also studied the toxicity of sodium bisulfite toward *Daphnia* at various concentrations of dissolved oxygen at 23°C, with the following results:

Dissolved Oxygen, mg/l	*Threshold Level of Toxicity, mg/l*
9.3	109
6.5	61
3.2	77
1.6	70

Le Clerc et al. (2942, 2943, 2944) exposed minnows for 6 hours to solutions of sodium bisulfite in distilled and hard water. The minimum lethal dose in distilled water at 19°C was 60-65 mg/l and in hard water at 18°C it was 80-85 mg/l. Using turbid water at 17-22°C and the mosquito-fish *(Gambusia affinis)* as the test organism, Wallen et al. (2940) found the 24-, 48-, and 96-hour TL_m values to be 240 mg/l. This concentration greatly reduced the turbidity.

SODIUM BORATE $Na_2B_4O_7 \cdot 10\,H_2O$

(see also Boron, Sodium Perborate)

Commonly known as borax, this compound is used extensively as a cleaning agent in homes and industry. It is also employed as a wood preservative, as a treatment against wood fungus, in tanning operation, and for the control of aquatic weeds (898). Certain other borates, such as phenyl mercury borate in a concentration of 10 mg/l, have been used effectively against the growth of slimes in pipes in paper mills (1346).

According to Le Clerc and Devlaminck (2942, 2943, 2944), the minimum lethal dose of sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) toward minnows was 3000 to 3300 mg/l at 19°C in distilled water and 7000 to 7500 mg/l at 17°C in hard water. Toward mosquito fish *(Gambusia affinis)* in turbid water, Wallen et al. (2940) found the 96-hour TL_m at 22-26°C to be 3600 mg/l.

With respect to lower aquatic life, sodium borate appears to be more toxic than towards fish. The threshold concentration for immobilization of *Daphnia magna* was reported (352) as being much less than 240 mg/l and was estimated at 120 mg/l (353). The toxic threshold concentration of sodium borate for *Polycelis nigra*, a planarian worm, was found to be 1530 mg/l as borate (354).

SODIUM BROMATE $NaBrO_3$

(see also Bromine, Sodium Bromide)

Highly soluble in water, this crystalline salt is used in ore processing and for promoting the growth of yeast cells. The oral MLD for rabbits is given at 580 mg/kg of body weight (364).

SODIUM BROMIDE NaBr

(see also Bromine)

Used primarily in photographic work, this salt of bromine is highly soluble in water. In water supplies used for preparing photographic solutions, bromides slow up the rate of the chemical reactions (242). The toxicity threshold concentrations of sodium bromide reported for *Daphnia magna* in Lake Erie water at 25°C range from 4100 to 8200 mg/l (352, 353). The toxic threshold concentration of sodium bromide for *Polycelis nigra*, a flatworm, was found to be 14,400 mg/l (354). To stimulate the movement of water beetles *(Laccophilus maculosis)*, a sodium bromide concentration of 14,400 mg/l was required (2956).

SODIUM p- BROMOBENZENESULFONATE
SODIUM BUTYL MERCAPTIDE C_4H_9SNa

This soluble compound ionizes in water to form sodium and mercaptide ions. In normal pH ranges, the mercaptide ion reacts with hydrogen ion to form butyl mercaptan. In testing the toxicity of various refinery materials toward bluegill sunfish, Turnbull et al. (2093) determined TL_m values as follows:

	TL_m Values in mg/l			
	as SH		*as C_4H_9SNa*	
	24-hour	*48-hour*	*24-hour*	*48-hour*
Without reoxygenation	7.4	5.5	25.1	18.7
With reoxygenation	4.1	3.5	13.9	11.9

SODIUM n - BUTYL SULFONATE

(see Chapter X)

SODIUM CARBONATE Na_2CO_3

1. General. Sources of sodium carbonate in water include natural minerals, and effluents from chemical plants and paper mills. Known also as "soda ash", this salt is highly soluble in water and dissociates into sodium and carbonate ions, with a resulting increase in alkalinity and a tendency to raise the pH value. The carbonate ions will react with hydrogen ions until an equilibrium is established (see Carbonates, Bicarbonates, and Alkalinity). Sodium carbonate is used in the manufacture of glass, soap, and other sodium salts, in textile scouring, in water treatment and in numerous other processes (364).

2. Cross References. Dissolved Solids, Carbonates, Bicarbonates, Chapter V.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The taste threshold of sodium carbonate in water is reported as 15 to 75 mg/l (621) and as 78 mg/l (3241). Waters containing 360, 430, and 500 mg/l of sodium carbonate have been considered wholesome and safe (32, 36).

b. Industrial Water Supplies. Sodium carbonate in water for making ice is about 1.25 times as objectionable as sulfates and chlorides (231). Sodium carbonate in water for preparing developer solutions for use in photography may speed up the action of weakly alkaline solutions (242). For brewing, a limit of 100 mg/l has been recommended (see Chapter V).

c. Irrigation. Sodium carbonate is extremely toxic to plants (264, 268). Sodium carbonate in irrigation water hardens soil and reduces its permeability (348) (see also Chapter V). On the other hand, it has been reported that 100 to 400 mg/l of sodium carbonate in the water was not injurious to various greenhouse plants over a period of six months of use (1205).

d. Stock and Wildlife Watering. Waters containing high concentrations of sodium carbonate cause diarrhea, symptoms of malnutrition, unsatisfactory growth, and may also interfere with reproduction of livestock. The following concentrations of sodium carbonate have been reported as injurious to animals:

Concentration in mg/l	*Animal*	*Effect*	*Reference*
1,025	General livestock	Diarrhea	2394
7,500	Poultry	Lethal in 7 days	2980
10,000–15,000	Rats	Lethal to young	287
10,000–19,000	Rats	Inhibited growth	2980
10,000–20,000	Rats	Lethal to young	284
20,000	Rats	Lethal to young	640

e. Fish and Other Aquatic Life. The threshold concentration of sodium carbonate for immobilization of *Daphnia magna* in Lake Erie water at 25°C was reported to be 424 mg/l (358); less than 424 mg/l (352); and 300 mg/l (574). The minimum lethal concentration for *Daphnia* was also shown to be 300 mg/l at 17°C and at 800 mg/l all animals were killed (3523). The 100-hour toxicity threshold at 23°C in double-distilled water for *Daphnia magna* has also been reported at 524 mg/l with a resulting pH value of 9.5. According to Fairchild (2946), the threshold of toxicity toward *Daphnia* depends on the dissolved oxygen content of the test water. At 23°C for a 100-hour exposure he found the threshold toxicity level to be 552 mg/l at a dissolved oxygen tension of 6.5 mg/l; but only 267 mg/l when the DO dropped to 1.53 mg/l.

The following concentrations of sodium carbonate have been reported to kill fish:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
68	Tap	120 hours	King salmon	3501
70	Tap	120 hours	Silver salmon	3501
80	Tap	120 hours	Cutthroat trout	3501
200	---	4.5 hours	Bass	1030
250	---	120 hours	Shiners	190
250-300	Tap	Few hours	Shiners, suckers, carp	313
300	Standard	96-hour TL_m	Sunfish	2933
500	---	4.5-11 hours	Sunfish	1030
500	---	7-9 hours	Bass	1030
500	---	½-3 days	Bass	1030
530	Tap	3 days	Bluegills	313
740	Turbid	24-hour TL_m	Mosquito-fish	2940
840	Turbid	48-hour TL_m	Mosquito-fish	2940
1200	Turbid	96-hour TL_m	Mosquito-fish	2940

The following concentrations of sodium carbonate have not been harmful within the time specified:

Concentration in mg/l	*Time of Exposure*	*Type of Fish*	*Reference*
33	5 days	Cutthroat trout	3501
44	5 days	Silver salmon	3501
58	5 days	King salmon	3501
100	7 days	Bass	1030
200	7 days	Sunfish	1030
200–500	7 days	Goldfish	1030
500	7 days	Some goldfish	1030

SODIUM CARBOXYMETHYL CELLULOSE

(see Chapter X)

SODIUM CHLORATE $NaClO_3$

(see also Chlorine)

This strong oxidizing compound dissolves readily in water and ionizes to Na^+ and ClO_3^-; but the anion is unstable in aqueous solution, releasing oxygen and forming OCl^-. (see Chlorine). Sodium chlorate is used for weed control, as well as in the manufacturing of matches and explosives, dyeing and printing of fabrics, and tanning and finishing of leather. It is also found in some effluents from chlorine and bromine works. Concentrations from 20,000 to 40,000 mg/l, as used for weed control, are considered non-toxic for man and animals, but create a fire hazard as the water evaporates (897). The oral LD_{50} in rats is given as 12,000 mg/kg of body weight (3009).

The threshold of toxicity for perch is given as 11,000 mg/l and for bleak as 13,000 mg/l in tap water (2977). A concentration of 1000 mg/l in hard water was not harmful to goldfish during a 5-day exposure (313).

With respect to lower organisms, the literature is not consistent. The toxic threshold of sodium chlorate toward *Polycelis nigra*, a flatworm, was found to be 16,000 mg/l (354) and toward *Daphnia magna* 4240 mg/l (352). Bringmann and Kuhn (2158) report the threshold concentration toward *Daphnia* to be greater than 1000 mg/l; but toward the alga *Scenedesmus* the threshold level was only 3 mg/l as chlorate (about 3.8 mg/l as sodium chlorate) and toward *E. coli* only 4 mg/l as chlorate (5.1 mg/l as sodium chlorate).

SODIUM CHLORIDE NaCl

1. General. Sodium chloride in water may be of natural origin, or it may be introduced as a component of sewage or industrial wastes, including effluents from salt and brine works, oil wells, dairies, spent irrigation waters, etc. The sodium and chloride ions in natural waters are frequently the most prevalent, and they constitute the bulk of the ions in sea water.

2. Cross References. Tastes, Chlorides, Dissolved Solids, Specific Conductance, Mercuric Chloride, Distilled Water, Chapter V.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Sodium chloride can be tasted in water by most people at a concentration of 550 mg/l (947); however, taste threshold values from 200 to 900 mg/l have also been reported (621, 3241, 3391,

3392). Water containing more than 500 mg/l of sodium chloride may be unpalatable and cause appetite disturbances (284, 948). Although an excess of sodium chloride induces thirst or can act as a diuretic (623), water containing up to 1410 mg/l has been used by some communities for many years without appreciable harm; however, 1000 to 1500 mg/l of sodium chloride generally renders water unpalatable (32, 36, 621). It has also been reported that 7500 mg/l of salt is harmless and that 10,000 mg/l causes vomiting (621, 579) (see also Sodium, Dissolved Salts, Chapter III, Chapter V).

b. Industrial Water Supplies. For ice making, as much as 342 mg/l of sodium chloride would probably not be detrimental, if it were the only salt present in the water (229). A concentration of 1000 to 1500 mg/l of sodium chloride in the process water causes tastes in canned foods, but does not affect the quality in other ways (224). For brewing, some sodium chloride is desirable and necessary in the water (171). It has been reported that 75 to 150 mg/l of sodium chloride is desirable in brewing water, but more than 500 mg/l is deleterious (170). It has also been recommended that for brewing waters the salt concentration should not exceed 200 mg/l (173); or 275 mg/l (152, 168). Other salt recommendations for brewing waters include the following values: pale ales I, 30 mg/l; pale ales II, 30 to 60 mg/l; mild ales, 60 to 120 mg/l; and stout, 120 to 175 mg/l (170). Good ale has been brewed, however, with water containing as much as 715 mg/l of sodium chloride (32). See Chapter V.

Larson and King (3675) conclude that the rates of corrosion of iron and steel in water are a function of the specific mineral quality as well as the alkalinity and pH values. They indict NaCl and other sodium salts as strong contributors to the rate of corrosion. For example, in a water with 400 mg/l of alkalinity (as $CaCO_3$) and pH 7, the corrosion rate will be zero at 100 mg/l of NaCl, but when the NaCl concentration is 400 mg/l, the corrosion rate will be about 100 mg per sq cm per day (3675).

c. Irrigation (see also Chapter V; Dissolved Solids). The effects of sodium chloride in irrigation water vary with the individual plants, the species, and environmental and climatic conditions. The presence of 10 milliequivalents or more of sodium chloride in nutrient solutions depressed root growth and water absorption by corn and tomato plants (1348). In irrigation water 100 to 200 mg/l of sodium chloride has caused injury to avocado trees in some soils, and also to some flowers, but many other plants are not sensitive to such concentrations (921). Water used for irrigation should not contain more than 700 mg/l of dissolved minerals in which sodium chloride predominates (638). Tomatoes have been killed by water containing 2000 mg/l of sodium chloride, but chrysanthemums have been resistant to as much as 3000 mg/l (921). To peas in liquid culture 4000 mg/l or more of sodium chloride was toxic and 9000 mg/l permitted almost no growth. The growth of wheat and barley plants in liquid culture was depressed by 8000 mg/l of sodium chloride; 10,500 mg/l appeared to be lethal over a long period, although some plants could tolerate this concentration for a limited period of a few weeks. In general, no plants grew to any appreciable extent when irrigated with water containing 15,000 mg/l of sodium chloride (1349).

Young rice is sensitive to the presence of chlorides in water, but develops resistance as the plant matures. According to Shutts (1693), the commonly accepted tolerances of rice to salt water are as follows:

Concentrations of Salts as NaCl, mg/l	*Tolerances*
600	Tolerable at all stages
1300	Rarely harmful, and only to seedlings in dry, hard soil
1700	Harmful before tillering; tolerable from jointing to heading
3400	Harmful before booting; tolerable from booting to heading
5100	Harmful at all stages

Chlorides tended to accumulate in plants and soil when wheat was grown 2 and 10 meters away from a ditch carrying water containing 103,000 mg/l sodium chloride. Increasing concentrations of salt caused progressively greater reductions in height, weight, leaf growth and seed number of affected plants (1694).

The effect of NaCl on plant growth has been demonstrated by culture in sand to which various concentrations of salt have been added to the normal nutrient solution. With sand culture, the effect of sodium on the soil structure is avoided as a contributing factor. Results are shown below:

NaCl Added				
Atmospheres	*mg/l*	*Crop Designation*	*Relative Dry Weight*	*Reference*
Control	---	All	100	---
1.0	1400	Rice straw	89.7	3379
		Rice grain	74.5	3379
		Rice roots	90.9	3379
1.5	2100	Bean plant	89.0	3376
		Bean leaves	87.3	3376
		Bean roots	93.3	3376
2.0	2800	Rice straw	46.1	3379
		Rice grain	12.0	3379
		Rice roots	49.6	3379
2.5	3500	Bean plant	73.8	3376
		Bean leaves	70.5	3376
		Bean roots	80.1	3376

This table demonstrates that NaCl affects the growth of plants and that rice is more sensitive than beans.

d. Stock and Wildlife Watering (see also Chapter V; Dissolved Solids). Salt poisoning has been reported among a great variety of livestock; its symptoms include malnutrition, wasting diseases, diarrhea, nervous disturbances, decreased milk or egg production, reduced reproduction, and sometimes death. The minimum lethal dose of sodium chloride for poultry has been reported as about 2 grams per pound (1350). The following doses of sodium chloride have been reported to be toxic to various animals: pheasants and rabbits, 3 grams (3676); ducks, 6 grams; dogs, 30 to 60 grams; sheep and pigs, 120 to 140 grams; cows, 2 to 3 kilograms; and horses, 1 to 2 kilograms (284).

The following concentrations of NaCl in drinking water have been reported to have caused the indicated effects on animals:

Reactions	*Reference*	*Concentration of NaCl, mg/l*	*Animal*
4000	Laying hens	Watery droppings	2398
5000	Young chicks	Nervousness, inability to stand	1351, 1551

Concentration of NaCl, mg/l	Animal	Reactions	Reference
7000	Swine	Slight scouring	2398
7000	Cattle	Reduction in weight	2398
7200	Sheep	Lethal to some animals	642, 1352
9000	Chicks	Lethal to two-thirds	2980
10000	Rats	Decreased reproduction	284, 287, 295
10000	Cattle, swine, poultry	Toxic effect	2398
10000	Laying hens	Loss of weight and egg production	2398
15000	Rats	Retarded growth, some deaths	284, 287, 295
15000	Cattle	Reduced water intake	3392
15000–17600	Cattle	Sickened or killed	293, 294, 1350
15000–25000	Cattle, hens, pigs, rabbits	Injurious or fatal	284
17500	Cattle	Lost weight	2396
20000	Young chicks	Death in three days	1551

On the other hand, the following concentrations of NaCl in drinking water have been reported to cause no apparent adverse effect on the designated animals:

Concentration of NaCl, in mg/l	Animal	Reference
1025	General livestock	2394
2000	Young chicks	1551
2450	Cattle, sheep, swine, poultry	3391
3000	Chicks	2980
4000	Cattle, swine, poultry	2398
5000	Rats	284, 287, 295
5720	Sheep, horses	642, 1352
9000-10000	Dairy cows	292, 293
9123	Horses, without working	642
11440	Cattle and sheep	642
11690	Rats	2398
12500	Cattle	2396
20000	Cattle not in milk production	288
25000	Sheep	288

e. Fish and Other Aquatic Life. (see Dissolved Solids; Chapter V). The following concentrations of sodium chloride have immobilized or killed fresh-water fish:

Concentration of NaCl, mg/l	Type of Water	Time of Exposure	Type of Fish	Reference
1270- 2540	Soft, distilled	--	Fish	1459
2500	--	120 hours	Shiners	190
2500	--	--	Fish	344
2500	Distilled	9-24 days	Minnows	313
3820- 5100	Hard, alkaline	--	Fresh-water fish	1459
3850	Natural	--	Whitefish, pickerel	598
5000	--	--	Newly hatched trout	1459
5000	Distilled	148 hours	Shiners	313, 645
5000	Distilled	200-250 hours	Bass	313, 645
8500	Distilled	4-8 days	Minnows, perch	1459
10000	--	97-148 hours	Shiners, bass	645
10000	Tap	4-8 days	Minnows, perch	1459
10000	River	4-10 days	Goldfish	313
10000	Distilled	6 hours	Minnows	2942, 2943, 2944
10000	--	--	Fish	603, 1070
11500-12000	Hard	6 hours	Minnows	2942, 2943, 2944
11680-14600	--	--	Stickleback	353
11700	Tap	--	Salmon eggs and fry	1459
11765	Distilled	17 hours	Goldfish	313
12946	Aerated	96-hour TL_m	Bluegills	3381
14000	Synthetic	24 hours	Bluegills	1459
14000	--	--	Goldfish	2920
15000	--	4.7 hours	Bass	645
15000-30000	--	24 hours	Fresh-water fish	1459
16500	Natural	--	Whitefish	598
17550	Turbid	96-hour TL_m	Mosquito-fish	2940
20000	--	1.3 hours	Shiners	645
50000	--	15 minutes	Trout	441

On the other hand, the following concentrations of NaCl have been reported as harmless in the time specified:

Concentration of NaCl, mg/l	Type of Water	Survival Time	Type of Fish	Reference
5000	River	25 days	Goldfish	313
5850	Tap	--	Salmon eggs and larvae and young stickleback	1459
9100-17500	--	30 days	Perch	644
11700	--	50 hours	Young eels	1459
12000	Distilled	--	Stickleback	1459
20000	Tap	--	Stickleback	1459
25000-30000	--	--	Older trout	1459
30000	--	30-60 minutes	Brook trout	441

The threshold concentrations of sodium chloride in natural waters reported for immobilization of *Daphnia* and other fish-food organisms range from 2100 to 6143 mg/l (352, 353, 358, 598, 2946, 2955). Yet in distilled water, one mg/l of NaCl killed *Daphnia magna* in three hours (313). The resistance of *Daphnia magna* to NaCl appears to vary with the oxygen tension (2946). At 6.4 mg/l of dissolved oxygen the threshold toxicity level of NaCl was 5093 mg/l, but at 1.48 mg/l of DO, the threshold for NaCl was only 3170 mg/l (2946). Many small crustacea and fish fry are immobilized by concentrations above 3100 mg/l (598). The toxic threshold for a flatworm, *Polycelis nigra*, was found to be 11,200 mg/l (354).

In Lake Erie water at 20-25°C, threshold limits for several fish-food organisms were reported (2955) as follows:

Organism	Threshold Concentration, mg/l
Daphnia magna, young	3700
Daphnia magna, adult	4600
Leptodora kindtii	3700
Cyclops vernalis	6100
Diaptomus oregonensis	3030

A Russian article (3677) indicates the following maximum NaCl tolerances for fresh-water organisms at two temperatures:

Organism	NaCl Tolerance in mg/l 20°C	3°C
Stictochironomus	788	1000
An oligochaete	1576	2000
Daphnia magna	200	800
Cyclops serrulatus	394	--
Leucaspisis delineatus	1970	--

Anderson considers sodium chloride concentrations above 3000 mg/l deleterious to both fish-food organisms and fish fry and recommends a permissible limit of 2000 mg/l in fresh waters (598). On the other hand, higher

concentrations of sodium chloride have been found to decrease somewhat the toxicity of some metallic compounds toward fish (1265) (see also Mercuric Chloride). Sodium chloride is the least toxic to fish of the chlorides of potassium, magnesium, calcium and sodium. Sodium chloride is antagonistic to the toxicity of calcium and potassium chlorides (353, 307). A concentration of 50 mg/l of sodium chloride has greatly increased the antagonistic effect of sodium nitrate toward copper sulfate poisoning of fish (311). The toxicity of sodium chloride toward fish is decreased by calcium chloride (307).

f. Shellfish Culture. The most favorable salt content of water for oyster growth is about 30,800 mg/l of salinity of which about 80 percent is sodium chloride (314).

SODIUM p-CHLOROBENZENE SULFONATE

(see Chapter X)

SODIUM CHROMATE

(see Chromium)

SODIUM CITRATE $C_3H_4OH(COONa)_3 \cdot 2H_2O$

(see also Citric Acid)

This highly soluble salt has many uses in medicine, especially as an anticoagulant. It is also used in photography and as a sequestering agent to remove trace metals (364).

Anderson (352) reports that the threshold concentration for immobilization of *Daphnia magna* in Lake Erie water at 25°C was 825 mg/l. For *Polycelis nigra,* a flatworm, the threshold for toxicity at 15 to 18°C was a 0.015 molar concentration, or 4420 mg/l of sodium citrate (354).

SODIUM CYANATE NaOCN

(see also Cyanides, Hydrogen Cyanide)

Cyanates are formed when cyanides in waste waters are treated by alkaline chlorination. According to Resnick et al. (2974), cyanates are stable in aerobic water at pH 7 and 20°C for at least 10 days. Eventually, cyanates undergo hydrolysis to yield ammonium carbonate. In anaerobic solutions cyanates are reduced not to cyanide but to a mixture of ammonia and formic acid.

According to Washburn (1070) the maximum tolerance limit of creek chub to sodium cyanate is 75 mg/l.

SODIUM CYANIDE NaCN

1. General. This highly soluble salt is used in electroplating baths, for extracting gold and silver from ores, and for generating HCN gas in fumigating operations (364). Upon solution in water, the cyanide ion combines with hydrogen ions in the water to form weakly dissociated hydrogen cyanide, thereby tending to raise the pH of the solution. For further explanation of the cyanides, see Hydrogen Cyanide.

2. Cross References. Cyanides, Cyanogen Chloride, Hydrogen Cyanide, Nitriles, and other cyanide salts.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The fatal dose of NaCN for man by ingestion is reported to be about 0.2 grams (353), but since the fatal dose of HCN has been given as 50 to 60 mg (364), the former figure may be high. See also Cyanides and Hydrogen Cyanide.

b. Stock and Wildlife Watering. The approximate minimum toxic dose of NaCN for sheep is given as 4.15 mg per kg of body weight, and a dose of 5.22 mg per kg is lethal (353).

c. Fish and Other Aquatic Life. The following concentrations of sodium cyanide have been shown to be harmful or toxic to fish:

Concentration of NaCN, in mg/l	*Time of Exposure*	*Type of Fish*	*Remarks*	*Reference*
0.05	124 hours	Trout	100 percent mortality	1115
0.28	96 hours	Bluegills	TL_m in soft water	2958
0.33-0.35	2.5 hours	Minnows	Lethal	1468
0.4	1 hour	Minnows	Stopped eating	362
0.43	96 hours	Fathead Minnow	TL_m in soft water	2958
0.44	48 hours	Fathead Minnow	TL_m	3335
0.5	4 to 6 hours	Goldfish and green sunfish	Lethal, 25°C	3678
0.5 to 0.7	24 hours	Minnows	25 percent mortality	362
0.66	96 hours	Fathead minnow	TL_m in hard water	2958
0.75	24 hours	Minnows	50 percent mortality	362
0.8	24 hours	Minnows	100 percent mortality	362
1.0	0.7 to 10 hours	Mixed fish	Lethal at 25°C	3678
1	20 minutes	Trout	100 percent mortality	1115
1.5	0.6 to 24 hours	Goldfish and green sunfish	Lethal	3678
2	47 minutes	Trout	Lethal	1116
2.0	--	Sticklebacks	Lethal at 17°C	2920
3.1	90 minutes	Fish	Depressed respiration	468
4.3		Hardy carp	Paralyzed	362
5	12 minutes	Shiner	Killed	1115
5	2 hours	Trout, bluegill	Killed	2976
10	4 minutes	Shiner	Killed	1115

Non-toxic or non-harmful concentrations have been reported as follows:

Concentration of NaCN, in mg/l	*Time of Exposure*	*Type of Fish*	*Remarks*	*Reference*
0.02	—	Trout fingerlings	No effect	1115
0.08	--	Trout	Non-toxic	1109
0.3	24 hours	Minnows	No effect	362
2.3	155 minutes	Fish	Survived	468
5.0	2 hours	Sea lamprey	Ill, but survived	2976

According to Anderson (365), the threshold concentration for immobilization of *Daphnia magna* in Lake Erie water at 25°C was found to be less than 3.4 mg/l of sodium cyanide and Jones (354) reports that the threshold concentration for *Polycelis nigra,* a flatworm, is 16 mg/l as cyanide (30 mg/l as NaCN). A concentration of 3.6 mg/l of NaCN inhibited BOD by 50 percent (2949).

SODIUM DECYLBENZENE SULFONATE

SODIUM n-DECYL SULFATE

SODIUM 2, 5-DICHLOROBENZENE SULFONATE

(see Chapter X)

SODIUM DICHROMATE

(see Chromium)

SODIUM DIHYDROGEN PHOSPHATE

(see Sodium Phosphates)

SODIUM DI (2-ETHYLHEXYL) SULFOSUCCINATE

SODIUM DIOCTYL SULFOSUCCINATE

SODIUM DODECYLBENZENE SULFONATE

SODIUM DODECYL SULFATE

SODIUM 2-ETHYLHEXYL SULFATE

(see Chapter X)

SODIUM FERROCYANIDE $Na_4Fe(CN)_6$

(see also Cyanides, Hydrogen Cyanide, Potassium Ferro- and Ferricyanide)

This compound, containing reduced iron, is highly soluble in water. It is used for blueprinting, in photography, pigments, dyes, and metallurgy (364). In aqueous solution it decomposes under the action of sunlight to release cyanide ion and HCN.

For *Daphnia magna* in Lake Erie water at 25°C, the toxic concentration was reported by Anderson (352) to be less than 600 mg/l, or about 540 mg/l (353). For *Polycelis nigra,* a planarium, Jones (354) found the toxic threshold to be 170 mg/l. It should be recognized, however, that under the action of sunlight with the resultant decomposition to cyanides, this compound may be much more lethal. For further details, see Potassium ferrocyanide.

SODIUM FLUORIDE NaF

(see also Fluorides)

Highly soluble in water, this crystalline solid is used as a pesticide, as a fluoridating agent in municipal water, and in many industrial processes. The LD_{50} for hamsters was found to be 70-80 mg/kg of body weight (3705). Much of the information described under "Fluorides" was derived from the use of sodium fluoride and will not be repeated here.

Bringmann and Kuhn (2158, 3343) found the threshold of NaF for *Daphnia* at 23°C to be 270 mg/l during a 2-day exposure. For *Scenedesmus* the threshold of toxic effect was 95 mg/l during 4 days at 24°C, for *Microregma* 226 mg/l, and for *Escherichia coli* at 27°C it was 180 mg/l. According to Wantland (3705), free-living protozoa and freshwater rotifers survived and reproduced in water containing 1000 mg/l of NaF, but were killed at 1700 mg/l.

SODIUM FORMATE

(see Formic Acid and Formates)

SODIUM HYDROCARBON SULFONATE

(see Chapter X)

SODIUM HYDROSULFIDE

(see Sodium Sulfhydrate)

SODIUM HYDROXIDE NaOH

1. General. This common caustic compound is highly soluble in water with dissociation to sodium and hydroxide ions, with a consequent effect upon alkalinity and pH. Sodium hydroxide may occur in wastes from the following industries: soap manufacture; cleaning compounds; pulp and paper; petroleum, mineral and vegetable oils; leather; reclaimed rubber; ink; dyes and textile dyeing; cotton; perfumes; water glass; coal distillation, etc.

2. Cross References. Sodium, Alkalinity, Hydroxides.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The taste threshold of sodium hydroxide in water is reported to be 1 to 50 mg/l (621). A dose of 1.95 grams of sodium hydroxide may cause death in man (353).

b. Stock and Wildlife Watering. Sodium hydroxide, in a concentration of 5000 mg/l, was almost harmless for rats; 10,000 mg/l caused nervousness, sore eyes, diarrhea, and retarded growth (284, 287).

c. Fish and Other Aquatic Life. The toxicity threshold concentration of sodium hydroxide for *Daphnia magna* has been reported to range from 40 to 240 mg/l (190, 352, 353, 358, 574, 690, 1466, 3500, 3523). Concentrations of sodium hydroxide from 125 to 1000 mg/l have been lethal to various insect larvae (190, 574, 690, 3500). A concentration of 100 mg/l has not been harmful to mayflies, and 700 mg/l has not been harmful to *Chironomous* larvae (690).

The following concentrations of sodium hydroxide have been reported to have killed fish:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
20	---	---	Silver salmon	3502, 3501
25	---	24 hours	Brook trout	359
35	---	---	Cutthroat trout	3502, 3501
40	Fresh	24 hours	Creek chub	1442
43	---	---	King salmon	3502, 3501
70	Stagnant	5 hours	Fish, crabs	823
71.5	---	---	Carp, shiners, suckers	359
90	Circulating	4.5 hours	Fish, oysters	823
96	Tap	2-10 minutes	Carp, shiners, suckers	313
99	Tap	48-hour TL_m	Bluegill sunfish	2093
100	---	---	Minnows	690, 190, 3500
100	---	---	Fish	658, 344
100	---	120 hours	Shiners	190
100	---	3-20 hours	Goldfish, bass	1030
125	Turbid	96-hour TL_m	Mosquito-fish	2940
180 (pH 12)	Circulating	23 hours	Fish, oysters	823

The following concentrations of sodium hydroxide have not harmed fish within the time specified:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
10	---	---	Cutthroat trout	3502, 3501
11	---	---	Silver salmon	3502, 3501
20	Fresh	24 hours	Creek chub	1442
27	---	---	King salmon	3502, 3501
50	Distilled	2 hours	Perch, roach	313
50	---	7 days	Goldfish, bass	1030
55.5	---	---	Carp, shiners, suckers	359
200	---	5 hours	Some fish	823

For roach and perch in sodium hydroxide solutions of 1:5000 strength, the time until loss of equilibrium was found to be about 30 minutes. In 1:10,000 dilutions, both kinds of fish survived for 40 minutes (1357).

According to Murdock (1460) sodium hydroxide has not been shown to be lethal to fully developed fish in natural fresh waters unless the pH value is well over 9. The lethal pH for goldfish is given by Jones (2920) as 10.9, and by Cairns and Scheier (2933, 2934) for bluegill sunfish as 10.5.

To stimulate the movement of water beetles *(Laccophilus maculosus),* Hodgson (2956) found that a concentration of 400 mg/l of NaOH was required.

SODIUM IODATE $NaIO_3$

(see also Iodine)

The threshold concentration of sodium iodate for the immobilization of *Daphnia magna* in Lake Erie water was found to be less than 158 mg/l (352). The toxic threshold concentration of sodium iodate for the flat-

worm *Polycelis nigra* was found to be 230 mg/l, as iodate (354).

SODIUM IODIDE NaI

(see also Iodine)

The taste threshold of sodium iodide in chlorinated water is reported to be about 0.005 mg/l (459). The threshold concentration of sodium iodide for the immobilization of *Daphnia magna* in Lake Erie water was found to be 3.3 mg/l (352). A concentration of 5 mg/l had no effect on the sea lamprey during a 24-hour exposure in Lake Huron water at 12°C (2976). The toxic threshold concentration of sodium iodide for *Polycelis nigra* a flatworm, was found to be 6600 mg/l (354). To stimulate movement of the water beetle *(Laccophilus maculosis)* a sodium iodide concentration of 14,700 mg/l was required (2956).

SODIUM ISOPROPYL NAPHTHALENE SULFONATE

SODIUM KERYLBENZENE SULFONATE

SODIUM LAURYL AND MYRISTYL COLLAMIDE SULFONATE

SODIUM LAURYL SULFATE

(see Chapter X)

SODIUM METABISULFITE $Na_2S_2O_5$

This highly soluble solid is used in medicine as an antioxidant (364). According to Le Clerc (2942) the minimum lethal dose to minnows during a 6-hour exposure was 60-65 mg/l in distilled water at 18°C and 75-80 mg/l in hard water at 17°C.

SODIUM MOLYBDATE

(see Molybdenum)

SODIUM MONOBUTYL PHENYLPHENOL MONOSULFONATE

SODIUM MONOETHYL PHENYLPHENOL MONOSULFONATE

SODIUM NAPHTHALENE -β- SULFATE

(see Chapter X)

SODIUM NITRATE $NaNO_3$

1. General. Known commonly as "Chile saltpeter", this compound was formerly used extensively for the manufacture of nitric acid. It is also used in making glass, enamels, matches, and cigarettes, and in pickling meats. The technical grade is employed in fertilizers as a source of nitrate. The compound is readily soluble in water, releasing sodium and nitrate ions.

2. Cross References. Sodium Nitrates.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The taste threshold of sodium nitrate in water is reported to be about 450 to 800 mg/l (621). Sodium nitrate acts as a diurectic (364, 623).

b. Industrial Water Supplies. Sodium nitrate in water interferes with fermentation processes and causes disagreeable tastes in beer (165).

c. Stock and Wildlife Watering. A dose of 240 grams of sodium nitrate has been fatal to cows; 20 grams, to sheep; and 6 grams to dogs (284). A concentration of 6200 mg/l of sodium nitrate in water has caused frenzy, paralysis, diuresis, cyanosis, and deaths among cows (284). Pigs fed with soup prepared with well water containing 1740-2970 mg/l of sodium nitrate died from methemoglobinemia. A total dose of 90 mg of sodium nitrate per kg of body weight is fatal to pigs (1695).

d. Fish and Other Aquatic Life. The toxicity threshold concentration of sodium nitrate for *Daphnia magna* has been reported to be 5000 mg/l and 8500 mg/l (352, 353, 358).

The following concentrations of sodium nitrate have been reported as toxic to fish:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
1,282	Distilled	14 hours	Goldfish	313
1,845	Tap	---	Stickleback	598, 1460
2,000	---	4 days	Goldfish	1035
2,000-4,000	---	---	Some sunfish	1035
2,210	Tap	7 days	Stickleback	1460
2,950	Tap	4 days	Stickleback	1460
4,000	Hard	80 hours	Goldfish	313
5,530	Tap	48 hours	Stickleback	1460
6,650	Turbid	96-hour TL_m	Mosquito-fish	2940
10,000	Standard	96-hour TL_m	Bluegill sunfish	2933, 2934
11,060	Tap	24 hours	Stickleback	1460
12,000	Aerated	96-hour TL_m	Bluegill sunfish	3381
13,600	---	---	Stickleback	2920

The following concentrations of sodium nitrate have not been harmful to fish within the time specified:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
1,000	Hard	---	Goldfish	313, 1035
2,000-4,000	Hard	---	Some sunfish	1035
3,000	Hard	100 hours	Goldfish	313
4,000	Hard	---	Bass	1035

SODIUM NITRATE $NaNO_2$

(see also Nitrates, Nitrites, Sodium)

This soluble salt is used in the manufacture of dyes and other chemicals, in textile processes, and in photography. In water it dissociates completely and under aerobic conditions the nitrite ions are oxidized to nitrates. The oral MLD for dogs is given as 330 mg/kg of body weight (364).

A concentration of 17.1 mg/l of $NaNO_2$ was apparently harmless to minnows during a 24-hour exposure (362); but 50 mg/l can be fatal in 14 days (3577). A massive dose of 10,000 mg/l is lethal to minnows in 1.5 hours (3577). Testing mosquito-fish (*Gambusia affinis*) in highly turbid water at 21-24°C, Wallen et al. (2940) found the 24-hour TL_m to be 8.1 mg/l while the 48- and 96-hour TL_m values were 7.5 mg/l. There was no decrease in turbidity by this dosage. Sodium nitrite has an antagonostic effect upon the toxicity of copper sulfate to goldfish. In cencentrations up to 5000 mg/l, sodium nitrite has decreased the toxic effect of 10 mg/l of copper sulfate toward goldfish (311).

The toxic threshold concentration of sodium nitrite for the flatworm *Polycelis nigra* was found to be 42 mg/l (354). Toward *Daphnia magna* in Lake Erie water, the threshold concentration for immobilization was reported as less than 28 mg/l (352); but in River Havel water at 23°C it was given (2158) as 99 mg/l of $NaNO_2$. Also in River Havel water, the toxic threshold for *E. coli* was found to be 195 mg/l but for *Scenedesmus* concentrations up to 1500 mg/l of $NaNO_2$ were harmless.

SODIUM m-NITROBENZENE SULFONATE

SODIUM 4-NITROCHLOROBENZENE -2-SULFONATE

(see Chapter X)

SODIUM NITROFERRICYANIDE $Na_2Fe(CN)_5NO$

(see also Cyanides, Sodium Ferricyanide)

This substance is used primarily in chemical laboratories for the detection of many organic compounds (364). According to Schaut (362), minnows exposed for one hour to 17.1 mg/l of sodium nitroferricyanide in stabilized tap water showed no effects. Toward *Polycelis nigra,* a flatworm, the toxic concentration was found to be 190 mg/l, as nitroferricyanide (354). Like the ferricyanides, this substance tends to decompose slowly in aqueous solution, but faster in sunlight, to release cyanide ions (see Cyanides).

SODIUM OLEATE

(see Fatty Acids, also Chapter X)

SODIUM OXALATE

(see Oxalic Acid, Oxalates)

SODIUM PALMITATE

SODIUM PENTACHLOROPHENATE

(see Chapter X)

SODIUM PERBORATE $NaBO_3 \cdot 4H_2O$

(see also Boron, Sodium Borate, Chapter X)

Relatively soluble in water, this white crystalline salt is used in bleaching operations, in soaps, and in dentifrices. When mixed with warm water it decomposes to liberate nascent oxygen.

The threshold concentration of sodium perborate for immobilization of *Daphnia magna* in Lake Erie water at 25°C was found to be very much less than 5.2 mg/l (352) and was estimated at 2.6 mg/l (353). Fingerling trout survived a sodium perborate concentration of 160 mg/l for 24 hours but 80 percent were killed by 320 mg/l.

SODIUM p-PHENOL SULFONATE

(see Chapter X)

SODIUM PHOSPHATES

(see also Phosphates, Phosphoric Acid)

Under this designation, six sodium phosphates are considered:

(a) Sodium phosphate, monobasic, $NaH_2PO_4 \cdot H_2O$ also known as sodium dehydrogen phosphate. Highly soluble in water, it is used in baking powders and treatment of boiler water. The aqueous solution is acid.

(b) Sodium phosphate, dibasic, $Na_2HPO_4 \cdot 7H_2$, or disodium hydrogen phosphate. It is highly soluble in water and gives an alkaline solution. It is used in human and veterinary medicine as a laxative.

(c) Sodium phosphate, tribasic, $Na_3PO_4 \cdot 12H_2O$, or trisodium phosphate. Also highly soluble in water, it is used in photographic developers, treatment of municipal and boiler water, in detergent compounds, and in tanneries, paper mills, and laundering plants.

(d) Tetrosodium pyrophosphate, $Na_4P_2O_7$, or sodium pyrophosphate. It is used as a sequestering agent in water treatment, cleaning compounds, oil well drilling, and other processes.

(e) Sodium hexametaphosphates are mixtures of $(NaPO_3)_6$ that occur in commercial detergents such as Calgon, Micromet and Quadrafos.

(f) Sodium tripolyphosphate, $Na_5P_3O_{10}$, used as a sequestering agent and meat preservative.

Campbell et al. (2059) indicate that the taste threshold for trisodium phosphate is 225 mg/l and for Calgon 200 mg/l. According to Lockhart et al. (3241) the taste threshold of trisodium phosphate in water is 180 mg/l. Cohen et al. (2049) found that as little as 0.1 mg/l of sodium tripolyphosphate inhibited the coagulation of turbid water by ferric sulfate.

For *Polycelis nigra,* a flatworm, the toxic threshold concentration of trisodium phosphate ($Na_3PO_4 \cdot 12H_2O$) was found to be 9860 mg/l (354). The threshold concentrations for immobilization of *Daphnia magna* in Lake Erie water at 25°C were found to be lower than the following values:

Compound	*Concentration in mg/l*
Sodium dehydrogen phosphate	1560
Disodium hydrogen phosphate	59
Trisodium phosphate	52

To stimulate the movement of water beetles (*Laccophilus maculosis*) in water, Hodgson (2956) found that a trisodium phosphate concentration of 179,000 mg/l was required.

Toward fish, sodium phosphates are not strongly toxic, the principal effect being a change in pH value. Using turbid water and the mosquito-fish (*Gambusia affinis*) as the test animal, Wallen et al. (2940) report the following results:

Compound	*Temperature °C*	*pH Range*	*TL_m in mg/l 24-hour*	*48-hour*	*96-hour*
NaH_2PO_4	19–23	7.6–11.0	720	720	720
$Na_4P_2O_7 \cdot 10H_2O$	19–24	8.2–9.8	1380	1380	1380
Na_3PO_4	17–22	7.4–10.3	467	467	151

The Water Pollution Research Board of England (2907) tested a mixture of sodium tripolyphosphates and tetrasodium pyrophosphate on rainbow trout. It was found that 560 mg/l as phosphate caused no deaths in 24 hours but 1120 mg/l as phosphate killed all of the fish.

Quadrafos, a sodium hexametaphosphate, was tolerated by fish in concentrations up to 3,500 mg/l, but this concentration killed oysters (3005).

SODIUM PYROPHOSPHATE

(see Sodium Phosphates)

SODIUM SALICYLATE

(see Salicylic Acids and Salicylates)

SODIUM SELENITE Na_2SeO_3, $Na_2SeO_3 \cdot 5H_2O$

1. General. Sodium selenite is found in some types of soil and in wastes from pottery works. It is freely soluble in water.

2. Cross References. Selenium, Selenious Acid.

3. Effects Upon Beneficial Uses.

a. Stock and Wildlife Watering. For most mammals the fatal dose of sodium selenite is about 4 to 6 mg per kilogram of body weight. Sodium selenate is about half as toxic as sodium selenite. The minimum lethal dose of sodium selenite for rabbits is about 1.5 mg per kg of body weight (353).

As sodium selenite, administered orally or as a drench, the minimum lethal single dose of selenium for horses is about 1.5 mg per kg of body weight; for cows and calves, 4.5 to 5.0 mg per kg of body weight; and for pigs, 6 to 8 mg per kg (1049).

b. Fish and Other Aquatic Life. The following concentrations of sodium selenite have been reported as toxic to fish.

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
10	Hard	98-144 hours	Goldfish	353, 313
100	Very soft	1-4 days	Goldfish	353
100	Hard	8-19.5 hours	Goldfish	353, 313

Bringmann and Kuhn (2158, 3343) investigated the toxicity of sodium selenite in River Havel water. They found the following thresholds of toxicity:

Organism	*Temperature °C*	*Time of Exposure*	*Threshold Concentrations in mg/l*
Daphnia	23	2 days	2.5
Scenedesmus	24	4 days	2.5
Escherichia coli	27	--	90
Microregma	--	--	183

SODIUM SILICATE Na_2SiO_3

This colorless crystalline salt is only slightly soluble in water, but in combination with other sodium silicates such as $Na_2Si_3O_7$ it is marketed as a solution known as "water glass," which has numerous commercial applications (3 4).

The 100-hour toxicity threshold of sodium silicate in double-distilled water at 23°C to *Daphnia magna* has been reported (1691) at 247 mg/l with a resulting pH value of 9.1, but in the presence of other sodium salts this threshold may be somewhat lower (see Sodium).

The Water Pollution Research Board of England determined that sodium silicate was not lethal to fingerling rainbow trout at a concentration of 256 mg/l. Using turbid water at 21-22°C, Wallen et al. (2940) found that the 96-hour TL_m for the mosquito-fish *(Gambusia affinis)* was 2320 mg/l.

SODIUM SILICOFLUORIDE Na_2SiF_6

(see also Fluorides)

This white granular powder, highly soluble in water, has been used extensively in the fluoridation of drinking water. The oral LD_{50} in rats has been reported (364) as 125 mg/kg of body weight. For guinea pigs, the oral LD_{50} is given as 250 mg/kg of body weight (3271). Toward the fish, *Tinca vulgaris,* Simonin (3271) reports the lethal dose to be 50 mg/l.

SODIUM STEARATE

(see Chapter X)

SODIUM SULFATE Na_2SO_4 and $Na_2SO_4 \cdot 10H_2O$

1. General. Sodium and sulfate ions may occur naturally in water, or they may be derived from various industrial wastes, such as those from paper mills. Sodium sulfate is used in manufacturing dyes, glass, soaps, freezing mixtures, and paper; and in dyeing and printing of textiles. It is readily soluble in water.

2. Cross References. Sulfates, Sodium.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The taste threshold of sodium sulfate in water is 250 to 500 mg/l (621). In high concentrations, sodium sulfate has a purgative effect (623). Water containing 500 mg/l of sodium sulfate and 1200 mg/l of magnesium sulfate occasionally has caused diarrhea among human beings; under ordinary conditions such water would be regarded as unsuitable for domestic use (36).

b. Industrial Water Supplies. For boiler waters, the ratio between the concentration of sodium sulfate and that of sodium sulfite is significant. The following ratios as given in Chapter V, are specified as limits:

Boiler Pressure in psi	*Ratio Na_2SO_4:Na_2SO_3*
0-150	1:1
150-250	2:1
More than 250	3:1

For brewing, waters should not contain more than about 100 mg/l of sodium sulfate, and for ice making, not more than 300 mg/l (see Chapter V).

Larson and King (3675) conclude that the rates of corrosion of iron and steel in water are a function of the specific mineral quality as well as the alkalinity and pH values. They indict sodium sulfate and other sodium salts as strong contributors to the rate of corrosion. For example, in a water with 400 mg/l of alkalinity (as $CaCO_3$) and pH 7, the corrosion rate will be zero at 200 mg/l of Na_2SO_4, but when the concentration of sodium sulfate is 400 mg/l, the corrosion rate will be about 100 mg per sq cm per day (3675).

c. Irrigation. A concentration of 4000 mg/l of sodium sulfate in the soil is injurious to plants (246). At 710 mg/l, sodium sulfate has depressed root growth and water absorption of plants grown in liquid culture (1348).

The effect of Na_2SO_4 on plant growth has been demonstrated by culture in sand to which various concentrations of this salt have been added to the normal nutrient solution. With sand culture, any alteration of the soil

structure is avoided as a factor contributing to sodium effects. Results are shown below:

Na_2SO_4 Added in mg/l	Portion of Crop	Relative Dry Weight	Reference
Control	--	100	--
2560	Rice straw	91.9	3379
	Rice grain	79.8	3379
	Rice roots	95.4	3379
2840	Tomato vine	88.2	3679
	Tomato root	85.0	3679
3840	Bean plant	87.5	3376
	Bean roots	90.2	3376
	Bean leaves	86.3	3376
5120	Rice straw	55.6	3379
	Rice grain	15.6	3379
	Rice roots	58.2	3379

d. Stock and Wildlife Watering. Water containing 4546 to 7369 mg/l of salts, mostly sodium sulfate, was not harmful to two cows over a two-year period; however, a sudden change from normal water to this water caused diarrhea (291). At a level of 7000 mg/l there was some evidence of weight reduction in cattle and at 10,000 mg/l weight losses were severe (2398). Rats were not harmed by water containing 15,000 mg/l of sodium sulfate (284). Poultry, however, experienced a 33 percent mortality after drinking for 15 days water containing 7500 mg/l of sodium sulfate. Ballantyne (2394) sets the threshold limit of Na_2SO_4 for all livestock at 2050 mg/l.

e. Fish and Other Aquatic Life. The following concentrations of sodium sulfate were reported to be toxic or lethal to fish and other aquatic life:

Concentration in mg/l	Time of Exposure	Type of Fish	Reference
100	120 hours	Shiners	190
100	—	Fish	344
1000	7 days	hydra	1696
4547	100 hours	*Daphnia magna*	1691
5000	7-28 days	Water snails	1696
5000	6-30 hours	Microcrustacea	1467
5000	1-6 days	Fingerling trout	1696
5000-7105	—	*Daphnia magna*	352, 358, 574
5200	48 hours	*Daphnia*	3523
6700	--	Cutthroat trout	3502
6820	--	*Polycelis nigra*	354
7000	1-2 days	Rainbow trout	1696
11,200-12,000	6 hours	Minnows, distilled water	2942
12,750	96-hour TL_m	Bluegill sunfish	2933
13,400-184,000	7-69 days	Perch	644
13,500	96-hour TL_m	Bluegill sunfish	2936, 3381
16,500	--	Silver salmon	3502
16,500	96-hour TL_m	Mosquito-fish	2940
16,700-17,600	6 hours	Minnows, hard water	2942
24,000	24-hour TL_m	Mosquito-fish	2940

The following concentrations of sodium sulfate were not harmful to aquatic life in the indicated time:

Concentration in mg/l	Time of Exposure	Type of Fish	Reference
100	--	Minnows	3523
500	24 hours	Goldfish	313
705	24 hours	Rainbow trout	2907
1000	5 days	Fish	609
2500	--	Cutthroat trout	3502
5000	6-16 days	Dace	1696
5000	--	*Daphnia*	3523
5000-10,000	--	Water plants	1696
7500	24 days	Perch	1696
10,000	--	Silver salmon	3502
15,000	24 days	Carp	1696
15,000	7 days	Roach	1696
Up to 15,000	--	Algae	1696
15,000-20,000	28 days	Protozoa	1696

To stimulate the movement of water beetles, *Laccophilus maculosis*, Hodgson (2956) found that 35,600 mg/l of sodium sulfate was required. Fairchild (2946) reported that the threshold toxicity value of sodium sulfate toward *Daphnia* depended on the dissolved oxygen concentration. At a DO value of 6.6 mg/l, the toxicity threshold of Na_2SO_4 was 5514 mg/l; but at a DO value of 1.46 mg/l, the toxicity threshold of Na_2SO_4 dropped to 2752 mg/l.

SODIUM SULFIDE Na_2S

1. General. Sodium sulfide is used in tanneries and may also be a constituent of Kraft mill wastes, and beet-sugar-factory effluents. It has a high chlorine demand and a high oxygen demand; a small continuous dose of sodium sulfide can result in oxygen depletion and fish deaths (313, 362). It is highly soluble in water, the sulfide ions tending to react with hydrogen ion to form HS^- or H_2S, depending on the pH value of the solution.

2. Cross References. Sulfide, Distilled Water, Hydrogen Sulfide.

3. Effects Upon Beneficial Uses.

a. Industrial Water Supplies. Even when present in very low concentrations, sodium or potassium sulfide in the water used for mixing developers for photographic use can result in chemical fogging of emulsions on the films (242).

b. Fish and Other Aquatic Life. The following concentrations of sodium sulfide have been reported as toxic or lethal to fish:

Concentration in mg/l	Type of Water	Time of Exposure	Type of Fish	Reference
0.55	pH 5.2	---	Young carp	1698
1.0 (threshold)	Aerated	---	Salmonoid fish	1582
1.8 (lethal)	Aerated	---	Salmonoid fish	1582
2.0	Distilled	---	Minnows	361
2.4	---	---	Brown trout	2920
3.0	---	---	Minnows	690
3.0	12°C	---	Cutthroat trout	3502
3.0	---	---	Shiners, minnows	344, 658, 690
3.1	15°C	---	Silver salmon	3502
3.2	---	48-hour TL_m	Fathead minnows	3335
3.3	pH 7.4	---	Young carp	1698
3.5	17.5°C	---	King salmon	3502
7.8	---	90 minutes	Minnows	468
10-11	Hard, 15°C	6 hours	Minnows	2942
11	---	---	Stickleback	2920
12-13	Distilled, 25°C	6 hours	Minnows	2942
39	---	6 minutes	Minnows	468
50	Hard	---	Minnows	361
61	Standard	48-hour TL_m	Bluegill sunfish	2093
750	Turbid	96-hour TL_m	Mosquito-fish	2940

The maximum concentration of sodium sulfide that produced no mortality has been given (3502) as 1.8 mg/l for king salmon at 17.5°C, 1.3 mg/l for silver salmon at 15°C, and 1.0 mg/l for cutthroat trout at 12°C. Fish tried to avoid solutions of sodium sulfide in concentrations of 3.1 to 39.0 mg/l (1046).

Fish removed from toxic concentrations of sodium sulfide before they stopped breathing recovered and survived (468). Minnows exposed to 17.1 mg/l of sodium sulfide in tap water have been disturbed for as long as five hours; within 24 hours, however, the sulfide had disappeared and the fish recovered (362). On the other hand, Ellis quotes a reference to the effect that exposure

for one hour to 1150 mg/l of sodium sulfide in tap water has resulted in the death of tench six days later (313).

When Na_2S is added to water it dissociates to release sulfide ions which then react with hydrogen ions to form HS^- or H_2S, depending on the pH value of the solution (see Hydrogen Sulfide). For that reason, the toxicity of sodium sulfide tends to increase as the pH of the water decreases from 9 to 6 (1046). This relationship has been shown by Tomiyama and Yamagawa (1698) for young carp as follows:

pH Value	*Critical Lethal Concentration, mg/l*
5.2	0.55
6.1	0.95
7.4	3.3
8.2	8.0

Other aquatic life have responded to sodium sulfide as shown below:

Concentration in mg/l	*Organism*	*Remarks*	*Reference*
1–2	Mayfly larvae	Killed	690
2–1000	Insect larvae	Killed	690
2.44	Bivalve larvae	Lethal	2989
3.2	*Mesocyclops leuckarti*	Toxic threshold	2955
9.4–10	*Daphnia magna*	Killed or immobilized	352, 690, 2955
10	*Daphnia magna*	48-hour MLD at 17°C	3523
34	*Polycelis nigra*	Toxic threshold	354
63	*Daphnia*	Toxic threshold	2158
97	*Scenedesmus*	Toxic threshold	2158
226	*E. coli*	Toxic threshold	2158
1000	Chironomus larvae	Resisted	690

SODIUM SULFHYDRATE NaHS

Completely and rapidly soluble in water, with an H_2S odor, this crystalline substance is used in tanneries for dehairing hides, in the manufacture of sulfur dyes, and for desulfurizing viscous rayon (364). It may also be found in Kraft mill wastes.

Using turbid water at 21-33°C, Wallen et al. (2940) studied the toxicity of NaHS toward the mosquito-fish, *Gambusia affinis*. They found the 24-, 48-, and 96-hour TL_m values to be 206 mg/l. The 144-hour TL_m was lowered to 138 mg/l. Haydu et al. (3501, 3502) report the minimum lethal concentrations of NaHS to be 3.3 mg/l for king salmon, 3.5 mg/l for silver salmon, 1.8 mg/l for cutthroat trout, and 0.5 mg/l for Wisconsin minnows. The maximum concentrations of sodium sulfhydrate tolerated by king salmon, silver salmon, and cutthroat trout are 2.1, 2.5, and 0.3 mg/l respectively (3501, 3502).

Gillette et al. (1442) tested the toxicity of this substance to creek chub, a fish considered to be average in tolerance. They found that the concentration below which all four test fish survived for 24 hours was 4 mg/l, while above 10 mg/l all fish died. The critical concentration of NaHS to salmonoid fish in water containing over 5 mg/l of dissolved oxygen ranged from 0.3 mg/l for threshold damage to 2.5 mg/l for lethality (1582).

SODIUM SULFITE Na_2SO_3

1. General. This soluble salt is used chiefly in photographic developers and fixing operations. It is also employed in bleaching operations and as a reducing agent in the manufacture of dyes (364). Sodium and sulfite ions may occur in various paper mill and beet-sugar-factory effluents (313).

2. Cross References. Sulfites, Sulfite Waste Liquor.

3. Effects Upon Beneficial Uses.

a. Stock and Wildlife Watering. A concentration of 15,000 mg/l of sodium sulfite in the drinking water of rats caused no apparent injury (287).

b. Fish and Other Aquatic Life. The lethal concentration of sodium sulfite to goldfish in 96 hours at 18-23°C has been reported (2920) as 100 mg/l; but on the other hand, concentrations up to 260 mg/l at pH 5 to 9 have been observed to be harmless to young carp (1698). Using turbid water at 18-26°C, Wallen et al. (2940) found the 24-, 48-, and 96-hour TL_m for mosquito-fish to be 2600 mg/l.

The toxic threshold concentration of sodium sulfite for the flatworm, *Polycelis nigra*, was found to be 7580 mg/l (354). The minimum lethal concentration for *Daphnia* has been reported to be 300 mg/l (574, 3523). For the immobilization of *Daphnia magna* in Lake Erie water at 25°C, the threshold concentration of sodium sulfite has been reported to be 440 mg/l (352) and 3784 mg/l (358).

SODIUM SULFONATES

(see Chapter X)

SODIUM TETRAPROPYLENE BENZENE SULFONATE

(see Chapter X)

SODIUM THIOCYANATE NaSCN

(see also Cyanides, Hydrogen Cyanide, Potassium Thiocyanate)

This highly soluble salt is used in the manufacture of other thiocyanates, especially the organic compounds, and in many of the same processes as potassium thiocyanate (364). When chlorinated, it breaks down to release cyanide ions (see KSCN).

According to Schaut (362), fish were killed in two hours by 3240 mg/l of sodium thiocyanate. For *Daphnia magna* in Lake Erie water at 25°C, however, the threshold concentration was found by Anderson (352) to be less than 11.3 mg/l or about 10.2 mg/l (353). The threshold concentration for *Polycelis nigra*, a planarian, was reported by Jones (354) at 700 mg/l as SCN^-.

Thiocyanate compounds have been used for the treatment of hypertension in human patients. The daily ingestion of less than approximately 120 mg of SCN has not given rise to reports of harmful effects in patients treated for 10 years (2963).

SODIUM THIOSULFATE $Na_2S_2O_3 \cdot 5H_2O$

Sodium and thiosulfate ions may occur as constituents of Kraft mill wastes. The toxic threshold concentration of sodium thiosulfate for the flatworm *Polycelis nigra* was found to be 8400 mg/l (354). The threshold concentration of sodium thiosulfate for the immobilization of *Daphnia magna* in Lake Erie water was found to be much less than 520 mg/l (352). The minimum lethal concentration of sodium thiosulfate for minnows has been reported to be 5.0 mg/l, for a 120-hour period (190, 344). Using highly turbid water at 22-24°C, Wallen et

SCHRADAN

(see Chapter IX)

SECONDARY SODIUM ALKYL SULFATE (Teepol)

(see Chapter X)

SELENIOUS ACID H_2SeO_3

(see also Selenium)

When selenium dioxide, SeO_2, is dissolved in water, selenious acid is formed. At normal pH values, i.e. 5 to 8, this acid is only partially dissociated in water, with most of the molecules occurring as $HSeO_3^-$ (911). In stabilized tap water, minnows were not harmed during a one-hour exposure to 17.1 mg/l of selenious acid (362).

SELENIUM Se

1. General. Analogous to sulfur in many of its chemical combinations, selenium is used in its elemental form and as several salts in a variety of industrial applications, such as pigmentation in paints, dyes, and glass production; as a component of rectifiers, semiconductors, photo-electric cells, and other electrical apparatus; as a supplement to sulfur in the rubber industry; as a component of alloys; and for insecticide sprays (364, 2121, 2129). Selenium occurs in some soils as basic ferric selenite, as calcium selenate, as elemental selenium, and in organic compounds derived from decayed plant tissue. In some areas of South Dakota and Wyoming, soils may contain up to 30 mg/kg of selenium (2121). Selenium may be expected in trace quantities in the municipal sewage from industrial communities. Although selenium is found in some natural waters, food appears to be a more likely source for human ingestion (1049, 1328, 1329).

2. Cross References. Arsenic, Selenious Acid, Sodium Selenite.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Proof of human injury by selenium is scanty and definite symptoms of selenium poisoning have not been identified; but it is widely believed that selenium is highly toxic to man (353, 997, 1329, 1330). It has been stated that the symptoms of selenium poisoning are similar to those of arsenic poisoning (555).

In areas where selenium poisoning of livestock is common, the toxic effects appear to be associated with the consumption of high concentrations of selenium in food rather than in the water (152, 1331). In food stuffs 4.0 mg/kg is considered an extreme tolerance limit; and 3 mg/kg is regarded as a safer limit (353).

Mild chronic selenium poisoning has been observed in humans living in areas where the soil and produce are rich in selenium. In addition, there have been cases of selenosis at industrial establishments that use or produce selenium compounds. Hydrogen selenide (H_2Se) in the air in concentrations as low as 0.2 parts per million by volume produces definite toxic symptoms (2129).

Selenium in trace amounts appears to be essential for the nutrition of animals, including man, although very little is known about the mechanism of its action (3217). Arsenic and selenium are apparently antagonistic in their toxicity, tending to counteract each other. Selenium salts are rapidly and efficiently absorbed from the gastro-intestinal tract and excreted largely through the urine. Retention is highest in the liver and kidney (2121, 2129).

Surveys have shown that dental caries rates of permanent teeth were significantly higher in seleniferous areas than in non-seliniferous areas (2253). There is also a tendency for increased malocclusion and gingivitis in seleniferous areas (2253).

The U.S. Food and Drug Administration placed a "zero tolerance" on selenium and seleniferous materials with respect to permissible residue on fruits and other edible crops (3651).

The USPHS Drinking Water Standards (2036) have restricted selenium to 0.05 mg/l on a mandatory basis for many years (See Table 5-1). In 1962, however, the new standards lowered the mandatory limit to 0.01 mg/l. The WHO International (2328) and European (2329) Drinking Water Standards prescribe a mandatory limit of 0.05 mg/l. These strict standards were undoubtedly set because of the similarity between arsenic and selenium poisoning, the dental effect, and the known toxicity to livestock, as described below.

b. Irrigation. In general, the soil in parts of the world where selenium poisoning occurs naturally contains 1 to 6 mg/kg of selenium in the top eight inches. However, plants vary in their ability to absorb selenium; the final selenium concentration in the plant will be determined by many factors, including the species and age of the plant, season of the year, and the concentration of soluble selenium compounds in the root zone (1049).

Plants can absorb relatively large amounts of selenium from irrigation water or soil without apparent injury to themselves. Certain weeds, such as *Astragulus*, may contain as much as 4500 mg/kg of selenium (1049). Other plants grown on seleniferous soil have been found to contain as much as, or more than, 1610 mg/kg of selenium (dry weight) (1003, 1328). Byers believes that any vegetation containing 5.0 mg/kg of selenium is potentially dangerous for livestock; and that any soil containing more than 0.5 mg/kg is also hazardous (1329). Russell has pointed out that fodder containing as little as one mg/kg of selenium has caused poisoning, and states that 4.0 mg/kg is the tolerance limit of livestock (1049).

Cereals and grains concentrate selenium from the soil, the amount depending on the chemical condition of the selenium, the type of crop, and the sulfate content of the soil. High sulfates diminish the uptake of selenium. Up to 63 mg/kg of selenium have been found in wheat and up to 17.8 mg/kg in onions (2121).

Plants have been injured by selenium in the soil; wheat plants have been severely damaged by 30 mg/kg of sodium selenate in the soil (1328). However, injury to plants can be reduced or prevented by sulfate ion, when it is present in a concentration about 12 times as high as that of selenium (1328). Klintworth (1493) believes that selenium is harmful to plants at all concentrations. Walker and Rigdon (1688) have prepared an extensive bibliography of papers on selenium toxicity to plants and animals.

The USDA Regional Salinity Laboratory at Riverside, California, has not established limits on selenium, but

Dr. Beath of the Research Chemistry Department, University of Wyoming, suggests the following tentative limits of selenium in water for irrigation (3652):

Irrigation Class	*Selenium in mg/l*	*Remarks*
1-low	0.00-0.10	No plant toxicity anticipated
2-medium	0.11-0.20	Usable, but with possible long-term accumulations under particular conditions should be watched
3-high	0.21-0.50	Doubtful-probable toxic accumulation in plants except under especially favorable conditions
4-very high	over 0.50	Non-usable under any conditions

c. Stock and Wildlife Watering. Selenium poisoning ("alkali disease" or "blind staggers") occurs frequently among livestock in the Great Plains regions of the United States and Canada, and also in Mexico. It can be produced in laboratory rats, as well as livestock, by feeding abnormal amounts of inorganic selenium compounds or seliniferous feed. In general, the soil in affected areas contains 1 to 6 mg/kg of selenium in the top eight inches, but higher values have also been found. Not all such soils give rise to toxic vegetation, partly because of differences in the solubility of the selenium compounds present and their availability to plants. Where poisoning is prevalent, samples of vegetation have been found to contain as little as one mg/kg of selenium. About four mg/kg appears to be the tolerance limit for livestock. Acute poisoning is caused by ingesting large amounts.

Selenium poisoning occurs naturally among cattle, sheep, horses, pigs, and even poultry, in both chronic and acute forms. It is characterized by loss of hair from mane and tail and soreness of the feet, as well as by deformity, loss of condition, and emaciation. Among poultry, the eggs give rise to abnormal or weak chicks (1049, 1328).

Impairment of vision, weakness of limbs, and respiratory death have resulted from livestock feeding on plants containing 100 to 1000 mg/kg of selenium. At lower concentrations in vegetation, up to 24 mg/kg, livestock is subject to "alkali disease" involving lack of vitality, loss of hair, sterility, lameness, and possibly death from anemia and malnutrition (2129).

Rats show toxic effects from diets containing 3 to 40 mg/kg of selenium. A dose of 4 grams of powdered selenium introduced into a dog's stomach appeared to be harmless; but 4 mg of soluble selenate per kilogram of body weight was quickly fatal (353).

The lethal dose of selenium salts for cattle is believed to be 2 mg per animal pound (1003). As sodium selenite administered by mouth, the minimum lethal dose of selenium for horses is about 1.5 mg per kilogram of body weight; for cows and calves, 4.5 to 5.0 mg per kg; and for pigs, 6 to 8 mg per kg. Chronic poisoning of cattle has been caused by as little as 1 to 4 mg/kg of selenium in fodder (1049). Milk from cows in poisoning areas contains 0.3 to 1.2 mg/l of selenium (1049).

In water, 0.4 to 0.5 mg/l of selenium is believed to be non-toxic to cattle. Such water may contribute to selenium poisoning, but the selenium content of the feed is a more critical factor (997, 1003).

The toxicity of 9 to 18 mg/kg of selenium in the dry ration of rats, pigs, and dogs has been counteracted by various arsenic compounds administered in the drinking water (see also Arsenic) (921, 1005).

Selenium in the dry ration at a concentration of 0, 2, or 4 mg/kg did not affect the health of poultry or the hatchability of eggs. Chicks fed 5-8 mg/kg of selenium showed no harmful effects. A concentration of 5 mg/kg (dry ration) should be considered the toxic threshold for poultry (1551).

Kohlmeyer and Moxan found that 5 mg/kg of selenium (dry ration) did not affect the hatchability of turkey eggs, but 9 mg/kg caused abnormalities in young turkeys (1551).

An increase in mortality among rats fed a ration containing 11 mg/kg of Se has been caused by the presence in drinking water at concentrations of 5 mg/l of various chemicals. The presence of 5 mg/l of soluble tungsten in the drinking water reduced the mortality slightly. For a comprehensive presentation of the selenium problem, particularly in feeds, the reader is referred to the paper by Moxan and Rhian (1481).

d. Fish and Other Aquatic Life. Added as sodium selenite, 2.0 mg/l of selenium has been toxic to goldfish in eight days, and lethal in 18 to 46 days (353). Minute concentrations of selenium appear not to be harmful to fish during an exposure period of several days; however, constant exposure to traces of selenium has caused disturbances of appetite and equilibrium, pathological changes, and even deaths of fish after several weeks. Concentrations considered safe for human beings over a period of weeks have been toxic to fish (770, 3203).

Barnhart (3653) describes a reservoir in Colorado that has been stocked repeatedly with a variety of game fishes, but the fish die in a few weeks. Bottom deposits in the reservoir contain high concentrations of selenium. It is believed that selenium is passed up through the food chain to the fish which accumulate this element in the liver in lethal concentrations.

Using water from the River Havel, from which the test organisms had been recovered, Bringmann and Kuhn (2158, 3343) studied the threshold effect of selenium added as sodium selenite. For *Daphnia* the median threshold effect for a 48-hour exposure at 23°C occurred at 2.5 mg/l of selenium; for *Scenedesmus* exposed at 24°C for 4 days, the median threshold level was also 2.5 mg/l as selenium; but for *Escherichia coli* at 27°C it was 90 mg/l of selenium, and for a protozoan (*Microregma*) it was 183 mg/l.

SESONE

(see Chapter IX)

SETTLEABLE SOLIDS

(see also Suspended Solids, Silt)

Settleable solids may consist of sewage and industrial sludges, including many kinds of suspended solids that tend to settle out slowly on the stream bottom, thereby smothering bottom organisms, covering and destroying spawning beds, blanketing bacteria, fungi, and decomposing organic wastes, and in general trapping and maintaining offensive and deleterious stream conditions. Deposits of solids interfere with recreation, navigation, fish and shellfish production, and destroy esthetic values

of water. They may decompose to produce putrefactive odors and they may exude products of decomposition to overlying waters (611, 664, 673, 684, 780, 781, 782, 783, 2407, 3654, 3655).

Ellis has recommended that the stream bottom should not be blanketed to a depth of more than one-quarter of an inch by sedimentary deposits in order to prevent destruction of bottom fauna and shellfish (347).

According to many authorities, settleable solids should be removed from sewage and effluents before discharge into streams or ocean, or on land for irrigation purposes (784, 785, 786, 787, 788). Several alternative recommendations or practices have been mentioned in the literature, and most standards or criteria for streams include a provision that wastes discharged thereto should not form sludge banks or deposits. In this connection it is significant to recognize that dissolved or colloidal solids may be synthesized by bacteria in a stream to form suspended and settleable sludge consisting primarily of bacterial and biological slimes. Sulfite waste liquors, for example, contain little or no settleable solids but they can result in sizeable sludge deposits as a result of biological action in the stream (7).

Specific limiting or threshold concentrations of settleable solids permitted in natural waters are not to be found in the literature. Instead, it is generally required that settleable solids be removed from sewage or industrial wastes.

SEVIN

(see Chapter IX)

SILICA AND SILICATES SiO_2, SiO_3^{--}

1. General. The element silicon is not found free in nature but it occurs as silica in sand or quartz and as silicates in feldspar, kaolinite, and other minerals. Silicon dioxide, or silica, is insoluble in water or acids, except hydrofluoric, but it may occur in natural waters as finely divided or colloidal suspended matter, in concentrations of one to 40 mg/l (32, 2342). Silicon is used in metallurgy and silica is widely employed in industry for making glass, silicates, ceramics, abrasives, enamels, petroleum products, etc. (364). Silicates have been used in water treatment as coagulants and corrosion inhibitors while sodium silicofluoride has been used as a fluoridating agent.

2. Cross Reference. Turbidity, Suspended Solids, Silt.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Sodium silicates have been used as coagulants for the removal of turbidity and iron (1332) and dosages of 4.0 to 8.0 mg/l have been used to form protective coatings on pipes to inhibit corrosion of iron (206, 1333, 1334, 3305, 3656) or lead (1335). In concentrations found in natural or treated waters, silica or silicates appear to have caused no adverse physiological effects (36). According to Raux (178), silica in a concentration greater than 50 mg/l may cause difficulties arising from turbidity. When municipal water is used for boiler feed water, however, the presence of silicates may be detrimental (see below).

b. Industrial Water Supplies. A summary of the recommended limits of silica in water for various industrial processes, as discussed in Chapter V, is presented below:

Industrial Use	*Recommended Limit in mg/l*
Brewing	50
Boiler feed water, 0-150 psi	40
150-250 psi	20
250-400 psi	5
400 psi and over	1
Pulp and paper mills:	
Groundwood pulp	50
Soda and sulfate pulp	20
Kraft pulp, bleached	50
Kraft pulp, unbleached	100
Fine paper	20
Rayon pulp production	25

Silica appears to be particularly undesirable in boiler feed waters (212, 1336, 1337, 1338) because it passes over in the steam and deposits in the tubes of heaters and on steam-turbine blades. Silica appears to have a volatility or solubility in steam (3657), for as much as 0.4 mg/l of silica per ml of steam has been found leaving the boilers, according to Schwartz (1339), who found that silica in boiler feed water gave the following concentrations in condensate:

Silica in Feedwater, mg/l	*Silica in Condensate, mg/l*
25	0.43
100	0.07 to 0.90
200	1.8

For the maximum concentration of silica in boiler feed water, Reichelt (1689) recommended 0.5 mg/l while Stassart (1690) preferred a limit of 0.3 mg/l for high-pressure boilers. Imhoff (1340) suggested that the silica content of steam be kept below 0.1 mg/l to control turbine-blade deposits at operating pressures of 350 psi.

c. Irrigation. Silica, per se, is generally considered to be of little importance in irrigation practice (268). Limits for several constituents suggested for irrigation water by the U. S. Department of Agriculture include silica at 10 to 50 mg/l.

d. Fish and Other Aquatic Life. An abundance of silica in water, along with other necessary nutrients, favors the growth of diatoms (36, 1287) (see also Silt, Turbidity, and Suspended Solids). Indeed, blooms of diatoms synthesize silica into the tests of the organisms and thereby lower the content in the water. The diatom *Asterionella*, and possibly other algae, require silica in concentrations above 0.5 mg/l for growth in ponds, and cannot utilize silica when present at lower concentrations (1453). For factors by which silicon (radioactive or otherwise) is concentrated from water into biological forms, see Chapter VIII.

SILICON

(see Silica and Silicates)

SILT

1. General. By the term "silt" is meant loose sedimentary material, rock particles, mud, or clay that settle readily and may be transported by bed-load movement. Silt results from erosion, logging, mining, dredging, or irrigation operations, steel mills, gravel washing, the

precipitation of certain industrial dusts, such as those from limestone saw-mills, and also from operations in cotton and wool-cleansing plants (779, 2194, 2879, 3655, 3658, 3659). Fine silt may stay in suspension and be classified as "suspended solids", while the larger particles settle to the bottom of the water course. Like other suspended solids in streams, silt may have far-reaching effects on the nature and biota of the stream by reducing the amount of light transmitted by the water, by altering the rate of temperature change in water strata, by adsorbing organic matter and other substances that create unfavorable conditions on the bottom, and by blanketing the stream bottom (2183). Although a moderate amount of silt may have a beneficial effect on aquatic life by increasing the amount of mineral nutrients available (789), excessive quantities have been reported to affect deleteriously all of the desirable uses of water (346, 780, 781, 790, 2879, 2895, 3654, 3660, 3661).

2. Cross References. Suspended Solids, Settleable Solids, Turbidity.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Silt and mud are undesirable in domestic and industrial water supplies, and the cost of removing mud from water may exceed all other costs in making water suitable for use (780).

b. Irrigation. Mud constituents of water can fertilize the soil and may also be valuable for closing up cracks in the earth channels of water. However, grit and sand may injure crops (791). Silt from fertile land is beneficial, but silt from the subsoil of eroded areas deposited on the surface of productive soils can markedly decrease their productivity (246). Silt consisting of clay-like matter can result in the packing of sediment on soil to form an impervious blanket, keeping air and water out of the soil (792). Over a three-year period of observation, silty water did not affect the yields of corn as compared with the yields from plots irrigated with clear water; however, oat fields were reduced when sediment was deposited in sufficient quantities before germination to prevent the emergence of the seedlings; and heavy silting killed bean plants grown in furrows, but not those grown on ridges (793).

c. Aquatic Life. Silt in water reduces light penetration, alters the rate of temperature change in different water levels, and carries organic wastes to the bottom where processes of decay may continue to operate over a long period, leading to oxygen depletion, changes in pH, and other unfavorable conditions. Erosion and silting hinder fish production, smother food organisms, destroy spawning grounds, and fill pools (308, 611, 781, 790, 794, 795, 796, 797). Smith has reported that silting has reduced the bottom fauna of the Rogue River by 25 to 50 percent (798). According to Ellis, hard particles cause abrasive injuries to the delicate external organs, such as gills, spiracles, and fins, of miscellaneous aquatic animals, and fine particles tend to coat and destroy fish eggs (347, 799, 800). Ellis has recommended, therefore, that suspensoids of a hardness of one or more should be so finely divided as to pass through a 1000-mesh screen, and that the stream bottom should not be blanketed to more than one-quarter of an inch (see also Turbidity) (313, 347, 801).

Salmon and trout can withstand a heavy load of silt for several weeks; nevertheless, by limiting successful reproduction and the food supply, silting is harmful to fish (800). Ward (560, 882) agrees that silt deposits may smother spawning beds and nests, but believes that natural and mining silts are not always inimical to fish life; they may possibly be beneficial because of the food organisms and particles they carry and because muddy waters afford protection to fish against predatory animals and anglers. Ward cites experiments in which young salmon were maintained for three to four weeks without harm in circulating water containing a suspension of mud in a concentration above 1000 mg/l. Griffin (802) has reported experiments of three to four weeks' duration in which trout and salmon fingerlings fed and grew apparently well in muddy water carrying a constant silt load of 300 to 750 mg/l, and for short intervals daily during stirring, a load as high as 2300 to 6500 mg/l (For comparison, the Willamette River in heavy flood, when it appeared muddy, carried a silt concentration of only 42 mg/l).

Ziebell (2687) and Wagner (3662) measured the productivity in terms of aquatic insect populations upstream and downstream from a gravel dragline operation. He found a productivity decline of 85 percent as a result of the gravel operation. Turbidities were increased from zero to 91 mg/l and suspended solids from 2 mg/l upstream to 103 mg/l downstream. Tarzwell (2407) cites an instance where bottom samples from a silted area averaged only 36 organisms per sq ft whereas in a clean stream bottom the average was 249 per sq ft.

d. Shellfish Culture. Most fresh-water mussels have been unable to maintain themselves in either sand or gravel bottoms when a layer of silt one-quarter to one inch deep was allowed to accumulate on the surface (790). Silt deposits can smother both mussels (331, 795, 798) and oysters (332, 790). Silt in suspension chokes the respiratory and intestinal passages of young shellfish (314, 332, 803). Silt concentrations from 1000 to 4000 mg/l have depressed the pumping and feeding rate of oysters as much as 94 percent; and in heavy suspensions oysters have been stopped feeding entirely (804). On the other hand, some species of oysters thrive in silt-laden water (315, 804), and silt from dredging operations in the Susquehanna River did not cause oyster deaths (795).

As little as 100 mg/l of silt or other turbidity-producing substance seriously reduces the pumping rate of oysters to as low as 40 percent; 1000 mg/l caused a drop to 20 percent of the normal rate; and 3000-4000 mg/l resulted in a reduction to 4 percent. Silt, kaolin, and chalk all gave comparable results. When the oysters were returned to clear water, however, they resumed their normal pumping (1448).

e. Recreation and Navigation. Silt and mud destroy esthetic values and produce deposits in harbors that cause significant economic losses (780, 781, 1033).

SILVER Ag

1. General. In nature, silver is found in the elemental state and combined in ores such as argentite, Ag_2S, horn silver, AgCl, proustite, Ag_3AsS_3, and pyrargyrite, Ag_3SbS_3. From these ores, silver ions may be leached into

ground waters and surface waters, but since many silver salts such as the chloride, sulfide, phosphate, and arsenate are insoluble, silver ions cannot be expected to occur in significant concentration in natural waters. Nine municipal water supplies in the U.S. have shown the presence of 0.05 mg/l of silver ion and at Denver the concentration is reported as 0.2 mg/l (152). Silver nitrate is highly soluble and silver sulfate moderately so (1224).

Silver metal is used in jewelry and silverware, in alloys, for electroplating, and in the processing of food and beverages. Silver nitrate is used in photography, ink manufacture, electroplating, coloring porcelain, and as an antiseptic (364, 2121). From such sources, traces of silver can be expected to reach natural waters.

2. Cross References. Nitrates.

3. Effects on Beneficial Uses.

a. Domestic Water Supplies. Silver oxide has been used for water purification at Warsaw, Poland, and other cities. There is abundant literature on the oligodynamic action of silver and its bactericidal power, but a review of such literature is beyond the scope of this report. Dosages of 0.000001 to 0.5 mg/l of silver have been reported as sufficient to sterilize water (577, 1341, 1342, 1343, 3408, 3663, 3664, 3665). At such dosage rates, silver is not an irritant, has no toxic action toward humans, and does not interfere with the taste (1344). The fatal dose of silver nitrate for humans has been reported as 10 grams (2129).

Silver occurs in soil humus up to 5 mg/kg, in grain and cereals up to 0.9 mg/kg, and in mushrooms up to several hundred mg/kg (2121). Silver nitrate solution has been used as an antiseptic, especially in the upper respiratory system.

Prior to 1962, no restriction was placed on silver in the USPHS Drinking Water Standards (2036), nor does any limitation presently appear in the WHO International or European standards (2328, 2329). The 1962 edition of the USPHS Standards (2036) sets a mandatory limit of 0.05 mg/l of silver. Presumably, this restriction was set not because of the danger of silver in natural sources of water supply but to prevent its intentional addition to water, in excessive amounts, for disinfection. The limit was based on cosmetic considerations rather than public-health effects.

The chronic administration of silver salts, not uncommon in the early part of the 20th century, led to a bluish permanent darkening of the skin called "argyria". With prolonged administration of silver salts, a small proportion is absorbed and deposited in the skin where it is reduced by light to metallic silver (2129). This impregnation of the tissues with silver causes no recognizable disturbance to health (3704), but is obviously undesirable from an esthetic viewpoint. Although the total dosage of ingested silver required to produce argyria is not known, the intravenous injection of 1 gram will cause this effect. Based on the USPHS limit of 0.05 mg/l of silver and two liters per day of water consumption, it would take over 27 years to ingest one gram of silver, a large part of which would pass through the intestinal tract without assimilation (2121). This calculation ignores the daily intake of silver from food, which might be appreciable for mushroom lovers.

b. Fish and Other Aquatic Life. Using silver nitrate, Marsh and Robertson (1459) found that 0.04 mg/l killed some (but not all) test salmon fry in tap water within 48 hours; 0.44 mg/l of silver nitrate proved decidedly toxic. A concentration of 0.033 mg/l of silver nitrate did not prove fatal in 48 hours. The highest concentration of silver nitrate tolerated by young eels for more than 24 hours was reported to be about 0.2 mg/l. Toward guppies, the LD_{50} value was found to be 0.0043 mg/l, as silver (2921).

Jones (1460) found that the lethal concentration limit of silver applied as silver nitrate, for sticklebacks at 15-18°C was 0.003 mg/l. In different concentrations of silver the average survival times of the fish were as follows: one week at 0.004 mg/l, four days at 0.01 mg/l and only one day at 0.1 mg/l, as silver. Anderson (598) reported that the toxic threshold of silver nitrate for sticklebacks was 0.0048 mg/l.

The level of toxicity of silver toward lower organisms has been reported by several observers. Using water from the River Havel from which the test organisms were recovered, Bringmann and Kuhn (2158, 3343) studied the threshold effects of silver, added as silver nitrate, on various species during a 4-day exposure, at 23-27°C. For *Daphnia* and for *Microregma* the median threshold effect occurred at 0.03 mg/l of silver, for *E. coli* at 0.04 mg/l, and for *Scenedesmus* at 0.05 mg/l. Jones (608) tested the toxicity of silver nitrate toward *Polycelis nigra*, a flatworm, and found the threshold concentration to be 0.15 mg/l of silver. Toward *Daphnia magna* in Lake Erie water at 25°C, the threshold for immobilization was reported (598) to be 0.0051 mg/l of silver nitrate. Hodgson (2956) studied the stimulating effects of several substances on the water beetle, *Laccophilus maculosus*. He found that 580 mg/l of silver nitrate stimulated 50 percent of the beetles into movement, but no lethal dose was reported.

The Severn and Mersey River Boards in England (2950) have set working standards of 1.0 mg/l on the total of all heavy metals, including silver.

SILVEX

(see Chapter IX)

SIMAZINE

(see Chapter IX)

SINOX

(see Chapter IX)

SKATOLE C_9H_9N

This constituent of coal tar, feces, and some plants is soluble in hot water, giving a fecal odor (364). The odor is detectable at a concentration of 0.0012 mg/l (1756).

SLAKED LIME

(see Calcium Hydroxide)

SOAPS

(see also Chapter X)

Soaps are salts of fatty acids, usually made by the saponification of a vegetable oil with sodium hydroxide.

They may be formed also by resinous acids in the Kraft process at paper mills.

Toward fish, crude sulfate soap from Kraft mill wastes had a minimum lethal concentration of 5.0 mg/l toward minnows and shiners, according to research by the National Council for Stream Improvement (190, 344, 574, 658, 690, 3500, 3523). LeClerc and Devlaminck (3666) report that for minnows the minimum lethal concentration of sodium palmitate and sodium oleate was 10-12 mg/l in distilled water and 900-1000 mg/l in hard water. For sodium stearate it was 10-12 mg/l in distilled water and 250-300 mg/l in hard water. The effect of water hardness was confirmed by Henderson et al. (1910) who found that the 96-hour TL_m values for three packaged soaps ranged from 29 to 42 mg/l in soft water but averaged 1470 mg/l in hard water. Soaps form insoluble complexes or precipitates in hard water which are apparently non-toxic to fish. The pure compound, sodium stearate, was somewhat less toxic than the packaged soap products (1910).

Cole (311) claims that dairy wastes contain soaps that are toxic to fish at 600 mg/l and Schaut (362) reports that soapy waste waters at a dilution of 1:240 had no effect on minnows. Turnbull et al. (2093) reported that the 48-hour TL_m of truck-washing soap solution toward bluegill sunfish at 20°C occurred at dilutions of 1:357 to 1:416.

Toward other aquatic organisms, crude sulfate soap from Kraft mills had a TL_m of 50 mg/l for *Chironomus* larvae, 10 mg/l for mayfly larvae, and 5.0 mg/l for *Daphnia magna* (190, 574, 690, 3500, 3523). Soaps in concentrations of 1000 to 2500 mg/l were lethal to mosquito pupae by reducing the surface tension of the water so that the pupae were unable to maintain their position and suffocated (805). Solutions containing 5,000 to 10,000 mg/l were directly toxic to the pupae. In another instance (756) soap in a concentration of 2000 mg/l completely destroyed mosquito larvae and pupae.

SODA PULP MILL WASTE

(see Kraft Pulp Mill Wastes)

SODIUM Na

1. General. This very active metal does not occur free in nature, but sodium compounds constitute 2.83 percent of the crust of the earth (364). Owing to the fact that most sodium salts are extremely soluble in water, any sodium that is leached from soil or discharged to streams by industrial wastes will remain in solution. Some sodium may be removed by ion-exchange phenomena in the passage of ground waters through certain soils. For other effects of sodium on soils, see Chapter V—Agricultural Supply.

Sodium is the cation of many salts used in industry and as such is one of the most common ions in process wastes. The information presented below pertains to literature in which the sodium ion, per se, was mentioned without reference to the sodium salt. Where concentrations were given in terms of the salt, the literature has been abstracted under the appropriate sodium compound.

2. Cross References. Sodium Salts, Potassium, Dissolved Solids, Chapter IV, Chapter V.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Sodium in drinking water may be harmful to persons suffering from cardiac, renal, and circulatory diseases (1242, 3667), and as much as 200 mg of sodium from drinking water may be injurious (152). Although it has been reported (627) that drinking water of good quality may contain up to 115 mg/l of sodium, Hibbard (250) recommends a limit of 10 mg/l as desirable. According to Lockhart et al. (3241) the taste threshold of sodium in distilled water varies with the sodium salt used, as follows:

Salt Tested	*Taste Threshold of Sodium in mg/l*
Sodium acetate	140
Sodium carbonate	34
Sodium bicarbonate	290
Sodium chloride	135
Trisodium phosphate	75

b. Industrial Water Supplies. More than 50 mg/l of sodium plus potassium in boiler waters may cause foaming (1214). Other limitations for sodium, as given in Chapter V, are expressed in terms of the sodium salts (see Sodium Chloride, Carbonate, and Sulfate).

c. Irrigation. Sodium is required in very limited amounts for most plant growth (3634). An exception is the desert plant, halogeton, which apparently needs sodium for optimum growth (3668). In high concentrations sodium is not only toxic to plants but deleterious to soil conditions (268, 635, 1322) (see also Chapter V—Irrigation). Sodium carbonate and bicarbonate are more harmful than other sodium salts to plants (273). On a weight basis, chlorides are generally more toxic to plants than sulfates, but at equal osmotic pressures, sodium sulfate appeared to be more injurious to flax than sodium chloride (1093).

High concentrations of sodium (e.g. 106-212 mg/l) in water used for sprinkler irrigation can, under certain conditions and with a few plants, cause deleterious accumulations of sodium in the foliage (3669, 3670). Sufficient sodium has been absorbed by citrus leaves from a single sprinkling with water containing 69 to 190 mg/l of sodium to cause serious leaf burn and defoliation (3670).

Sodium-saturated soils are greasy to feel. Sodium soil colloids swell, closing the pores of the soil, reducing soil permeability to water and air, and increasing the pH of the soil solution to dangerous levels. The deterioration of soil quality because of sodium in the irrigation water is a steadily cumulative process, with impaired drainage resulting in even more highly concentrated soil solutions (280).

Kelley reported that an average concentration of 82 mg/l of sodium in water from the Colorado River was not expected to affect seriously either the growth of citrus trees or soil conditions in the South Coastal Basin of California (1060). For an instance where sodium figured prominently in a court case, see *Barakis vs American Cyanamid Co.* in Chapter IV.

d. Stock and Wildlife Watering. Excess concentrations of various sodium salts in the drinking water are deleterious to various animals. Well waters containing 7000 mg/l of sodium were toxic to chicks (1551). A threshold limit of 2000 mg/l of sodium for livestock has been suggested by Stander (3373). For further details,

see Sodium Salts, Dissolved Solids, Chapter V. Sodium bicarbonate is less injurious than sodium carbonate (640).

e. Fish and Other Aquatic Life. Of the United States waters supporting a good fish fauna, ordinarily the concentration of sodium plus potassium is less than 6 mg/l in about 5 percent; less than 10 mg/l in about 50 percent; and less than 85 mg/l in about 95 percent (310).

The toxicity threshold of sodium for *Polycelis nigra* is 4370 mg/l from sodium chloride, and 1000 mg/l from sodium nitrate (353, 608). A sodium concentration of 500 mg/l is lethal to stickleback (353). On the other hand, moderate amounts of sodium in water may reduce the toxicity of potassium and ammonium salts toward fish (306).

The possible accumulation of radioactive sodium (Na-24) in aquatic life, fish, or oysters is not considered to be hazardous owing to the short half life (about 15 hours) of radiosodium (2442).

Several investigators have reported independently that 500-1000 mg/l of sodium was toxic to fish in distilled and soft waters when sodium chloride or nitrate was tested 1450, 2941). However, Gueylard reported as harmless to sticklebacks concentrations up to 4720 mg/l of sodium in distilled water and up to 7870 mg/l in tap water when sodium chloride was added (1459).

Freeman and Fowler (1691), using a standardized double-distilled water, studied the toxicity of five inorganic sodium salts to *Daphnia magna* at 23°C. The 100-hour toxicity thresholds were determined for each salt alone, in combinations of two, and in combinations of three. The 100-hour toxicity threshold was defined as the concentration that will immobilize 50 percent of the organisms in 100 hours. Rather than list their results separately under the specific sodium salts involved, all of their data are presented under this heading in order that the magnitudes can be compared. For each of the five salts tested singly, the 100-hour toxicity thresholds and the resulting pH values are shown in the following tabulation:

Compound Tested	*100-Hour Toxicity Threshold in mg/l*	*pH at Threshold*
Sodium bisulfite	102	7.3
Sodium carbonate	524	9.5
Sodium chromate	0.42	7.8
Sodium silicate	247	9.1
Sodium sulfate	4547	7.7

When these salts were tested in combinations of two, the results were as follows:

Compounds Tested	*100-Hour Toxicity Thresholds in mg/l*	*pH at Threshold*
Sodium bisulfite Sodium carbonate	85 436	9.2
Sodium bisulfite Sodium chromate	70 0.286	7.3
Sodium bisulfite Sodium silicate	177 427	7.5
Sodium bisulfite Sodium sulfate	82 3642	7.5
Sodium carbonate Sodium chromate	408 0.33	9.3
Sodium carbonate Sodium silicate	180 85	9.3
Sodium carbonate Sodium sulfate	221 1918	9.2
Sodium chromate Sodium silicate	0.159 93	8.7
Sodium chromate Sodium sulfate	0.276 2984	7.9
Sodium silicate Sodium sulfate	158 2899	9.0

In combinations of three, the toxicities of the sodium salts were as follows:

Compounds Tested	*100-Hour Toxicity Thresholds in mg/l*	*pH at Threshold*
Sodium bisulfite Sodium carbonate Sodium chromate	87 440 0.35	9.3
Sodium bisulfite Sodium carbonate Sodium silicate	38 194 92	8.8
Sodium bisulfite Sodium carbonate Sodium sulfate	58 295 2562	9.0
Sodium bisulfite Sodium chromate Sodium silicate	144 0.861 506	6.9
Sodium bisulfite Sodium chromate Sodium sulfate	75 0.306 3312	6.9
Sodium bisulfite Sodium silicate Sodium sulfate	52 126 2308	8.5
Sodium carbonate Sodium chromate Sodium silicate	182 0.146 86	9.1
Sodium carbonate Sodium chromate Sodium sulfate	240 0.192 2079	9.3
Sodium carbonate Sodium silicate Sodium sulfate	155 73 1343	8.8
Sodium chromate Sodium silicate Sodium sulfate	0.201 119 2180	8.5

From the foregoing tabulations it is noted that the toxicity of sodium salts depends largely on the anion involved, the chromate being exceedingly toxic and the sulfate least so. Moreover, there appears to be no correlation between the toxicity displayed and the pH value, but since *Daphnia magna* is known to live within the pH range 6.2-9.5, which embraces all of the foregoing results, pH was apparently not the sole cause of mortality. It is also noted that the thresholds for specific compounds are lower when tested in combinations of two or three with the exception of sodium bisulfite with sodium silicate, or with sodium silicate and sodium chromate. This latter variation may be attributable to the possible oxidizing action of the chromate on the bisulfite (1691).

SODIUM ACETATE $NaC_2H_3O_2 \cdot 3H_2O$

Highly soluble in water, this compound is used as a mordant in textile dyeing, as a buffering agent in chemical production, in photography, and in medicine as a diuretic, expectorant, or alkalizer (364). According to Lockhart et al. (3241), the taste threshold of the anhydrous salt is 500 mg/l, or for the crystalline form 830 mg/l. Hodgson (2956) studied the stimulating effects of

sodium acetate on the water beetle (*Laccophilus maculosis*) and found that a concentration of 32,600 mg/l was required to stimulate the movement of 50 percent of the beetles.

SODIUM ACID PYROPHOSPHATE $Na_2H_2P_2O_7$

Soluble in water with an acid reaction, this white fused substance is used chiefly in baking powder (364). A concentration of 400 mg/l in sea water caused a decrease in irritability of oysters, but 500 mg/l caused oyster deaths within 44 hours (1448, 1467).

SODIUM ALKYL ARYL SULFONATE

SODIUM ALKYL BENZENE SULFONATE

SODIUM ALKYL SULFATE

(see Chapter X)

SODIUM ALUMINATE $NaAlO_2$

This highly soluble salt is used in water treatment, for sizing in paper mills, and for printing in textile plants (364). Wallen et al. (2940) tested its toxicity toward mosquito-fish (*Gambusia affinis*) in turbid water at 17-19°C. They found 24-, 48-, and 96-hour TL_m values of 199, 126, and 111 mg/l respectively. This salt coagulated the water and reduced turbidity.

SODIUM ANTHRAQUINONE-α-SULFONATE

SODIUM ARYL ALKYL POLYETHER SULFONATE

(see Chapter X)

SODIUM ARSENATE Na_2HAsO_4

(see also Arsenic, Arsenic Trioxide, Sodium Arsenite)

This colorless, odorless salt, readily soluble in water, is used in dyeing and printing fabrics and in various fungicides and insecticides.

It is not highly toxic to fish or other aquatic life. According to Jones (2120) the lethal concentration of sodium arsenate toward minnows at 16-20°C is 234 mg/l, as arsenic. It has also been reported (617) that minnows survived for 16 hours in a solution of sodium arsenite equivalent to 250 mg/l of arsenic.

Toward lower forms of aquatic life, the toxicity of sodium arsenate is variable. The threshold concentration for immobilization of *Daphnia magna* in Lake Erie water has varied from 18 to 31 mg/l as sodium arsenate, or 4.3 to 7.5 mg/l as arsenic (352, 353, 358). The toxic threshold for the flatworm *Polycelis nigra*, is reported to be 670 mg/l as AsO_4, or 361 mg/l as arsenic (354).

SODIUM ARSENITE $NaAsO_2$

(see also Arsenic, Arsenic Trioxide, Sodium Arsenite)

Freely soluble in water, sodium arsenite is used in dyeing, in arsenical soap making, in termite extermination, and in weed-killers. As a commercial preparation it is often a mixture of several sodium and arsenic compounds. Therefore, references to sodium arsenite solutions are often reported in terms of arsenic concentration or as arsenic trioxide equivalents.

Sodium arsenite has been used extensively as a herbicide for the control of mixed submerged vegetation in static water. Commercial sodium arsenite contains varying amounts of other arsenic compounds and impurities; hence it is labeled in terms of equivalent arsenic trioxide (As_2O_3). For the control of submerged vegetation in ponds and lakes, applications of 2 to 5 mg/l as arsenic trioxide (1.5 to 3.8 mg/l as As) have been found effective. Although these concentrations are generally considered to be safe for fish and other aquatic animals, it is advisable to spray only a part of the pond or lake at one time so that fish may avoid the sprayed area. Fish are reported to be sensitive to sub-lethal doses of sodium arsenite and will generally swim away into fresh water (890, 2861, 3671). Commercial sodium arsenite in concentrations up to 10 mg/l (arsenic content not stated) have been used for weed control in Wisconsin lakes without harm to the fish population (1006).

The following concentrations of sodium arsenite, expressed as arsenic, have been reported as lethal, toxic, or otherwise deleterious to aquatic organisms:

Concentration, as Arsenic, mg/l	*Time of Exposure*	*Organism*	*Reference*
1.0-2.0	--	Fish	1692
1.4-2.3	--	Fish food organisms	574, 1007
1.9-3.0	--	Midges, mayflies, amphipods	3009
4.6	48 hours	*Daphnia*	2158
5.0	10 days	Pink salmon	2091
5.0	48 hours	*Microregma* (protozoan)	3343
5.2	--	*Daphnia magna*	352
8.4	48-hour TL_m	Chum salmon fry	2900
15	48 & 72-hour TL_m	Fingerling channel catfish	2981
17.8	--	Minnow	2920
20	36 hours	Rainbow trout, minnows	3005
20	36 hours	Minnows	617
27	72-hour TL_m	Minnows	3672
27.6	24-hour TL_m	Fingerling channel catfish	2981
29	48-hour TL_m	Minnows	3672
35-46	48 hours	*Scenedesmus*	2158
45	24-hour TL_m	Minnows	3672
290	48 hours	*Escherichia coli*	2158

The literature on weed control contains statements that applications of sodium arsenite up to 10 mg/l as As_2O_3 (7.6 mg/l as As) are not harmful to fish, but the foregoing table would indicate that the threshold level is somewhat lower than expected. Furthermore, fish-food organisms are susceptible at concentrations as low as 1.0 mg/l.

On the other hand, the following concentrations of sodium arsenite, expressed as arsenic, have been reported to have no harmful effects on the organism noted:

Concentration, as Arsenic, mg/l	*Time of Exposure*	*Organism*	*Reference*
1.3	---	Fish or fish-food organism	1007
1.3-1.5	---	Fish-food organisms	1007, 1008
1.4-2.9	---	Fish	416
2.9	---	Chironomus larvae	574
5.0	24 hours	Trout, bluegills, sea lamprey	2976
8-16	---	Damselflies, dragonflies, sowbugs, water mites	3009
15	96 hours	Minnows	3672

Lawrence (3673, 3674) reports that two applications of 4 mg/l of sodium arsenite applied one month apart in experimental ponds reduced the number of bottom organisms an average of 34 percent and reduced bluegill production an average of 42 percent as compared with control ponds. Two applications of 8 mg/l of sodium arsenite applied one month apart reduced the number of bottom organisms an average of 45 percent and bluegills 65 percent as compared with control ponds. An application of 4 mg/l killed all microcrustacea and greatly reduced the population of rotifers.

SODIUM AZIDE NaN_3

Highly soluble in water, this crystalline solid is used in chemical manufacturing and in medicine as a hypotensive agent (364). Using water of the River Havel, Bringmann and Kuhn (2158, 3343) determined the thresholds of toxicity to be 0.3 mg/l for *Daphnia,* 3 mg/l for *Microregma* (a protozoan), 4 mg/l for *Scenedesmus* (an alga), and 19 mg/l for *Escherichia coli.* Toward trout, bluegills, and sea lamprey in Lake Huron water at 12°C, 5 mg/l of sodium azide had no apparent effect during a 24-hour exposure (2976).

SODIUM BENZENE SULFONATE

(see Chapter X)

SODIUM BENZOATE C_6H_5COONa

(see also Benzoic Acid)

This highly soluble salt is used as a preservative in food products with concentrations up to 1000 mg/kg being permitted. The oral LD_{50} for rats is given as 4100 mg/kg of body weight (364).

In Lake Erie water at 25°C, the threshold concentration of sodium benzoate for immobilization of *Daphnia magna* with 48 hours of exposure was less than 650 mg/l, and Anderson (352) estimated the true threshold concentration to be about 585 mg/l. Toxicity was due to factors other than osmotic pressure.

SODIUM BICARBONATE $NaHCO_3$

1. General. This freely soluble salt dissociates completely in water into sodium and bicarbonate ions. The concentration of bicarbonate ions, in turn, depends upon the resulting pH value (see Bicarbonates, Carbonates). The bicarbonate acts as a powerful buffering agent. Sodium bicarbonate is used in the manufacture of many sodium salts, as a source of carbon dioxide, in fire extinguishers, in effervescent salts and baking powder, in cleaning compounds, and in many industrial processes.

Goudey (992) suggested classification of ground waters and included the following recommendations as to sodium bicarbonate limits: Class AA (domestic), 0 mg/l; Class A (irrigation), 0 to 50 mg/l; Class B (irrigation), 50 to 100 mg/l; and Class C (irrigation and industry), more than 100 mg/l.

2. Cross References. Sodium Carbonate, Bicarbonates, Chapter V.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The taste threshold of sodium bicarbonate in water is reported (3241) to be 1060 mg/l.

b. Industrial Water Supplies. It has been recommended (173) that the sodium bicarbonate concentration of raw water to be used for making ice not exceed 35 mg/l.

c. Irrigation. Water containing sodium carbonate or bicarbonate as the predominant salts may be more harmful to plants than water with other sodium salts (273). Sodium bicarbonate, although it is an important buffer in water, may be directly or indirectly harmful to plants. It may be toxic, it tends to increase soil alkalinity, and in addition, by precipitating calcium carbonate out of solution, it reduces calcium concentration of the soil and soil permeability (264, 268). A concentration of 1500 to 2000 mg/l of sodium bicarbonate in the water has caused a 50-percent reduction in the dry weight of tomatoes grown in liquid culture (1345). In irrigation water 4000 mg/l has rendered soils alkaline and impaired their fertility (921).

d. Stock and Wildlife Watering. The following concentrations of sodium bicarbonate in drinking water have been reported as injurious to animals by causing thirst, diarrhea, and impaired growth:

Concentration in mg/l	*Animal*	*Reference*
6000	Chicks	287, 284, 2980
15000	Rats	287, 284
20000	Rats	287, 284

Concentrations from 12,000 to 24,000 mg/l have caused deaths among chicks (284).

e. Fish and Other Aquatic Life. The toxic threshold concentration of sodium bicarbonate for *Polycelis nigra,* a flatworm, was found to be 7140 mg/l (354). The threshold concentration of sodium bicarbonate for immobilization of *Daphnia magna* in Lake Erie water was reported to be 2350 mg/l (352) and 4200 mg/l (358). Cairns et al. (2933, 2934, 2935) found the 96-hour TL_m values for bluegill sunfish in standard dilution water at 20°C to be 8250 to 9000 mg/l depending on the size of the fish. Using highly turbid water at 20-22°C and the mosquito-fish as the test organism, Wallen et al. (2940) found the 96-hour TL_m to be 7550 mg/l. The 24-hour TL_m was 7700 mg/l.

SODIUM BIFLUORIDE $NaF \cdot HF$

(see also Sodium Fluoride)

This highly soluble powder produces an acid reaction in solution; hence it is used as a "sour" in laundrying (364). According to Simonin (3271) the fish *Tinca vulgaris* is killed by 100 mg/l of this compound.

SODIUM BISULFATE $NaHSO_4$

(see also Sulfates, Sulfuric Acid)

Known also as sodium acid sulfate, this compound is freely soluble in water and dissociates into Na^+ and HSO_4^- ions. The concentration of the bisulfate ion in solution depends upon the resulting pH value and above pH4 almost all bisulfate will be converted to sulfate ion.

For immobilization of *Daphnia magna,* the threshold concentration of sodium bisulfate in Lake Erie water was found to be less than 145 mg/l (352). Later work by Fairchild (2946) showed that the threshold toxicity value is dependent on dissolved oxygen. For *Daphnia*

exposed 100 hours at 23°C, the threshold concentrations were as follows:

Dissolved Oxygen, mg/l	*Threshold Level of Toxicity, mg/l*
6.5	153
8.9	145
1.55	106

SODIUM BISULFITE $NaHSO_3$

Used as a disinfectant and bleach in textile, laundering, paper, and fermentation industries, this compound is freely soluble in water, dissociating into Na^+ and HSO_3^- ions. The concentration of the undissociated bisulfite ion depends upon the resulting pH value; furthermore, it is readily oxidized to sulfate (364).

The toxicity threshold (100-hours at 23°C) to *Daphnia magna* has been reported at 102 mg/l (1691), but in the presence of other sodium salts, this threshold may be somewhat lower (see Sodium). Fairchild (2946) also studied the toxicity of sodium bisulfite toward *Daphnia* at various concentrations of dissolved oxygen at 23°C, with the following results:

Dissolved Oxygen, mg/l	*Threshold Level of Toxicity, mg/l*
9.3	109
6.5	61
3.2	77
1.6	70

Le Clerc et al. (2942, 2943, 2944) exposed minnows for 6 hours to solutions of sodium bisulfite in distilled and hard water. The minimum lethal dose in distilled water at 19°C was 60-65 mg/l and in hard water at 18°C it was 80-85 mg/l. Using turbid water at 17-22°C and the mosquito-fish *(Gambusia affinis)* as the test organism, Wallen et al. (2940) found the 24-, 48-, and 96-hour TL_m values to be 240 mg/l. This concentration greatly reduced the turbidity.

SODIUM BORATE $Na_2B_4O_7 \cdot 10\,H_2O$

(see also Boron, Sodium Perborate)

Commonly known as borax, this compound is used extensively as a cleaning agent in homes and industry. It is also employed as a wood preservative, as a treatment against wood fungus, in tanning operation, and for the control of aquatic weeds (898). Certain other borates, such as phenyl mercury borate in a concentration of 10 mg/l, have been used effectively against the growth of slimes in pipes in paper mills (1346).

According to Le Clerc and Devlaminck (2942, 2943, 2944), the minimum lethal dose of sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) toward minnows was 3000 to 3300 mg/l at 19°C in distilled water and 7000 to 7500 mg/l at 17°C in hard water. Toward mosquito fish *(Gambusia affinis)* in turbid water, Wallen et al. (2940) found the 96-hour TL_m at 22-26°C to be 3600 mg/l.

With respect to lower aquatic life, sodium borate appears to be more toxic than towards fish. The threshold concentration for immobilization of *Daphnia magna* was reported (352) as being much less than 240 mg/l and was estimated at 120 mg/l (353). The toxic threshold concentration of sodium borate for *Polycelis nigra,* a planarian worm, was found to be 1530 mg/l as borate (354).

SODIUM BROMATE $NaBrO_3$

(see also Bromine, Sodium Bromide)

Highly soluble in water, this crystalline salt is used in ore processing and for promoting the growth of yeast cells. The oral MLD for rabbits is given at 580 mg/kg of body weight (364).

SODIUM BROMIDE NaBr

(see also Bromine)

Used primarily in photographic work, this salt of bromine is highly soluble in water. In water supplies used for preparing photographic solutions, bromides slow up the rate of the chemical reactions (242). The toxicity threshold concentrations of sodium bromide reported for *Daphnia magna* in Lake Erie water at 25°C range from 4100 to 8200 mg/l (352, 353). The toxic threshold concentration of sodium bromide for *Polycelis nigra,* a flatworm, was found to be 14,400 mg/l (354). To stimulate the movement of water beetles *(Laccophilus maculosis),* a sodium bromide concentration of 14,400 mg/l was required (2956).

SODIUM p- BROMOBENZENESULFONATE
SODIUM BUTYL MERCAPTIDE C_4H_9SNa

This soluble compound ionizes in water to form sodium and mercaptide ions. In normal pH ranges, the mercaptide ion reacts with hydrogen ion to form butyl mercaptan. In testing the toxicity of various refinery materials toward bluegill sunfish, Turnbull et al. (2093) determined TL_m values as follows:

	TL_m Values in mg/l			
	as SH		*as C_4H_9SNa*	
	24-hour	*48-hour*	*24-hour*	*48-hour*
Without reoxygenation	7.4	5.5	25.1	18.7
With reoxygenation	4.1	3.5	13.9	11.9

SODIUM n - BUTYL SULFONATE

(see Chapter X)

SODIUM CARBONATE Na_2CO_3

1. General. Sources of sodium carbonate in water include natural minerals, and effluents from chemical plants and paper mills. Known also as "soda ash", this salt is highly soluble in water and dissociates into sodium and carbonate ions, with a resulting increase in alkalinity and a tendency to raise the pH value. The carbonate ions will react with hydrogen ions until an equilibrium is established (see Carbonates, Bicarbonates, and Alkalinity). Sodium carbonate is used in the manufacture of glass, soap, and other sodium salts, in textile scouring, in water treatment and in numerous other processes (364).

2. Cross References. Dissolved Solids, Carbonates, Bicarbonates, Chapter V.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The taste threshold of sodium carbonate in water is reported as 15 to 75 mg/l (621) and as 78 mg/l (3241). Waters containing 360, 430, and 500 mg/l of sodium carbonate have been considered wholesome and safe (32, 36).

b. Industrial Water Supplies. Sodium carbonate in water for making ice is about 1.25 times as objectionable as sulfates and chlorides (231). Sodium carbonate in water for preparing developer solutions for use in photography may speed up the action of weakly alkaline solutions (242). For brewing, a limit of 100 mg/l has been recommended (see Chapter V).

c. Irrigation. Sodium carbonate is extremely toxic to plants (264, 268). Sodium carbonate in irrigation water hardens soil and reduces its permeability (348) (see also Chapter V). On the other hand, it has been reported that 100 to 400 mg/l of sodium carbonate in the water was not injurious to various greenhouse plants over a period of six months of use (1205).

d. Stock and Wildlife Watering. Waters containing high concentrations of sodium carbonate cause diarrhea, symptoms of malnutrition, unsatisfactory growth, and may also interfere with reproduction of livestock. The following concentrations of sodium carbonate have been reported as injurious to animals:

Concentration in mg/l	*Animal*	*Effect*	*Reference*
1,025	General livestock	Diarrhea	2394
7,500	Poultry	Lethal in 7 days	2980
10,000–15,000	Rats	Lethal to young	287
10,000–19,000	Rats	Inhibited growth	2980
10,000–20,000	Rats	Lethal to young	284
20,000	Rats	Lethal to young	640

e. Fish and Other Aquatic Life. The threshold concentration of sodium carbonate for immobilization of *Daphnia magna* in Lake Erie water at 25°C was reported to be 424 mg/l (358); less than 424 mg/l (352); and 300 mg/l (574). The minimum lethal concentration for *Daphnia* was also shown to be 300 mg/l at 17°C and at 800 mg/l all animals were killed (3523). The 100-hour toxicity threshold at 23°C in double-distilled water for *Daphnia magna* has also been reported at 524 mg/l with a resulting pH value of 9.5. According to Fairchild (2946), the threshold of toxicity toward *Daphnia* depends on the dissolved oxygen content of the test water. At 23°C for a 100-hour exposure he found the threshold toxicity level to be 552 mg/l at a dissolved oxygen tension of 6.5 mg/l; but only 267 mg/l when the DO dropped to 1.53 mg/l.

The following concentrations of sodium carbonate have been reported to kill fish:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
68	Tap	120 hours	King salmon	3501
70	Tap	120 hours	Silver salmon	3501
80	Tap	120 hours	Cutthroat trout	3501
200	---	4.5 hours	Bass	1030
250	---	120 hours	Shiners	190
250-300	Tap	Few hours	Shiners, suckers, carp	313
300	Standard	96-hour TL_m	Sunfish	2933
500	---	4.5-11 hours	Sunfish	1030
500	---	7-9 hours	Bass	1030
500	---	½-3 days	Bass	1030
530	Tap	3 days	Bluegills	313
740	Turbid	24-hour TL_m	Mosquito-fish	2940
840	Turbid	48-hour TL_m	Mosquito-fish	2940
1200	Turbid	96-hour TL_m	Mosquito-fish	2940

The following concentrations of sodium carbonate have not been harmful within the time specified:

Concentration in mg/l	*Time of Exposure*	*Type of Fish*	*Reference*
33	5 days	Cutthroat trout	3501
44	5 days	Silver salmon	3501
58	5 days	King salmon	3501
100	7 days	Bass	1030
200	7 days	Sunfish	1030
200–500	7 days	Goldfish	1030
500	7 days	Some goldfish	1030

SODIUM CARBOXYMETHYL CELLULOSE

(see Chapter X)

SODIUM CHLORATE $NaClO_3$

(see also Chlorine)

This strong oxidizing compound dissolves readily in water and ionizes to Na^+ and ClO_3^-; but the anion is unstable in aqueous solution, releasing oxygen and forming OCl^-. (see Chlorine). Sodium chlorate is used for weed control, as well as in the manufacturing of matches and explosives, dyeing and printing of fabrics, and tanning and finishing of leather. It is also found in some effluents from chlorine and bromine works. Concentrations from 20,000 to 40,000 mg/l, as used for weed control, are considered non-toxic for man and animals, but create a fire hazard as the water evaporates (897). The oral LD_{50} in rats is given as 12,000 mg/kg of body weight (3009).

The threshold of toxicity for perch is given as 11,000 mg/l and for bleak as 13,000 mg/l in tap water (2977). A concentration of 1000 mg/l in hard water was not harmful to goldfish during a 5-day exposure (313).

With respect to lower organisms, the literature is not consistent. The toxic threshold of sodium chlorate toward *Polycelis nigra*, a flatworm, was found to be 16,000 mg/l (354) and toward *Daphnia magna* 4240 mg/l (352). Bringmann and Kuhn (2158) report the threshold concentration toward *Daphnia* to be greater than 1000 mg/l; but toward the alga *Scenedesmus* the threshold level was only 3 mg/l as chlorate (about 3.8 mg/l as sodium chlorate) and toward *E. coli* only 4 mg/l as chlorate (5.1 mg/l as sodium chlorate).

SODIUM CHLORIDE NaCl

1. General. Sodium chloride in water may be of natural origin, or it may be introduced as a component of sewage or industrial wastes, including effluents from salt and brine works, oil wells, dairies, spent irrigation waters, etc. The sodium and chloride ions in natural waters are frequently the most prevalent, and they constitute the bulk of the ions in sea water.

2. Cross References. Tastes, Chlorides, Dissolved Solids, Specific Conductance, Mercuric Chloride, Distilled Water, Chapter V.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Sodium chloride can be tasted in water by most people at a concentration of 550 mg/l (947); however, taste threshold values from 200 to 900 mg/l have also been reported (621, 3241, 3391,

3392). Water containing more than 500 mg/l of sodium chloride may be unpalatable and cause appetite disturbances (284, 948). Although an excess of sodium chloride induces thirst or can act as a diuretic (623), water containing up to 1410 mg/l has been used by some communities for many years without appreciable harm; however, 1000 to 1500 mg/l of sodium chloride generally renders water unpalatable (32, 36, 621). It has also been reported that 7500 mg/l of salt is harmless and that 10,000 mg/l causes vomiting (621, 579) (see also Sodium, Dissolved Salts, Chapter III, Chapter V).

b. Industrial Water Supplies. For ice making, as much as 342 mg/l of sodium chloride would probably not be detrimental, if it were the only salt present in the water (229). A concentration of 1000 to 1500 mg/l of sodium chloride in the process water causes tastes in canned foods, but does not affect the quality in other ways (224). For brewing, some sodium chloride is desirable and necessary in the water (171). It has been reported that 75 to 150 mg/l of sodium chloride is desirable in brewing water, but more than 500 mg/l is deleterious (170). It has also been recommended that for brewing waters the salt concentration should not exceed 200 mg/l (173); or 275 mg/l (152, 168). Other salt recommendations for brewing waters include the following values: pale ales I, 30 mg/l; pale ales II, 30 to 60 mg/l; mild ales, 60 to 120 mg/l; and stout, 120 to 175 mg/l (170). Good ale has been brewed, however, with water containing as much as 715 mg/l of sodium chloride (32). See Chapter V.

Larson and King (3675) conclude that the rates of corrosion of iron and steel in water are a function of the specific mineral quality as well as the alkalinity and pH values. They indict NaCl and other sodium salts as strong contributors to the rate of corrosion. For example, in a water with 400 mg/l of alkalinity (as $CaCO_3$) and pH 7, the corrosion rate will be zero at 100 mg/l of NaCl, but when the NaCl concentration is 400 mg/l, the corrosion rate will be about 100 mg per sq cm per day (3675).

c. Irrigation (see also Chapter V; Dissolved Solids). The effects of sodium chloride in irrigation water vary with the individual plants, the species, and environmental and climatic conditions. The presence of 10 milliequivalents or more of sodium chloride in nutrient solutions depressed root growth and water absorption by corn and tomato plants (1348). In irrigation water 100 to 200 mg/l of sodium chloride has caused injury to avocado trees in some soils, and also to some flowers, but many other plants are not sensitive to such concentrations (921). Water used for irrigation should not contain more than 700 mg/l of dissolved minerals in which sodium chloride predominates (638). Tomatoes have been killed by water containing 2000 mg/l of sodium chloride, but chrysanthemums have been resistant to as much as 3000 mg/l (921). To peas in liquid culture 4000 mg/l or more of sodium chloride was toxic and 9000 mg/l permitted almost no growth. The growth of wheat and barley plants in liquid culture was depressed by 8000 mg/l of sodium chloride; 10,500 mg/l appeared to be lethal over a long period, although some plants could tolerate this concentration for a limited period of a few weeks. In general, no plants grew to any appreciable extent when irrigated with water containing 15,000 mg/l of sodium chloride (1349).

Young rice is sensitive to the presence of chlorides in water, but develops resistance as the plant matures. According to Shutts (1693), the commonly accepted tolerances of rice to salt water are as follows:

Concentrations of Salts as NaCl, mg/l	*Tolerances*
600	Tolerable at all stages
1300	Rarely harmful, and only to seedlings in dry, hard soil
1700	Harmful before tillering; tolerable from jointing to heading
3400	Harmful before booting; tolerable from booting to heading
5100	Harmful at all stages

Chlorides tended to accumulate in plants and soil when wheat was grown 2 and 10 meters away from a ditch carrying water containing 103,000 mg/l sodium chloride. Increasing concentrations of salt caused progressively greater reductions in height, weight, leaf growth and seed number of affected plants (1694).

The effect of NaCl on plant growth has been demonstrated by culture in sand to which various concentrations of salt have been added to the normal nutrient solution. With sand culture, the effect of sodium on the soil structure is avoided as a contributing factor. Results are shown below:

NaCl Added				
Atmospheres	*mg/l*	*Crop Designation*	*Relative Dry Weight*	*Reference*
Control	---	All	100	---
1.0	1400	Rice straw	89.7	3379
		Rice grain	74.5	3379
		Rice roots	90.9	3379
1.5	2100	Bean plant	89.0	3376
		Bean leaves	87.3	3376
		Bean roots	93.3	3376
2.0	2800	Rice straw	46.1	3379
		Rice grain	12.0	3379
		Rice roots	49.6	3379
2.5	3500	Bean plant	73.8	3376
		Bean leaves	70.5	3376
		Bean roots	80.1	3376

This table demonstrates that NaCl affects the growth of plants and that rice is more sensitive than beans.

d. Stock and Wildlife Watering (see also Chapter V; Dissolved Solids). Salt poisoning has been reported among a great variety of livestock; its symptoms include malnutrition, wasting diseases, diarrhea, nervous disturbances, decreased milk or egg production, reduced reproduction, and sometimes death. The minimum lethal dose of sodium chloride for poultry has been reported as about 2 grams per pound (1350). The following doses of sodium chloride have been reported to be toxic to various animals: pheasants and rabbits, 3 grams (3676); ducks, 6 grams; dogs, 30 to 60 grams; sheep and pigs, 120 to 140 grams; cows, 2 to 3 kilograms; and horses, 1 to 2 kilograms (284).

The following concentrations of NaCl in drinking water have been reported to have caused the indicated effects on animals:

Reactions	*Reference*	*Concentration of NaCl, mg/l*	*Animal*
4000	Laying hens	Watery droppings	2398
5000	Young chicks	Nervousness, inability to stand	1351, 1551

Concentration of NaCl, mg/l	Animal	Reactions	Reference
7000	Swine	Slight scouring	2398
7000	Cattle	Reduction in weight	2398
7200	Sheep	Lethal to some animals	642, 1352
9000	Chicks	Lethal to two-thirds	2980
10000	Rats	Decreased reproduction	284, 287, 295
10000	Cattle, swine, poultry	Toxic effect	2398
10000	Laying hens	Loss of weight and egg production	2398
15000	Rats	Retarded growth, some deaths	284, 287, 295
15000	Cattle	Reduced water intake	3392
15000–17600	Cattle	Sickened or killed	293, 294, 1350
15000–25000	Cattle, hens, pigs, rabbits	Injurious or fatal	284
17500	Cattle	Lost weight	2396
20000	Young chicks	Death in three days	1551

On the other hand, the following concentrations of NaCl in drinking water have been reported to cause no apparent adverse effect on the designated animals:

Concentration of NaCl, in mg/l	Animal	Reference
1025	General livestock	2394
2000	Young chicks	1551
2450	Cattle, sheep, swine, poultry	3391
3000	Chicks	2980
4000	Cattle, swine, poultry	2398
5000	Rats	284, 287, 295
5720	Sheep, horses	642, 1352
9000-10000	Dairy cows	292, 293
9123	Horses, without working	642
11440	Cattle and sheep	642
11690	Rats	2398
12500	Cattle	2396
20000	Cattle not in milk production	288
25000	Sheep	288

e. Fish and Other Aquatic Life. (see Dissolved Solids; Chapter V). The following concentrations of sodium chloride have immobilized or killed fresh-water fish:

Concentration of NaCl, mg/l	Type of Water	Time of Exposure	Type of Fish	Reference
1270- 2540	Soft, distilled	--	Fish	1459
2500	--	120 hours	Shiners	190
2500	--	--	Fish	344
2500	Distilled	9-24 days	Minnows	313
3820- 5100	Hard, alkaline	--	Fresh-water fish	1459
3850	Natural	--	Whitefish, pickerel	598
5000	--	--	Newly hatched trout	1459
5000	Distilled	148 hours	Shiners	313, 645
5000	Distilled	200-250 hours	Bass	313, 645
8500	Distilled	4-8 days	Minnows, perch	1459
10000	--	97-148 hours	Shiners, bass	645
10000	Tap	4-8 days	Minnows, perch	1459
10000	River	4-10 days	Goldfish	313
10000	Distilled	6 hours	Minnows	2942, 2943, 2944
10000	--	--	Fish	603, 1070
11500-12000	Hard	6 hours	Minnows	2942, 2943, 2944
11680-14600	--	--	Stickleback	353
11700	Tap	--	Salmon eggs and fry	1459
11765	Distilled	17 hours	Goldfish	313
12946	Aerated	96-hour TL_m	Bluegills	3381
14000	Synthetic	24 hours	Bluegills	1459
14000	--	--	Goldfish	2920
15000	--	4.7 hours	Bass	645
15000-30000	--	24 hours	Fresh-water fish	1459
16500	Natural	--	Whitefish	598
17550	Turbid	96-hour TL_m	Mosquito-fish	2940
20000	--	1.3 hours	Shiners	645
50000	--	15 minutes	Trout	441

On the other hand, the following concentrations of NaCl have been reported as harmless in the time specified:

Concentration of NaCl, mg/l	Type of Water	Survival Time	Type of Fish	Reference
5000	River	25 days	Goldfish	313
5850	Tap	--	Salmon eggs and larvae and young stickleback	1459
9100-17500	--	30 days	Perch	644
11700	--	50 hours	Young eels	1459
12000	Distilled	--	Stickleback	1459
20000	Tap	--	Stickleback	1459
25000-30000	--	--	Older trout	1459
30000	--	30-60 minutes	Brook trout	441

The threshold concentrations of sodium chloride in natural waters reported for immobilization of *Daphnia* and other fish-food organisms range from 2100 to 6143 mg/l (352, 353, 358, 598, 2946, 2955). Yet in distilled water, one mg/l of NaCl killed *Daphnia magna* in three hours (313). The resistance of *Daphnia magna* to NaCl appears to vary with the oxygen tension (2946). At 6.4 mg/l of dissolved oxygen the threshold toxicity level of NaCl was 5093 mg/l, but at 1.48 mg/l of DO, the threshold for NaCl was only 3170 mg/l (2946). Many small crustacea and fish fry are immobilized by concentrations above 3100 mg/l (598). The toxic threshold for a flatworm, *Polycelis nigra*, was found to be 11,200 mg/l (354).

In Lake Erie water at 20-25°C, threshold limits for several fish-food organisms were reported (2955) as follows:

Organism	Threshold Concentration, mg/l
Daphnia magna, young	3700
Daphnia magna, adult	4600
Leptodora kindtii	3700
Cyclops vernalis	6100
Diaptomus oregonensis	3030

A Russian article (3677) indicates the following maximum NaCl tolerances for fresh-water organisms at two temperatures:

Organism	NaCl Tolerance in mg/l 20°C	3°C
Stictochironomus	788	1000
An oligochaete	1576	2000
Daphnia magna	200	800
Cyclops serrulatus	394	--
Leucaspisis delineatus	1970	--

Anderson considers sodium chloride concentrations above 3000 mg/l deleterious to both fish-food organisms and fish fry and recommends a permissible limit of 2000 mg/l in fresh waters (598). On the other hand, higher

concentrations of sodium chloride have been found to decrease somewhat the toxicity of some metallic compounds toward fish (1265) (see also Mercuric Chloride). Sodium chloride is the least toxic to fish of the chlorides of potassium, magnesium, calcium and sodium. Sodium chloride is antagonistic to the toxicity of calcium and potassium chlorides (353, 307). A concentration of 50 mg/l of sodium chloride has greatly increased the antagonistic effect of sodium nitrate toward copper sulfate poisoning of fish (311). The toxicity of sodium chloride toward fish is decreased by calcium chloride (307).

f. Shellfish Culture. The most favorable salt content of water for oyster growth is about 30,800 mg/l of salinity of which about 80 percent is sodium chloride (314).

SODIUM p-CHLOROBENZENE SULFONATE

(see Chapter X)

SODIUM CHROMATE

(see Chromium)

SODIUM CITRATE $C_3H_4OH(COONa)_3 \cdot 2H_2O$

(see also Citric Acid)

This highly soluble salt has many uses in medicine, especially as an anticoagulant. It is also used in photography and as a sequestering agent to remove trace metals (364).

Anderson (352) reports that the threshold concentration for immobilization of *Daphnia magna* in Lake Erie water at 25°C was 825 mg/l. For *Polycelis nigra,* a flatworm, the threshold for toxicity at 15 to 18°C was a 0.015 molar concentration, or 4420 mg/l of sodium citrate (354).

SODIUM CYANATE NaOCN

(see also Cyanides, Hydrogen Cyanide)

Cyanates are formed when cyanides in waste waters are treated by alkaline chlorination. According to Resnick et al. (2974), cyanates are stable in aerobic water at pH 7 and 20°C for at least 10 days. Eventually, cyanates undergo hydrolysis to yield ammonium carbonate. In anaerobic solutions cyanates are reduced not to cyanide but to a mixture of ammonia and formic acid.

According to Washburn (1070) the maximum tolerance limit of creek chub to sodium cyanate is 75 mg/l.

SODIUM CYANIDE NaCN

1. General. This highly soluble salt is used in electroplating baths, for extracting gold and silver from ores, and for generating HCN gas in fumigating operations (364). Upon solution in water, the cyanide ion combines with hydrogen ions in the water to form weakly dissociated hydrogen cyanide, thereby tending to raise the pH of the solution. For further explanation of the cyanides, see Hydrogen Cyanide.

2. Cross References. Cyanides, Cyanogen Chloride, Hydrogen Cyanide, Nitriles, and other cyanide salts.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The fatal dose of NaCN for man by ingestion is reported to be about 0.2 grams (353), but since the fatal dose of HCN has been given as 50 to 60 mg (364), the former figure may be high. See also Cyanides and Hydrogen Cyanide.

b. Stock and Wildlife Watering. The approximate minimum toxic dose of NaCN for sheep is given as 4.15 mg per kg of body weight, and a dose of 5.22 mg per kg is lethal (353).

c. Fish and Other Aquatic Life. The following concentrations of sodium cyanide have been shown to be harmful or toxic to fish:

Concentration of NaCN, in mg/l	*Time of Exposure*	*Type of Fish*	*Remarks*	*Reference*
0.05	124 hours	Trout	100 percent mortality	1115
0.28	96 hours	Bluegills	TL_m in soft water	2958
0.33-0.35	2.5 hours	Minnows	Lethal	1468
0.4	1 hour	Minnows	Stopped eating	362
0.43	96 hours	Fathead Minnow	TL_m in soft water	2958
0.44	48 hours	Fathead Minnow	TL_m	3335
0.5	4 to 6 hours	Goldfish and green sunfish	Lethal, 25°C	3678
0.5 to 0.7	24 hours	Minnows	25 percent mortality	362
0.66	96 hours	Fathead minnow	TL_m in hard water	2958
0.75	24 hours	Minnows	50 percent mortality	362
0.8	24 hours	Minnows	100 percent mortality	362
1.0	0.7 to 10 hours	Mixed fish	Lethal at 25°C	3678
1	20 minutes	Trout	100 percent mortality	1115
1.5	0.6 to 24 hours	Goldfish and green sunfish	Lethal	3678
2	47 minutes	Trout	Lethal	1116
2.0	--	Sticklebacks	Lethal at 17°C	2920
3.1	90 minutes	Fish	Depressed respiration	468
4.3		Hardy carp	Paralyzed	362
5	12 minutes	Shiner	Killed	1115
5	2 hours	Trout, bluegill	Killed	2976
10	4 minutes	Shiner	Killed	1115

Non-toxic or non-harmful concentrations have been reported as follows:

Concentration of NaCN, in mg/l	*Time of Exposure*	*Type of Fish*	*Remarks*	*Reference*
0.02	--	Trout fingerlings	No effect	1115
0.08	--	Trout	Non-toxic	1109
0.3	24 hours	Minnows	No effect	362
2.3	155 minutes	Fish	Survived	468
5.0	2 hours	Sea lamprey	Ill, but survived	2976

According to Anderson (365), the threshold concentration for immobilization of *Daphnia magna* in Lake Erie water at 25°C was found to be less than 3.4 mg/l of sodium cyanide and Jones (354) reports that the threshold concentration for *Polycelis nigra,* a flatworm, is 16 mg/l as cyanide (30 mg/l as NaCN). A concentration of 3.6 mg/l of NaCN inhibited BOD by 50 percent (2949).

SODIUM DECYLBENZENE SULFONATE

SODIUM n-DECYL SULFATE

SODIUM 2, 5-DICHLOROBENZENE SULFONATE

(see Chapter X)

SODIUM DICHROMATE

(see Chromium)

SODIUM DIHYDROGEN PHOSPHATE

(see Sodium Phosphates)

SODIUM DI (2-ETHYLHEXYL) SULFOSUCCINATE

SODIUM DIOCTYL SULFOSUCCINATE

SODIUM DODECYLBENZENE SULFONATE

SODIUM DODECYL SULFATE

SODIUM 2-ETHYLHEXYL SULFATE

(see Chapter X)

SODIUM FERROCYANIDE $Na_4Fe(CN)_6$

(see also Cyanides, Hydrogen Cyanide, Potassium Ferro- and Ferricyanide)

This compound, containing reduced iron, is highly soluble in water. It is used for blueprinting, in photography, pigments, dyes, and metallurgy (364). In aqueous solution it decomposes under the action of sunlight to release cyanide ion and HCN.

For *Daphnia magna* in Lake Erie water at 25°C, the toxic concentration was reported by Anderson (352) to be less than 600 mg/l, or about 540 mg/l (353). For *Polycelis nigra,* a planarium, Jones (354) found the toxic threshold to be 170 mg/l. It should be recognized, however, that under the action of sunlight with the resultant decomposition to cyanides, this compound may be much more lethal. For further details, see Potassium ferrocyanide.

SODIUM FLUORIDE NaF

(see also Fluorides)

Highly soluble in water, this crystalline solid is used as a pesticide, as a fluoridating agent in municipal water, and in many industrial processes. The LD_{50} for hamsters was found to be 70-80 mg/kg of body weight (3705). Much of the information described under "Fluorides" was derived from the use of sodium fluoride and will not be repeated here.

Bringmann and Kuhn (2158, 3343) found the threshold of NaF for *Daphnia* at 23°C to be 270 mg/l during a 2-day exposure. For *Scenedesmus* the threshold of toxic effect was 95 mg/l during 4 days at 24°C, for *Microregma* 226 mg/l, and for *Escherichia coli* at 27°C it was 180 mg/l. According to Wantland (3705), free-living protozoa and freshwater rotifers survived and reproduced in water containing 1000 mg/l of NaF, but were killed at 1700 mg/l.

SODIUM FORMATE

(see Formic Acid and Formates)

SODIUM HYDROCARBON SULFONATE

(see Chapter X)

SODIUM HYDROSULFIDE

(see Sodium Sulfhydrate)

SODIUM HYDROXIDE NaOH

1. General. This common caustic compound is highly soluble in water with dissociation to sodium and hydroxide ions, with a consequent effect upon alkalinity and pH. Sodium hydroxide may occur in wastes from the following industries: soap manufacture; cleaning compounds; pulp and paper; petroleum, mineral and vegetable oils; leather; reclaimed rubber; ink; dyes and textile dyeing; cotton; perfumes; water glass; coal distillation, etc.

2. Cross References. Sodium, Alkalinity, Hydroxides.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The taste threshold of sodium hydroxide in water is reported to be 1 to 50 mg/l (621). A dose of 1.95 grams of sodium hydroxide may cause death in man (353).

b. Stock and Wildlife Watering. Sodium hydroxide, in a concentration of 5000 mg/l, was almost harmless for rats; 10,000 mg/l caused nervousness, sore eyes, diarrhea, and retarded growth (284, 287).

c. Fish and Other Aquatic Life. The toxicity threshold concentration of sodium hydroxide for *Daphnia magna* has been reported to range from 40 to 240 mg/l (190, 352, 353, 358, 574, 690, 1466, 3500, 3523). Concentrations of sodium hydroxide from 125 to 1000 mg/l have been lethal to various insect larvae (190, 574, 690, 3500). A concentration of 100 mg/l has not been harmful to mayflies, and 700 mg/l has not been harmful to *Chironomous* larvae (690).

The following concentrations of sodium hydroxide have been reported to have killed fish:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
20	---	---	Silver salmon	3502, 3501
25	---	24 hours	Brook trout	359
35	---	---	Cutthroat trout	3502, 3501
40	Fresh	24 hours	Creek chub	1442
48	---	---	King salmon	3502, 3501
70	Stagnant	5 hours	Fish, crabs	823
71.5	---	---	Carp, shiners, suckers	359
90	Circulating	4.5 hours	Fish, oysters	823
96	Tap	2-10 minutes	Carp, shiners, suckers	313
99	Tap	48-hour TLm	Bluegill sunfish	2093
100	---	---	Minnows	690, 190, 3500
100	---	---	Fish	658, 344
100	---	120 hours	Shiners	190
100	---	3-20 hours	Goldfish, bass	1030
125	Turbid	96-hour TLm	Mosquito-fish	2940
180 (pH 12)	Circulating	23 hours	Fish, oysters	823

The following concentrations of sodium hydroxide have not harmed fish within the time specified:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
10	---	---	Cutthroat trout	3502, 3501
11	---	---	Silver salmon	3502, 3501
20	Fresh	24 hours	Creek chub	1442
27	---	---	King salmon	3502, 3501
50	Distilled	2 hours	Perch, roach	313
50	---	7 days	Goldfish, bass	1030
55.5	---	---	Carp, shiners, suckers	359
200	---	5 hours	Some fish	823

For roach and perch in sodium hydroxide solutions of 1:5000 strength, the time until loss of equilibrium was found to be about 30 minutes. In 1:10,000 dilutions, both kinds of fish survived for 40 minutes (1357).

According to Murdock (1460) sodium hydroxide has not been shown to be lethal to fully developed fish in natural fresh waters unless the pH value is well over 9. The lethal pH for goldfish is given by Jones (2920) as 10.9, and by Cairns and Scheier (2933, 2934) for bluegill sunfish as 10.5.

To stimulate the movement of water beetles *(Laccophilus maculosus),* Hodgson (2956) found that a concentration of 400 mg/l of NaOH was required.

SODIUM IODATE $NaIO_3$

(see also Iodine)

The threshold concentration of sodium iodate for the immobilization of *Daphnia magna* in Lake Erie water was found to be less than 158 mg/l (352). The toxic threshold concentration of sodium iodate for the flat-

worm *Polycelis nigra* was found to be 230 mg/l, as iodate (354).

SODIUM IODIDE NaI

(see also Iodine)

The taste threshold of sodium iodide in chlorinated water is reported to be about 0.005 mg/l (459). The threshold concentration of sodium iodide for the immobilization of *Daphnia magna* in Lake Erie water was found to be 3.3 mg/l (352). A concentration of 5 mg/l had no effect on the sea lamprey during a 24-hour exposure in Lake Huron water at 12°C (2976). The toxic threshold concentration of sodium iodide for *Polycelis nigra* a flatworm, was found to be 6600 mg/l (354). To stimulate movement of the water beetle *(Laccophilus maculosis)* a sodium iodide concentration of 14,700 mg/l was required (2956).

SODIUM ISOPROPYL NAPHTHALENE SULFONATE

SODIUM KERYLBENZENE SULFONATE

SODIUM LAURYL AND MYRISTYL COLLAMIDE SULFONATE

SODIUM LAURYL SULFATE

(see Chapter X)

SODIUM METABISULFITE $Na_2S_2O_5$

This highly soluble solid is used in medicine as an antioxidant (364). According to Le Clerc (2942) the minimum lethal dose to minnows during a 6-hour exposure was 60-65 mg/l in distilled water at 18°C and 75-80 mg/l in hard water at 17°C.

SODIUM MOLYBDATE

(see Molybdenum)

SODIUM MONOBUTYL PHENYLPHENOL MONOSULFONATE

SODIUM MONOETHYL PHENYLPHENOL MONOSULFONATE

SODIUM NAPHTHALENE -β- SULFATE

(see Chapter X)

SODIUM NITRATE $NaNO_3$

1. General. Known commonly as "Chile saltpeter", this compound was formerly used extensively for the manufacture of nitric acid. It is also used in making glass, enamels, matches, and cigarettes, and in pickling meats. The technical grade is employed in fertilizers as a source of nitrate. The compound is readily soluble in water, releasing sodium and nitrate ions.

2. Cross References. Sodium Nitrates.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The taste threshold of sodium nitrate in water is reported to be about 450 to 800 mg/l (621). Sodium nitrate acts as a diurectic (364, 623).

b. Industrial Water Supplies. Sodium nitrate in water interferes with fermentation processes and causes disagreeable tastes in beer (165).

c. Stock and Wildlife Watering. A dose of 240 grams of sodium nitrate has been fatal to cows; 20 grams, to sheep; and 6 grams to dogs (284). A concentration of 6200 mg/l of sodium nitrate in water has caused frenzy, paralysis, diuresis, cyanosis, and deaths among cows (284). Pigs fed with soup prepared with well water containing 1740-2970 mg/l of sodium nitrate died from methemoglobinemia. A total dose of 90 mg of sodium nitrate per kg of body weight is fatal to pigs (1695).

d. Fish and Other Aquatic Life. The toxicity threshold concentration of sodium nitrate for *Daphnia magna* has been reported to be 5000 mg/l and 8500 mg/l (352, 353, 358).

The following concentrations of sodium nitrate have been reported as toxic to fish:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
1,282	Distilled	14 hours	Goldfish	313
1,845	Tap	---	Stickleback	598, 1460
2,000	---	4 days	Goldfish	1035
2,000-4,000	---	---	Some sunfish	1035
2,210	Tap	7 days	Stickleback	1460
2,950	Tap	4 days	Stickleback	1460
4,000	Hard	80 hours	Goldfish	313
5,530	Tap	48 hours	Stickleback	1460
6,650	Turbid	96-hour TL_m	Mosquito-fish	2940
10,000	Standard	96-hour TL_m	Bluegill sunfish	2933, 2934
11,060	Tap	24 hours	Stickleback	1460
12,000	Aerated	96-hour TL_m	Bluegill sunfish	3381
13,600	---	---	Stickleback	2920

The following concentrations of sodium nitrate have not been harmful to fish within the time specified:

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
1,000	Hard	---	Goldfish	313, 1035
2,000-4,000	Hard	---	Some sunfish	1035
3,000	Hard	100 hours	Goldfish	313
4,000	Hard	---	Bass	1035

SODIUM NITRATE $NaNO_2$

(see also Nitrates, Nitrites, Sodium)

This soluble salt is used in the manufacture of dyes and other chemicals, in textile processes, and in photography. In water it dissociates completely and under aerobic conditions the nitrite ions are oxidized to nitrates. The oral MLD for dogs is given as 330 mg/kg of body weight (364).

A concentration of 17.1 mg/l of $NaNO_2$ was apparently harmless to minnows during a 24-hour exposure (362); but 50 mg/l can be fatal in 14 days (3577). A massive dose of 10,000 mg/l is lethal to minnows in 1.5 hours (3577). Testing mosquito-fish (*Gambusia affinis*) in highly turbid water at 21-24°C, Wallen et al. (2940) found the 24-hour TL_m to be 8.1 mg/l while the 48- and 96-hour TL_m values were 7.5 mg/l. There was no decrease in turbidity by this dosage. Sodium nitrite has an antagonostic effect upon the toxicity of copper sulfate to goldfish. In cencentrations up to 5000 mg/l, sodium nitrite has decreased the toxic effect of 10 mg/l of copper sulfate toward goldfish (311).

The toxic threshold concentration of sodium nitrite for the flatworm *Polycelis nigra* was found to be 42 mg/l (354). Toward *Daphnia magna* in Lake Erie water, the threshold concentration for immobilization was reported as less than 28 mg/l (352); but in River Havel water at 23°C it was given (2158) as 99 mg/l of $NaNO_2$. Also in River Havel water, the toxic threshold for *E. coli* was found to be 195 mg/l but for *Scenedesmus* concentrations up to 1500 mg/l of $NaNO_2$ were harmless.

SODIUM m-NITROBENZENE SULFONATE

SODIUM 4-NITROCHLOROBENZENE -2-SULFONATE

(see Chapter X)

SODIUM NITROFERRICYANIDE $Na_2Fe(CN)_5NO$

(see also Cyanides, Sodium Ferricyanide)

This substance is used primarily in chemical laboratories for the detection of many organic compounds (364). According to Schaut (362), minnows exposed for one hour to 17.1 mg/l of sodium nitroferricyanide in stabilized tap water showed no effects. Toward *Polycelis nigra,* a flatworm, the toxic concentration was found to be 190 mg/l, as nitroferricyanide (354). Like the ferricyanides, this substance tends to decompose slowly in aqueous solution, but faster in sunlight, to release cyanide ions (see Cyanides).

SODIUM OLEATE

(see Fatty Acids, also Chapter X)

SODIUM OXALATE

(see Oxalic Acid, Oxalates)

SODIUM PALMITATE

SODIUM PENTACHLOROPHENATE

(see Chapter X)

SODIUM PERBORATE $NaBO_3 \cdot 4H_2O$

(see also Boron, Sodium Borate, Chapter X)

Relatively soluble in water, this white crystalline salt is used in bleaching operations, in soaps, and in dentifrices. When mixed with warm water it decomposes to liberate nascent oxygen.

The threshold concentration of sodium perborate for immobilization of *Daphnia magna* in Lake Erie water at 25°C was found to be very much less than 5.2 mg/l (352) and was estimated at 2.6 mg/l (353). Fingerling trout survived a sodium perborate concentration of 160 mg/l for 24 hours but 80 percent were killed by 320 mg/l.

SODIUM p-PHENOL SULFONATE

(see Chapter X)

SODIUM PHOSPHATES

(see also Phosphates, Phosphoric Acid)

Under this designation, six sodium phosphates are considered:

(a) Sodium phosphate, monobasic, $NaH_2PO_4 \cdot H_2O$ also known as sodium dehydrogen phosphate. Highly soluble in water, it is used in baking powders and treatment of boiler water. The aqueous solution is acid.

(b) Sodium phosphate, dibasic, $Na_2HPO_4 \cdot 7H_2$, or disodium hydrogen phosphate. It is highly soluble in water and gives an alkaline solution. It is used in human and veterinary medicine as a laxative.

(c) Sodium phosphate, tribasic, $Na_3PO_4 \cdot 12H_2O$, or trisodium phosphate. Also highly soluble in water, it is used in photographic developers, treatment of municipal and boiler water, in detergent compounds, and in tanneries, paper mills, and laundering plants.

(d) Tetrosodium pyrophosphate, $Na_4P_2O_7$, or sodium pyrophosphate. It is used as a sequestering agent in water treatment, cleaning compounds, oil well drilling, and other processes.

(e) Sodium hexametaphosphates are mixtures of $(NaPO_3)_6$ that occur in commercial detergents such as Calgon, Micromet and Quadrafos.

(f) Sodium tripolyphosphate, $Na_5P_3O_{10}$, used as a sequestering agent and meat preservative.

Campbell et al. (2059) indicate that the taste threshold for trisodium phosphate is 225 mg/l and for Calgon 200 mg/l. According to Lockhart et al. (3241) the taste threshold of trisodium phosphate in water is 180 mg/l. Cohen et al. (2049) found that as little as 0.1 mg/l of sodium tripolyphosphate inhibited the coagulation of turbid water by ferric sulfate.

For *Polycelis nigra,* a flatworm, the toxic threshold concentration of trisodium phosphate ($Na_3PO_4 \cdot 12H_2O$) was found to be 9860 mg/l (354). The threshold concentrations for immobilization of *Daphnia magna* in Lake Erie water at 25°C were found to be lower than the following values:

Compound	*Concentration in mg/l*
Sodium dehydrogen phosphate	1560
Disodium hydrogen phosphate	59
Trisodium phosphate	52

To stimulate the movement of water beetles (*Laccophilus maculosis*) in water, Hodgson (2956) found that a trisodium phosphate concentration of 179,000 mg/l was required.

Toward fish, sodium phosphates are not strongly toxic, the principal effect being a change in pH value. Using turbid water and the mosquito-fish (*Gambusia affinis*) as the test animal, Wallen et al. (2940) report the following results:

	Temperature	*pH*	*TL_m in mg/l*		
Compound	*°C*	*Range*	*24-hour*	*48-hour*	*96-hour*
NaH_2PO_4	19–23	7.6–11.0	720	720	720
$Na_4P_2O_7 \cdot 10H_2O$	19–24	8.2–9.8	1380	1380	1380
Na_3PO_4	17–22	7.4–10.3	467	467	151

The Water Pollution Research Board of England (2907) tested a mixture of sodium tripolyphosphates and tetrasodium pyrophosphate on rainbow trout. It was found that 560 mg/l as phosphate caused no deaths in 24 hours but 1120 mg/l as phosphate killed all of the fish.

Quadrafos, a sodium hexametaphosphate, was tolerated by fish in concentrations up to 3,500 mg/l, but this concentration killed oysters (3005).

SODIUM PYROPHOSPHATE

(see Sodium Phosphates)

SODIUM SALICYLATE

(see Salicylic Acids and Salicylates)

SODIUM SELENITE Na_2SeO_3, $Na_2SeO_3 \cdot 5H_2O$

1. General. Sodium selenite is found in some types of soil and in wastes from pottery works. It is freely soluble in water.

2. Cross References. Selenium, Selenious Acid.

3. Effects Upon Beneficial Uses.

a. Stock and Wildlife Watering. For most mammals the fatal dose of sodium selenite is about 4 to 6 mg per kilogram of body weight. Sodium selenate is about half as toxic as sodium selenite. The minimum lethal dose of sodium selenite for rabbits is about 1.5 mg per kg of body weight (353).

As sodium selenite, administered orally or as a drench, the minimum lethal single dose of selenium for horses is about 1.5 mg per kg of body weight; for cows and calves, 4.5 to 5.0 mg per kg of body weight; and for pigs, 6 to 8 mg per kg (1049).

b. Fish and Other Aquatic Life. The following concentrations of sodium selenite have been reported as toxic to fish.

Concentration in mg/l	*Type of Water*	*Time of Exposure*	*Type of Fish*	*Reference*
10	Hard	98-144 hours	Goldfish	353, 313
100	Very soft	1-4 days	Goldfish	353
100	Hard	8-19.5 hours	Goldfish	353, 313

Bringmann and Kuhn (2158, 3343) investigated the toxicity of sodium selenite in River Havel water. They found the following thresholds of toxicity:

Organism	*Temperature °C*	*Time of Exposure*	*Threshold Concentrations in mg/l*
Daphnia	23	2 days	2.5
Scenedesmus	24	4 days	2.5
Escherichia coli	27	--	90
Microregma	--	--	183

SODIUM SILICATE Na_2SiO_3

This colorless crystalline salt is only slightly soluble in water, but in combination with other sodium silicates such as $Na_2Si_3O_7$ it is marketed as a solution known as "water glass," which has numerous commercial applications (3 4).

The 100-hour toxicity threshold of sodium silicate in double-distilled water at 23°C to *Daphnia magna* has been reported (1691) at 247 mg/l with a resulting pH value of 9.1, but in the presence of other sodium salts this threshold may be somewhat lower (see Sodium).

The Water Pollution Research Board of England determined that sodium silicate was not lethal to fingerling rainbow trout at a concentration of 256 mg/l. Using turbid water at 21-22°C, Wallen et al. (2940) found that the 96-hour TL_m for the mosquito-fish *(Gambusia affinis)* was 2320 mg/l.

SODIUM SILICOFLUORIDE Na_2SiF_6

(see also Fluorides)

This white granular powder, highly soluble in water, has been used extensively in the fluoridation of drinking water. The oral LD_{50} in rats has been reported (364) as 125 mg/kg of body weight. For guinea pigs, the oral LD_{50} is given as 250 mg/kg of body weight (3271). Toward the fish, *Tinca vulgaris,* Simonin (3271) reports the lethal dose to be 50 mg/l.

SODIUM STEARATE

(see Chapter X)

SODIUM SULFATE Na_2SO_4 and $Na_2SO_4 \cdot 10H_2O$

1. General. Sodium and sulfate ions may occur naturally in water, or they may be derived from various industrial wastes, such as those from paper mills. Sodium sulfate is used in manufacturing dyes, glass, soaps, freezing mixtures, and paper; and in dyeing and printing of textiles. It is readily soluble in water.

2. Cross References. Sulfates, Sodium.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The taste threshold of sodium sulfate in water is 250 to 500 mg/l (621). In high concentrations, sodium sulfate has a purgative effect (623). Water containing 500 mg/l of sodium sulfate and 1200 mg/l of magnesium sulfate occasionally has caused diarrhea among human beings; under ordinary conditions such water would be regarded as unsuitable for domestic use (36).

b. Industrial Water Supplies. For boiler waters, the ratio between the concentration of sodium sulfate and that of sodium sulfite is significant. The following ratios as given in Chapter V, are specified as limits:

Boiler Pressure in psi	*Ratio Na_2SO_4:Na_2SO_3*
0-150	1:1
150-250	2:1
More than 250	3:1

For brewing, waters should not contain more than about 100 mg/l of sodium sulfate, and for ice making, not more than 300 mg/l (see Chapter V).

Larson and King (3675) conclude that the rates of corrosion of iron and steel in water are a function of the specific mineral quality as well as the alkalinity and pH values. They indict sodium sulfate and other sodium salts as strong contributors to the rate of corrosion. For example, in a water with 400 mg/l of alkalinity (as $CaCO_3$) and pH 7, the corrosion rate will be zero at 200 mg/l of Na_2SO_4, but when the concentration of sodium sulfate is 400 mg/l, the corrosion rate will be about 100 mg per sq cm per day (3675).

c. Irrigation. A concentration of 4000 mg/l of sodium sulfate in the soil is injurious to plants (246). At 710 mg/l, sodium sulfate has depressed root growth and water absorption of plants grown in liquid culture (1348).

The effect of Na_2SO_4 on plant growth has been demonstrated by culture in sand to which various concentrations of this salt have been added to the normal nutrient solution. With sand culture, any alteration of the soil

structure is avoided as a factor contributing to sodium effects. Results are shown below:

Na_2SO_4 Added in mg/l	Portion of Crop	Relative Dry Weight	Reference
Control	--	100	--
2560	Rice straw	91.9	3379
	Rice grain	79.8	3379
	Rice roots	95.4	3379
2840	Tomato vine	88.2	3679
	Tomato root	85.0	3679
3840	Bean plant	87.5	3376
	Bean roots	90.2	3376
	Bean leaves	86.3	3376
5120	Rice straw	55.6	3379
	Rice grain	15.6	3379
	Rice roots	58.2	3379

d. Stock and Wildlife Watering. Water containing 4546 to 7369 mg/l of salts, mostly sodium sulfate, was not harmful to two cows over a two-year period; however, a sudden change from normal water to this water caused diarrhea (291). At a level of 7000 mg/l there was some evidence of weight reduction in cattle and at 10,000 mg/l weight losses were severe (2398). Rats were not harmed by water containing 15,000 mg/l of sodium sulfate (284). Poultry, however, experienced a 33 percent mortality after drinking for 15 days water containing 7500 mg/l of sodium sulfate. Ballantyne (2394) sets the threshold limit of Na_2SO_4 for all livestock at 2050 mg/l.

e. Fish and Other Aquatic Life. The following concentrations of sodium sulfate were reported to be toxic or lethal to fish and other aquatic life:

Concentration in mg/l	Time of Exposure	Type of Fish	Reference
100	120 hours	Shiners	190
100	—	Fish	344
1000	7 days	hydra	1696
4547	100 hours	*Daphnia magna*	1691
5000	7-28 days	Water snails	1696
5000	6-30 hours	Microcrustacea	1467
5000	1-6 days	Fingerling trout	1696
5000-7105	—	*Daphnia magna*	352, 358, 574
5200	48 hours	*Daphnia*	3523
6700	--	Cutthroat trout	3502
6820	--	*Polycelis nigra*	354
7000	1-2 days	Rainbow trout	1696
11,200-12,000	6 hours	Minnows, distilled water	2942
12,750	96-hour TL_m	Bluegill sunfish	2933
13,400-184,000	7-69 days	Perch	644
13,500	96-hour TL_m	Bluegill sunfish	2936, 3381
16,500	--	Silver salmon	3502
16,500	96-hour TL_m	Mosquito-fish	2940
16,700-17,600	6 hours	Minnows, hard water	2942
24,000	24-hour TL_m	Mosquito-fish	2940

The following concentrations of sodium sulfate were not harmful to aquatic life in the indicated time:

Concentration in mg/l	Time of Exposure	Type of Fish	Reference
100	--	Minnows	3523
500	24 hours	Goldfish	313
705	24 hours	Rainbow trout	2907
1000	5 days	Fish	609
2500	--	Cutthroat trout	3502
5000	6-16 days	Dace	1696
5000	--	*Daphnia*	3523
5000-10,000	--	Water plants	1696
7500	24 days	Perch	1696
10,000	--	Silver salmon	3502
15,000	24 days	Carp	1696
15,000	7 days	Roach	1696
Up to 15,000	--	Algae	1696
15,000-20,000	28 days	Protozoa	1696

To stimulate the movement of water beetles, *Laccophilus maculosis*, Hodgson (2956) found that 35,600 mg/l of sodium sulfate was required. Fairchild (2946) reported that the threshold toxicity value of sodium sulfate toward *Daphnia* depended on the dissolved oxygen concentration. At a DO value of 6.6 mg/l, the toxicity threshold of Na_2SO_4 was 5514 mg/l; but at a DO value of 1.46 mg/l, the toxicity threshold of Na_2SO_4 dropped to 2752 mg/l.

SODIUM SULFIDE Na_2S

1. General. Sodium sulfide is used in tanneries and may also be a constituent of Kraft mill wastes, and beet-sugar-factory effluents. It has a high chlorine demand and a high oxygen demand; a small continuous dose of sodium sulfide can result in oxygen depletion and fish deaths (313, 362). It is highly soluble in water, the sulfide ions tending to react with hydrogen ion to form HS^- or H_2S, depending on the pH value of the solution.

2. Cross References. Sulfide, Distilled Water, Hydrogen Sulfide.

3. Effects Upon Beneficial Uses.

a. Industrial Water Supplies. Even when present in very low concentrations, sodium or potassium sulfide in the water used for mixing developers for photographic use can result in chemical fogging of emulsions on the films (242).

b. Fish and Other Aquatic Life. The following concentrations of sodium sulfide have been reported as toxic or lethal to fish:

Concentration in mg/l	Type of Water	Time of Exposure	Type of Fish	Reference
0.55	pH 5.2	---	Young carp	1698
1.0 (threshold)	Aerated	---	Salmonoid fish	1582
1.8 (lethal)	Aerated	---	Salmonoid fish	1582
2.0	Distilled	---	Minnows	361
2.4	---	---	Brown trout	2920
3.0	---	---	Minnows	690
3.0	12°C	---	Cutthroat trout	3502
3.0	---	---	Shiners, minnows	344, 658, 690
3.1	15°C	---	Silver salmon	3502
3.2	---	48-hour TL_m	Fathead minnows	3335
3.3	pH 7.4	---	Young carp	1698
3.5	17.5°C	---	King salmon	3502
7.8	---	90 minutes	Minnows	468
10-11	Hard, 15°C	6 hours	Minnows	2942
11	---	---	Stickleback	2920
12-13	Distilled, 25°C	6 hours	Minnows	2942
39	---	6 minutes	Minnows	468
50	Hard	---	Minnows	361
61	Standard	48-hour TL_m	Bluegill sunfish	2093
750	Turbid	96-hour TL_m	Mosquito-fish	2940

The maximum concentration of sodium sulfide that produced no mortality has been given (3502) as 1.8 mg/l for king salmon at 17.5°C, 1.3 mg/l for silver salmon at 15°C, and 1.0 mg/l for cutthroat trout at 12°C. Fish tried to avoid solutions of sodium sulfide in concentrations of 3.1 to 39.0 mg/l (1046).

Fish removed from toxic concentrations of sodium sulfide before they stopped breathing recovered and survived (468). Minnows exposed to 17.1 mg/l of sodium sulfide in tap water have been disturbed for as long as five hours; within 24 hours, however, the sulfide had disappeared and the fish recovered (362). On the other hand, Ellis quotes a reference to the effect that exposure

for one hour to 1150 mg/l of sodium sulfide in tap water has resulted in the death of tench six days later (313).

When Na_2S is added to water it dissociates to release sulfide ions which then react with hydrogen ions to form HS^- or H_2S, depending on the pH value of the solution (see Hydrogen Sulfide). For that reason, the toxicity of sodium sulfide tends to increase as the pH of the water decreases from 9 to 6 (1046). This relationship has been shown by Tomiyama and Yamagawa (1698) for young carp as follows:

pH Value	*Critical Lethal Concentration, mg/l*
5.2	0.55
6.1	0.95
7.4	3.3
8.2	8.0

Other aquatic life have responded to sodium sulfide as shown below:

Concentration in mg/l	*Organism*	*Remarks*	*Reference*
1–2	Mayfly larvae	Killed	690
2–1000	Insect larvae	Killed	690
2.44	Bivalve larvae	Lethal	2989
3.2	*Mesocyclops leuckarti*	Toxic threshold	2955
9.4–10	*Daphnia magna*	Killed or immobilized	352, 690, 2955
10	*Daphnia magna*	48-hour MLD at 17°C	3523
34	*Polycelis nigra*	Toxic threshold	354
63	*Daphnia*	Toxic threshold	2158
97	*Scenedesmus*	Toxic threshold	2158
226	*E. coli*	Toxic threshold	2158
1000	Chironomus larvae	Resisted	690

SODIUM SULFHYDRATE NaHS

Completely and rapidly soluble in water, with an H_2S odor, this crystalline substance is used in tanneries for dehairing hides, in the manufacture of sulfur dyes, and for desulfurizing viscous rayon (364). It may also be found in Kraft mill wastes.

Using turbid water at 21-33°C, Wallen et al. (2940) studied the toxicity of NaHS toward the mosquito-fish, *Gambusia affinis*. They found the 24-, 48-, and 96-hour TL_m values to be 206 mg/l. The 144-hour TL_m was lowered to 138 mg/l. Haydu et al. (3501, 3502) report the minimum lethal concentrations of NaHS to be 3.3 mg/l for king salmon, 3.5 mg/l for silver salmon, 1.8 mg/l for cutthroat trout, and 0.5 mg/l for Wisconsin minnows. The maximum concentrations of sodium sulfhydrate tolerated by king salmon, silver salmon, and cutthroat trout are 2.1, 2.5, and 0.3 mg/l respectively (3501, 3502).

Gillette et al. (1442) tested the toxicity of this substance to creek chub, a fish considered to be average in tolerance. They found that the concentration below which all four test fish survived for 24 hours was 4 mg/l, while above 10 mg/l all fish died. The critical concentration of NaHS to salmonoid fish in water containing over 5 mg/l of dissolved oxygen ranged from 0.3 mg/l for threshold damage to 2.5 mg/l for lethality (1582).

SODIUM SULFITE Na_2SO_3

1. General. This soluble salt is used chiefly in photographic developers and fixing operations. It is also employed in bleaching operations and as a reducing agent in the manufacture of dyes (364). Sodium and sulfite ions may occur in various paper mill and beet-sugar-factory effluents (313).

2. Cross References. Sulfites, Sulfite Waste Liquor.

3. Effects Upon Beneficial Uses.

a. Stock and Wildlife Watering. A concentration of 15,000 mg/l of sodium sulfite in the drinking water of rats caused no apparent injury (287).

b. Fish and Other Aquatic Life. The lethal concentration of sodium sulfite to goldfish in 96 hours at 18-23°C has been reported (2920) as 100 mg/l; but on the other hand, concentrations up to 260 mg/l at pH 5 to 9 have been observed to be harmless to young carp (1698). Using turbid water at 18-26°C, Wallen et al. (2940) found the 24-, 48-, and 96-hour TL_m for mosquito-fish to be 2600 mg/l.

The toxic threshold concentration of sodium sulfite for the flatworm, *Polycelis nigra*, was found to be 7580 mg/l (354). The minimum lethal concentration for *Daphnia* has been reported to be 300 mg/l (574, 3523). For the immobilization of *Daphnia magna* in Lake Erie water at 25°C, the threshold concentration of sodium sulfite has been reported to be 440 mg/l (352) and 3784 mg/l (358).

SODIUM SULFONATES

(see Chapter X)

SODIUM TETRAPROPYLENE BENZENE SULFONATE

(see Chapter X)

SODIUM THIOCYANATE NaSCN

(see also Cyanides, Hydrogen Cyanide, Potassium Thiocyanate)

This highly soluble salt is used in the manufacture of other thiocyanates, especially the organic compounds, and in many of the same processes as potassium thiocyanate (364). When chlorinated, it breaks down to release cyanide ions (see KSCN).

According to Schaut (362), fish were killed in two hours by 3240 mg/l of sodium thiocyanate. For *Daphnia magna* in Lake Erie water at 25°C, however, the threshold concentration was found by Anderson (352) to be less than 11.3 mg/l or about 10.2 mg/l (353). The threshold concentration for *Polycelis nigra*, a planarian, was reported by Jones (354) at 700 mg/l as SCN^-.

Thiocyanate compounds have been used for the treatment of hypertension in human patients. The daily ingestion of less than approximately 120 mg of SCN has not given rise to reports of harmful effects in patients treated for 10 years (2963).

SODIUM THIOSULFATE $Na_2S_2O_3 \cdot 5H_2O$

Sodium and thiosulfate ions may occur as constituents of Kraft mill wastes. The toxic threshold concentration of sodium thiosulfate for the flatworm *Polycelis nigra* was found to be 8400 mg/l (354). The threshold concentration of sodium thiosulfate for the immobilization of *Daphnia magna* in Lake Erie water was found to be much less than 520 mg/l (352). The minimum lethal concentration of sodium thiosulfate for minnows has been reported to be 5.0 mg/l, for a 120-hour period (190, 344). Using highly turbid water at 22-24°C, Wallen et

al. (2940) found the 96-hour TL_m toward the mosquitofish *(Gambusia affinis)* to be 24,000 mg/l.

SODIUM TRIPOLYPHOSPHATE

(see Sodium Phosphates)

SODIUM TUNGSTATE

(see Tungsten)

SOLIDS

(see also Dissolved Solids, Specific Electrical Conductance, Suspended Solids, Silt, Settleable Solids, Turbidity, and Distilled Water)

Solids in water are classified as either "dissolved", i.e., capable of passing a fine mat of asbestos fiber in a Gooch crucible, or "suspended", i.e., retained on the asbestos mat. Both dissolved and suspended solids may be differentiated further as "fixed", i.e., inorganic, and "volatile", i.e., organic material or volatile matter driven off by ignition at about 600°C. In this report, no attempt is made to segregate fixed and volatile solids (469).

Another test for solids, readily conducted by means of the Imhoff cone, is the determination of "settleable" solids, i.e., the volume in ml per liter that will settle in one hour. The total effect of dissolved solids is also measured by the effect of the dissociated ions on the conductivity of the solution. This test for total dissolved solids is known as the "specific electrical conductance".

In this report each of the aforementioned categories of solids is considered separately and the reader is referred, therefore, to each type as itemized above.

SORBITAN MONOLAURATE

(see Chapter X)

SORBITAN MONOSTEARATE

(see Chapter X)

SPECIFIC ELECTRICAL CONDUCTANCE

1. General. Natural inland waters usually contain in solution relatively small quantities of mineral salts, but in waters polluted by brines and various chemical wastes the salt concentration may rise to levels harmful to living organisms because of the increase in osmotic pressure. In studies of waters for use in irrigation and fish production, the salinity is often expressed as specific electrical conductance. The determination of conductivity is a quick method for measuring the ion concentration of water. Procedures and equipment are described by Standard Methods for the Examination of Water and Waste Water (469), by Ellis and coworkers (247), and by Wilcox (635). Conductivity is reported as specific electrical conductance, the reciprocal of the resistance in ohms of a column of solution one centimeter long and with a cross section of one square centimeter, at a specified temperature, usually 25°C.

In fish studies, specific electrical conductance is reported directly as reciprocal ohms (mhos) at 25°C. Thus, a 0.005N solution of NaCl, containing 292.2 mg/l, has a specific electrical conductance, K, of 0.000604 mhos or 604×10^{-6} mhos at 25°C. In irrigation studies, however, it is customary to report specific electrical conductance as $K \times 10^5$ or as $EC \times 10^6$ at 25°C. For the 0.005N solution of NaCl, irrigation literature would report the conductance as $K \times 10^5 = 60.4$ or as $EC \times 10^6 = 604$. Considering the combined effects of all of the dissolved salts, it has been found that $K \times 10^5$ values times 6.5, and $EC \times 10^6$ values times 0.65, are approximately equal to the concentrations of dissolved salts in mg/l. Values of $K \times 10^5$ divided by 10 are approximately equal to the concentration of cations or anions in equivalents per million (269). Where NaCl is the only salt, $K \times 10^5$ values should be multiplied by 4.7 to 5.7 to give concentrations in mg/l, depending on the concentration.

2. Cross References. Dissolved Solids.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supply. See Dissolved Solids.

b. Irrigation Waters. Conductance is one of the most important criteria for the classification of irrigation waters (see Chapter V—Irrigation). The limits suggested by various authorities (246, 263, 269, 273, 274) are summarized in the following table:

SPECIFIC ELECTRICAL CONDUCTANCE OF IRRIGATION WATERS

Designation	*$EC \times 10^6$ at 25°C in Micromhos per cm*	*Reference*
Class I, suitable under most conditions	<1000	246, 273, 274, 1912
	< 750	267, 1733
Class II, suitability on crop, climate, etc.	1000–3000	246, 273, 274, 1912
	750–3000	267, 1733
Class III, unsuitable under most conditions	>3000	246, 267, 274, 273 1733, 1912
Excellent	< 250	269, 263
Good	250– 750	269, 263
Permissible	750–2000	269, 263
Doubtful	2000–3000	269, 263
Unsuitable	>3000	269, 263

b. Fish and Other Aquatic Life. All substances in solution in the water collectively exert osmotic pressure on the organisms living in it, and the aquatic life is adapted to the conditions imposed upon the water by its dissolved constituents. Most species can tolerate some changes in the relative amounts of salts normally present if the total concentration is not exceeded. However, wide variations in the total salinity or in the concentrations of individual salts can have far-reaching effects upon stream fauna, resulting even in the elimination of species.

When the osmotic pressure is sufficiently high, owing to salts in solution, water may be drawn from gills and other delicate external organs, with considerable damage to cells, or even death. High concentrations of many kinds of pollutants present this danger apart from any other toxic or corrosive effects they may have.

Ellis' studies of inland fresh waters indicated that the specific conductance of streams and rivers supporting a good mixed fish fauna lay, in general, between 150 and 500 mhos $\times 10^{-6}$ at 25°C, except in streams of the western plains and desert areas, particularly those carrying the more alkaline natural waters. Such waters, draining land rich in soluble alkalis, were found to have a conductance as high as 5000 mhos $\times 10^{-6}$ at 25°C. However, good mixed fish fauna were usually not found in waters with

a specific conductance greater than 2000 mhos $\times 10^{-6}$ at 25°C. On the basis of his studies Ellis had concluded that conductances in excess of 1000 mhos $\times 10^{-6}$ at 25°C in most types of stream, or in excess of 2000 mhos in the alkaline western streams are probably indicative of the presence of acid or salt pollution of various kinds (247, 313, 345, 347, 801).

Using Ellis' data, Hart et al. (310) have reported that among United States waters supporting a good fish fauna about 5 percent have a specific conductivity under 50 $\times 10^{-6}$ mhos at 25°C; about 50 percent under 270 $\times 10^{-6}$ mhos; and about 95 percent under 1100 $\times 10^{-6}$ mhos.

Ellis (611) has also found that a specific conductance of 4000 $\times 10^{-6}$ mhos at 25°C is approximately the upper limit of ionizable salts tolerated by fish in mixtures of sodium, magnesium, and calcium compounds in gas-well waters.

STANNIC AND STANNOUS SALTS

(see Tin)

STRONTIUM Sr

(see also Chapter VIII)

Related to calcium, magnesium, and barium, this element is not found free in nature. It occurs largely with calcium or barium minerals, in the form of the sulfate (celestite) or the carbonate (strontianite). The carbonate is soluble only to the extent of about 10 mg/l and the sulfate to about 114 mg/l (364). Hence, strontium ions are not likely to occur in high concentrations in surface waters. The concentration in sea water has been given as 10 mg/l (2121) and 8.5 mg/l (3345). It is not adsorbed readily by soils and hence strontium may be expected to travel readily along with ground waters (1976). Strontium metal is used in some alloys of tin and lead. The salts are used in pyrotechnics, in refining beet sugar, in manufacturing glass and paints, in ceramics, and in some medicines (364, 2121).

Strontium is present in plant and animal tissues in trace concentrations, but it occurs in much higher concentrations in bone structures. There is no evidence that strontium is essential for plant nutrition, but there are indications that it is necessary for the growth of animals and especially for calcification of bones and teeth. In humans, the strontium content of bones has been given as 120 to 234 mg/kg, in contrast with a tissue concentration of less than 0.1 mg/kg. Toxicologically, no evidence was uncovered to show that non-radioactive strontium salts taken orally by man or animals produces deleterious action. Its toxicity is probably on the same order of magnitude as calcium (2121). Indeed, strontium bromide has been used medicinally as a sedative and antiepileptic; strontium iodide has been used in iodine therapy; and strontium lactate for osteoporosis (364).

With the advent of nuclear fission and weapons testing, strontium came to the forefront of publicity as one of the inevitable constituents of fall-out. Owing to its long half-life and its tendency to accumulate in bone structure, Sr-90 is ranked second only to Ra-226 as a hazard to human health. The 1962 Drinking Water Standards of the USPHS (2036) limit the Sr-90 content to 10 pc/l. The WHO Standards, both European and International (2328, 2329) limit the total beta-emitters, including Sr-90, to 10 pc/l. As a result of nuclear testing, rain water has been reported to contain from 2 to 8 pc/l (3680, 3681). In lake waters, the Sr-90 activity has reached 0.45 pc/l, in river waters 0.24 pc/l, but in ground waters less than 0.01 pc/l (3680). Food, however, and not water is considered to be the major vehicle by which radiostrontium reaches humans. It is estimated that less than 10 percent of the Sr-90 ingestion comes from consumption of water (3682).

Crop plants tend to accumulate radiostrontium from soils and water contaminated with fallout from nuclear explosions or waste products from nuclear reactors (3683). Plants do not appear to discriminate between calcium and strontium in soil moisture and hence the ratio of these two elements in plant tissue tends to be the same as their ratio in the soil solution. The uptake of strontium by plants can be reduced by adding calcium to the soil or applied water (3683, 3684, 3685, 3686).

Owens et al. (3689) measured the uptake of radiostrontium by aquatic macrophytes during 7 days exposure in hard river water containing Sr-89 with an activity of about 0.01 $\times 10^{-3}$ c/l. Concentration factors for 6 species varied from 30 to 74. In soft water, however, watercress had a concentration factor of 200 after 28 days in contrast to 55 in hard water. Other experiments (1816) showed concentration factors for watercress in the order of 600 to 800 in soft water (20 mg/l of $CaCO_3$) and less than 100 in hard water (300 mg/l $CaCO_3$). Again, the increase in calcium and magnesium ions appears to militate against the uptake of strontium. For *Porphyra*, an edible seaweed in South Wales (2435) the concentration factor was only 0.05-0.3.

Planktonic algae have also been shown to concentrate radiostrontium. The greatest part of the radioactivity in the cells appears to be due to Y-90, the radioactive daughter of Sr-90 (3690). Concentration factors for algae have been reported at values ranging from 8 to 3060 (2442, 3385, 3386, 3690). For marine bacteria, a concentration factor of about 100 has been given (3386).

For fish, Foreman and Bidwell (3691) observed that Sr-90 concentrated in the flesh, bones, and scales of rudd by factors of 2.6, 71, and 209 respectively. For lobsters, Templeton (2435) reported a concentration factor for non-radioactive strontium of 300 in the shell and 2.0 in the flesh. He indicated a probable accumulation factor of 150 for goldfish (the whole fish). Krumholz (3692) found that fish in a lake that received radioactive waste effluents at Oak Ridge concentrated radiostrontium by factors of 20,000 to 30,000 in hard tissues.

It is evident that the major hazard to humans of radiostrontium in water lies not in direct consumption but in plants and fish that accumulate this element.

STRONTIUM CHLORIDE $SrCl_2 \cdot 6H_2O$

(see also Strontium)

This highly soluble salt is used in pyrotechnics, in the manufacture of other strontium salts, and in freezing mixtures for refrigerators (364, 2121). Toward fish, strontium chloride has a low order of toxicity. Ellis (313) reported that 15,384 mg/l in distilled water killed goldfish in 17 to 31 hours. According to Jones (2920)

is the lethal concentration of $SrCl_2$ at 20°C was 18,000 mg/l for goldfish. Toward a small fresh-water fish (*Orizias*), the lethal concentration was given (1459) as about 20,000 mg/l for a 24-hour exposure.

Toward smaller aquatic life, the lethal dose may be somewhat lower. According to Anderson (598), the threshold concentration for immobilization of *Daphnia magna* in Lake Erie water at 25°C was found to be 114 mg/l in 64 hours. Bringmann and Kuhn (2158, 3343) found the threshold concentration for *Daphnia* at 23°C to be 210 mg/l of strontium; and for the protozoan, *Microregma,* it was 329 mg/l as strontium. Jones (608) reported that the threshold for *Polycelis nigra,* a flatworm, during a 48-hour exposure was 6600 mg/l. Toward the alga, *Scenedesmus,* and the bacterium, *E. coli,* 1000 mg/l as strontium had no noticeable effect (2158).

STRONTIUM FLUORIDE SrF_2

This salt is soluble in water to the extent of only 117 mg/l at 18°C (364). Yet, Simonin (3271) reports that the lethal dose for tench is 30,000 mg/l.

STRONTIUM NITRATE $Sr(NO_3)_2$

(see also Strontium)

This highly soluble salt is used for pyrotechnics, signal lights, flares, and matches. At a concentration of 9615 mg/l in distilled water, it was reported (313) to kill goldfish in 32 to 146 hours. For sticklebacks, the lethal dose was found to be 1200 mg/l during prolonged exposure. The average survival times at other concentrations were as follows: one week at 1500 mg/l, four days at 3000 mg/l, two days at 7000 mg/l, and one day at 10,000 mg/l, all expressed as strontium (1460). Goldfish were reported (1459) to have survived exposure for four days to strontium nitrate at concentrations of 3200 mg/l as strontium. According to Jones (608) the threshold concentration for *Polycelis nigra,* a flatworm, was 3500 mg/l as strontium.

STRYCHNINE $C_{21}H_{22}N_2O_2$

Strychnine and its salts are used for destroying rodents and predatory animals, and for trapping fur-bearing animals (364). The likelihood that it will reach natural waters in significant concentrations is small, Smith (922) reports that adults may safely drink daily 0.078 to 0.25 gallons of water containing 10 mg/l of strychnine (i.e., 2.94 to 9.45 mg per day) and a fatal dose of such water would be 1.5 to 3.0 gallons (56.7 to 113.4 mg). Carson (1483) states that strychnine in water is detectable because of its bitter taste in concentrations of more than 2 or 3 mg/l.

SUCROSE $C_{12}H_{22}O_{11}$

(see also Sugars)

Sucrose, obtained from sugar cane and sugar beet, is used as a sweetening agent and food, for fermentation, in pharmacy, and in many industrial processes. Rohlich and Sarles (759) report that the threshold concentration for tasting sucrose in water is about 5000 mg/l.

For fish and other aquatic life, the primary detrimental effect of sucrose arises from its high BOD. Ellis (313) reports that concentrations of 34 to 218 mg/l in distilled water caused the death of minnows in 24 to 48 hours. This effect, however, may have been due more to the distilled water than to the sucrose. Garrey (307) writes that cane-sugar solutions can be directly toxic to fish, being least toxic when the osmotic pressure of the water equals that of the blood. He reports that 137,000 mg/l of cane sugar in solution killed fish in 12 hours and 85,000 to 100,000 mg/l killed in three days.

SUGARS

(see also Beet-Sugar Wastes, Dextrose, Sucrose)

In addition to the possible harmful effects in streams, arising from deoxygenation and osmotic action, sugars are sometimes detrimental in industrial process waters. Hewlett (806) states that boiler feed water should contain not more than 5.0 mg/l of sugar, inasmuch as sugar causes foaming and the deposition of a film of decomposition products on the walls.

Sugar is one of the most detrimental substances that can be introduced in a mixture containing portland cement. Very small quantities in the mixing water may prevent portland cement mixtures from attaining their potential strength, but dry sugar has no effect on the hardened concrete. At 0.05 percent by weight of the sand (or 3400 mg/l in a 1:3 mix) sugar caused nearly all specimens to disintegrate in water (193, 360).

Oysters were harmed and even killed by feeding on sugar, oatmeal, or flour, according to Boyce and Herdman (807). Oysters thrive best on living plankton and live well in sewage-polluted water; but they appear unable to feed on carbohydrates and sugars.

Sugars in water can be tasted in a concentration of 2000 mg/l (1578).

SULFATED AMINE

(see Chapter X)

SULFATED FATTY ESTER

(see Chapter X)

SULFATED ESTERS

(see Chapter X)

SULFATED MONOGLYCERIDES

(see Chapter X)

SULFATE PULP MILL WASTES

(see Kraft Pulp Mill Wastes)

SULFATES SO_4^{--}

1. General. Sulfates occur naturally in waters, particularly in the western United States, as a result of leachings from gypsum and other common minerals. They may also occur as the final oxidized stage of sulfides, sulfites, and thiosulfates: e.g., iron pyrite FeS may be leached from abandoned coal mines and the sulfide ions converted in surface streams to sulfates (1358). Sulfates may also occur as the oxidized state of organic matter in the sulfur cycle, but they, in turn, may serve as sources of energy for sulfate-splitting bacteria (see

Sulfur Bacteria). Finally, sulfates may be discharged in numerous industrial wastes, such as those from tanneries, sulfate-pulp mills, textile mills, and other plants that use sulfates or sulfuric acid.

The sulfates of lead, barium strontium and calcium are relatively insoluble (1224), but sodium, potassium, and ammonium sulfate are highly soluble.

2. Cross References. Sulfur, Sulfides, Sulfur Dioxide, Various sulfate salts, Chapter V, Chapter VII—Sulfur Bacteria.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The 1962 Drinking Water Standards of the USPHS (2036) recommend that sulfates do not exceed 250 mg/l, except where a more suitable supply is not available. This limit does not appear to be based on taste or physiological effects other than a laxative action toward new users. Public water supplies with sulfate contents above this limit are commonly and constantly used without adverse effects (152). The 1958 WHO International Standards (2328) established a "permissible limit" of 200 mg/l and an "excessive limit" of 400 mg/l, but set no maximum allowable limit. The 1961 WHO European Standards (2329) include a recommended limit of 250 mg/l for sulfates.

Korotchenok (622) reported on heavily mineralized drinking waters in western Turkmenia (USSR), noting that no outbreaks of disease have been ascribed to waters in which the content of sulfate did not exceed 1295 mg/l. In British Somaliland well waters used for human consumption contain very high sulfates, many having 2000-3000 mg/l. One village is using water of 4400 mg/l sulfates (1546). A survey in North Dakota (1628a) indicated that water containing less than 600 mg/l of sulfates is usually safe. In reviewing the literature, Moore (621) quotes Sollman to the effect that concentration of 1000 mg/l of sulfates in water is harmless. A cathartic dose is 1.0 to 2.0 grams, or a liter of water containing 1000 to 2000 mg/l of sulfates.

Sulfates appear to have no detrimental effect on the corrosion of brass fittings in domestic water systems (1359), nor do concentrations less than 200 mg/l increase plumbo-solvency (1073). However, for the effects of higher concentrations, see Sodium Sulfate. Whipple is quoted by Moore (621) to the effect that the taste thresholds of sulfate salts were as follows:

Sodium sulfate	200-500 mg/l
Calcium sulfate	250-900 mg/l
Magnesium sulfate	400-600 mg/l

b. Industrial Water Supplies. Limiting or threshold concentrations, or optimum ranges, as described in Chapter V for sulfates are assembled below:

	Limits of Recommended Values, mg/l			
Industrial Process	SO_4^{--}	$CaSO_4$	$MgSO_4$	Na_2SO_4
Brewing	---	100-500	100-200	100
Carbonated beverages	250	---	---	---
Concrete corrosion	25	---	---	---
Ice making	---	300	130-300	300
Milk industry	60	---	---	---
Photographic processes	---	100	---	---
Sugar making	20	---	---	---
Textiles	100	---	---	---

Ice appears to be particularly susceptible to excessive sulfates, since they become trapped in the entire block instead of migrating toward the core where they can be removed (534). A widely quoted formula is used by some ice makers in which the sulfates are weighted as 1.00, the chlorides as 0.75, and sodium carbonate as 1.25. When the sum of these weighted concentrations is less than 171 mg/l, first quality ice can be made readily; at higher values added precautions are needed and if the total is more than 684 mg/l merchantable ice cannot be expected (164, 168, 228, 231). In concentrations of 257 mg/l or less, sodium sulfate is reported to cause a white shell in ice (229).

Sulfates appear to increase the corrosiveness of water toward concrete (see Chapter V—Concrete Mixing and Concrete Corrosion). Kellam (196) states, "If the SO_3 is less than 25 mg/l, injury from this source will be mild and inconsequential. When the SO_3 is in the hundreds of mg/l, it is a possible source of great harm and protection must be provided". Hammerton (360) claims that there will be little attack if the sulfate concentration is less than 300 mg/l (as SO_3), but if the sulfate is greater than 1000 mg/l attack will be rapid (855). As pointed out hereinbefore, the disintegration of concrete is a function of several factors, including pH, carbonic acid, sulfates, and biological action (2355). According to Szigligeti (1699) sulfates in excess of 300 mg/l in soil and water are detrimental to asbestos-cement and asbestos-concrete pipes.

c. Irrigation. Sulfates are somewhat less toxic than chlorides in irrigation waters (275). In high concentrations, sulfate ions may cause the precipitation of calcium and may in themselves be toxic to plants (281). Scofield (263) gave examples of permissible limits of sulfates for a definite region of irrigation waters as follows:

Excellent	less than 192 mg/l
Good	192 to 336 mg/l
Permissible	336 to 576 mg/l
Doubtful	576 to 960 mg/l
Unsuitable	over 960 mg/l

Eaton, McGallum and Mayhugh (264) condensed these same values into three classifications: less than 192 mg/l, from 192 to 576 mg/l, and over 576 mg/l. Hinman (219) points out that concentrations over 500 mg/l are generally hazardous. The 10-year weighted average sulfate concentration of Colorado River water is 230 mg/l and Kelley (1060) did not feel that this concentration will be deleterious to underground waters or citrus trees in southern California.

d. Stock and Wildlife Watering. In Minnesota, cattle that drank water containing 3590 mg/l and 2104 mg/l of sulfate developed run-down, ragged appearances and eventually weakened and died (643). However, sulfate seems to offset in part the toxicity of selenium toward cattle (1328). At concentrations of 2500 mg/l, sulfates caused diarrhea in dogs (3693). A threshold limit of 1000 mg/l has been suggested by Stander (3373).

e. Fish and Other Aquatic Life. In U.S. waters that support good game fish, 5 percent of the waters contain less than 11 mg/l of sulfates, 50 percent less than 32 mg/l, and 95 percent less than 90 mg/l (310). Experiments indicate that water containing less than 0.5 mg/l of sulfate will not support growth of algae (2203). For further data on specific sulfates, see sodium sulfate, copper sulfate, potassium sulfate, etc.

4. Summary. On the basis of the information gleaned from literature, it appears that the following concentrations of sulfate will not be detrimental for the indicated beneficial use:

a. Domestic water supply ..... 500 mg/l
b. Irrigation ..... 200 mg/l
c. Stock watering ..... 500 mg/l

SULFATE SUBSTITUTED AMIDE

(see Chapter X)

SULFIDES S^-

(see also Hydrogen Sulfide, Sulfur, Salts of Sulfides and Chapter VII—Sulfur Bacteria)

Sulfides are constitutents of many industrial wastes such as those from tanneries, paper mills, chemical plants, and gas works; but they are also generated in sewage and some natural waters by the anaerobic decomposition of organic matter. When added to water, soluble sulfide salts such as Na_2S dissociate into sulfide ions which in turn react with the hydrogen ions in the water to form HS^- or H_2S, the proportion of each depending upon the resulting pH value (see Hydrogen sulfide). Thus, when reference is made to sulfides in water, the reader should bear in mind that the sulfide is probably in the form of HS^- or H_2S.

Owing to the unpleasant taste and odor which result when sulfides occur in water, it is unlikely that any person or animals will consume a harmful dose (1077). The thresholds of taste and smell were reported (686) to be 0.2 mg/l of sulfides in pulp-mill wastes. For industrial uses, however, even small traces of sulfides are often detrimental (see Hydrogen sulfide). Sulfides are of little importance in irrigation waters (268).

The toxicity of solutions of sulfides toward fish increases as the pH value is lowered, i.e., the H_2S or HS^-, rather than the sulfide ion, appears to be the toxicity principle (346). In water containing 3.2 mg/l of sodium sulfide, trout overturned in two hours at pH 9.0, in 10 minutes at pH 7.8, and in four minutes at pH 6.0, as reported by Southgate (346). According to Doudoroff (2409) inorganic sulfides have proved fatal to sensitive fishes such as trout at concentrations between 0.5 and 1.0 mg/l as sulfide, even in neutral and somewhat alkaline solutions.

SULFITES

(see Sulfur Dioxide)

SULFITE WASTE LIQUORS

1. General. The waste water resulting from the pulping of wood with calcium on magnesium bisulfite liquor with steam under pressure are known as sulfite waste liquors (SWL) or spent sulfite liquors (SSL). They contain in dissolved or very finely divided suspension approximately half of the weight of the wood used for pulping, and comprise fiber-binding substances such as lignin and pectin, hemicelluloses, sulfur dioxide, sulfites, polythionates, organic acids, calcium or magnesium, and numerous other organic and inorganic substances. Acid in reaction, they are high in BOD and dissolved organic matter, with a persistent pungent odor and a tendency to foam in receiving streams.

Gunter and McKee (2415) have summarized the chemical and physical characteristics of SWL. They point out that most SWL contains 10 to 14 percent total solids, with 5-day BOD values of about 30,000 mg/l. The lignin component of SWL comprises a great variety of high-molecular-weight compounds that include benzene or phenol complexes to which sulfonate has been added for pulping purposes. SWL can be detected in dilute concentrations in receiving waters by the Pearl-Benson test, which is precise and reproducible but not specific. Many natural phenolic substances will produce positive tests. The Pearl-Benson test is calibrated in terms of SWL having 10 percent solids. In effect, then, it expresses the dilution of 10 percent SWL expressed in parts per million by volume. Hence, a P-B reading of 15 ppm represents only 1.5 mg/l of total solids in the original SWL. For this reason, Gunter and McKee (2415) proposed the term "Pearl-Benson Index" or "PBI" to describe the concentrations measured by this test.

2. Cross References. Sulfur and Sulfur Compounds.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The controlling factor for SWL in streams that may be used for domestic water supply appears to be taste and odor. To eliminate the taste of SWL, a dilution of 1:25,000 appears to be necessary (7). At such dilutions, the concentrations of other potential pollutants from SWL are insignificant.

b. Fish and Other Aquatic Life. The principal deleterious effect of SWL towards fish appears to be its high BOD, with resultant oxygen depletion. Dilutions of 1:200 or better are required to prevent serious oxygen depletion. (673). Compared with Kraft mill effluents, SWL is relatively non-toxic (683). A second deleterious effect arises from the fact that the sugars in SWL stimulate the growth of *Sphaerotilus* in streams (2500, 2642).

Most of the references dealing with the effect of SWL on fish express their findings in terms of dilution. Since the strength of the SWL is seldom given, results cannot be compared exactly; but it is logical to assume that the SWL was approximately 10 percent solids. The following table summarizes the observed effects of SWL on fish:

Given Dilution (ppm by volume)	*Type of Fish*	*Observed Effect*	*Reference*
50-75	--	No effect	3503
500-600	--	Threshold of lethality, 30 days	2642
530-1550	Pink salmon	5 percent kill	3695
560-1175	Chinook salmon	5 percent kill	3695
599-1022	Young herring	Tolerance level	3694
805	Chinook salmon	TL_m for 30 days	3524
880	Chum salmon	Toxic threshold	2091
1000	Trout	Lethal in a few days	673
1015-1230	Silver salmon	5 percent kill	3695
1330-2000	Trout	Lethal	359
1700	Coho salmon	TL_m for 30 days	3524
2000	Salmon	Avoidance reaction	3524
2000-5000	Warm-water fish	Survived 10-20 days	673
5000	Mixed	No deaths in 28 days	311, 688
5000	Fish	Toxic	687
8330-20,000	Trout fry, perch, and bass	Killed in 29-113 hours in non-aerated water	808

Given Dilution (ppm by volume)	*Type of Fish*	*Observed Effect*	*Reference*
10,000	Bass	Not killed in 17 days in aerated water	808
20,000	Salmon fry	Killed in 28 hours	311, 688
100,000	Fish	Lethal	540
100,000	Rock bass	Lethal	359

Long exposures to SWL affect the internal organs of fish, with definite damage at dilutions of 1:100,000. Moreover, SWL gives a taste to fish flesh (7).

Very little quantitative information is available upon which to assess the effects of SWL on fish-food organisms. In general, it appears that SWL exerts no direct toxic action except at concentrations in excess of 1000 ppm by volume of 10 percent SWL. The sparse literature in this subject has been reviewed and summarized by Gunter and McKee (2415). Lasater (3694) found that some copepods could not tolerate SWL, with significant mortalities being reported at 50-157 ppm of 10 percent SWL in 2 to 14 days.

c. Shellfish Culture. On the basis of a comprehensive literature review, Gunter and McKee (2415) recommended that the Pearl-Benson index attributable to SWL in waters overlying beds for the growing of Olympia oysters should not exceed 10 during the months of April through October and 20 during the months of November through March. In waters overlying beds of the Pacific oyster, the PBI should not exceed 40 during April through October nor 80 during November through March. There appears to be some evidence that PBI concentrations of 8 to 16 are inimical to the adult Olympia oyster and perhaps the threshold concentration for adverse effects is even lower. Adult-Pacific oysters appear to be far more tolerant of SWL than Olympias. It is apparent that Pacifics can withstand PBI concentrations of 50 to 100, or perhaps even more, over long periods of time without mortality.

Toward oyster larvae, on the other hand, SWL may be more adverse toward Pacifics than towards Olympia, owing to the difference in spawning mechanisms. For Pacific larvae, PBI values of 8 to 16 represent a tentative threshold of toxicity. In recent work, Lindsay, Westley, and Woelke (3696) point out that the reproductive cycle of Olympia oysters was affected by fresh ammonia-base SWL at PBI values of 2, 4, and 8; but these effects were not demonstrated as being necessarily adverse. The development of Pacific larvae was affected at PBI values of 2 and greater, and at 18 almost 100 percent of the larvae were abnormal. These authors also point out that SWL is lethal to *Monas sp,* an important oyster food organism at P-B indices of 1,000 to 10,000. Over several months, SWL appears to have a depressing effect on this food organism at PBI values as low as 2.5.

SULFOETHYL METHYL OLYAMIDE

(see Chapter X)

SULFONATED AMIDE

(see Chapter X)

SULFONATED ESTERS

(see Chapter X)

SULFONATED CASTOR OIL

(see Chapter X)

SULFOXIDE

(see Chapter IX)

SULFUR S

(see also Hydrogen Sulfide, the various sulfur compounds, Chapter VII—Sulfur Bacteria)

Elemental sulfur occurs in a free state in nature and it may also result in water from the oxidation of sulfides (see Chapter VII—Sulfur Bacteria). It is insoluble in water but it may be present in suspension in the colloidal state. It is used in the manufacture of sulfur compounds, insecticides, fungicides, matches, plastics, and enamels, in the vulcanizing of rubber, and in the synthesis of dyes (364, 1224). Pharmaceutical sulfur has been used internally as a laxative and parasiticide in doses of 1 to 4 gms (364).

Traces of sulfur in water used for plating baths will affect the smoothness or brightness of the deposits (214), and in photographic developers colloidal sulfur will react and cause fog (242). References are quoted by Ellis (313) to the effect that 1600 mg/l of colloidal sulfur in tap water was fatal to goldfish in 3.5 to 5.25 hours and 2100 mg/l in 48 to 71 minutes. According to Harukawa (1360), 16,000 mg/l of colloidal sulfur killed goldfish in five hours and 200,000 mg/l in less than one hour, but the author points out that the theoretical threshold concentration for goldfish is probably about 10 to 80 mg/l (time is not specified). In highly turbid water, Wallen et al. (2940) found the 96-hour TL_m for the mosquitofish to be over 10,000 mg/l.

Radioactive sulfur, S-35, is reported to concentrate by a factor of 5 in the soft tissues of vertebrates and invertebrates (3375). For more detail on concentration factor, see Chapter VIII.

SULFUR DIOXIDE SO_2

(see also Sulfur, Sulfates)

A colorless, nonflammable gas with a suffocating odor, sulfur dioxide is soluble in water to the extent of over 100,000 mg/l at 20°C. The dissolved gas combines with water to form sulfurous acid, thus $SO_2 + H_2O \rightleftharpoons H_2SO_3$ and the sulfurous acid dissociates according to the equations:

Equations	*K* at 25°C
$H_2SO_3 \rightleftharpoons H^+ + HSO_3^-$	1.7×10^{-2}
and $HSO_3^- \rightleftharpoons H^+ + SO_3^{--}$	5×10^{-6}

The percentages of undissociated sulfurous acid, HSO_3^-, and sulfite ion at various pH values are shown by the following tabulation:

pH	H_2SO_3	Percent as HSO_3^-	SO_3^{--}
4	0.56	94.7	4.74
5	0.04	66.5	33.46
6	---	16.7	83.3
7	---	2.0	98.0
8	---	0.2	99.8
9	---	0.02	99.98

Very little of the sulfurous acid remains undissociated in the normal pH ranges; at pH 5 and below, HSO_3^- predominates and above 5 the bivalent sulfite ion prevails (911).

In the presence of oxidizing agents such as dissolved oxygen and chlorine, the sulfite ion is gradually oxidized to sulfate, the sulfur changing from a + 4 valence to + 6 (364).

In domestic water, sulfur dioxide and sulfites are deleterious primarily in that they lower the pH value and increase corrosivity. By being oxidized and utilizing dissolved oxygen, on the other hand, sulfites may retard corrosion. Konrich and Haase (1312) fear that a high concentration of sulfite ion in water may cause exzema.

In 1917, Shelford (363) reported that 16 to 19 mg/l of sulfur dioxide killed orange-spotted sunfish in one hour. Ellis (313) quotes references to the effect that concentrations of SO_2 of 10 mg/l in tap water caused trout to float helplessly within 10 minutes, and Ellis (609) also reported that 5.0 mg/l of SO_2 kills trout in one hour. According to Bandt (656), 0.5 mg/l of sulfurous acid is toxic to fish. Sulfurous acid is lethal to tench at a concentration of 1.0 mg/l for two hours (2977).

SULFURIC ACID H_2SO_4

1. General. Like hydrochloric, nitric, and other strong acids, sulfuric acid dissociates almost completely in water to release sulfate ions and hydrogen ions, thereby tending to lower the pH value. At very low pH values (3 and lower), some of the sulfate ion will remain as acid sulfate, HSO_4^-, but at higher pH values dissociation to sulfate ions is practically complete (911). In considering the effects of sulfuric acid upon the beneficial uses of water, therefore, it must be recognized that an increase in sulfate ions and a lowering of pH are involved.

Sulfuric acid is the most important and widely used acid. It is employed in nearly all chemical processes and in many other industries, e.g., the production of fertilizers, other acids, explosives, and dyestuffs, for the purification of oils, and for pickling of metals (364, 1224). As a result of its wide use, increased sulfate ions and lowered pH values are likely to result in many types of industrial wastes.

2. Cross References. Acidity, pH, Sulfates.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. (see Sulfates, Acidity, and pH)

b. Fish and Other Aquatic Life. The following concentrations of sulfuric acid in water have been reported as lethal or toxic toward fish:

Concentration of H_2SO_4 in mg/l	Time of exposure	Type of water	Species of fish	Reference
1.2	--	--	Sunfish	359
6.0-8.0	6 hours	Distilled, 20°C	Minnows	2942
6.25	24 hours	--	Trout	359
7.36	60 hours	Distilled	Bluegills	313
10.0	--	--	Gamefish	1010, 541
24.5	24 hours	--	Bluegills	359
26.0	15 minutes	Tap	Minnows	362
42	96-hour TL_m	Turbid	Mosquito-fish	2940
49	48-hour TL_m	Tap, 20°C	Bluegill sunfish	2093
59.0	1-1.25 hours	Soft, pH 3.2	Goldfish	313
71.2	--	--	Pickerel	353
80.1	--	--	Whitefish	353
110-120	6 hours	Hard, 20°C	Minnows	2942
138.0	4 hours	Hard, pH 4	Goldfish	313
167	48 hours	--	Fish	3706

Non-toxic or harmless concentrations of sulfuric acid have been reported as follows:

3.68	1 month	Distilled	Bluegills	313
17.0	Over 4 days	Soft	Goldfish	353
20.0	24 hours	--	Minnows	359
100.0	100 hours	Hard	Goldfish	313

Toward other aquatic life, the following concentrations of sulfuric acid were harmful:

29	24-72 hours	Soft, pH 5	*Daphnia magna*	313
33.11	--	--	Bivalve larvae	2989
50	--	--	All aquatic life	541
88	64 hours	Lake Erie	*Daphnia magna*	358
138	--	--	*Daphnia magna*	353

The foregoing tabulations illustrate the fact that the toxicity of sulfuric acid toward aquatic life is a function of the resulting pH, i.e., a dose that would be lethal in distilled water or soft water might be harmless in a highly buffered water. Doudoroff and Katz (361) report that strong mineral acids such as sulfuric can be directly lethal to fully developed fish in most natural waters only when the pH is reduced to 5.0 or lower. Ellis (611) confirms this observation by stating that sulfuric acid must be diluted so as to give a pH of 4.5 or higher in order for fish to survive, and to pH 5.5 to make it possible for other aquatic organisms to thrive and provide food for fish. Death of trout has been reported (2920) at pH 3.9, while for sunfish, bass, and carp (3697) a pH of 3.5 caused death and 4.0 gave pronounced gill irritation. For *Polycelis nigra*, the toxic threshold of sulfuric acid has been reported (353) as 0.63 mg/l of hydrogen ion, or at pH 3.2 in distilled water. When sulfuric acid in streams depresses the pH below 5.0, specialized flora and fauna develop (3608).

c. Shellfish Culture. Oysters are sensitive to changes in pH. A dose of 100 mg/l of sulfuric acid killed 18 percent of oyster spat in five days (317, 523).

SULFUROUS ACID

(see Sulfur Dioxide)

SURFACE ACTIVE AGENTS

(see Chapter X)

SUSPENDED SOLIDS

1. General. In natural waters, suspended solids consist normally of erosion silt, organic detritus, and plankton. The impact of man's activities, however, alter and augment the suspended solids in surface waters by the

discharge of liquid wastes from communities and industries, by increased erosion from deforested and cultivated areas, by gravel washings and mine tailings, by steel mill wastes, and by dusts that are blown into streams (308, 347, 473, 540, 541, 611, 664, 673, 684, 701, 779, 780, 799, 814, 1552, 1700, 3655, 3658).

To differentiate between suspended solids and settleable solids is often difficult, because these two terms are often confused in the literature, although the method of determination of each is clearly described in Standard Methods for the Examination of Water and Wastewater (469). Strictly speaking, until they have settled to the bottom of a water course (or a laboratory sample bottle), all settleable solids are suspended solids. On the other hand, only a fraction of the suspended solids are settleable and this fraction is dependent upon quiescence, temperature, density, flocculation, and many other factors. In this section of the report only those references that deal strictly with the term "suspended solids" are considered.

Where stream classifications and standards have been promulgated, or where effluent standards are used, the test for suspended solids is generally employed as a significant criterion. For details of standards used by state and interstate agencies, see Chapter III.

2. Cross References. Turbidity, Silt, Settleable Solids, and Chapters III and V.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The 1962 USPHS Drinking Water Standards (2036) do not specify limiting concentrations for suspended solids, but such concentrations are indirectly controlled by the limits on turbidity (5 units) and total solids (500 mg/l). Bean (1745) has proposed that, for drinking water of ideal quality, suspended matter by limited to 0.1 mg/l or less. In raw water sources for domestic use, state and regional agencies generally specify that suspended solids in streams shall not be in sufficient concentration to be objectionable or to interfere with normal treatment processes (see Chapter III for details). Those agencies that employ effluent standards, however, are more specific in delineating concentrations (or percentage removals) of suspended solids in effluents.

b. Industrial Water Supplies. Suspended solids in water interfere with many industrial processes, and cause foaming in boilers (815), or incrustations on equipment exposed to water, especially as the temperature rises (1361, 3698). Water for steel manufacturing should contain not over 25 mg/l of suspended solids (249). Suspended solids are undesirable in water for textile industries (185, 212, 258, 2377); paper and pulp (212, 402); beverages (180, 181); dairy products (816); butadience (251); laundries, dyeing, photography, cooling systems, and power plants (369). For further details, see specific industries as listed in Chapter V.

c. Irrigation Water. Paperboard mill waste waters containing 240 mg/l of suspended solids have been successfully used for spray irrigation of alfalfa. There was no problem from suspended paper fibers (3699).

d. Fish and Other Aquatic Life. Disregarding any possible toxic effects attributable to substances leached out by water, suspended solids may kill fish and shellfish by causing abrasive injuries; by clogging the gills and respiratory passages of various aquatic fauna; and by blanketing the stream bottom, killing eggs, young, and food organisms, and destroying spawning beds (3660). Indirectly, suspended solids are inimical to aquatic life because they screen out light (see Turbidity) and because, by carrying down and trapping bacteria and decomposing organic wastes on the bottom, they promote and maintain the development of noxious conditions and oxygen depletion, killing fish, shellfish and fish food organisms, and reducing the recreational value of the water.

While it appears to be debatable whether wood fibers (673, 688, 3675) and other suspended solids cause the death of healthy fish by clogging their gills, nevertheless, there is agreement that they are harmful to the extent that they blanket the stream bed, prevent normal growth of food organisms and contribute to conditions of decay. It has been pointed out that healthy fish may swim through heavy suspensions of solids without significant injury (2546); yet, in the presence of even low concentrations of toxic substances, fish may be so weakened that the abrasive and clogging actions of suspended solids become more effective and dangerous, and even lethal (313).

Ellis (313, 347, 801) has recommended that all cellulose pulps and sawdust be excluded from streams; that suspended solids of a hardness of one or more (mineralogical scale) be so finely divided as to pass through a 1000-mesh screen; and that the stream bottom not be blanketed to a depth of more than one-quarter inch.

Tests on the survival of rainbow trout in suspensions of inert solids (kaolin and diatomaceous earth) showed that concentrations of 30 mg/l had no observable effect, a few fish died at 90 mg/l, while at 270 mg/l more than half of the fish died in 2 to 12 weeks. No difference could be detected between the lethal effects of kaolin and diatomaceous earth, although the particle size of the former lay mostly in the range of 0.13 to 5.0 microns, and of the latter in the range of 1 to 6 microns (88, 3321). In concurrent field tests in England, it was found that a stream containing 60 mg/l of suspended solids had just as many trout and invertebrates as a clear control stream; but in the River Fal, with 1000 mg/l of suspended matter, trout were only one-seventh and invertebrates only one-third of the densities in control streams. In the polluted River Par, with about 6000 mg/l of suspended solids, trout were one-seventh and invertebrates one-nineteenth of the densities in the control streams (88). Based on the laboratory phase of the investigation, it seems unlikely that reductions in trout populations were caused solely by reductions in food supply.

Waste fibers from a wood grinding mill, built in 1915, have covered and destroyed certain spawning grounds of whitefish in a Swedish river. Since 1923 the total annual catch of fish from this river has been reduced to about 1 pound per acre. Laboratory experiments showed that fish eggs in aquaria whose bottoms were covered with this material had a 36-75 percent mortality rate, whereas in the control aquaria the mortality rate was 3-15 percent (1701).

Destruction of fish and plankton in a long stretch of river polluted by mine wastes was attributed not to the

effects of any toxic chemicals, but to the deposition on skin and gills of particles of iron hydroxide (1702).

According to Robinson (2614) concentrations of some suspended materials as high as 1458 mg/l were filtered and passed through the gut of *Daphnia magna* with no apparent harm; but charcoal and montmorillonite were toxic at 100 mg/l, ground glass at 98 mg/l, chlorite at 120 mg/l, and illite at 264 mg/l.

e. Shellfish Culture. In concentrations of suspended matter and silt higher than 750 mg/l, the development of eggs and larvae of the venus clam was decreased (3700).

SYNTHETIC DETERGENTS

(see Chapter X)

SYSTOX

(see Chapter IX)

TANNIC ACID $C_{76}H_{52}O_{46}$

1. General. Tannic acid occurs naturally in the bark and fruit of many plants. In the pure state it is a yellowish brown powder with a faint characteristic odor and an astringent taste. Highly soluble in water and alcohol, it is used as a mordant in dyeing, in the manufacture of ink, for sizing paper and silk, in tanning, for clarifying beer and wine, and for many other commercial purposes.

2. Cross References. Acids, Organic, General.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Tannic acid has a high chlorine demand and when chlorinated a concentration of 17.1 mg/l gave a sweetish odor (362). Concentrations of 2.0 to 4.0 mg/l give water a woody taste and odor (701).

b. Industrial Water Supplies. To prevent the formation of scale in boilers, 3.0 to 5.0 mg/l of tannic acid have been added to boiler feed waters (1363) and tannins such as quebracho have proved satisfactory for prevention of embrittlement of locomotive boilers (824).

c. Fish and Other Aquatic Life. The lethal doses reported for fish vary widely, depending on the species tested and the conditions of the experiment. At least 0.4 mg/l is required to affect most fish and some species can survive 10 mg/l or more, according to Westfall and Ellis (701); yet Ellis (313) reports that 10 mg/l in hard water was not injurious in 100 hours to goldfish, but that 100 mg/l killed in 9 to 20 hours. Belding (359) quotes references to the effect that the lethal doses to kill in 24 hours were 6.25 mg/l for trout, 71 mg/l for minnows and 142 mg/l for goldfish. Schaut (362) found that minnows lost their appetite in 17.1 mg/l of tannic acid but did not die.

Using highly turbid water and the mosquito-fish (*Gambusia affinis*) as the test organism, Wallen et al. (2940) found the 24- and 48-hour TL_m for tannic acid to be 41 mg/l while the 96-hour TL_m was 37 mg/l. The addition of tannic acid increased the turbidity from an initial 135 mg/l to 170 mg/l, but lowered the pH only from 7.3 to 6.7. The State of Washington Department of Fisheries (2091) found the critical level for young chinook salmon to be 1.73 to 2.85 mg/l for a 72-hour exposure in flowing fresh water and below 1.70 mg/l in flowing sea water.

According to Doudoroff and Katz (361), tannic acid can be toxic to fish without lowering the pH to 5.0. Toxicity is then attributable chiefly to the undissociated acid in solution and hence pH is not a reliable index of dangerous pollution.

In Lake Erie water, according to Anderson (358) the threshold concentration for immobilization of *Daphnia magna* after prolonged exposure at 25°C was 26 mg/l, but it may be as low as 3.0 mg/l.

TARTARIC ACID AND TARTRATES $COOH(CHOH)_2COOH$

Tartaric acid occurs in many fruits, generally in the form of calcium, magnesium, or potassium tartrate. It is produced commercially from argols or wine-lees that are formed in the production of wine. Highly soluble in water, it is used in baking powders, dyeing and printing fabrics, tanning, and many other commercial processes.

The toxicity of tartaric acid to fish and aquatic life depends on the hardness and buffering action of the water. Doudoroff and Katz (361) maintain that tartaric acid can be directly toxic to fully developed fish only when it lowers the pH value to 5.0 or lower and Cambosu (833) points out that the disinfecting action of tartaric acid is lowest in hard water owing to the precipitation of insoluble calcium tartrate.

In hard water, 200 mg/l of tartaric acid was not lethal to goldfish after 100 hours of exposure but in soft water 100 mg/l killed them in three hours. In hard water, 1000 mg/l killed in three to four hours, according to Ellis (313).

In Lake Erie water at 25°C, Anderson (358) shows that the threshold concentration of tartaric acid for immobilization of *Daphnia magna* after prolonged exposure was 135 mg/l. For sodium tartrate, the threshold concentration was 3150 mg/l (352), and the toxicity was attributed to factors other than osmotic pressure. Because tartrates are salts of a weak acid, their addition to water would be expected to raise rather than lower the pH value.

The threshold of toxicity of sodium tartrate to the flatworm *Polycelis nigra* at 15 to 18°C was about 9600 mg/l of tartrate, according to Jones (354).

TARS

1. General. The references abstracted herein deal primarily with tar on tarred roads and the effects of such tar upon nearby streams as a result of rainfall, leaching action, and drainage. For a more thorough treatment of coal tar and its constituents, see the cross references, especially Oil-Petroleum.

2. Cross References. Acridine, Cresols, Cresylic Acid, Hydrocarbons, Naphthalene, Oil-Petroleum, Phenanthrene, Phenol, Pyridine, Quinoline, Thiophene, Xylene, and similar compounds.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Test sections of tarred roads were washed with distilled water equal to one inch of rainfall and the chlorinated run-off waters were tested for tastes and odors, according to Carpenter and

Klinger (825). They concluded that chlorophenolic tastes were liable to appear in water supplies if heavy rain fell within two hours after surfacing a road with primer coat, but the danger was less pronounced with aging or with resurfacing.

b. Fish and Other Aquatic Life. The death of fish in the Tees estuary was attributed in part to tar acids which were present in concentrations of 0.1 to 0.3 mg/l (826). Commercial tar products, especially the less-viscous types, contain large amounts of water-soluble poisons (827). Undiluted samples of drainage from tarred roads caused a loss of equilibrium in perch after 10 minutes but they recovered later in fresh water. Drainage after the first rainfall on a newly tarred road killed all six test perch in 13 minutes (365). One such lethal effluent contained 18.5 mg/l of tar acids and 2.0 mg/l of tar bases. In other tests, saturated extracts of coal-tar pitch killed trout within one hour.

Shelford (363) reported that sunfish were killed in one hour by liquid tar acids at a concentration of 70 mg/l, while Gutsell (463) found that 66 mg/l was fatal in one hour and 13 to 33 mg/l in one to three days. One liquid tar paste killed sunfish in one day at a concentration of only 2.0 mg/l. Yearling trout were susceptible to aqueous extracts of tar, with 20 mg/l proving lethal in one hour (828).

Two independent investigators, Schuman (829) and Herrman (830) contend that the amounts of phenols, cresols, and xylenols that could possibly be leached from tar-surfaced roads would not injure fish and other aquatic life. Another anonymous article (831) points out that young perch and roach showed no ill effects after three hours with an extract from bituminous road material and young brown trout were unharmed after 19 weeks in a pond receiving actual drainage from a bituminous road.

From the differences in results and conclusions presented above, it is probable that the lethality of washings from tarred roads depends on the type and composition of tar employed, its age, the action of soil and material processes on the waters as they flow from the road to the stream, the temperature, and numerous other variables that cannot be evaluated readily.

A study was made of the toxic limit toward yearling trout (3245) of the acidic, basic, and neutral fractions of tar. The toxic limit was defined as the concentration resulting in a loss of fish equilibrium in 60 minutes at 12°C. Toxicity in relation to boiling point and solubility is shown in the following tabulation:

Tar Fraction	*Boiling Point, °C*	*Toxic Limit, mg/l*	*Solubility mg/l*	*Effective Toxicity**
Tar acids	197-210	6.0	16,500	27.5
	240-270	3.0	4,125	13.8
	270-300	2.0	1,375	6.88
	300-325	1.5	825	5.50
Tar bases	205-235	7.5	1,274	1.70
	230-250	7.5	951	1.27
	275-305	3.0	438	1.46
	320-345	1.0	238	2.38
Neutral oils	200-240	3.75	35	0.935
	240-280	3.75	25.3	0.675
	290-344	3.75	14.1	0.376

*Effective toxicity is given as solubility $\times 10^{-2}$ divided by toxic limit.

The toxic limit alone is not definitive of the hazard of tar fractions for all of the substances would not necessarily be abstracted from the tar by water to the same extent. The "effectivity toxicity" takes into account the fact that the hazard rises with the solubility and is inversely related to the toxic limit, i.e. the lower the toxic limit, the more toxic is the substance.

The tabulation shows that the toxicity of tar acids and tar bases increases with the boiling point, while the toxicity of neutral oils is relatively independent of the boiling point. The variations in solubility are much greater than the variations in toxic limits; hence solubility must be considered as a major factor in determining the harmfulness to fish. It is significant to note in all cases, however, that the solubilities far exceed the toxic limits; hence all of these tar ingredients are potentially harmful to fish.

Albersmeyer and Erichsen (3404) studied the effects of tar constituents in waste waters, but they used pure phenolic compounds. Their results are included under cresols, phenols, and xylenols.

c. Shellfish. According to Mitchell (832) there was no noticeable effect of water-gas tar on oysters in constantly renewed sea water; but large amounts of tar in stagnant sea water causes serious or fatal effects. The danger of tar depends on the ability of the oyster to rid itself of such foreign matter.

TASTES

(see also Odors, Chapters III, VII, IX, and X)

While taste responses are often difficult to differentiate from odor responses because the senses of taste and smell are closely inter-related, certain non-volatile substances dissolved in water can cause tastes without causing odors. To some extent, therefore, the subject of taste-producing substances in water may be discussed independently. Although no procedures for taste determinations are recommended by Standard Methods for the Examination of Water and Wastewater (469), threshold taste tests are made using techniques similar to those used for odor tests (459).

It is not practicable to retabulate here the taste thresholds of all of the potential pollutants covered in this chapter and in Chapters VII, IX, and X. Where taste thresholds of specific substances were revealed in the literature, they are included in the description of the designated pollutant. Taste thresholds for several compounds have been tabulated by Lockhart et al. (3241), Baker (2058), Filicky et al. (2040), Cohen et al. (3301), and Campbell et al. (2059).

Rohlich and Sarles (759) have stated that volatile substances can generally be detected by smell in much lower concentrations than by taste. However, it has been reported that the extremely disagreeable tastes imparted to chlorinated water by phenol, cresol hydrocarbons of the benzene series, anthracene, and naphthalene can be detected at concentrations far below the odor threshold; possibly from one-fiftieth to one-hundredth of the quantity tetected by odor can be distinguished by taste. The taste characteristics have been described as (a) very similar to iodoform as typified by chlorinated phenol; (b) intermediate between that given by o-cresol and p-cresol

on chlorination; and (c) similar to that given by p-cresol which more nearly resembles p-cresol itself than a chlorinated product. This subject is covered in some detail by Ruchoft and Ettinger (459), who have assembled from various sources the taste thresholds for phenolic compounds when chlorinated in very dilute solution. Their studies indicate that this taste problem is by no means a simple one, and that usually the effect of chlorine on phenols and cresols is first to increase the taste, and then to reduce it to zero as further chlorine is added.

Dissolved gases and minerals make water palatable and distilled water has a flat taste. The tolerance of salts in water, according to their taste, is partly a matter of individual reaction, and taste tolerances can be developed, when it becomes necessary to use a comparatively unsatisfactory water supply.

The flesh of fish can acquire a taste from organic compounds in the water. This subject has been studied by Albersmeyer and Erichsen (3404), Boetius (3492), Bandt (2827), Hendersen et al. (2958) and Viehl (3707). For details and concentrations, see the specific potential pollutants, but especially phenol, cresol, xylenol, oils, nitriles, and similar compounds.

TBA

TDE

TEDION

(see Chapter IX)

TEEPOL (Secondary Sodium Alkyl Sulfate)

(see Chapter X)

TEMPERATURE

1. General. Temperature changes in bodies or streams of water may result from natural climatic phenomena or from the introduction of industrial wastes, such as distillery effluents or discharges of cooling waters used by many industries. The temperature is important, and sometimes critical, for many uses of water. It affects the palatability of water, treatment processes, the value of water for many industrial uses, including cooling processes and its suitability as a habitat for aquatic life (199, 834, 835, 836).

Many state and interstate water-pollution-control agencies place restrictions on temperatures or allowable temperature increases. For a description of such standards or criteria, see Chapter III and its appendices.

The elevation of stream temperatures, especially as the result of spent cooling waters, may become a serious pollution problem. Additional heat may contribute to undesirable stream conditions such as decreased oxygen capacity, increased oxygen demand, anaerobic zones, putrefication of sludge deposits, and the growth of sewage fungus (1464, 1652, 1714, 1715, 1716, 3708, 3709, 3710). Stream temperatures may also be increased by irrigation practices and the return of agricultural drainage. Temperature rises of 10 to 20°C have been reported (2032, 2340). The return of cooling waters to underground aquifers has also been known to increase groundwater temperatures by 20°C (3711, 3712).

2. Cross References. Oxygen and other specific substances in Chapter VI, also Chapter III for state and interstate criteria, and Chapter VII for effects on biological pollutants.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. For drinking purposes, water with a temperature of 10°C is usually satisfactory. Temperatures of 15°C or higher are usually objectionable. Whipple is reported to have found that temperatures above 19°C cause complaints from most consumers (837).

Increased temperatures may stimulate growth of taste- and odor-producing organisms in lakes and impoundments (379, 388, 422, 537, 663, 838). Sometimes a drop in temperature has stimulated growth of some organisms (207).

In general, the survival time of infectious bacteria, cysts of *E. histolytica,* and ova of parasitic worms is reduced as temperature of the contaminated water increases. Cultures of *Leptospira* in river water have survived for 9 days at 5-6°C, but for only 4 days at 31°C (710). *E. Typhosa* and coliform organisms survive for longer periods at lower temperatures (302, 488, 715, 716), and pathogenic organisms have survived for a year under ice (718). *Schistosoma* ova die in 9 days at 29-32°C, in three weeks at 15-24°C, and in three months or longer at 7°C (840).

The efficiency of water purification and treatment methods may be markedly affected by the water temperature. It has been found that filtration is more effective in winter than in summer (841) and that chlorination is more effective in summer (841). It has also been reported that filtration is more effective at summer temperatures than at winter temperatures (837). The alum dose required to reduce the color of raw water was observed to be as much as 2.2 times greater at 14°-24°C than at 8-14°C (842); on the other hand, less alum has been found necessary at higher temperatures (837). Flocculation and sedimentation rates are increased as the temperature is increased. It was found that at 2°C the settling period was 73 percent longer than at 20°C (843). The exchange capacity of base-exchange materials used in water-softening processes varies with temperature, but not in a uniform direction; for some substances rising temperatures increase the exchange capacity, for others they reduce it (844).

The bactericidal effects of disinfectants are generally increased by an increase in the temperature of the water. For example, for a given dose of free chlorine the period required to disinfect water at 8°C is more than nine times as great as at 40°C (845) (also see 490, 493, 846, 847, 848). However, a Russian investigator has reported that temperature changes from 1° to 20°C have had no effect on the bactericidal efficiency of chlorine (849) and the killing effect of ozone upon cysts of *E. histolytica* was not changed by temperature variations from 10° to 27°C (850).

b. Industrial Water Supplies. For various industrial uses of water, the water temperature may be highly significant, possibly the factor of greatest importance, for example, in the pulp and paper industry (851). Water for barley malting purposes should have a uniform tem-

perature of about 12°C (240). For use on farms and in dairy factories the temperature should be 16°C or less (549). For use in steel mills, water must have a temperature below 24°C (249). For use in rayon manufacturing the water must be cool (852). Streams have lost their value for cooling purposes as the water temperature has risen as much as 27°C during the course of discharge and reuse of the water by many industries in succession (1652, 1714).

Warm water accelerates corrosion in pipe lines and cooling systems.

c. Irrigation Water. Below 10°C water weeds grow very sparsely, between 10-15°C growth is prolific, reaching a maximum above 15°C (858). One report indicates that rice irrigation was not harmed by cooling water that raised the temperature by 11°C (1717), but another article (3713) indicates that water of 32°C virtually destroyed rice plants.

d. Fish and Other Aquatic Life. Changes in water temperature as a result of human activity are generally upward. An exception is the release of cold bottom water from stratified artificial impoundments, which may alter the flora and fauna for many miles downstream from the dam. Other activities of man such as the discharge of cooling waters, warm industrial wastes, and return irrigation water generally raise the temperature of receiving waters, with the following concomitant effects: (a) higher temperatures diminish the solubility of dissolved oxygen and thus decrease the availability of this essential gas, (b) elevated temperatures increase the metabolism, respiration, and oxygen demand of fish and other aquatic life, approximately doubling the respiration for a 10°C rise in temperature; hence the demand for oxygen is increased under conditions where the supply is lowered, (c) the toxicity of many substances is intensified as the temperature rises, (d) higher temperatures militate against desirable fish life by favoring the growth of sewage fungus and the putrefaction of sludge deposits, and finally (e) even with adequate dissolved oxygen and the absence of any toxic substances, there is a maximum temperature that each species of fish or other organism can tolerate; higher temperatures produce death in 24 hours or less.

The maximum temperatures that adult fish can tolerate vary with the species of fish, prior acclimatization, oxygen availability, and the synergistic effects of other pollutants. Median tolerance limits (TL_m) have been reported by several authors, as shown in the following tabulation. Species have been arranged in order of heat tolerance.

LETHAL TEMPERATURES FOR FISH

Common Name	*Scientific Name*	*Exposure Time, Hours*	*TL_m °C*	*Reference*
Goldfish	*Carassius auratus*	--	38.6	3714
Mosquito-fish	*Gambusia affinis*	--	37.3	3715
Gizzard shad	*Dorosoma cepedianum*	--	36.5	3715
Largemouth bass	*Micropterus salmoides*	--	36.4	3715
		24	28.9	3716
Carp	*Cyprinus carpio*	24	35.7	3716
Roach	*Rutilus rutilus*	--	35.3	3720
Brown bullhead	*Ameiurus nebulosis*	12	35.1	3717
		--	34.5	3715
Northern black bullhead	*Ameiurus melas melas*	24	35.0	3716
Golden shiner	*Notomigonus crysoleucas*	--	34.7	3715
Bluegill sunfish	*Lepomis machrochirus*	--	35.0	3719
		--	33.8	3715
Pumpkinseed	*Lepomis gibbosus*	12	34.5	3717
		24	30.2	3716
Bluntnose minnow	*Hyborhynchus notatus*	--	34.0	3715
		--	33.3	3714
Fathead minnow	*Pimephales promelas*	12	33.8	3717
Northern channel catfish	*Ictalurus lacustris*	--	33.5	3715
Northern redbelly dace	*Chrosomus eos*	12	33.1	3717
Common shiner	*Notropis cornutus*	--	33.0	3715
		12	32.0	3717
		--	31.0	3714
Creek chub	*Semotilus atromaculatus*	12	32.6	3717
		--	31.5	3715
		--	30.3	3714
Finescale dace	*Pfrille neogaea*	12	32.3	3717
Yellow perch	*Perca flavescens*	--	32.3	3715
		12	30.9	3717
		--	29.7	3714
		12	29.2	3716
Saltwater perch	--------	--	32.2	3719
Guppy	*Lebistes reticulatus*	--	32.0	3721
Common white sucker	*Catostomus commersonni*	12	31.2	3717
		--	29.3	3714
Pearl dace	*Margariscus margarita*	12	31.1	3717
Lake emerald shiner	*Notropis atherinoides*	--	30.7	3714, 3715
Brook stickleback	*Eucalia inconstans*	12	30.6	3717
Blacknose dace	*Rhinichthys atratulus*	--	29.5	3715
		--	29.3	3714
Squaw fish	*Ptychocheilus oregonensis*	24	29.3	3716
Common brook trout	*Salvelinus fontinalus*	12	26.1	3717
		--	25.3	3714
		--	25.0	2623
Salmon (spring, pink, sockeye, chum, coho)	*Oncorhychus* sp.	168	25.1	3718
Lake trout	*Lalvelinus namaycush*	--	23.5	3722

As shown by this table, goldfish, bass, and carp are relatively tolerant of high temperatures whereas trout and salmon are sensitive. These temperatures, however, apply to adult fish.

For spawning and hatching of eggs, much lower temperatures are required. Trout eggs will not hatch over 14.4°C (2650); Chinook salmon eggs fare well at 16°C but suffer mortalities at 18°C (3723); Rocky Mountain whitefish eggs are affected at 20-21°C (3724); and the TL_m for herring larvae varies from 22 to 24°C (3725). It is apparent, therefore, that many fish are unable to complete their life cycles unless the temperatures at the time of spawning and hatching are 10 to 15°C below the median tolerance limits (2414).

Fish have optimum temperatures for rate of growth and reproduction. Given a choice, they also show a preference for water of a definite temperature range. The

following optimum or preferendum temperatures have been reported:

Common Name of Fish	*Optimum or Preferred Temperature, °C*	*Reference*
Rainbow trout	13	3726
Chum salmon	13.5	3726
Sockeye salmon	15	3729
Lake trout	15-17	3722
Coho salmon	20	3729
Greenthroat darter	20-23	3730
Largemouth bass	22-25	3731
Roach	23-24	3728
Guppy	23-25	3727
Carp	32	3732

If the temperature of a reach of stream is raised by 5-10°C, it is probable that cold-water game fish will avoid this reach and that they will be replaced by course warm-water fish. Thus, without any direct mortality, the character of the fish life will change. It will also change because of effects upon spawning and hatching of eggs.

Sudden changes in temperature are believed to be deleterious to fish life, with abrupt changes of 5°C or greater likely to be harmful (2414). Rainbow trout could not stand a temperature shock of 11°C above an acclimatization temperature of 12°C, but could tolerate a shock of 8°C above an acclimatization at 10°C (3733).

The Aquatic Life Advisory Committee of ORSANCO (see Chapter III) has recommended (2414) that waste discharges be so controlled that the temperature of the receiving water: (a) shall not be raised above 34°C at any place or at any time, (b) shall not be raised above 23°C at any place or at any time during the months of December through April, and (c) shall not be raised whatsoever in streams suitable for trout propagation.

Increased temperature is also an important consideration when toxic substances are present in a stream. Many substances (e.g. cyanides, phenol, xylene, zinc) exhibit increased toxicity at elevated temperatures. For details, see the specific pollutants in Chapters VI, IX, and X. This synergistic effect is aggravated at low tensions of dissolved oxygen. See "Dissolved Oxygen" for its relationship with temperature.

Changes in temperature also affect lower aquatic and marine life. For each organism there is a thermal death point and also a range of temperature of optimum growth. Temperature is one of the environmental factors that determines which organisms will thrive and which will diminish in numbers and size. There is relatively little information in the literature about optimum temperatures for aquatic flora and fauna. Diatoms appear to grow best at 15-25°C, green algae at 25-35°C, and blue-green algae at 30-40°C (2190, 2621, 3734).

e. Shellfish Culture. Among oysters, the genus *Crassostrea* will survive temperatures of —5°C to 35°C; but the genus *Ostrea* will survive only from —1°C to 30°C. The Olympia oyster (*O. lurida*) will spawn at 15-16°C but the Pacific oyster (*C. gigas*) reqires a temperature of about 20°C for successful spawning (315, 317, 2415). Temperature has an important influence on the pumping rate of oysters. Below 3°C only insignificant quantities of water are pumped, for the oyster is almost dormant; from 8° to 16°C, the pumping rate increases rapidly; above 20°C the metabolism consumes protoplasm faster than it can be synthesized from food; and at 30°-35°C, the oyster is in distress (1448). The low rate of pumping in cold water enables an oyster to avoid bacterial contamination from overlying waters so that the bacterial quality of the oyster is generally better than that of the water. In some states, therefore, the harvesting of oysters from moderately polluted areas is permitted when the temperature of the water is consistently below 5°C (317, 523, 869).

Scallops accustomed to water between 5 and 20°C may be killed by sudden changes to temperatures of 21 to 23°C (3735). The rate of growth of the clam, *Venus mercenaria*, is negligible below 10°C but reaches its optimum at 23°C, probably because higher pumping rates at that temperature are combined with increased productivity of phytoplankton (2416). The fresh-water mussel, *Dreissenia polymorpha*, is destroyed in 15 minutes by a temperature of 40°C (2426).

f. Swimming and Other Recreational Uses. Rising temperatures stimulate the decomposition of sludge, formation of sludge gas, multiplication of saprophytic bacteria and fungi (particularly in the presence of organic wastes), and the consumption of oxygen by putrefactive processes, thus affecting the esthetic value of a water course (835). On the other hand, increased temperatures may be beneficial to recreation by lengthening the swimming season (3736).

TEPP

(see Chapter IX)

TETRACHLOROPHENOL

(see Halogenated Phenols)

TETRASODIUM PYROPHOSPHATE

(see Sodium Phosphates)

TETRYL $(NO_2)_3C_6H_2N(CH_3)NO_2$

This yellow crystalline substance, known also as Nitramine, is insoluble in water. It is used primarily as an indicator but it may also occur in wastes from munitions works. Such wastes were found to kill minnows in 25 to 40 minutes when diluted 1:1.5 with tap water, according to Degani (544).

TEXTILE WASTES

(see Dyes)

TRIBROMOPHENOL

(see Halogenated Phenols)

TETRAETHYL LEAD

(see Lead Tetraethyl)

TETROSAN

(see Chapter X)

THALLIUM Tl

Pure thallium is a bluish-white, soft, inelastic, heavy metal which oxidizes readily in air to thallium oxide. Its salts are used as poisons for rats and other rodents

(364), and as ant bait. Thallium salts are also used in dyes and pigments in fireworks, in optical glass, and as a depilatory. It is a cumulative poison, four times as toxic as arsenious oxide, and it affects the sympathetic nervous system, causes muscular pains, endocrine disturbances, and loss of hair (677). It does not occur normally in animal tissues, but when taken into animal bodies it is cumulative (2121). Thallium salts, for the most part, are highly soluble in water and consequently any industrial discharges of this element are not likely to form precipitates as carbonates, hydroxides, or other common compounds.

Landauer (933) tested the effects of thallium salts on fowl and their molting mechanism. Of several thallium salts tested, the acetate was more toxic than the fluoride, sulfate, or carbonate. A daily dose of 50 ml of a 400 mg/l solution (20 mg) of thallium acetate caused death of test birds in 11 days and daily doses of 10 mg in water killed birds in 19 days. Chicks died in 11 weeks of chronic poisoning as a result of doses varying from 0.05 mg at two days old to 6 mg at seven weeks of age. The LD_{50} concentration for thallous acetate administered orally to rats, rabbits and dogs was found to be 32 mg/kg as thallium and for thallic oxide it was 39 mg/kg (3737).

A nutrient solution containing 102 mg/l of thallium was slightly injurious to sugar beets in sand culture (1473), and 341-2730 mg/l completely inhibited the germination of cress and mustard seeds during an 18-day exposure period (1479).

THIOCRESOL

(see Mercaptan, General)

THIOCYANATES

(see Ammonium, Potassium and sodium Thiocyanates)

THIOPHENE C_4H_4S

(see also Coal Tar)

Thiophene is found in coal-tar and gas-plant wastes. It is a liquid with a slight aromatic odor resembling that of benzene. Insoluble in water, it is in itself a solvent and is used in the manufacture of resins, dyes, and pharmaceuticals. According to Shelford (313, 363, 463), 27 mg/l of thiophene in tap water killed orange-spotted sunfish in one hour.

THIOPHENOL

(see Mercaptan, General)

THIRAM

(see Chapter IX)

THORIUM Th

This grayish-white lustrous metal is used in the manufacture of incandescent mantles and filaments for lamps (364). It is also used in nuclear reactors in conjuction with U-235. It occurs naturally in several minerals, such as the silicate and oxide, with a valence of 4. A concentration of 775 mg/l of thorium completely inhibited the germination of cress and mustard seeds during an 18-day exposure, and 97-388 mg/l considerably delayed germination and retarded growth (1479).

The lethal concentration of thorium chloride for three mature small fresh-water fish (*Orizias*), exposed for 24 hours to only 20 ml of test solution, was reported (1459) to be about 18 mg/l. Using water from the River Havel from which the test organism were recovered, Bringmann and Kuhn (2158, 3343) studied the threshold effects of thorium nitrate on four organisms. For *Scenedesmus* the median threshold effect for a 4-day exposure at 24°C occurred at 0.4-0.8 mg/l as thorium; for *E. coli* at 27°C, the median toxic level was at 0.8 mg/l as Th; for a protozoan (*Microregma*), 25 mg/l was deleterious, but for *Daphnia* at 23°C for 48 hours, 1000 mg/l of thorium was not toxic.

THURICIDE

(see Chapter IX)

TIN Sn

1. General. Tin is not present in natural waters, but it may occur in industrial wastes or in trace amounts when water is stored for any length of time in tin-coated metal containers (152). Stannic and stannous chloride are used as mordants for reviving colors, and dyeing of fabrics, weighting of silk, and tinning of vessels. Stannic chromate is used in decorating porcelain, and stannic oxide is used in glass works, dye houses, and for fingernail polishes. Stannic sulfide is used in some lacquers and varnishes (364). Tin compounds are also used in fungicides, insecticides, and antihelminthics (2121). From many of these processes, tin salts may reach surface waters or ground water; but because many of the salts are insoluble in water, it is unlikely that much of the tin will remain in solution or suspension.

2. Cross References. Fluorides

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. No reports have been uncovered to indicate that tin can be detrimental in domestic water supplies. Traces of tin occur in the human diet from canned foods and it has been estimated that the average diet contains 17.14 mg of tin per day. Man can apparently tolerate 850 to 1000 mg per day of free tin in the diet (353). There is no definite evidence that tin plays any essential biological role in human nutrition (2121).

b. Irrigation. In nutrient solution at a concentration of about 40 mg/l, stannous ions were not harmful to sugar beets in sand culture (1473).

c. Stock and Wildlife Watering. Rats have tolerated 25 mg or more of sodium stannous tartrate in the diet over a period of 4 to 12 months without ill effects (2121). Cats fed 20 mg of tin, as stannous tartrate or stannous chloride, per kg of body weight, showed no effect over a 3-month period but dosages of 30 and 50 mg per kg apparently caused some loss of body weight (3738). Guinea pigs survived on a diet containing 770 mg/kg of tin salts (2121). On the basis of these feeding experiments it is unlikely that any concentration of tin that would occur in water could be detrimental to livestock.

d. Fish and Other Aquatic Life. It is apparent that trace concentrations of tin are beneficial to fish. Stannous ions at a concentration of about 0.6 mg/l accelerated the growth of goldfish (3770). It has been re-

ported that goldfish survived 1000 mg/l of $SnCl_2$ (626 mg/l of tin) in very soft water at pH 3.5 for 1.0 to 1.5 hours and in hard water at pH 3.8 for 4 to 5 hours (598, 1459, 1466). Young eels have survived stannous chloride at a concentration of 1.2 mg/l of tin for over 50 hours, but succumbed to 6.0 mg/l in 2.8 hours (1459).

Threshold concentrations for the immobilization of crustacea in Lake Erie water to which stannic chloride ($SnCl_4$) was added were reported (2955) as follows:

Test Organism	*Threshold Concentration of $SnCl_4$ in mg/l*
Leptodora kindtii	114
Cyclops vernalis	125
Daphnia magna (young)	146

In contrast, the toxicity threshold of stannous chloride ($SnCl_2$) toward *Daphnia* in Lake Erie water was given as 25 mg/l (598).

TITANIUM Ti

Abundantly distributed in the earth's crust, titanium ores and salts constitute 0.5 to as much as 10 percent of soils. It serves as an inert element, similar to silicon in chemical and biological behavior. It is present in plants chiefly in the leaves and trace amounts are found in most animal tissues and organs (364, 2121).

Titanium metal is used as a constituent of several alloys. Titanium salts, primarily the oxide, are used as pigments in the paint industry, as filler in paper making, as mordants in the dyeing industry, in the manufacture of electronic apparatus, and in the glass and ceramics industry. Although several of the salts, e.g. the ammonium oxalate, nitrate, sulfate, and trichloride, are highly soluble, titanium dioxide is insoluble and tends to precipitate as a granular material (364, 2121, 2972).

There is no evidence that titanium is essential for plant or animal nutrition. It is not absorbed to any measurable degree by the human intestine, but appears to be almost completely inert and innocuous in the human alimentary system (2121). In nutrient solution, about 12 mg/l of titanium was reported to be slightly injurious to sugar beets in sand culture (1473).

When dense blooms of *Gymnodinium* occurred off the Florida west coast, spectrographic analyses of the water showed 3.37-33.7 mg/l of titanium. The role and origin of the titanium were not discovered (1720, 2722). Tarzwell and Henderson (2154) conducted exploratory tests to find the 96-hour TL_m of titanium sulfate toward the fathead minnow. In soft water, they found 8.2 mg/l as titanium while in hard water the TL_m value was 120 mg/l. Using water of the River Havel from which the test organisms had been recovered, Bringmann and Kuhn (2158, 3343) found the median threshold effect of titanium chloride toward *Scenedesmus* exposed at 24°C for 4 days occurred at 2.0 mg/l of titanium. For *Daphnia* held for 48 hours at 23°C, the median threshold level was 4.6 mg/l of titanium and for a protozoan (*Microregma*) it was 4.0 mg/l.

Titanium is accumulated in aquatic organisms from the surrounding water. Lackey (2442) reported concentration factors of 200 to 10,000 for six species of brown algae. For further details about concentration factors, see Chapter VII—Radioactivity.

TOLUENE $C_6H_5CH_3$

This flammable liquid with an odor of benzene is a constituent of coal tar. It is used in the manufacture of organic substances and as a solvent in the extractions of various principles from plants. The oral LD_{50} for white rats was reported as 7000 mg/kg of body weight (3348).

The concentrations of toluene that proved lethal to fish in clear water have been reported from 10 to over 90 mg/l, depending on the temperature and the species of fish (363, 461, 463, 544, 2977). Using highly turbid water, and the mosquito-fish (*Gambusia affinis*) Wallen et al. (2940) found the 24-, 48-, and 96-hour TL_m values at 17-22°C to be 1340, 1260, and 1180 mg/l respectively. The toluene had little effect on the turbidity of the water.

Toward lower organisms, Bringmann and Kuhn (2158) found that the threshold of toxicity for *Daphnia* occurred at 60 mg/l, for the diatom *Scenedesmus* at 120 mg/l, and for *E. coli* at 200 mg/l. North et al. (2108) reported that 10 mg/l of toluene gave a large reduction in the photosynthetic activity of the giant kelp (*Macrocytsis pyrifera*) in a 96-hour exposure.

TOLUIDINE $C_6H_4CH_3NH_2$

This aminotoluene occurs in three forms: m-toluidine, a colorless liquid slightly soluble in water, o-toluidine, a light yellow liquid slightly soluble in water, and p-toluidine, a white solid highly soluble in water. All three isomers are used in the manufacture of dyes and dye products (364).

The lethal level for fish (isomer not specified) is reported as 100 mg/l (2977). Concentrations of 1000 mg/l of o-, m-, and p-toluidine have no apparent effect on *Escherichia coli* at 27°C. For the diatom *Scenedesmus*, the threshold of toxicity at 24°C of o- and p-toluidine lies at 10 mg/l and for m-toluidine at 8 mg/l. For *Daphnia* at 23°C, the toxic threshold is reported at 8 mg/l with o-toluidine but for m- and p-toluidine it is only 0.6 mg/l (2158).

TOLYL MERCAPTAN

(see Mercaptan, General)

TOTAL SOLIDS

(see Solids)

TOXAPHENE

(see Chapter IX)

TOXIC SUBSTANCES

The terms "toxic substance" or "toxic minerals" occur in standards of criteria that have proposed or promulgated by state and interstate water-pollution-control agencies, but unfortunately the terms are not well defined. Furthermore, where either term is used, qualitative rather than quantitative limits are set. The New England Interstate Water Pollution Control Commission, for example, specified that "substances potentially toxic" shall be "none" in Classes A and B, and "not in toxic concentrations or combinations" in Classes C and D (see Chapter III).

Goudey (992) suggested specifications for ground waters including limits for "toxic minerals" that ranged from 0.05 mg/l for domestic water supplies to 1.00 mg/l for irrigation waters. In this report, toxic substances are not grouped by that classification; rather each potentially toxic material is treated separately. The Mersey River Board (England) proposed that trade effluents discharged to the River Alt contain no "substances toxic to fish or fish food" (2950).

TRICHLOROACETIC ACID (TCA)

(see Chapter IX)

TRICHLOROETHANE $C_2H_3Cl_3$

This nonflammable liquid with a pleasant odor is insoluble in water. It is used as a solvent for fats, waxes, resins, and alkaloids, and for cleaning metal and plastic molds (364). According to Garrett (2959) the TL_m toward the marine pinperch is 150-175 mg/l for 1,1,2-trichloroethane and 75-100 mg/l for 1,1,1-trichloroethane.

TRICHLOROETHYLENE $ClCH{=}CCl_2$

This nonflammable liquid with a chloroform-like odor is practically insoluble in water. It is used as a solvent and in solvent extraction by several industries, in degreasing and dry cleaning, and in the manufacture of chemicals and pharmaceuticals. The oral LD_{50} for dogs is given as 5.86 grams/kg of body weight (364). A concentration of 55 mg/l stupified fish within 10 minutes (2977). A concentration of 660 mg/l killed *Daphnia* in 40 hours, but 99 mg/l had no effect (2977).

TRICHLOROPHENOL

(see Halogenated Phenols)

TRICHLOROPHENOXYACETIC ACID

(see Chapter IX, 2, 4, 5-T)

TRICON OIL - SPILL ERADICTOR

TRIDECYL METHYLHYDROXY ETHYLIMIDAZOLINIUM CHLORIDE

(see Chapter X)

TRIETHYLAMINE $(C_2H_5)_3N$

Slightly soluble in water with an alkaline reaction, this liquid is used in the preparation of quaternary ammonium compounds. According to Gillette et al. (1442) the critical range for creek chub, a fish of moderate tolerance, exposed for 24 hours in well-aerated water at 15-21°C, was 50 to 80 mg/l, i.e. all fish survived 50 mg/l and all died at 80 mg/l. Bringmann and Kuhn (2158, 3343) reported that the threshold of toxicity toward *Scenedesmus* during 4 days of exposure at 24°C occurred at a triethylamine concentration of only 1.0 mg/l; but for *Daphnia* the threshold concentration was 200 mg/l for a 2 day exposure at 23°C and for *Microregma* 90 mg/l. There was no apparent effect on *E. coli* at concentrations up to 1000 mg/l.

TRIIODOPHENOL

(see Halogenated Phenols)

TRIMETHYLAMINE $(CH_3)_3N$

Readily soluble in water, this gas has a pungent, fishy, ammoniacal odor. It is widely found in nature as a degradation product of nitrogenous matter, especially fish Industrially, it is used in the manufacture of quaternary ammonium compounds (364). Corti (146) tested rainbow trout (*Salmo irideous*) at a trimethylamine concentration of 268 mg/l. The resulting pH was 9.62 and the temperature was 13.0-13.5°C. The fish responded instantly in an adverse fashion, turned over in about 4 minutes, and made their last motion in 11 to 14 minutes.

TRIMETHYL AMMONIUM CHLORIDE

TRIMETHYL OCTADECADIENYL AMMONIUM CHLORIDE

(see Chapter X)

TRINITROBENZENE $C_6H_3(NO_2)_3$

Symmetrical trinitrobenzene, soluble in water to the extent of about 350 mg/l, is used in explosives. According to Hiatt et al. (3350), marine fish show a slight irritant activity at a concentration of 0.1 mg/l, moderate irritant activity at 1.0 mg/l, and violent activity at 10 mg/l.

TRINITROPHENOL $(NO_2)_3C_6H_2OH$

1. General. 2,4,6-Trinitrophenol, more commonly known as picric acid, is a pale-yellow, odorless, intensely bitter, crystalline substance which explodes when heated rapidly or struck. It is used in explosives, matches, tanneries, textile mills, glass manufacture, and chemical industries.

2. Cross References. Nitrophenol, Dinitrophenol, Explosives, Phenol.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The ingestion of one to two grams of 2,4,6-trinitrophenol causes severe poisoning in humans (364), but the likelihood of such ingestion is slight inasmuch as small concentrations of the substance are objectionable. The minimum concentration detected by taste in drinking water has been given as 0.5 to 1.0 mgl (460). The salt, ammonium picrate, from shell-filling factories can be detected in drinking water at 0.5 mg/l, while 1.0 mg/l is objectionable (655). In addition to the bad taste, picric acid imparts a yellow color to the water, which is not removed by ordinary treatment processes (871). Furthermore, passage through the soil into ground-water reservoirs does not appear to neutralize or remove picric acid (872).

Confiscated Japanese picric acid, dumped into a harbor in Japan and picked up in water used for distillation by American ships, was believed to be the cause for hematuria in seamen (873).

b. Fish and Other Aquatic Life. Minnows can withstand aproximately 30 mg/l of picric acid in neutral solution, but at a concentration of 200 mg/l they overturned in 26 hours (617). Concentrations of 20 mg/l

have been reported (3740) as non-toxic, but high concentrations may be deleterious. Fish showed a preference for water containing trinitrophenol and prolonged their stay in higher concentrations of the chemical; but concentrations greater than 65.7 mg/l appeared to be repellant and fish in this solution showed signs of distress (3739).

For the alga *Scenedesmus* the threshold toxicity occurs at 240 mg/l after 4 days exposure at 24°C; for the protozoan *Microregma* at 900 mg/l; for the crustacean *Daphnia* at 88 mg/l after 2 days at 23°C; but for *E. coli*, 1000 mg/l was not toxic (2158, 3343).

TRINITROTOLUENE $(NO_2)_3C_6H_2CH_3$

(see also Explosives, Nitrotoluene)

TNT, or 2,4,6-trinitrotoluene, is a crystalline solid that is soluble in water to the extent of 200 mg/l at 15°C. It may occur in wastes from munitions and shell-loading plants. According to Schott and Ruchhoft (543), a typical waste from a TNT plant contained 1700 mg/l of total solids, 892 mg/l of ash, and only 29 mg/l of suspended solids. The acidity was 1700 mg/l, the sulfate ion content 610 mg/l, and the nitrate nitrogen was 27 mg/l. When diluted 1:1000 by a stream, this waste still had a color greater than that permitted by the U.S.P.H.S. standard and gave rise to objectionable tastes when the water was chlorinated. During World War II, according to Mohlman (542), drinking water standards were relaxed to permit the color of TNT-polluted water to be as high as 20 mg/l in the treated water. In river waters, color from TNT as high as 75 mg/l was accepted.

Degani (544) reported that about 40 mg/l of TNT in tap water killed minnows in 87 to 110 minutes, carp in 455 minutes, and bullheads in 556 minutes. In distilled water, however, the same concentration killed the various fish in about two-thirds the time required for tap water. According to Orton (874), a block of TNT in sea water exercised no recognizable effect on organisms within a few inches of it (concentrations not given).

According to Mohlman (542), TNT wastes in a river at dilutions necessary to reduce color to 20 mg/l caused no appreciable reduction in alkalinity, no odor, no killing of fish, no toxic effect in drinking water, and no objectionable tastes and odors. Dilutions of about 1:10,000 were necessary for drinking water, and 1:3000 for non-drinking water. Fish lived in combined wastes of TNT processes at a 1:800 dilution but not at a 1:400 dilution.

LeClerc (2942) observed that the minimum lethal concentration of TNT for minnows during a 6-hour exposure was 4.0-5.0 mg/l in either distilled or hard water at 23°C. The lethal concentration for fish has also been reported as lying between 1.5 and 2.0 mg/l (2977) and between 2.0 and 3.0 mg/l (3347).

In weak concentrations, TNT is subject to biochemical degradation. In concentrations of 0.5-1.0 mg/l the self-purification of water is slightly retarded; but beyond 1.0 mg/l deleterious effects occur (1721). The Soviet Union has established a maximum permissible concentration of 1.0 mg/l of TNT in surface waters (3107).

According to Mathews et al. (3347), waste waters from explosives-loading plants rapidly develop color when exposed to sunlight, owing to the formation to a TNT complex brought about by photochemical reactions. The maximum concentration of this TNT complex that was not harmful to fish was found to be 3 mg/l whereas the upper limit of harmlessness for the regular TNT was 2 mg/l (3347).

TRIPOTASSIUM PHOSPHATE

(see Potassium Phosphate, Tribasic)

TRISODIUM PHOSPHATE

(see Sodium Phosphates)

TRIVALENT CHROMIUM

(see Chromium)

TRYPTOPHANE $C_{11}H_{12}N_2O_2$

This amino acid is used medically as a nutrient, especially for the treatment of pellagra. Schaut (362) found that 17.1 mg/l of tryptophane in stabilized tap water produced no noticeable effect on minnows during a 24-hour exposure; but the dissolved-oxygen content of the water was decreased 53 percent. In Lake Huron water at 12-13°C, 5.0 mg/l of tryptophane had no effect on rainbow trout, bluegill sunfish, or sea lamprey during a 24-hour exposure (2976).

TUNGSTEN W

1. General. Also known as wolfram, tungsten occurs in nature in the minerals scheelite, wolframite, tungstite, tunstenite, and ferritungstite. Salts of tungsten are used in metallurgy, in the filaments for incandescent lights, as plating materials, for fireproofing and waterproofing fabrics in production of pigments, and phonograph needles (364, 2121). Calcium, magnesium, and iron salts of tungsten are insoluble in water; hence they are not likely to occure in natural waters or to remain in solution in waste waters from industry. Sodium and potassium tungstates are highly soluble.

2. Cross References. Selenium

3. Effects upon Beneficial Uses.

a. Irrigation. Hewitt (1473) has shown that 62 mg/l of tungstate ion (46 mg/l of tungsten) in a nutrient solution was harmless to sugar beets in sand culture.

b. Stock and Wildlife Watering. The addition of 2.5 to 5.0 mg/l of soluble tungsten in the drinking water reduced slightly the toxicity of selenium to rats fed a ration containing 11 mg/l of selenium (1481). The tungstate ion reduced the typical liver damage caused by seleniferous diets and decreased the mortality of rats. All other elements used in this regard tended to increase mortality. It appears, therefore, that tungsten is antagonistic toward the toxicity of selenium to animals and in low concentrations may be beneficial in certain waters (921).

According to Kinard and van de Erve (3741) complete mortality of rats was produced by 2 percent of tungsten in sodium tungstate and 5 percent of tungsten in ammonium paratungstate in the diet. On the other hand, 1000 to 5000 mg/l was not acutely toxic to rabbits but did retard growth.

c. Fish and Other Aquatic Life. Using water from the River Havel, from which the test organisms were recovered, Bringmann and Kuhn (2158, 3343) studied the threshold effects of tungsten added as sodium tungstate. For *Daphnia* the median threshold effect during 48-hour exposure at 23°C occurred at 350 mg/l of tungsten; for *Scenedesmus* at 24°C for 4 days the median threshold level was 110 mg/l as tungsten; for *Microregma* it was 502 mg/l as tungsten; and for *Escherichia coli* at 27°C it was at 167 mg/l of tungsten.

TURBIDITY

1. General. The turbidity of water is attributable to suspended and colloidal matter, the effect of which is to disturb clearness and diminish the penetration of light. Turbidity may be caused by micro-organisms or organic detritus; silica or other mineral substances including zinc, iron, and manganese compounds; clay or silt; or sawdust, fibers, or other materials, either as a result of natural processes or erosion or as a result of the addition of domestic sewage or wastes from various industries, such as mining, dredging, logging, pulp and paper manufacturing, and others.

The turbidity of a water sample is a measure of the extent to which the intensity of light passing through is reduced by the suspended matter. Although in some of the literature the terms "turbidity" and "suspended solids" (or other equivalent words, such as "suspended matter" and "suspensoids") are used almost synonymously, the degree of turbidity is not equal to the concentration (or quantity) of suspended solids, but is an expression of only one effect of suspended solids upon the character of the water.

The measurement of turbidity recommended by Standard Methods for the Examination of Water and Wastewater (469) is based upon a comparison of the amount of light passing through the given water sample with that passing through a standard sample. Turbidity is measured in standard units, defined in terms of the depth of water to which a candle flame can be clearly distinguished.

In his studies of waters suitable for fish, Ellis has introduced another system of measurement based upon the determination of the depth at which the light that penetrates the water is reduced to one-millionth of its intensity at the surface. This depth is designated the "millionth intensity depth" of light penetration, abbreviated as m.i.d. (313, 790, 801). The penetration of light, is also measured by the Secchi disk and by the hydrophotometer.

2. Cross References. Suspended Solids, Silt, Settleable Solids.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The 1962 USPHS Drinking Water Standards (2036) specify that turbidity shall not exceed 5 units. Bean (1745) has proposed a much stricter limit, viz, 0.1 units for ideal drinking water. State and interstate agencies that have adopted systems of stream classification and standards sometimes designate limits for turbidity in streams that are used for domestic water supply, with values ranging from 5 to 250 units but most commonly the limits of turbidity are expressed in general terms. For further details of state and interstate standards, see Chapter III.

b. Industrial Water Supplies. Turbidity is undesirable in waters for laundries, ice making, bottled beverages and brewing, textiles, pulp and paper, steam boilers and turbine operation, and other industrial processes.

A summary of the limiting values as recommended in the literature is shown in the following table:

RECOMMENDED LIMITS OF TURBIDITY FOR VARIOUS INDUSTRIAL USES OF WATER

Industrial Use	*Turbidity Units*	*Reference*
Beverages	1–2	463, 184, 152
Food products	10	173
Breweries	1–10	186, 152
Boiler feed water	1–20 (according to pressure)	159
Paper and pulp		
Alkaline pulps	25	245
High-grade paper	5–25	552, 879
Fine writing and book paper	10	350, 551
Unbleached kraft paper	100	351
Bleached kraft paper	40	351
Groundwood paper	50	244
Textiles		
Nitrocellulose	0.5	2405
Rayon	1	550, 405
Cotton	25	256
Cotton (callaway mills)	0.3–0.5	255
Baking	10	152
Cooling water	50	152
Ice making	1–5	152
Tanning	20	152

c. Fish and Other Aquatic Life. Excessive turbidity in water affects fish in the following ways:

1. By interfering with the penetration of light, it militates against photosynthesis and thereby decreases the primary productivity upon which the fish-food organisms depend (313, 347, 664, 684, 781, 790, 800, 880, 881, 2780, 3742, 3743). As a consequence, fish production is diminished.

2. At very high concentrations, the particulate matter that produces turbidity can be directly lethal (780, 881, 2409, 3210, 3659, 3745).

3. By excluding light, turbidity makes it difficult for fishes to find food. Conversely, smaller fish may be similarly protected from predators (560, 882, 2780, 3743, 3746).

4. Turbidity modifies the temperature structure of ponds. Bottom temperatures are generally lower in turbid ponds than in clear ones (2731). In many ponds, lower temperatures mean lower productivity.

With respect to productivity, Buck (3742) investigated several farm ponds, hatchery ponds, and reservoirs over a two-year period. For farm ponds, the following results were obtained:

	Clear Ponds	*Intermediate Ponds*	*Muddy Ponds*
Average turbidity, units	less than 25	25-100	over 100
Fish yield, lbs per acre	161.5	94	29.3
Ratio of volumes of net plankton	12.8	1.6	1

In reservoirs, the average volume of plankton in surface waters was 13.8 times greater in the clear reservoir than in the turbid one.

With respect to direct lethality van Oosten (881) concluded from a literature survey that average turbidities

as high as 200 units are harmless to fish. Ward (560, 882) has reported that turbidity as high as 245 units is not harmful to fish. Van Oosten (881) maintains that fish thrive in water with turbidities that range over 400 units and average 200 units. Kemp (780) states that turbidities of 3000 units are considered dangerous to fish when maintained over a 10-day period. In the constantly turbid waters of the Powder River, Oregon (from 1000 to 2500 units), eyed rainbow trout eggs were destroyed within a 6-day period (3659).

Wallen (3745) made a comprehensive study of the effects of turbidity produced from montmorillonite clay with respect to 16 species of fish. His results are summarized as follows:

Common Name of Fish	*Range of Temperature °C*	*Average Time of Test, Days*	*Fatal Turbidity in mg/l*		
			Minimum	*Average*	*Maximum*
Golden shiner	20-29	7.1	55,000	166,000	200,000
Mosquito fish	20-28	16.5	120,000	181,500	225,000
Goldfish	24-32	12.0	90,000	197,000	270,000
Green sunfish	20-29	5.5	50,000	166,500	225,000
Black bullhead	22-32	17.0	175,000	222,000	270,000
Red shiner	22-32	9.0	175,000	183,000	190,000
River carpsucker	24-32	9.6	105,000	165,000	250,000
Largemouth bass	16-32	7.6	52,000	101,000	150,000
Pumpkin seed	16-22	13.0	16,500	69,000	120,000
Orangespotted sunfish	22-32	10.0	100,000	157,000	200,000
Channel catfish	24-32	9.3	--	85,000	--
Blackstrip top-minnow	22-26	19.3	--	175,000	--
Black crappie	28-29	2.0	--	145,000	--
Rock bass	--	3.5	--	38,250	--

At turbidities causing death, the opercular cavities were found to be matted with soil and the gills had a layer of soil on them. Symptoms of distress generally appeared at turbidities much lower than those producing death. Harmful effects were observed as the turbidity approached 20,000 units.

Wallen (2780) also found that bullfrog tadpoles and crayfish can withstand over 150,000 units of turbidity without death under laboratory conditions.

Ellis' study of more than 5000 samples of interior waters has indicated a general range of turbidity values from about 84 to 53,887 millimeters, as millionth intensity depth. Only in very clear mountain streams, however, was the figure 53,887 mm; in polluted streams protected from undue erosion, the m.i.d. never dropped below 6000 *mm* even after heavy rains. In swamp areas, with little erosion silt, the m.i.d. varied from 1000 to 20,000 mm. Ellis has recommended that the millionth intensity depth of water for good fish production should exceed 5000 mm (5 meters) (308, 313, 790, 801).

d. Shellfish Culture. It has been reported that excessive turbidity inhibits feeding and restricts the growth of some species of oysters and fresh-water mussels (331, 804). On the other hand, studies by Lunz have indicated that increased turbidity of water in the vicinity of dredging operations in no way increased the mortality of oysters (795). It is also a fact that some oysters live and propagate successfully in waters carrying large quantities of silt and clay (804). Yet, according to Korringa (1448), silt and other turbidity-producing substances such as kaolin or chalk at concentrations by weight as low as 0.1 mg/l reduced the rate of pumping by oysters.

TURPENTINE

(see Resins)

URANIUM U

1. General. Uranium and many of its salts are reported to be highly toxic (364, 2129, 2972), but the literature does not always confirm this observation. In addition to its significance in atomic energy, uranium has been used in photography, in glazing and painting porcelain, and in chemical processes. Many of the salts of uranium are freely soluble in water and, hence, they may constitute a health hazard if present in a water supply. Roubault et al. (3744), however, imply from a limited study of certain areas that natural uranium in the soil, which is absorbed by persons through the water and foodstuffs grown on the land, may be a limiting factor in the incidence of leukemia.

Most of the literature concerning uranium in water relates to the radiological hazards. Results are expressed in terms of radiation parameters rather than in concentrations of uranium in mg/l. Such references are covered in Chapter VIII, Radioactivity. The criteria in the following paragraphs deal with chemical effects without reference to radiological hazards.

2. Cross References. See Radioactivity, Chapter VIII.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supply. The Division of Industrial Hygiene, Ontario Department of Health, has suggested a maximum allowable concentration for neutral uranium in drinking water of 500 to 1000 micrograms per liter (3747).

b. Stock and Wildlife Watering. In tests using two uranium-fluorine compounds, the insoluble uranium tetrafluoride, UF_4, and and soluble uranyl fluoride, UO_2F_2, Maynard et al. (3748) found that 2 percent of UF_4 and 0.05 percent of UO_2F_2 had no toxic effect in the diet of rats. Interference with growth and a depressed body weight were observable during the second year in animals on dietary concentrations of 20 percent UF_4 and 0.15 percent of UO_2F_2. Some tendency toward anemia was discovered in male rats fed diets containing 0.25 and 0.5 percent UO_2F_2. While only mild injury was found in the kidneys of rats whose diets contained 20 percent of UF_4, definite renal tubular damage was found in rats fed diets of 0.15 percent or more of UO_2F_2. In a one-year feeding experiment on dogs the same authors observed doses of 0.01 g/kg UO_2F_2 and 1.0 g/kg UF_4 were without effect on body weight, but animals fed 5 g/kg of UF_4 did not gain weight as almost all other animals did. The minimum dose given for one year that provided unmistakable renal damage in dogs was 0.025 g/kg/day for UO_2F_2 and 0.2 g/kg/day for UF_4. Moxan and Rhian (1481) reported that a concentration of 5 mg/l of uranium in the drinking water increased mortality among rats being fed a ration containing 11 mg/l of selenium.

c. Fish and Other Aquatic Life. Hoffman (769) examined the water of the River March between Czechoslovakia and Germany and found that it contained 0.001 mg/l of uranium. Much of this uranium was absorbed by algae but it was not toxic to them. The alga *Ochromonas* was reported to concentrate natural uranium (U-238) by a factor of 330 from water in 48 hours (3386). In a study of the Black Sea, Pshenim (2919) found yeasts to be more active than bacteria in taking up uranium.

Tarzwell and Henderson (2154) found the 96-hour TL_m of fathead minnow in soft water (pH = 7.4, alkalinity = 18 mg/l, hardness = 20 mg/l) was 2.8 mg/l for uranyl sulfate, $UO_2SO_4.3H_2O$, 3.1 mg/l for uranyl nitrate, $UO_2(NO_3)_2.6H_2O$, and 3.7 mg/l for uranyl acetate, $UO_2(C_2H_3O_2)_2.2H_2O$. In hard water (pH-8.2, total alkalinity = 360 mg/l, hardness = 400 mg/l), the 96-hour TL_m of uranyl sulfate was increased to 135 mg/l. Thus it appears that uranium compounds are considerably more toxic in soft water than in hard water (2407, 2410, 2973).

In Japan, it was observed that 250 mg/l of uranyl nitrate in sea water inhibited the formation of the fertilization membrane in *Urechis* eggs and led to polyspermy (3749). Bringmann and Kuhn (2158, 3343) using River Havel water, reported the threshold effect of uranyl nitrate on *Daphnia* to be 13 mg/l, on *Scenedesmus*, 22 mg/l, on *E. coli* 1.7 to 2.2 mg/l, and on a protozoan (*Microregma*) 28 mg/l, all expressed as uranium.

UREA H_2NCOH_2N

This product of the decomposition of protein is used for fertilizer, organic syntheses, and the production of resins and plastics. Schaut (362) reported that 17.1 mg/l of urea in stabilized tap water had no noticeable effect on minnows during 24 hours of exposure. The dissolved-oxygen content of the water dropped only 2.0 percent. According to Gillette et al. (1442) the critical range for creek chub, a fish of average tolerance, exposed for 24 hours in well-aerated water at 15-21°C, was 16,000 to 30,000 mg/l, i.e. all fish survived at 16,000 mg/l but all died at 30,000 mg/l.

VANADIUM V

Metallic vanadium does not occur free in nature but minerals containing vanadium are widespread. The metal is used in metallurgy to increase the hardness and malleability of steel, and the salts are used in the manufacture of glass, in photography, and as a mordant in dyeing and printing fabrics (364, 2121, 3750).

In its salts, vanadium may have valences of 2, 3, 4, and 5. The tetra- and penta-salts are generally soluble, but the trifluoride, trioxide, and trisulfide are insoluble. Vanadium forms the vanadyl cation (VO) the salts of which are soluble. It also forms the anion vanadate (VO_4). It is likely, therefore, that any vanadium salts, occurring in waste water will remain in solution.

Trace amounts of vanadium (0.02-0.15 mg/l) have been found in the waters of certain areas of New Mexico (3751). Vanadium occurs in many soils, with a concentration as high as 470 mg/kg being reported. It also occurs in vegetation grown in such soils, with some plants containing as much as 10 mg/l. In animal tissues, it is widely distributed in trace amounts. Although vanadium has not been demonstrated to be essential in human nutrition, there is evidence that it has certain beneficial biological functions (2121).

According to Mountain et al. (3752), 100 mg/kg of vanadium in the form of V_2O_5 in the diet lowered the free cholesterol and phospholipid content of the liver of rabbits. Vanadium appear to inhibit the synthesis of cholesterol and accelerate its catabolism. Workmen exposed to vanadium dust were found to have a lower serum chloresterol than a control group (3753, 3754). Stockinger (3751) reported that the incidence of coronary heart disease is lowest in New Mexico, where natural waters contain trace amounts of vanadium. He also noted that vanadium lowered the serum cholesterol levels in men by 20 percent, at which time the urinary excretion of vanadium averaged 0.046 mg/l.

It has also been reported that vanadium in drinking water at concentrations of 0.03 to 0.22 mg/l reduced the incidence of dental caries in both primary and permanent teeth (2253).

Vanadium fed as calcium vanadate in the diet of chicks inhibited growth at levels of 20 mg/kg (as vanadium), while the LD_{50} level was between 300 and 350 mg/l as vanadium (2927). In the drinking water administered to rats on a ration containing 11 mg/l of selenium, the addition of 5 mg/l of vanadium increased mortality (1481).

It is believed that small quantities of vanadium stimulates the growth of plants and may reduce the effects of iron deficiency (2121). In nutrient solution 0.1 mg/l of vanadium had no effect on the growth of soybeans and flax, but 10-20 mg/l was harmful (1595). According to Hewitt (1473) a concentration of about 50 mg/l of metavanadate ion, VO_3^-, equivalent to about 26 mg/l of vanadium was slightly injurious to sugar beets grown in sand culture. Up to 0.1 mg/l of vanadium increased the growth of the green alga *Scenedesmus*, and it is possible that vanadium is an essential micro-nutrient for all green plants (1602).

In exploratory tests, Tarzwell and Henderson (2154) investigated the toxicity of vanadium compounds to the fathead minnow. They found the 96-hour TL_m, expressed in terms of vanadium, to be 4.8 mg/l in soft water and 30 mg/l in hard water when vanadyl sulfate was used. The corresponding values when vanadium pentoxide was employed were 13 and 55 mg/l. Similar tests with the bluegill sunfish gave 96-hour TL_m values of 6 mg/l in soft water and 55 mg/l in hard water for vanadyl sulfate, expressed as vanadium.

VANILLIN $C_6H_3OHOCH_3CHO$

This 4-hydroxy-3 methoxybenzaldehyde solid is highly soluble in water. It is used as a flavoring agent in beverages, confectionery, foods, and perfumes. It also occurs in the raffinate wastes from pulp mills. According to Helbig (3494), the odor of vanillin becomes perceptible when its concentration in water reaches 0.15 mg/l. The State of Washington Department of Fisheries (2091) conducted tests with fingerling chinook salmon exposed to raffinate wastes containing 22.1 percent solids (vanillin content not specified). The critical tolerance level was between 500 and 100 ppm by volume. For yearling silver salmon in aerated sea water, it was between 2082 and 2549 ppm. According to Palmer and Maloney (2041) vanillin at a concentration of 2.0 mg/l is slightly inhibitory to the growth of the alga *Gomphonema* but not toxic to 5 other species of algae at this concentration.

VERSENE ACID

(see Ethylenediaminetetraacetic Acid)

WEED KILLERS

(see Chapter IX)

WETTING AGENTS

(see Chapter X)

WINTERGREEN OIL

(see Methyl Salicylate)

WOLFRAM

(see Tungsten)

WOOL-SCOURING WASTES

(see also Soaps, Synthetic Detergents, Alkalinity)

Wool-scouring wastes contain the detergents and alkalies used for cleansing and the waxes, fats, dung, blood, urea, sweat, and soil from the wool. Schaut (362) tested a dilution of 1:240 in stabilized tap water and found that it distressed fish but did not kill them, despite the increased turbidity and alkalinity. At a dilution of 1:2400, fish were normal.

XYLENE $C_6H_4(CH_3)_2$

Xylene is a constituent of coal tar, with the commercial form being a mixture of the three isomers, o-, m-, and p-xylene. It is a flammable liquid that is insoluble in water. Commercially it is used as a solvent and cleaning agent, and in the manufacture of dyes and organics. The oral LD_{50} for white rats is reported (3348) as 4300 mg/kg of body weight. According to Middleton et al. (2983) a concentration of 0.3 to 1.0 mg/l of xylene causes a detectable taste or odor in water.

The concentrations of xylene reported to kill fish vary from 10 to 90 mg/l, depending on the species, temperature, and isomer. In general, m-xylene appears to be most toxic and o-xylene least toxic (363, 461, 463, 2977). In Lake Huron water at 12°C, a concentration of 5 mg/l of m-xylene had no apparent effect on rainbow trout or sea lampreys during a 24-hour exposure, but this concentration caused illness to the bluegill sunfish within 10 hours (2976).

XYLENOLS $(CH_3)_2C_6H_3OH$

(see also Cresols, Cresylic Acid, and Phenols)

As a diemthyl phenol, this crystalline compound has six isomers, which are only slightly soluble in water. As constituents of cresylic acid, xylenols are used in the manufacture of resins and coal-tar disinfectants.

Ruchhoft (459) quotes references to the effect that, in chlorinated water, 1, 3, 4-xylenol could be tasted at a concentration of 0.001 mg/l and 1,2,5-xylenol at 0.002 mg/l.

The toxocity of xylenols toward fish and other aquatic life varies with the exposed species and the isomer of xylenol, as well as with temperature, time of exposure, and the synergism or antagonism of other substances. The following information on the toxic or harmful effects of specific xylenols has been reported:

Concentration in mg/l	*Time of Exposure*	*Type of Fish*	*Effect*	*Reference*
A. 1, 2, 4-xylenol (m-xylenol)				
4.0	24 hours	salmonide embryos	TL_m at 13°C	3404
5.0	--	bream, bleak	threshold of toxicity	2904
10	--	carp	threshold of toxicity	2904
11	--	perch	lower toxic limit	3405
17.8	24 hours	tench	TL_m at 18°C	3404
20	61 minutes	minnows	lethal limit	3405
21.1	24 hours	carp	TL_m at 18°C	3404
26	10 minutes	perch	lethal limit	3405
B. 1, 3, 4-xylenol (o-xylenol)				
4	--	perch	lower toxic limit	3405
8	--	bream, bleak	threshold of toxicity	2904
10	--	carp	threshold of toxicity	2904
13	24 hours	tench	TL_m at 18°C	3404
16	10 minutes	perch	lethal limit	3405
20	15.9 minutes	minnows	lethal limit	3405
27.7	24 hours	embryos	TL_m at 13°C	3404
30	24 hours	carp	TL_m at 18°C	3404
C. 1, 2, 5-xylenol (p-xylenol)				
2	--	perch	lower toxic limit	3405
5	--	bream, bleak	threshold of toxicity	2904
10	--	carp	threshold of toxicity	2904
12	10 minutes	perch	lethal limit	3405
20	5.7 minutes	minnows	lethal limit	3405
20	31.9 minutes	eels	lethal limit	3405
D. 1, 2, 6-xylenol				
7-9	--	trout	toxic level	346
E. 1, 3, 5-xylenol				
15	--	perch	threshold of toxicity	2904
18	--	bleak	threshold of toxicity	2904
20	--	bream	threshold of toxicity	2904
50	24 hours	salmonide embryos	TL_m at 13°C	3404
51.5	24 hours	tench	TL_m at 18°C	3404
53	24 hours	carp	TL_m at 18°C	3404
70	4.5 hours	roach	lethal at 9°C	910
F. 1, 4, 5-xylenol				
2	24 hours	salmonide embryos	TL_m at 13°C	3404
9	24 hours	tench	TL_m at 18°C	3404
10	24 hours	carp	TL_m at 18°C	3404
35	4.5 hours	roach	lethal at 11°C	910

Southgate (610) reports that sub-lethal concentrations of 1, 2, 6-xylenol increase the toxicity of p-cresol, but sub-lethal concentrations of p-cresol do not appreciably affect the toxicity of xylenol.

The sperm of carp and the eggs of that fish and of tench were found to be more resistant than the adult fish to 1, 2, 4-, 1, 3, 4-, 1, 3, 5-, and 1, 4, 5-xylenol (3404).

Xylenols begin to effect the taste of bleak at 1.0 mg/l and carp at 5.0 mg/l (2904).

Toward lower aquatic life, the xylenols do not appear to be as toxic as they are toward fish. In River Havel water with exposures of 48 to 96 hours, Bringmann and Kuhn (2158, 3343) found that the beginning of threshold effects occurred at the following concentrations:

	Threshold Concentration in mg/l for			
Isomer	*Daphnia at 23°C*	*Scenedesmus at 24°C*	*Microregma*	*E. coli at 27°C*
1, 2, 4-xylenol	24	40	70	>100
1, 3, 4-xylenol	16	40	10	500
1, 2, 5-xylenol	10	40	50	>100

Bandt (2904) reports that the toxic effects of 1, 3, 4-xylenol occur at 77 mg/l for ciliates and rotatoria, 46 mg/l for crustacea, 108 mg/l for molluscs, and 154 mg/l for mastigophora.

YTTRIUM

(see Chapter VIII)

ZDD (ZINC DIMETHYL DITHIOCARBAMATE)

(see Chapter IX)

ZEPHIRAN CHLORIDE

ZEPHIRAN LEAD

(see Chapter X)

ZINC Zn

1. General. Occurring abundantly in rocks and ores, zinc is readily refined into a stable pure metal and it is used extensively for galvanizing, in alloys, for electrical purposes, in printing plates, for dye-manufacture and for dyeing processes, and for many other industrial purposes. Zinc salts are used in paint pigments, cosmetics, pharmaceutics, dyes, insecticides, and other products too numerous to list herein (364, 2121). Many of these salts (e.g., zinc chloride and zinc sulfate) are highly soluble in water; hence it is to be expected that zinc might occur in many industrial wastes. On the other hand, some zinc salts (e.g., zinc carbonate, zinc oxide, zinc sulfide) are insoluble in water and consequently it is to be expected that some zinc will precipitate and be removed readily in most natural waters.

In zinc-mining areas, zinc has been found in natural waters in concentrations as high as 50 mg/l (152) and in effluents from metal-plating works and small-arms ammunition plants it may occur in significant concentrations (657, 1031); but, in most surface and ground waters, it is present only in trace amounts. There is some evidence that zinc ions are adsorbed strongly and permanently on silt, with a resultant inactivation of the zinc (3755).

2. Cross References. See also Zinc Salts, Chapter VIII (Radioactivity) Chapter IX (Pesticides).

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The U.S. Public Health Service Drinking Water Standards of 1962 set a limit of 5 mg/l of zinc in acceptable water supplies when no alternate sources are available (2036). From 1942 until 1962, the limit had been 15.0 mg/l, but this standard was lowered because the taste threshold for zinc occurs at about 5 mg/l. Furthermore, the WHO International and European standards for drinking water prescribe a permissible or recommended limit of 5.0 mg/l (2328, 2329).

Zinc has no known adverse physiological effects upon man except at very high concentrations. An emetic concentration requires 675-2280 mg/l. In fact, zinc is an essential and beneficial element in human nutrition (2121, 2129). The normal human intake of zinc is estimated at 10-15 mg per day (2121). A summary of literature relating to the toxicity of zinc (977, 978) reveals that families and communities have used waters containing 11.2, 17, 18.5 and 26.6 mg/l with no ill effects. One water supply containing 23.8 to 40.8 mg/l of zinc gave no harmful effects to 200 persons stationed at a depot. On the other hand, another supply containing 30.8 mg/l of zinc caused nausea and fainting spells. Hinman (219) reported that spring water in Missouri containing 50 mg/l of zinc was used for a long time without harmful effect. From these references, it would appear that the WHO and USPHS limits are conservative insofar as physiological effects are concerned.

From esthetic considerations, however, high concentrations of zinc in domestic water are undesirable. At a concentration of 30 mg/l, zinc gives water a milky appearance (555) and causes a greasy film on boiling (980). Indeed, concentrations as low as 5.0 mg/l are characterized by a greasy film on boiling (969).

All readily soluble salts of zinc have an unpleasant, astringent taste and can be detected in less-than-dangerous amounts in drinking water (979). Taste thresholds have been reported at 40 mg/l (555, 3756), 25 mg/l, and as low as 2 mg/l (3300). In tests performed by a taste panel at the R. A. Taft Sanitary Engineering Center (3301) with three zinc salts in distilled water and in mineralized natural spring water, the taste threshold was lower with zinc sulfate than with the nitrate or chloride. In the case of zinc sulfate in distilled water, the estimated taste threshold of 50 percent of the panel was 17.6 mg/l of zinc, but the most sensitive 5 percent of the panel were able to detect zinc at 4.3 mg/l. The taste was less noticeable in spring water, the median threshold being 27.2 mg/l and the 5 percentile at about 6 mg/l. These tests were partly instrumental in changing the USPHS Drinking Water Standard from 15 to 5 mg/l of zinc.

b. Industrial Water Supplies. According to Negus (633) zinc-bearing water should not be used in acid drinks like lemonade because zinc citrate and other organic zinc compounds that will result may be poisonous.

c. Irrigation Water. Kelley and Brown (277) report that small amounts of zinc are needed for nutrition by most crops, but toxicity results when concentrations exceed a very low level. A deficiency of zinc causes poor growth, a dwarfing of the leaves of fruit trees, and chlorosis in corn. Soils vary widely in their zinc content, from near zero to over 100 mg/kg. The addition of 2 mg of zinc per kg of dry soil increased the nitrogen content of peas (2926). Zinc is present in most plants in amounts varying from 1 to 10 mg/kg and in cereals up to 140 mg/kg (2121).

Wilson (1730) found that two species of pine tree (as seedlings) each required about 0.1 mg/l of zinc in nutrient solution for normal growth.

The following concentrations of zinc in nutrient solutions have been reported to be toxic to plants:

Concentration of Zinc, mg/l	*Compound Tested*	*Plant*	*Reference*
3	--	Orange and mandarin seedlings	1524
≥5	--	Flax	1458
≥10		Water hyacinths	1525
25-100	$ZnSO_4$	Oats	1462

On the other hand, 10 mg/l of zinc caused no apparent injury to oat plants (1462). The presence of 2 mg/l as zinc in water suppressed a fungus disease that infected the roots of watercress, without harming the plants (2658).

During an observation period of 18 days, 54-436 mg/l of zinc in nutrient solution delayed germination and greatly retarded the growth of cress and mustard seeds in solution culture (1479).

d. Stock and Wildlife Watering. Rats fed on water containing 50 mg/l of zinc showed no harmful effects (977), and an average daily dose of 2 mg was not detrimental (353). A total of 390 grams of zinc per pig over a period of three months (about 3.9 grams per day) caused no ill effects, but lameness and malnutrition were caused by feeding milk containing 1000 mg/l of zinc as

lactate for two months (353). Cats were not injured by doses of 50 mg of zinc as malate per day for 10 days to two months. Zinc in concentrations of 2500 mg/kg fed as chloride or carbonate in the diet was completely without effect on rats (2121). In another test, 1000 mg/kg of zinc carbonate in the diet of rats produced no effect; 5000 mg/kg was slightly toxic; and 10,000 mg/kg caused a cessation of growth and some deaths (2129). On the other hand, a concentration of only 5 mg/l of zinc in the drinking water increased the mortality among rats on a high selenium diet (1481). When 10,000 mg/l of water soluble zinc sulfate (2320 mg/l of zinc) was added to the drinking water of six laying hens, egg production dropped, water consumption decreased, and there was a loss of body weight (3761).

The radioisotope Zn-65 is concentrated on pasture grass irrigated with Columbia River water and subsequently transferred to grazing animals and milk (3762, 3763). In rats fed concentrated reactor effluent water for periods up to one year, the radioisotope accumulated in highest concentration was Zn-65 (3764).

e. Fish and Other Aquatic Life. It is toward fish and aquatic organisms that zinc exhibits its greatest toxicity. In soft water, concentrations of zinc ranging from 0.1 to 1.0 mg/l have been reported to be lethal, but calcium is antagonistic toward such toxicity. Jones (573) reports that for mature fish the lethal limit for zinc in water containing one mg/l of calcium is only 0.3 mg/l but in water with 50 mg/l of calcium, as much as 2.0 mg/l of zinc is not toxic (see also zinc sulfate and other zinc salts). The antagonistic effect of hardness toward zinc toxicity has been confirmed by Cairns and his colleagues (2934, 2936, 3519, 3759). Zinc is thought to exert its toxic action by forming insoluble compounds with the mucous that covers the gills (2411, 2920, 2941), by damage to the gill epithelium (3757), or possibly as an internal poison (2112, 3758).

The sensitivity of fish to zinc varies with species, age and condition of the fish, as well as with the physical and chemical characteristics of the water. Some acclimatization to the presence of zinc is possible, and survivors from batches of fish subjected to dissolved zinc have been less susceptible to additional toxic concentrations than fish not previously exposed. It has also been observed that the effects of zinc poisoning may not become apparent immediately, so that fish removed from zinc-contaminated to zinc-free water (after 4-6 hours of exposure to zinc) may die 48 hours later (1444, 1731).

The presence of copper appears to have a synergistic effect on the toxicity of zinc. Doudoroff observed that test fish in soft water could tolerate a concentration of 8 mg/l of zinc alone for 8 hours; however, most of the fish died within eight hours when exposed to a solution containing only 1 mg/l of zinc plus 0.025 mg/l of copper (1459, 1528, 2409, 2410, 3104, 3561, 3760).

The Water Pollution Research Board of England tested the synergistic action of copper and zinc salts in hard water (320 mg/l as $CaCO_3$) with rainbow trout, at a 6 to 1 ratio of zinc sulfate to copper sulfate (74). In contrast to Doudoroff's findings for soft water, it was shown that there is no synergism between zinc and copper in hard water. Indeed, in soft water (15-20 mg/l as $CaCO_3$), synergism between zinc and copper was evident at high concentrations; but the threshold concentration for the mixture (0.56 mg/l for zinc and 0.44 mg/l for copper) is about that to be expected on the assumption that there is no synergism.

The Water Pollution Research Board also found that there was little toxic action of the zinc precipitated from solution in alkaline water, almost all of the toxicity being attributable to the zinc remaining in solution (2112). They also showed that the toxicity of zinc salts to sticklebacks in soft water is reduced by the addition of calcium chloride, even more so than the addition of calcium carbonate; hence the calcium ion rather than the carbonate ion appears to be the antagonistic factor (2112, 3757).

The toxicity of zinc salts is increased at lower concentrations of dissolved oxygen in about the same proportion as for lead, copper, and phenols, e.g., the lethal concentration at 60 percent saturation of dissolved oxygen is only about 0.85 that at 100 percent saturation (1816, 2954, 3321, 3322, 3757).

An increase in temperature decreased the survival time of rainbow trout in solutions of zinc sulfate in hard water, but the threshold concentration was not appreciably affected by changes in temperature (3757) nor was the 96-hour TL_m (2936, 3519, 3759).

Sticklebacks develop an avoiding reaction to dilute solutions of zinc sulfate and will stay out of 0.0003 N zinc sulfate (about 10 mg/l of Zn) (2920).

Affleck (1444) found that 0.04 mg/l of zinc in soft water prevented the hatching of rainbow-trout eggs, but Goodman (1731) reported that young rainbow trout hatched successfully in the presence of 1.0 mg/l of zinc.

Doudoroff (2157, 3561) experimented with the toxicity of metal cyanide complexes toward minnows. He found that the zinc-cyanide complex, unlike nickel cyanide, dissociated in very dilute solutions, which have been found to be even more toxic than comparable solutions of cyanide without zinc, apparently because of cynergism (2109, 3409).

The following concentrations of zinc have been reported as lethal to fish in the time specified:

Concentration as Zinc, mg/l	*Type of Water*	*Temperature °C*	*Exposure Time*	*Fish*	*Reference*
0.01	---	---	---	Trout ova and young	1444
0.01-0.4	---	---	---	Young rainbow trout	1459
0.13	---	---	12-24 hrs	Rainbow fingerlings	1444
0.13	---	---	---	Guppy	2921
0.15	---	---	---	Salmon fry	2153
0.15	---	---	---	Trout	1732
0.3	Soft	---	---	Mature fish	573
0.3	---	---	---	Sticklebacks	1444, 2941
0.3-0.7	---	---	---	Fish	1459
0.4	Soft	---	6 days	Fish	1459
0.5	---	---	---	Mixed warmwater fish	3319
0.5	Soft	---	3 days	Fingerling rainbow trout	2112
0.5	Soft	---	3 days	Rainbow trout	3057
0.65	---	---	12 hrs	Eels	1459
1.0	Soft	---	24 hrs	Sticklebacks	2920
1.9-3.6	Soft	30	96-hr TL_m	Bluegill sunfish	3759, 3519
2.0	Soft	---	18 hrs	Fish	1459
2.9-3.8	Soft	20	96-hr TL_m	Bluegill sunfish	3759, 3519
3.0	Soft	---	8 hrs	Fingerling rainbow trout	2112
3.0	---	---	48 hrs	Rainbow fry	1731
3.5	Soft	30	96-hr TL_m	Bluegill sunfish	2936
3.5	Std. dil. water	20	96-hr TL_m	Bluegill sunfish (medium)	2934

Concentration as Zinc, mg/l	Type of Water	Temperature °C	Exposure Time	Fish	Reference
4.0	Hard	---	3 days	Rainbow trout	3057
4.2	Soft	20	96-hr TL_m	Bluegill sunfish	2936
5-15	---	---	---	Fish	1459
6.0	---	---	48 hrs	Young trout	1731
8.0	Soft	---	8 hrs	Fish	1528
8.02	Std. dil. water	---	96-hr TL_m	Bluegill sunfish	2957
8-11	---	---	---	Trout	1731
10.1-12.5	Hard	18 & 30	96-hr TL_m	Bluegill sunfish	3759, 3519
12.5-12.9	Hard	20 & 30	96-hr. TL_m	Bluegill sunfish	2936
15.0	---	---	8 hrs	Fish	1731
20.0	Soft	---	<6 hrs	Fish	1459
25-50	Tap	---	2 hrs	Rainbow trout	1459
200	Soft	---	3.5 hrs	Fish	1459

The following concentrations of zinc have been reported not to have caused harm to fish during the time specified:

Concentration as Zinc, mg/l	Type of Water	Exposure Time	Fish	Reference
0.003	----	28 days	Rainbow alevins	1444
0.003	----	20 days	Brown trout fingerlings	1444
0.13	Hard	20 days	Brown trout fingerlings	1444
1.0	Hard	10 days	Sticklebacks	2920
2.0	Hard	2 days	Mature fish	573
2.5–3.5	Hard	14 days	Rainbow trout	3757
3.0	Hard	10 days	Fingerling rainbow trout	2112
4.0	----	48 hours	Young trout	1731
8.0–11.0	----	----	Some individual trout	1731

Daphnia and other crustacea are susceptible to zinc but most of the literature surveyed gives the results in terms of the zinc salts; therefore, see zinc chloride, zinc sulfate, etc. Zinc, however, is somewhat less toxic than copper (575).

Zinc has a toxic effect toward protozoa and bacteria, but not nearly as pronounced as copper (3323). As little as 0.1 mg/l of zinc will cause an effect upon biochemical oxygen demand and 62.5 mg/l of Zn will cause a 50 percent drop in the 5-day BOD (2922). In concentrations up to 1.0 mg/l, zinc is reported to stimulate nitrification, but 10 mg/l is inhibitory (2977).

The Mersey and Severn River Boards in England have adopted working standards that limit the concentration of zinc, in combination with all heavy metals, to 1.0 mg/l (1756, 2950).

For effects of zinc on kelp, see zinc sulfate.

f. Shellfish Culture. In very small amounts, zinc has been reported to be dangerous for oysters and in large amounts to impart a blue-green color (317). Toward snails, toxic action by zinc has been reported at 1.0 mg/l in natural water (3765) and as low as 0.05 to 0.10 mg/l in distilled water (2733). Toward the pond snail *(Physa heterostropha)*, Cairns and Scheier (3759) found the 96-hour TL_m for zinc in soft water to be 0.79 to 1.27 mg/l at 20°C and 0.62 to 0.78 mg/l at 30°C. In hard water the results were 2.67 to 5.57 mg/l at 20°C and 2.36 to 6.36 mg/l at 30°C. Hardness of the dilution water had a greater effect than did the temperature.

Oysters and other shellfish concentrate zinc-65 from their environment, often by a factor of 100,000 (2438, 2442, 2443, 2446, 3766). A large part of this concentration occurs in the plankton on which oysters feed (2440, 2442, 2447, 3386). For more detail, see Chapter VIII, Radioactivity.

ZINC ACETATE $Zn(CH_3COO)_2$

This white crystalline substance, highly soluble in water, has a faint acetous odor and an astringent taste. It is used as a mordant in dyeing and for veterinary treatments (364). Daily doses of 10 to 15 mg of zinc acetate for four months caused no injury to rats (353).

ZINC CHLORIDE $ZnCl_2$

Zinc chloride is highly soluble in water, one gram dissolving in 0.5 ml of water (364). It is used as a deodorant, disinfecting, and embalming material and in the manufacture of paper, dyes, glues, and many other processes.

A dose of six grams of zinc chloride has been reported as fatal to man (353). Practically all recorded cases of poisoning and deaths from zinc have involved either the chloride or the sulfate (969).

Young carp were killed within 24 hours by 1.0 mg/l of zinc chloride in tap water (1459). The highest concentration of zinc chloride tolerated by young eels for more than 50 hours was 0.14 mg/l, and 0.65 mg/l as zinc killed eels in about 12 hours (1459). For medium-sized bluegill sunfish in standard dilution water at 20°C, the 96-hour TL_m for zinc chloride was 7.20 mg/l (2933, 2934, 2935). Another observer (1731) reported that 15 mg/l of zinc chloride killed fish within 8 hours. On the other hand, exposure for one hour to 17.1 mg/l of zinc chloride in stabilized tap water had no detrimental effect on minnows (362).

According to Cairns and Scheier (2957) the 96-hour TL_m values for bluegill sunfish exposed to zinc chloride were 8.02 mg/l of zinc at normal oxygen tensions, but when the dissolved oxygen was periodically lowered to 2.0 mg/l, the TL_m was only 4.9 mg/l as zinc.

Anderson (598) quotes references to the effect that 1.36 mg/l of zinc chloride in pond water killed *Daphnia magna* in less than five days, and in Lake Erie water at 25°C the threshold concentration for immobilization of *Daphnia magna* was found to be very much less than 0.15 mg/l of zinc chloride. The hardness of water appears to affect markedly the toxicity of such zinc salts.

ZINC NITRATE $Zn(NO_3)_2$

Highly soluble in water, zinc nitrate is a colorless, odorless salt used as a mordant in dyeing (364). Ellis (313) quotes references to the effect that tadpoles survived a three-month exposure to 1.89 mg/l of zinc nitrate but failed to develop limb buds, that 5.7 mg/l killed most tadpoles, and that 94.7 mg/l killed tadpoles quickly. Anderson (598) reports that zinc nitrate concentrations of 189 mg/l in well water killed *Daphnia magna* in 15 hours, but for stickleback fish the toxic threshold was only 0.87 mg/l.

ZINC OXIDE ZnO

This white, or yellowish-white, amorphous, odorless powder is insoluble in water (364). It is used extensively as a pigment in white paints, in cosmetics, tires, printing inks, and other products. It is also employed as a mild astringent and antiseptic in skin diseases (364).

A concentration of 900 mg/l of zinc oxide in the nutrient solution retarded the germination of maize and barley seeds (1479). In a review of the literature, Anderson, Reinhard, and Hammel (977) report that well water containing 32.4 mg/l of zinc oxide caused vegetables to

turn green on cooking and intestinal distress on drinking. Dosages of 0.5 to 34.4 mg of zinc oxide per day for one month to a year did not harm rats and 175 to 1000 mg of zinc oxide per day for 3 to 53 weeks showed little evidence of harm to dogs and cats (353).

ZINC SULFATE

$ZnSO_4 \cdot 7H_2O$ or $ZnSO_4 \cdot H_2O$

1. General. This colorless, odorless, crystalline or amorphous substances, highly soluble in water, is used as a mordant in calico printing, for preserving wood and skins, and for electroplating of zinc (364). It has been employed as an emetic, but 45 gms are reported to be fatal.

2. Cross References. Zinc, other zinc salts, Sulfate.

3. Effects Upon Beneficial Uses.

a. Irrigation. Davies (990) reports that zinc sulfate is toxic to plants. Dwarf French beans grown in solutions of 100 mg/l of zinc sulfate died in a few weeks. Brenchley found that 10 mg/l of zinc sulfate in nutrient solution was toxic to pea plants (1479). However, 24.7 mg/l of zinc sulfate caused no apparent injury to oats (1462).

b. Fish and Other Aquatic Life. Using zinc sulfate, Jones (1460) found the lethal concentration limit for sticklebacks to be 0.3 mg/l as zinc. In different concentrations, the average survival times were as follows: four days at 0.7 mg/l and one week at 0.4 mg/l as zinc. Jones (467) claims that fish detect and avoid concentrations of zinc sulfate as low as 24 mg/l, although the salt is not extremely toxic.

The following concentrations of zinc sulfate, as the salt or as zinc, have been reported lethal to fish in the stated time of exposure:

Concentration of Zinc Sulfate, mg/l	*Type of Water*	*Time of Exposure*	*Fish*	*Reference*
0.13 (as Zn)	--	----	Guppy	2921
0.3 (as Zn)	--	Long term	Sticklebacks	1460
0.4 (as Zn)	--	7 days	Sticklebacks	1460
0.7 (as Zn)	--	4 days	Sticklebacks	1460
0.8	--	144 minutes	Minnows	991
1.5	Soft	24 hours	Sticklebacks	2977
3-6 (as Zn)	Tap	48 hours	Young trout	1731
4.0	--	144 minutes	Minnows	991
6.0 (as Zn)	Tap	14 hours	Trout fingerlings	1731
8.1	--	72 minutes	Minnows	991
10	Fresh	----	Fish	609
10	--	30 hours	Trout	2977
10	Fresh	48 hours	Minnows	1459
16*	.	20 hours	Young eels	1459
25	Distilled	133 minutes	Rainbow trout	617
25-50 (as Zn)	Tap	2 hours	Rainbow trout	1731
100		5 days	Goldfish	313, 598
400	Distilled	200 minutes	Minnows	313
1000	Hard	1-4 hours	Goldfish	313

*Approximately

The following concentrations have been reported as not harmful in the time specified:

Concentration in mg/l	*Reported as*	*Type of Water*	*Time of Exposure*	*Fish*	*Reference*
2.0	Zinc	--	24 hours	Young trout	1731
2.5-3.5	Zinc	Hard	14 days	Rainbow trout	3757
4.0	Zinc	--	24 hours	Old trout	1731
100	Zinc sulfate	Hard	4 days	Fish	1459
200	Zinc sulfate	Sea	--	Minnows	1459
1000	Zinc sulfate	--	1 day	Trout	1459

According to Rudolfs, Barnes, et al. (574), studies by different people indicate that the effects of zinc sulfate on plankton forms in soft water were as follows: concentrations up to 500 mg/l were tolerated by larvae of stonefly, caddis, and water boatman but 10 mg/l killed many small crustacea. *Daphnia* in an Idaho lake were killed by 0.65 mg/l in three days and 0.3 mg/l was the minimum tolerance for mayfly nymphs, 0.2 mg/l for fresh-water snails and shrimps, and 0.1 mg/l was toxic to some organisms during long exposures. Anderson (598) reported that 0.024 mg/l of zinc sulfate killed *Daphnia magna* in hard water but some survived 0.24 mg/l in soft water. In Lake Erie water at 25°C the threshold concentration for immobilization of *Daphnia magna* was found to be less than 48 mg/l (358). Naumann (654) claims that 10 mg/l of zinc sulfate had no effect on *Daphnia magna* during the first five hours of exposure, but thereafter signs of poisoning appeared. The animals may live for some days but their color fades and they lose their power of reproduction.

Using water of the River Havel from which the test organisms were recovered, Bringmann and Kuhn (2158, 3343) studied the threshold effects of zinc added as $ZnSO_4.7H_2O$ on various species during an exposure of 2-4 days. For *Daphnia* the median threshold effect occurred at 1.8 mg/l of zinc, for *E. coli* at 1.4-2.3 mg/l, for *Scenedesmus* at 1.0-1.4 mg/l, and for *Microregma* at 0.33 mg/l.

Cleland (3328) found various abnormalities in the fertilization and cleavage of eggs of sea urchins when zinc sulfate was added to the sea water at concentrations as low as 0.16 mg/l of zinc.

Clendenning and North (2106, 2107) tested the effect of zinc sulfate on the giant kelp, *Macrocystis pyrifera*. During a 4-day exposure, 1.31 mg/l, as zinc, showed no appreciable effect on the rate of photosynthesis but 10 mg/l caused a 50 percent inactivation of bottom kelp fronds.

ZIRCONIUM

Zr

Generally regarded as a rare metal, zirconium actually constitutes a higher percentage of the earth's crust than do copper, lead, or zinc. It occurs as zirconium silicate (zircon) primarily in beach sands from volcanic areas. It is used in metallurgy to increase the wear resistance of alloys, in electronic manufacturing, in nuclear reactors as a shielding material, in tanneries, as a pigment in plastics, and in the ceramic and glass industry (364, 2121).

Although zirconium chloride, nitrate, and sulfates are soluble in water, the carbonate, hydroxide, oxide, and silicate are highly insoluble. As a consequence, any dissolved zirconium that reaches natural waters will be precipitated quickly and removed by adsorption or sedimentation.

Animal studies reported by Rothstein (2129) indicated neither acute nor chronic injury in response to zirconium compounds given orally for as long as two years. Even 20 percent zirconium oxide in the diet was not harmful (2129). Cochran et al. (3527) found the LD_{50} values for oral administration of zirconium salts to rats, expressed as zirconium, to range from 853 mg/kg of body weight for the nitrate to 2290 mg/kg for sodium zirconyl sulfate.

Palange et al. (3767), 3768) conducted bioassays with fathead minnows to determine the toxicity of waste

waters from a plant producing zirconium. They found that the 48-hour TL_m occurred at a 0.28 percent concentration of the zirconium phthalate filtrate and at 0.16 percent of the zirconium hydroxide filtrate. Concentrations of zirconium or pure compounds were not specified.

In exploratory tests with the fathead minnow, Tarzwell and Henderson (2154) found the 96-hour TL_m for zirconium sulfate to be 14 mg/l, as zirconium in soft water and 115 mg/l, as zirconium, in hard water. For zirconium oxychloride, the corresponding concentrations were 18 and 240 mg/l. This latter compound was also tested against bluegill sunfish, with 96-hour TL_m values of 15 mg/l in soft water and 270 mg/l in hard water, all expressed as zirconium.

Collier (1720) reported that during a dense bloom of *Gymnodium* off the Florida west coast water samples analyzed spectrographically showed the presence of zirconium at concentrations of 0.34-3.4 mg/l. The origin and role of the zirconium, like titanium, was not determined.

CHAPTER VII

BIOLOGICAL POLLUTANTS

This chapter is concerned primarily with the effects of living material, both plant and animal, upon the quality and beneficial uses of water. Such biological agents may be primary pollutants or corollary pollutants. Primary pollutants comprise biota that are added directly to water as a result of man's activities, e.g., enteric bacteria or viruses from sewage. These biological forms are not native to the water environment and they seldom multiply or prosper there; indeed they normally die off rapidly. Corollary pollutants represent indigenous living material that interferes with beneficial uses of the water, either by natural processes and growth (e.g., weeds in irrigation ditches) or by stimulation from man's activities (e.g., algal blooms stimulated by nitrates and phosphates from waste discharges). In the case of corollary pollutants, the interfering biological agent has not been added directly by man, but its prominence or its interference may be attributable to human endeavors.

Biological substances are significant in the evaluation of water quality in two other ways, viz, the impact of pollutants upon natural and beneficial aquatic and marine life, and the role of biota in the biochemical and biological stabilization of waste products. The effects of potential pollutants upon fauna and flora comprise a major part of this survey of water-quality criteria, as described by beneficial use in Chapter V and under many of the specific substances in Chapters VI-X inclusive.

The role of living material in the stabilization of waste material is a subject far too vast to be covered in this volume. Moreover, it is not directly relevant, for this study is concerned with the concentrations of pollutants, whether physical, chemical, biological, or radiological, on the beneficial uses of water, and not with the ultimate fate of these pollutants. Aspects of stabilization enter into the discussion of biological pollutants, however, when their levels of concentration are being related to beneficial uses and the reasons for these levels are being evaluated.

For further information the reader is referred to the classics of Ward and Whipple (15), and Whipple and Fair (16) mentioned in Chapter II. Of more pertinence to this work, however, are the English publications by Hynes (2602) and Yapp (2126). Also highly pertinent are the triennial symposia conducted by the U. S. Public Health Service at the Robert A. Taft Sanitary Engineering Center in Cincinnati, Ohio (2127, 2128).

This chapter is organized in the same manner as Chapter VI, as explained in the introduction thereof, with appropriate cross-referencing, both internally and with other chapters. Biological substances are listed alphabetically by the most common designation; but where other terms are in common use they have been listed with cross-referencing.

ACTINOMYCETES

(see also Fungi, Bacteria—General, and Algae)

The mold-like bacteria of the genera *Actinomyces* and *Streptomyces* (Bergey includes others) are commonly referred to as actinomycetes. They play an active part in the decomposition of organic matter and are largely responsible for the musty odor of freshly turned soil (2289).

According to Silvey (2516) the primary stages of the aquatic actinomycetes develop submerged in company with various plankton or organic matter rich in nitrogen. The growth requirements for the secondary (mycelial) stage include available oxygen, temperatures of 10° to 32°C, potassium, a source of organic carbon, and some form of nitrogen. As potassium is increased from minimal amounts of 2 to 4 mg/l, luxuriousness of growth is quite evident.

The aquatic actinomycetes are responsible for tastes and odors in water supplies (152, 662, 663, 1572, 1574, 2058, 2241, 2278, 2504). The primary stages are said to be associated with rather mild odors described as fishy, grassy, or marshy. The secondary stages appear to be more elaborate in the type of odor compounds they produce. These compounds include such metabolic products as aromatic amines, ketones, aldehydes, saturated fatty acids, and unsaturated aromatics (2058, 2516, 2517).

In culture medium, copper has a stimulating effect on all types of aquatic actinomycetes (2240). Calcium, sodium, and magnesium stimulate the growth of some species. Four types of actinomycetes from Los Angeles were found to require some available nitrogen in order to give rise to musty or earthy odors (2517).

Ives (2248) mentions a symbiotic relationship between *Streptomyces* and the alga *Cladophora* that causes tastes in water. However, many tastes and odors mistakenly attributed to algae are reported to be due to actinomycetes (2241). The spores of *Streptomyces* are very small and therefore may pass through rapid sand filters. Such spores can lodge in deposits in the distribution system and cause tastes and odors not found in water leaving the treatment plant (2058).

Silvey studied the relation of irrigation run-off to tastes and odors (1573). Results indicated an imperfect relation between algae and actinomycetes which, in the absence of sufficient phosphate and nitrates, causes rapid destruction of the algae and release of the by-products of metabolism of the actinomycetes.

An obligate autotrophic actinomycete is reported as being capable of destroying cyanide (2518).

ADENOVIRUSES

(see also Viruses and Chapter V—Swimming and Bathing Waters)

The adenoviruses are associated with upper respiratory infections, particularly in children. These spherical viruses are somewhat larger than most enteroviruses, being 60 to 80 mμ in diameter. They are excreted in large numbers in the stools of infected individuals (2159). The adenovirus is also known as the APC virus after adenoidal—pharyngeal—conjunctival.

Although adenoviruses have been isolated from sewage, no outbreaks of human infection from drinking water have been reported. Two papers have been published concerning adenovirus outbreaks that appear to be associated with swimming pools. One was manifest in an epidemic of conjunctival fever in which 74 of the 112 cases studied originated in a swimming pool (2160, 2161).

ALGAE

1. General. In 1956, Palmer and Ingram pointed out the confusion that reigns in classifying of algal forms (2163). The same article offered a system of classification to the water-pollution specialist based upon the oxygen-consuming or oxygen-producing characteristics of the organisms. Palmer, in subsequent publications (2164, 2165, 2166), has made use of a four-part classification; (a) blue-green algae; (b) green algae; (c) diatoms; and (d) flagellates. The algae, in this second edition of WATER QUALITY CRITERIA, have been divided into three groups based largely on the convenience of Palmer's latter organization: (a) algae; (b) diatoms; and (c) marine flagellates.

The algae constitute a group of life forms that may be unicellular or multicellular, motile or immobile, of which practically all have photosynthetic pigments. None posess a multicellular sex organ with a sterile jacket such as the bryophytes (15). Since the algae occur naturally in surface waters, they fall into the category of corollary pollutants. They can be beneficial to waters because they add oxygen, remove carbon dioxide, are significant in self-purification processes, and serve as food for certain aquatic fauna (16, 366, 367, 368, 369). However, Heukelekian (2144) notes that since algae require inorganic materials such as phosphate, ammonia, nitrate, and carbon dioxide which are then photosynthetically transformed into cellular organic materials and oxygen, the algae should be considered as producers rather than destroyers of organic matter.

Growth or types are reported to be influenced by pH (2167, 2168), turbidity (370, 2169), sunlight (371, 2162), sulfates—more than 0.5 mg/l being needed (2203), temperature, water velocity, silica, nitrates, carbonates, phosphates, and manganese (373, 374, 375, 376, 377, 378, 379, 439, 2171). Others have cited protein-derived ammonia, oxygen, oxidizable organic content (2172), alkalinity, iron, and the relative concentrations of carbon, nitrogen, and phosphorus (1451, 1452, 2173, 2174, 2202). Phosphorus is repeatedly cited as a critical factor in plankton growth (2170, 2173, 2175, 2176, 2177, 2178, 2179, 2180, 2181). However, turnover of inorganic phosphate under natural conditions appears to be caused by aquatic bacteria. Bacteria and algae may compete for inorganic phosphate (2140). Sawyer studied the relationship between the production of algal blooms and the nitrogen: phosphorus ratios in a group of Wisconsin lakes. For some algae, N : P ratios of 30 : 1 were found to be optimal; for other algae, ratios of 15-18 :1 appeared to be most favorable. Sawyer suggested 0.01 mg/l of inorganic phosphorus as the maximum concentration that can be permitted without danger of supporting undesirable growths (1450, 2182). If the assets of inorganic nitrogen and phosphorus from wastes exceed 0.3 and 0.01-0.015 mg/l respectively at the start of the growing season, nuisance blooms of algae may occur (2183, 2519). Tucker (2184) has suggested that nutrient elements (other than phosphorus) combined with ferric complexes may be responsible for the blooms that occur following overturn in deeper lakes. In a study of a small artificial pond, chrysomonads were not found when the water contained more than 0.02 mg/l phosphate but cryptomonads increased at this concentration (2185). Inorganic nitrogen stimulated several green algal species but did not affect Eugleninae. Similarly, green algae development was related to phosphate concentration, but Eugleninae were not affected. Organisms of questionable algal status, Cyanophyceae and Cryptomonadinae, reacted similarly to Eugleninae. Algae can absorb and concentrate radioactive substances from water. For various concentration factors, see Chapter VIII, Radioactivity.

An Israeli study of fishpond phytoplankton showed that, although blooms of blue-green algae occurred in all ponds, diatoms predominated in ponds of high salinity, green algae in ponds of low salinity. Organic manure was an effective source of nutrients over a long period; chemical fertilizers had more rapid but less lasting effect (2186).

Primary effluent from a small sewage treatment plant in an Ohio stream characteristically causes an increase in some green flagellates, Euglenophyceae, and the disappearance of yellow-brown flagellates, Chrysophyceae (2520).

Temperature effects in relation to algae have been the subject of several papers. Release of cold impounded water affects stream biota. Plant populations in some tailwater areas is dominated entirely by algae (2187). Elevated temperatures of stream water of 2.8°-5.5°C caused no significant increase in plankton, but differences in fish populations were noted (2188). However, another report on a stream subject only to temperature pollution indicated that blue-green algae became more abundant, green algae less abundant. Marked irregularities in the behavior of the periphyton population not related to temperature were believed due to chlorination of condenser water. Hale (422) reports that blue-greens favor 15°-27°C, green algae 10°-21°C, and yellow-green algae 4°-10°C (2189). Cairns cites the best growth temperature of blue-green algae as 30-40°C, and green algae 25-35°C. He also notes that in mixed populations from a normal healthy stream, a large number of species of diatoms were present at 20°C, dominating the culture. But at 30°C, only two species of diatoms were numerous and blue-green algae predominated (2190). Ingram and Towne (2197) state that high temperature may alter the entire biosphere, eliminating many organisms and allow-

ing a few heat resistant blue-green algae to predominate, thereby giving rise to unfavorable stream conditions. Early seasonal development of *Cladophora glomerata* owing to warmer water temperatures occurred on the Tween and Teviot Rivers in England (2181). Some green algae have adapted to extremes of temperature. *Protococcus nivalis* is found in arctic regions, and *Tribonema* has been noted in water at 45°C. *Phormidium,* a blue-green alga, is heat tolerant (2183). *Anabaena* and *Coelosphaerium* seldom give any trouble unless water temperatures exceed 21°C. *Aphanizomenon* apparently prefers colder water than most Cyanophyceae. It is present in some ponds even when the surface is frozen (16). In a discussion of temperature, light, and nutrient affects on the green alga *Chlamydomonas reinhardi* Dangeard, McCombie (2191) states that temperature acts as a typical controlling factor governing the rate of growth by determining the rate of metabolism. The concentration of nutrient salt shifts the temperature optimum for growth. According to Smith (2192), temperature rarely plays a direct role in acclimatization of algae, but has an important effect on rate of growth. However, it can be a limiting factor as is shown by cases where tropical algae have not been able to survive overwintering conditions.

Fichter (2193) reports that plankton were 13 times more abundant in clear, and 1.5 times more abundant in moderately turbid water than in muddy Oklahoma ponds. Algae were significantly fewer in samples from silted zones of the Truckee River in California (2194). Nigrosine dye was used in Arizona to limit light intensity for aquatic weed control. This change was reflected in a lower bottom temperature and a temporary increase in plankton (2195). Neel, however, reports that in observations made on the Missouri River after the reservoir system went into effect, algae began active growth soon after the water cleared. Attached filamentous forms, previously absent, became common. Algae concentrations in the nuisance range caused tastes and odors at Yankton, S.D., and extended all the way to Omaha (2169). Light exclusion is one of the most important effects of suspended matter on algae, for it interferes with their photosynthetic action, depriving them of active growth (2196).

In a study of productivity of British Columbia lakes, it was stated that the concentration of total dissolved solids was the most important factor (2198), although this factor could not be used to predict the numbers of plankton, bottom fauna, nor fish. Casey and Webb (2199) observed a fair correlation between total dissolved solids and amount of plankton, noting that greatest plankton populations were present in lakes having total dissolved solids over 100 mg/l. Smith (2192) states that algae of soft-water regions are largely desmids and certain species of Chrysophyceae, Myxophyceae, and Chlorophyceae, that pH of water is limiting, and that Birge and Juday related the greater abundance of algae in hard-water lakes as compared to soft-water lakes to the bicarbonate content.

Truly halophilic algae have been found that will develop in concentrations of sodium chloride greater than three molar (2192). Yet, the alga responsible for red patches on fresh-snow surfaces resides in near-distilled water (2200). Studies are underway on the feasibility of using algae that concentrate sodium chloride as a means of desalting sea water (2201). This study has revealed that both fresh and marine algae have surprisingly wide salinity tolerances. Fresh water species grew well in 50 percent sea water whereas marine species grew well in 25 percent sea water.

In studies of nitrogen and phosphorus removal from sewage plant effluents with *Chlorella* and *Scenedesmus,* the removal of phosphorus was never complete but continued long after the absorption of nitrogen had ceased (2204). The complex phosphate additives of synthetic detergents (2205) undergo a slow hydrolysis that tends to yield a continuously renewed supply of available phosphate for algal nutrition and prevents phosphate concentrations in streams from reaching low levels (2172).

Negative relationships of algae to some environmental factors are not lacking in the literature. A thesis noted that no correlations could be made between phytoplankton numbers and changes in the chemical environment in a study of 18 consecutive months duration (2206). No relation between phytoplankton pulses and concentrations of bound phosphorus were observed on four ponds in North Carolina (2207). Lund (2208) states that phosphate shows no variation related to algal production, but nevertheless, addition of phosphate is often followed by an increase in algae. He then describes avenues by which phosphate may be secondarily involved in such blooms. Lund also cites similar arguments with respect to nitrogen and other essential nutrients.

Gerloff and Skoog (2209) note that *Microcystis* can store nitrogen and phosphorus in excess of its needs. Hence, the amount of nitrogen and phosphorus in the water may be a poor measure of the alga's reproduction capacity. This factor also casts doubt upon the effectiveness of phosphorus removal processes for sewage treatment plant effluents to limit algal blooms (2209). Critical levels—the minimum cell contents required for maximum growth—of nitrogen and phosphorus are to some extent a function of the carbohydrate content of the cells (*Microcystis aeruginosa*) (2210). Sodium is required for the normal growth of *M. aeruginosa.* Another effect of sodium is to reverse the inhibitory effects of excess levels of potassium. Concentrations of manganese that are toxic in synthetic culture media were found to be non-inhibitory in lake water owing to antagonistic effects of calcium (2211). Critical levels for maximum growth for iron and manganese were 100 and 4 mg/l respectively (2212). Waters low in calcium but high in nitrogen are likely to develop large numbers of blue-green algae (2213).

Some algae are believed capable of excreting substances toxic to bacteria (2012, 2214, 2215, 2216, 2217, 2218) or other algae (2219, 2220). Coliforms have been reported to be suppressed by algal growth (99). A bacterial contaminant has also been mentioned in connection with a slow-death factor in cultures of *Microcystis aeruginosa* (2219). The blue-green alga *Anabaena cylindrica,* as well as other species, excretes a polypeptide that can form complexes with other ions including copper, zinc, ferric iron, phosphate, and certain organic substances. This phenomenon may be important in making phosphates available to algae and in making toxic

ions less toxic (2222). For an excellent summary of algal nutrition see Provasoli's review (2202).

The blue-green alga *Oscillatoria rubescens* is reported to be a notorious indicator of pollution in many lakes (2133, 3351). Lackey cautions investigators in the use of the terminology "characteristic of" and "indicative of" as applied to algae and organic enrichment. He concludes that the indicator value of the algae is still not clear (2230). However, Bucka reports studies that successfully correlated phytoplanktonic flora with the trophic classification system (2521). Palmer (2223) writes that a particular species or genera is not a reliable indicator of pollution, but when the whole community of algae is considered, the group may be reliable—even to the extent of indicating the degree of purification that has occurred. An interesting study of marine plankton as indicators of pollution has been published in Japan (2224). In this work B.I.P. is defined as 100 $\frac{B}{A+B}$, where A is the number of chlorophyll-containing organisms in a volume of water, and B is the number of organisms containing no chlorophyll. Highly polluted sea water gives a B.I.P. of over 80; slightly polluted water of 51 to 80; normal water of 21 to 50; and clear water less than 21. For a discussion of algae in the recovery of polluted streams, see Bartsch and Ingram (2143).

The decomposition of organic matter and the drainage from rich farmlands serve to stimulate algal blooms (16, 346, 380, 381, 382, 1992). Pollution from a duck farm entering an estuary favored the growth of *Nannochloris* and *Stichococcus* over native estuarine phytoplankton. However, low salinities are also mentioned as a possible mechanism (2225).

An important chemical effect of algae is the removal of carbon dioxide from the water during daylight hours. *Chlorella* metabolizes as a phototrophic organism in the light, but in the dark it obtains energy from reduced carbon compounds, thereby competing with bacteria for food. The Euglenophyceae do likewise (2012). The daytime process brings about an alteration in the relative amounts of soluble (unbound) carbonic acid, intermediately soluble (half bound) bicarbonates, and the nearly insoluble (bound) monocarbonates, often causing precipitation of some of the latter. All of this produces a decrease in total hardness (2166, 2226). Vigorous growths have been known to reduce hardness by as much as one-third. These activities also result in pH variations (2126, 2181, 2226, 2227, 2228), the pH increasing in the daylight, decreasing at night. These extremes have been recorded as high as 10.5 at 4 p.m. and 7.8 at night (2229).

A most important action of algae, of course, is its effect on dissolved oxygen (2126). Indeed, Hull concludes that during the critical warm months the amount of photosynthetic oxygenation is relatively more important than atmospheric oxygenation in Baltimore Harbor (2522). Heavy crops may produce oxygen supersaturation in the daylight and depletion at night (2012). This metabolic oxygen depletion can result in fish kills (2183). The highest figure noted in the literature was 300 percent supersaturation (418). A report from the Tweed River Purification Board in England notes repeated daytime supersaturation with oxygen to the extent of 150-200 percent, accompanied by night values of 50 percent —or even less in the vicinity of sewage outfalls (2181). A net photosynthetic gain of 1.7 mg/l of dissolved oxygen per mile of river has been observed, the figure being a daily average (not just daytime changes). Bottle tests show that oxygen production exceeds oxygen utilization in BOD reactions, resulting in a net gain (1992). Westlake also notes that pH and dissolved oxygen can be raised to levels that may be detrimental to the blooms that created the conditions. *Cladophora* is cited as an example of species capable of adding considerable dissolved oxygen to a stream (2126).

Phenol has been reported as coming from algal blooms (2232). Algae may interfere with disinfection (2227, 2234), coagulation (2166), and filtration (2227, 2234). They add a very large organic burden in heavy concentrations (1992), but in lower concentrations may be an asset rather than a liability (2166). When masses of algae die, dissolved oxygen is depleted faster than it can be absorbed from the air (2226). The electrokinetic (zeta) potential of algae has been studied (2234). Under all conditions studied, 14 genera of naturally occurring fresh-water algae and 8 genera of artificially cultured algae exhibited a negative electric charge.

Algae have been implicated in the disintegration of submerged concrete blocks (2233), and in corrosion (2227) of metal sedimentation tanks (397, 399, 400, 401, 2166). The Department of Agriculture and the Bureau of Reclamation jointly studied the biological corrosion of concrete (2235). Algae, lichens, and fungi were involved in studies over a five-year period. When the sulfite content of the mortar was 0.6 percent or greater, pitting by the plants was very pronounced. Where the sulfite content was only 0.2 percent, pitting was much reduced. The report suggests that gelatin present in the plants plus their organic acids, viz., carbonic, oxalic, and salicic acids, corroded the surfaces with plants growing on them when sulfite content was high.

Lackey (2012) recognizes three benefits of algae: (1) reoxygenation; (2) mineralization; and (3) production of a food chain; and three ills: (1) algal toxicity; (2) esthetic harm, including tastes and odors; and (3) buildup of a BOD. Also, many blue-green algae produce mucilaginous envelopes that harbor bacteria and may protect coliforms and possibly pathogens such as *Salmonella* from chlorination.

2. Cross References. Protozoa, Oxygen, Ammonia, Nitrates, Phosphates, Polioviruses, Diatoms, Marine Flagellates, Coliform Bacteria, Salmonella, Actinomycetes, Gastroenteritis Organisms, Bacteria-General, Pathogenic Bacteria, Settleable Solids, Nitrogen, Weeds, and Chapters VII, IX, and X.

3. Effects on Beneficial Uses.

a. Domestic Water Supplies. Algae have been frequently cited in the literature as responsible for tastes and odors in water supplies (16, 210, 369, 383, 384, 385, 386, 422, 428, 429, 430, 738, 1573, 1755, 2058, 2164, 2166, 2169, 2182, 2189, 2196, 2213, 2221, 2226, 2234, 2236, 2237, 2238, 2239, 2241, 2242, 2243, 2244, 2245, 2246, 2247, 2248, 2249, 2250, 2251, 2252, 2253, 2254, 2523,

2524). The reader is referred to Palmer's book "Algae in Water Supplies" (2166), which gives algae species involved, type of taste or odor produced, and color illustrations of selected species. Taste- and odor-producing algae are said to be as common to rivers as to hard-water lakes and reservoirs (2221). At one time, according to Fox (2250), odors in water from organisms were believed to be due only to microbial decay. Decomposition products from organic matter, including dead microorganisms, were listed as among the most significant causes of tastes and odors in water (2213). Now it is well known that many living organisms through their metabolic products impart an odor to water (2213, 2250). Palmer notes that sometimes certain types of algae are responsible for tastes and odors in small or moderate concentration, citing *Synura* and *Euglena* in the State of Texas. When tastes and odors result from the decomposing of large quantities of algal material, the algae are likely—in the south-central states—to be a limited genera of blue-greens during the summer months, and a mixed growth of greens and flagellates in the spring and fall (2213).

Although it is difficult to establish limiting or threshold values for algae, the Metropolitan Water Commission of Boston sets the following allowable limits at which treatment is begun (2255):

Organism	*Standard Units per ml*
Aphanizomenon	1000
Anabaena	600
Cryptomonas	200
Chlamydomonas	10
Dinobryon	500
Synura	200
Ureglenopsis	200

Fox (2250) and Swartz (2189) report that low (500-1000 Standard Areal Units/ml) concentrations of blue-green algae cause a grassy or moldy odor, while at higher concentrations a pigpen odor is evident. The green algae produce a grassy or nasturtium odor at high (2000 units/ml) concentration, and the yellow-green algae can cause noticeable odor even at low 100-500 units/ml) concentrations.

Palmer and Poston (2247) report that as few as five colonies of *Synura* per ml can cause taste and odor trouble. See Palmer's book (2166) for more complete information. Ives (2248), in a discussion on the effects of algae in water, reports that the symbiotic relationship between *Streptomyces* (an actinomycete) and *Cladophora* causes tastes and odors in water. In studies on *Oscillatoria,* Russian workers reported that *O. splendida* gave an odor when the cells were damaged mechanically, dried, or frozen, and also when there was insufficient nitrate. *O. agarhdii* gave an odor under unfavorable environmental conditions, including an accumulation of wastes, insufficient nitrogen supply, lack of ferrous iron, etc. The acrid taste imparted by this organism reportedly disappears in the presence of added nitrate and an organic carbon source (2256). In 1953, Silvey published his work on the algae-actinomycetes relationship and the production of tastes and odors in water (1573).

Algae growths can impart color or turbidity to water (369, 2164, 2182, 2213, 2226, 2249). Reservoirs in Glendale, Pasadena, and Los Angeles, California, have had to be roofed because of public complaint about green water (2257).

Algal contamination of drinking water has been suspected as a factor in outbreaks of gastroenteritis of unknown etiology (390, 424, 2166, 2429, 2523). Moreover, the accumulation of dead and dying plankton in filter sand offers an excellent substrate for the growth of such bacteria as *Pseudomonas.* Because some species of *Pseudomonas* are concerned with gastroenteric disturbances in man, algae may be indirectly connected with waterborne gastroenteritis (2182). Also, two cases of allergy to blue-greens of the subgroup Oscillatoriaceae (genera not mentioned) have been reported by Heise (2258). Wheeler, et al. (391) have pointed out that algal poisons usually act on the central nervous system and skin rather than on the gastrointestinal tract; on the other hand, Steyn, of South Africa, believes that algal poison is one of the most virulent poisons known to the science of toxicology and is capable of producing a "mysterious" cirrhosis of the liver and/or of reducing the resistance to disease of many human beings (381, 391, 392, 393, 406). The possible relationships of such compounds to cancer and geriatric disorders are currently under study (2523).

Algae may interfere with water treatment processes, including rapid sand filtration and disinfection (210, 369, 385, 395, 396, 397, 398, 2164, 2165, 2166, 2182, 2226, 2234, 2239, 2248, 2251, 2254, 2259, 2260). Long filamentous organisms such as *Oscillatoria* are more effective in clogging filters than shorter or nearly spherical organisms (2254). Plankton such as *Cryptomonas* and *Euglena* disintegrate readily on coagulation and filtration and do not clog the filters (2182). At Chicago, plankton counts of 60,500 per square foot on filter surfaces resulted in 41-hour runs and 4,572,700 in 3.5-hour runs. In another case, water became impossible to filter owing to a massive crop of *Ceratium hirundinella,* the numbers of which exceeded 10,000 per ml (2261). Records of plankton counts as high as 100,000 per ml have been obtained on Indiana's White River (2182). Burnson (2253), however, considered filters to be more efficient in the summer when microscopic plant and animal life are abundant than in the winter.

In studying tastes due to *Oscillatoria,* Uspenskaya (2239) noted that their growth varied with the organic content of the bottom muds, and that the more oxidizable the mud, the stronger was the taste imparted to the water. To avoid such tastes, reservoir mud should not contain over one percent nitrogen.

The increased organic content in water resulting from algae may serve as nutrient for organisms in the distribution system. Algae also reduce the useful capacity of reservoirs by concentrating at certain depths in the water or along the shallow margins or bottom (2226, 2238, 2249). Other problems caused by algae in domestic water supply include clogging of intake screens and reduced pipeflow (2166, 2213, 2262).

b. Industrial Water Supplies. Algae are undesirable in water for a variety of industrial uses, including butadiene manufacture (251); condensers, cooling towers, and sprayers (208, 210, 2263); paper manufacture (402, 2524); laundries, dyeing, photography, and chemical in-

dustries (369, 385); beverage bottling (403, 404); and rayon processing (405).

A heavy growth of green algae in an impounding reservoir resulted in the incrustation of pipe lines and the failure of pumps (2264). The incrustation was largely calcium carbonate caused by the algae.

c. Irrigation Waters. Interference by algal growth in irrigation distribution systems has been the only information published linking algae with this beneficial use of water (2213, 2226, 2265, 2525). *Cladophora* and *Rhizoclonium* are by far the most important algae in this respect (2265).

d. Stock and Wildlife Watering. From many different parts of the world, including the United States, there have been reports of rapid deaths of a great variety of animals after drinking water containing high concentrations of blue-green algae, such as *Anacystis, Aphanizomenon, Nostoc rivulare, Nodularia, Gloeotrichia, Gomphosphaeria,* and *Anabaena.* The first recorded occurrence of a waterbloom toxic to domestic animals is attributed to Francis in Australia (2266). The organism responsible was *Nodularia spumigena.* Fresh algae have been reported to be more toxic than decaying algae. Fatal poisoning has occurred among cattle, pigs, sheep, dogs, horses, turkeys, ducks, geese, and chickens, and also among experimental animals, such as rabbits, rats, guinea pigs, and mice. It is believed that such algae may be toxic to all warm-blooded animals. Algae poisoning is considered a menace to migratory waterfowl, and has been suspected as a cause of mass deaths of ducks. Fatal doses can be very small, of the order of a few milliliters of water suspension taken orally, but animals vary in their susceptibility. Waterbloom poisoning is reported to be very similar to shellfish poisoning (2429).

At least two poisons appear to be involved, and efforts are being made to isolate and identify them. One affects the nervous system and liver; another, the algal pigment phycocan, becomes concentrated in the skin, where it absorbs ultra-violet light, causing burns, sores, and possibly death (381, 391, 395, 406, 407, 408, 409, 410, 411, 1454, 1455, 1456, 2012, 2166, 2213, 2267, 2268, 2269, 2270, 2271, 2272, 2273, 2274, 2275, 2276, 2277, 2278).

An *Anacystis* toxin has been isolated and identified as an alkaloid (2278). A *Nostoc rivulare* toxin administered to mice in food and drink gave the same results as intraperitoneal injections. Heat-toxicity studies of *N. rivulare* suspensions showed that autoclaving destroyed toxicity, and the minimum lethal dose to mice is 0.0933 mg of algae per gram of body weight. The toxic factor is soluble in ethanol, and affects the neuromuscular and respiratory systems (2277).

Samples of blue-green algae were randomly collected at different times from two Canadian lakes. Present were *Anacystis aeruginosa, Coelosphaerium, Anabaena, Aphanizomenon, Gloeotrichia,* and *Lyngliya.* These were injected into mice for different incubation periods. All samples produced symptoms of toxicity. Fresh material had no lethal effect. Toxicity increased to a maximum after 18-26.5 hours incubation. Incubation for longer periods reduced toxicity. The toxicity of one sample was markedly reduced by storage at 3°C for 18 days, followed by incubation at 27°C for 18 hours. The authors conclude that toxicity develops only gradually in blooms, and decreases again as the algae decompose (2276).

Aphanizomenon, Anabaena, and *Anacystis* blooms were found in water samples taken from a Canadian lake. Animals had reportedly died very shortly after drinking water from this lake. The relative numbers of the three algae were 99 : 0.9 : 0.1 respectively. The mixture killed laboratory mice and rats within 20 hours. The filtrate was not lethal. The residue, resuspended in distilled water, was toxic. Chromatographs of acetone and ether extracts yielded definite green, yellow, and brown bands, but the toxic material was not isolated and identified. Although it is not known which species was more important, it is believed that *Aphanizomenon* (flos-aquae) would be the more important because of its numerical predominance (2267).

e. Fish and Other Aquatic Life. Algae can be severe pollutants to fish in two respects: (a) they can cause heavy fish mortality through direct poisoning or (b) they can be responsible for oxygen imbalance, thereby killing fish through oxygen depletion or oxygen supersaturation of the waters (373, 410, 412, 413, 414, 415, 416, 418, 427, 2012, 2269, 2279, 2280, 2281, 2282, 2283, 2524).

It has been suggested that possibly Haff's disease (acute attacks involving muscle pain, vomiting, etc.), a European disease always associated with consumption of fish or fish liver, may be the result of absorption by the fish of toxic substances from algal-infested water (406).

A fresh-water dinoflagellate, identified as either a *Gymnodinium* or *Glenodinium,* is believed to be responsible for the first recorded mass death of fish (plankton-feeding shad) in fresh water. The water reportedly had a blood-red color (2213).

Prescott found that fish introduced into a water containing decaying blue-green algae died within two hours. An analysis showed that both hydroxylamine and hydrogen sulfide were present in sufficient concentrations to kill fish (2274).

Dead algae have caused the death of fish by clogging their gills (419, 2495), and by accumulating in a mat on the surface so dense as to prevent a barrier to the penetration of oxygen under the algae (2166).

Anacystis (formerly *Microcystis*) suspensions and extracts were tested on frogs and carp (2526). The toxin was found to be soluble in water, 96 percent ethanol, and methanol, but insoluble in chloroform, ether, acetone, and absolute alcohol. In neutral solution it was stable to heat at 120°C for 30 minutes, but was readily destroyed by alkali. The minimum lethal dose of dried *Anacystis* for carp weighing 8-10 gms was 1-2.5 mg; 60-80 gms, 5-10 mg; and 300 to 500 gms, 25-50 mg.

Fish poisoned by algae and in turn consumed by grebes have been assumed to be responsible for the death of grebes (2285). Algae growths reportedly foster increased growth of insect larvae and water fleas in ponds and reservoirs (388, 389, 2213). Bottom fauna have been seriously affected by the smothering effect of thick growths of algae. Welch reports the physical effects of dense growths of the green algae *Cladophora* as possibly inhibiting the supply of desirable fish-food organisms (2286).

Oscillatoria in water can give fish a muddy taste (414). Cyanogen compounds from blue-green algae have been considered as being involved in fish deaths (413).

Chlorella effected a change in the pumping rate of oysters at concentrations greater than $2x10^5$ cells per ml, or aproximately 15 mg/l (2985). Another report cites $2x10^6$ cells per ml (1448), or 3000 *Euglena* per ml.

Poisonous plankton organisms consumed by mussels are the cause of paralytic shell-fish poisoning in humans (421). However, the mussel is apparently not readily harmed by the algae or their toxins. See Marine Flagellates and Chapter V—Shellfish Culture.

f. Recreational Use. Excessive growths of algae destroy recreational and esthetic values of lakes, and also cause accidents (210, 369, 378, 395, 2012, 2262, 2524). The filamentous marine alga, *Lyngbya majuscula*, was believed to have been responsible for numerous cases of dermatitis in beach areas of Hawaii (2287). Although the toxin's chemical nature is not known, it was found to be fairly stable.

Algae can be an esthetic nuisance in another way. Wave action may concentrate a large mass of algae on shore where, if not removed immediately, decomposition will cause the usual annoyances of septic odors (2288).

APC VIRUSES

(see Adenoviruses)

BACTERIA, GENERAL, SAPROPHYTIC

1. General. Although a few bacteria, viz, the pathogens, are inimical to man's welfare, the vast majority of bacteria are beneficial. Together with the molds and yeasts, they are responsible for the dissolution of organic matter and for the concentration of vital elements without which the green plants, and hence man himself, could not survive (2289). The dissolution of organic matter is the prime role of bacteria in the self-purification of streams. Secondarily, they also serve as food for protozoa and other aquatic animals, and therefore are the very foundation or beginning of the food chain that terminates in man.

Although bacteria are unicellular, they may occur singularly or in aggregates. Each cell in an aggregate or colony is physiologically independent. Nevertheless, it is influenced by the environmental changes effected by its neighboring cells. The study of bacterial associations and antagonisms is a fascinating and difficult area. Weindling (2152) listed some of the antagonistic factors, including competition, inhibition, antibiosis, parasitism, and predation (all of which may affect other life forms) (2219), and also some associated effects, including balanced growth, succession, stimulation, and symbiosis. The various associations and antagonisms are important factors in studies of viability of pathogens.

Bacteria have an extraordinarily broad range of biochemical activity. They perform a major role in the life processes on earth by concentrating the elements essential to life itself for use by other species and other forms. The use of radioactive isotopes in bacterial metabolic studies is yielding enlightening information regarding this complex science. The reader is referred to Chapter VIII for some information on concentration factors of certain elements by bacterial species.

Bacteria also make available again essential nutritional factors that have been stored until death in the bodies of plants and animals. Because of this role, they are the workhorses of biological sewage treatment plants and of the natural purification phenomena of lakes and streams (2144).

Insofar as water quality is concerned, bacterial pollution can be divided into two types: (1) primary pollution, which is manifest by the direct introduction of pathogens or other organisms into a body of water, and (2) corollary pollution, in which the naturally occurring aquatic bacterial saprophytes proliferate, thereby becoming pollutants because of:

a. The introduction of toxic chemicals which may cause bacterial increases by providing additional food substrates in the form of dead plants and/or animals.

b. The introduction of certain materials and chemicals, such as organic matter and phosphorus (432), which results directly in their extreme proliferation.

c. The introduction of material, such as an oxygen-demanding waste, that will alter the properties of the stream thereby enabling less desirable bacterial species to proliferate.

Bacteria, in addition to being categorized as pathogenic or saprophytic, are also grouped on the basis of their reaction to the presence of nascent oxygen in the immediate environment. Those that require oxygen are aerobic, those to which oxygen is toxic are anaerobic. Organisms that can adapt to either environment are facultative. This grouping is of importance to the water pollution worker. A stream or body of water into which wastes are discharged must remain aerobic for reasons other than appearance, smell, corrosivity, effect on fish and aquatic life, etc., since the aerobic stabilization of organic matter proceeds at a more rapid rate than does anaerobic stabilization.

The use of monomolecular films of hexadecanol to retard evaporation has led to the finding that saprophytic bacteria of the genera *Pseudomonas*, *Flavobacterium*, and *Alcaligenes* attack these films. The incorporation of a bactericide in the film in order that the film may last longer has been suggested (2290). In one lake studied, *Pseudomonas* and *Alcaligenes* species increased in numbers but the extent of the increase was not considered sufficient to affect water quality adversely (2291, 2292, 2293). The rate at which hexadecanol films are consumed by bacteria at 26°C increases geometrically with three-week intervals of time. When the film is removed, the numbers of organisms drop off again, this period also being about three weeks. *Pseudomonas* and *Alcaligenes* consume most monomolecular films at the rate of 0.03 to 0.4 lb/acre/day based on laboratory work (2294), a more median rate appearing to be 0.2 to 0.3 lb/acre/day.

Dratchev et al. (2295) studied the distribution of saprophytes in water and foam. They found that the surface film of water has 2 to 10 times, and foams have 100 to 1000 times, the bacterial concentration as the water below.

In lake sediment studies, no significant reduction in bacterial numbers with depth was found in the top five cm. Below this, however, a gradual reduction occurs (2296).

Rigler (2140) noted that turnover of phosphate under natural conditions appears to be caused by aquatic bacteria, and that bacteria may compete with algae for the available inorganic phosphate. Watt (2141) added an antibiotic and found that the increase in phosphate turnover time was accompanied by an increase in radioactive phosphorus accumulated in algal tissues. This finding suggests that the aquatic bacteria take up dissolved inorganic phosphorus and release it again in an organic form not immediately available to the alga.

Bacteria that produce vitamin B-12 have been mentioned as a possible triggering mechanism in the blooms of planktonic organisms such as the "red tide" (2297). *Bacillus subtilis* and *Proteus vulgaris* are reported to deactivate rapidly the toxin of the phytoflagellate *Prymnesium parvum* (2298).

The bifid bacteria, *Lactobacillus bifidus,* were investigated as an indicator of fecal pollution. Bifid bacteria are 10 to 100 times more numerous in the feces of man than the coliforms and enterococci. At 4°-6°C, fecal suspensions inoculated with the three types of organisms showed the enterococci to have the highest survival. At 10°-20°C, for the first two days, coliforms and enterococci multiplied, but the bifid organisms dropped in numbers. The third day the coliforms and bifids both dropped at the same rate, whereas the rate of decline of enterococci was lower. The investigators said that water composition and size of inoculum did not influence the survival tests (2300).

Four types of bacteria, *Serratia marcesans,* a variety of *Streptococcus faecalis* (*S. zymogenes*), *Chromobacterium violaceum,* and two strains of *Bacillus globigii* were tested as ground-water tracers. *S. zymogenes* was found most suitable, being isolated over 50 feet away from the dosing well. It was also found to survive over a year in dialyzing sacs, although the number dropped from 150×10^6 to 5×10^3 per ml (2151).

Spores of the nonpathogenic (to man) *Bacillus thuringiensis* are utilized as a living pesticide that is known as Thuricide (2299, 2527) (see also Chapter IX, Pesticides).

Joseph (2301) made a microbiological study of acid mine drainage streams with pH of 2.0 to 2.9. There was a clear paucity of numbers of bacteria. However, the genera *Escherichia, Aerobacter, Bacillus, Micrococcus, Sarcina, Thiobacillus, Crenothrix,* and *Microsporium* were identified.

2. Cross References. Pathogenic Bacteria, Coliform Bacteria, Iron Bacteria, Sulfur Bacteria, Slime Bacteria, Fecal Streptococci, Algae, Protozoa, Insects, Crustaceae, Marine Flagellates, and Chapters V, VIII, and IX.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Bacteria antagonistic to *E. coli* have been isolated from surface waters. They include *Micrococcus, Sarcina, Pseudomonas, Flavobacterium,* and others. Their presence serves to reduce the numbers of *E. coli* (435). *Pseudomonas,* of which most species are not pathogenic, is sometimes associated with gastroenteritis. Two papers have been published urging that *P. aeruginosa* be included in the sanitary examination of drinking water (2302, 2303). Other saprophytic bacteria such as *Pseudomonas, Flavobacterium,* and *Chromobacterium* can discolor water supplies, but otherwise they are generally not harmful. Bacterial growths can also impart tastes and odors to water either because of waste products of their own metabolism or by transformation of sulfate, proteins, or other chemicals (152).

Many surface and ground waters are said to contain sufficient organic matter to support abundant growths in filters. Such growths have been mentioned as a principal cause of caking and short circuiting (2304). The accumulation of dead and dying plankton in filter sand is said to offer an excellent substrate for the growth of such forms as *Pseudomonas.* Hence, because some members of this genus may be associated with gastroenteritis (2305), rich plankton growth may be indirectly connected with intestinal disturbance in man (2182). *Proteus* has been mentioned as a possible pathogen (2305).

Russian workers, in a report on microorganisms causing the appearance and disappearance of tastes and odors in water, report that the odorous gas produced by *Actinomyces* can be used as a carbon source by other soil and water organisms. The periodic disappearance of odor from water was attributed to these forms, all of which were *Pseudomonas* strains (2256) (see also Actinomycetes).

In water below a thermocline, the dissolved CO_2 resulting from decomposition processes can redissolve settling, precipitated normal carbonates, thereby producing an increase in hardness (2304).

b. Industrial Water Supplies. Growth of bacteria in water may interfere with many industrial processes such as the manufacturing of leather products (254), paper (212, 436), textiles (212, 405, 450), breweries (170, 171, 175, 449, 2308) and food processing (179, 223, 224, 437).

Iron, manganese, nitrogen, and sulfur bacteria are a nuisance in waterworks, boilers, and many other industries because of their corrosive action on metals, concrete, and rubber (198, 208, 438, 440, 442, 443, 444, 448, 449, 1050) (see also Iron Bacteria and Sulfur Bacteria).

A *Pseudomonas* infestation of nuclear reactor cooling water has been reported (2309). Concentrations of 10^8 organisms per ml were thriving on the carbon and nitrogen released by the disintegrating resin of the deionizers used in treating the water.

In an investigation of organic fouling of anion exchange resins, Frisch and Kunin (2310) inoculated such resins with various water microorganisms in trace salt solutions but found the rate of attack to be negligible.

The fall in pH of cooling water has been reported as due to the formation of nitrous and nitric acids from ammonia through the action of nitrosofying and nitrifying bacteria (2311).

c. Irrigation Waters. Although saprophytic bacteria play an essential role in the growth of all plants, no publications pertaining to the effects upon irrigation farming of any sewage saprophytes in polluted waters have been encountered. Most such work appears to be concerned with the ultimate fate of pathogens. Biological growths in soil are a factor that influence soil permeability, hence rate of percolation (2312).

d. Stock and Wildlife Watering. No papers dealing with the effects of saprophytic bacterial pollutants of water upon animal watering were found. However, in eggs experimentally infected with *Pseudomonas,* subsequent washing of the eggs in waters containing one to

five mg/l of iron may introduce iron into the egg in sufficient quantity to increase the rate of spoilage (2306).

e. Fish and Aquatic Life. Occasionally bacteria that are ordinarily considered saprophytic have infected fish and caused disease (445, 446). Oxygen utilized by bacteria during decomposition may significantly deplete dissolved oxygen and result in fish kills, especially in winter with an ice cover (447). Sewage decomposition by bacteria stimulates development of plant growth (such as algae) increasing the supply of fish-food organisms (367). *Bacillus subtilis* was preferred by ciliates as a food over other bacteria tested (see Protozoa) (2313).

f. Recreational Use. *Alcaligenes faecalis* is reported to have been found in a swimming pool where it was highly resistant to halogen disinfectants. This nonpathogen is widely distributed in decomposing organic matter and has no significance in the sanitary analysis of swimming water (2315).

Levine et al. (2316) suggest that *Streptococcus salivarius, Neisseria catarrhalis,* and *N. sicca* (also *Staphylococcus aureus*—see Pathogenic Bacteria) are worthy of consideration as indicator organisms for swimming pools.

A report to the British Ministry of Health in 1953 recommended that 75 percent of 37°C total plate counts should not exceed 10 colonies and that the remainder should not exceed 100 colonies (2317). The same report found that *Neisseria catarrhalis* was seldom isolated where free chlorine residuals exceeded 0.1 mg/l. Alpha-hemolytic streptococci and staphylococci were too resistant to chlorine to be suitable indicators.

Russian standards for swimming pool waters include a total bacterial count requirement of less than 1000/ml (2592).

BACTERIOPHAGES

(see also Viruses and Coliform Bacteria)

Bacteriophages are submicroscopic parasites of bacterial cells. They are highly host specific, and this property is used in the laboratory to differentiate between various strains of the same bacterial species. Specific bacteriophages active against many pathogenic bacteria have been found in sewage-polluted waters. This relationship has prompted a number of investigations to explore the use of phage as an indicator of pollution. Results show that bacteriophage counts cannot be correlated with water potability (456), nor with *Escherichia coli* content (coliphage not studied) (2318). An interesting observation has been reported from Europe where, in polluted stream stretches, the Vi-phage curve was similar to that of chemical and bacterial criteria of pollution (2319). One report mentions agreement between coliform counts and salmonella phage content (2320). Four reports cite the insignificant role played by bacteriophage in the reduction of bacterial numbers or in the purification process (451, 453, 455, and 740).

With respect to bacteriophages in sea water, Guelin has published two papers, both of which concluded that a direct relation exists between the amount of bacteriophage and numbers of bacteria, hence fecal pollution of the sea (452, 2321). A recent study by Carlucci and Pramer, however, in which phages active against *E. coli, Aerobacter aerogenes,* and *Serratia marinorubra* were isolated without difficulty with suitable enrichment procedures, observed that the effectiveness of coliphages was dependent on the presence in sea water of sufficient nutrients to support growth and multiplication of host cells (2156). The same workers reported that coliphages were inactivated rapidly in natural sea water but persisted in filter and heat-sterilized sea water. Diernert (454) in 1944 stated that a bathing water was usually satisfactory if no bacteriophages were present in 20 ml of the water. Gilcreas and Kelly report that at 60°C, phage is more resistant than either animal viruses or coliforms. Differences in survival rate between coliforms and phage is dependent on temperature rather than upon response to pH levels (2322).

BEAVERS

A study of beaver activities on water quality concluded that such impoundments have considerable organic matter, marked color, increased coliform and total bacteria counts, increased chlorine demand, increased water temperatures in summer months, but no apparent effect on palatability (2307, 2314).

B. COLI

(see Coliform Bacteria)

BIRDS

The Ninth Pacific Northwest Symposium on Water Pollution Research pointed out that the covering of all finished waters in the Pacific Northwest is long overdue and the problem of bacterial contamination by birds of such waters often underestimated. Five cities in Washington could not meet bacteriological standards during 1960 because of contamination by birds (2528).

CARP

One report has named carp as a pollution problem in some rivers because they tend to dominate the waters they inhabit. Since they are bottom feeders they agitate bottom muds making it more difficult for other fish to find food, and making the water unfit to drink and unpleasant for swimming (2529).

CHIRONOMIDS

(see also Insects, Mosquitoes, and Chapters VIII, IX, and X)

Chironomids have appeared frequently in the water pollution literature. They are sometimes mentioned as pests in potable supplies, sometimes related with varying types and densities of pollution, and sometimes mentioned as a part of the beneficial fauna of polluted waters. There is a question, apparently, as to their location in the scheme of biological classification (2420). However, it is the larvae of Chironomus and not the adult midge that has attracted so much attention in the water field. It is a red-colored larva commonly called a bloodworm since many species have hemoglobin in their blood (15). Since some entomologists assign them to the genus *Tendipes* in the order Diptera of Insecta, the names *Chironomus* and *Tendipes* can be used interchangeably. Unidentified or non-chironomid midges are discussed under Insects.

Chironomid infestation of potable water supplies is discussed by Ingram and Bartsch (2420) and by Palmer (2213). A Cincinnati case resulted from migration of chironomids from an uncovered clear well into the distribution system. The clear well had an accumulation of leaves and algae on the bottom, forming an ideal habitat for the bloodworms. Chironomids in a glass of drinking water are probably more revolting than nemotodes because of their red color. Bloodworms in finished water tanks have been observed in other locales also—but all were believed to originate from the direct ovipositing of eggs by the adult female midge and not by penetration of the worm or larvae through the filtering system. A very strong taste and odor from minute red worms (presumably *Chironomus*) has been reported (2530). *Chironomus* larvae are tube-building creatures, and in slow sand filters these tubes allow water to pass through upper sand layers without filtration (2420). *Chironomus* is quite sensitive to chlorinated hydrocarbon and organic phosphorous insecticides, Silvey quoting toxic values in the range of 0.006 to 0.04 mg/l. However, they are quite resistant to chlorine, Silvey noting that seven mg/l in 24 hours is necessary for destruction (2531). Ritter describes controlling a slight infestation with 100 mg/l of chlorine as hypochlorite in one year, and the following year a severe infestation with 0.01 mg/l DDT. Small amounts of chlorinated hydrocarbons reportedly prevent *Chironomus* from building tubes (0.005 mg/l BHC, 0.02 mg/l DDT) (2532). Nevertheless, Averett (2533) notes a report by Webb (2534) that chironomids were the first to reappear in a stream that had been polluted with DDT. See Chapter IX, Pesticides, for more information on specific insecticides.

Chironomids, as do all aquatic life, concentrate certain elements (2535). For various concentration factors and further information in this respect, see Chapter VIII, Radioactivity. However, *Tendipes plumosus* and *T. attenuatus* fourth instar larvae cannot be used as indicators for P-32 or F-59 because their rate of uptake is too low (2536).

Effects of *Chironomus riparius* on activated sludge that had been allowed to settle and consolidate for six months was to raise the pH value and the oxidation-reduction potential. The pH of the water above was increased, as well as the concentration of ammonia, and the concentration of oxidized nitrogen reduced (2537). Experiments showed that the addition of chironomids increased the decomposition of cellulose (2139). Midge breeding (*Chironomus novaezealandiae*) is reported to be confined to environments of low salinity (0.47 percent chloride) or fresh water, but is unrelated to pH (2538). *Chironomus* are among the most sensitive fish-food animals tested against synthetic detergents (2116). See Chapter X, Surface-Active Agents.

In Florida, the adult midge is called a "blind mosquito"—even though it doesn't bite. Overproduction of chironomids has been linked to excessive nutrients such as come from a sewage treatment plant effluent. The major chironomid pest, *Glyptotendipes paripes*, breeds on sand bottoms irrespective of depth, turbidity, pH and alkalinity, and feeds on algae non-selectively to the extent of 99 percent (2539).

The presence of certain chironomid larvae in large numbers is characteristic of grossly polluted areas (2540, 2541, 2542). Predatory chironomids such as *Anatopynia dyari* and *Pelopia stellata* have a wider range and their use as indicator organisms is limited. Four red-blooded chironomids showed a considerable difference in pollution tolerance, *C. riparius* confined to septic and recovery zones, and *Stictochironomus varius* and *Microtendipes pedellus* to clean water areas, but *C. decorus* were ubiquitous (2543). The tendipedids make their appearance in the sequence of recovery sooner than the mayflies and stoneflies (2544).

Larvae of *C. riparius* have been recorded in densities exceeding 100,000 per square meter (74). Butcher (2545) reports that organisms associated with gross pollution include "sewage fungus," tubificid worms, and *Chironomus* larvae. Sediment is least likely to bother tube-building creatures such as chironomids (2546).

The oxygen consumption rates of *C. riparius* have been studied (2547). In badly polluted waters they can lower the dissolved oxygen in a river by over 1.0 mg/l per mile at 15°C (2548) or 1.5 mg/l per mile (2126).

Bartsch and Ingram note that sludge worms such as *Tubifex* and *Limnodrilus* are found in great numbers closer to the source of pollution than any other bottom dwelling animals. In turn, they are replaced by *Chironomus*, and they by *Asellus* (2143). Since chironomids require at least a very small amount of dissolved oxygen, they will not be found in completely septic waters.

COLIFORM BACTERIA

1. General. The direct search for the presence of a specific pathogen in water is too uneconomical, slow, and unwieldy for routine control purposes. Instead, water is examined for an indication of fecal contamination, and when such indication is found, it is assumed that the water is potentially dangerous.

Several chemical and bacteriological tests for sewage pollution have been tried and found inadequate, including the total bacterial plate count on agar at 37°C. Now, however, the demonstration of the presence of coliform organisms in a water sample is regarded as evidence of such pollution and has served for many years as a basis for water-quality criteria (2549). For judicial expression in this regard, see Chapter IV.

The coliform group of organisms includes, by definition, "all aerobic and facultative anaerobic, Gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas formation within 48 hours at 35°C," and is equivalent to the *B. coli* and coli-aerogenes groups of early editions of Standard Methods for the Examination of Water and Wastewater (469). It embraces organisms of diverse origin among which the best known are (a) *Escherichia coli* strains, which are usually, but not always of fecal origin, and (b) intermediate and *Aerobacter aerogenes* strains, which are usually, but not always, of soil, vegetable, or other non-fecal origin. *E. coli* has been frequently proposed as a more specific and reliable indicator of fecal pollution than the broader coliform group (470, 471, 472). Delaney recommends thorough comparative studies between *E. coli* and coliform tests for any individual supply, and as an interim

standard in all water except drinking water suggests that the maximum limit for *E. coli* be one-half the present coliform standard (2550).

Nevertheless, there is still enough doubt as to the significance of the various types of coliform organisms that their differentiation in routine water analysis is unwarranted. At times, however, it may be advantageous or desirable to identify the organisms further as, for example, in a study of unexpected coliform densities that might be explained by the multiplication of non-fecal forms on materials such as jute packing in the distribution system (473, 2551).

The effect of the use of the coliform group instead of *E. coli* as the basis for standards is to make the water relatively safer. However, since the coliforms are only indicative of pollution rather than specific, there is some question as to whether they can stand the test of rigid court examination (481, 482). An Illinois court decision (482) held that rather than being positive proof of contamination, high counts of coliforms occurring over a long period of time without outbreaks of typhoid fever might even indicate safe water (see Chapter IV). In doubtful cases, therefore, it may be advisable to complete further tests for *E. coli* for legal protection.

Until 1936, the results of the coliform tests were expressed officially as the coliform index, or *B. coli* index, the reciprocal of the highest dilution in which a positive result was obtained when a geometric series of samples was planted. Since 1936, the number of coliforms present have been expressed generally in terms of the most probable number (MPN), a value now regarded as more precise though still subject to error (334, 469). One result of the change in terminology has been some confusion in the literature, especially engineering review articles; in many papers, also, there is insufficient comprehension of the difference between *B. coli group* (coliform organisms) and *E. coli* (152, 330, 334, 469, 715). The British use the term *B. coli* group to refer to the coliform group, of which *E. coli* is a member.

The effectiveness of the coliform standards has been demonstrated by the decrease in incidence of waterborne enteric diseases, although the standards adopted have often been criticized as arbitrary. Their use has also been justified by studies of the relative death rates of coliforms and enteric pathogens under natural conditions (487, 488, 489) and when exposed to various bactericides (302). Under certain conditions, such as at different pH values and temperatures, some strains of *Salmonella, Shigella* and *M. tuberculosis* (301) may be more resistant to disinfectants than the coliforms, particularly *E. coli.* Such data are suggestive that occasionally, as a measure of the effectiveness of purification processes, the significance of the absence of coliforms may be open to doubt (490, 491, 492, 493, 494). The increasing importance placed upon virus-transmitted illness in water supplies serves to cast further doubt (121, 2528, 2552). A study of the bacteriophage titer and *E. coli* counts indicated no relation (2318). The enterococci were reported to be useful when *E. coli* were absent or present only in small numbers (1503, 2553). In a discussion of coli determinations, it has been suggested that other organisms (germs of the coli group) be considered (2554).

While a discussion of the efficiency of treatment methods is beyond the scope of this report, it is believed that the subject should be briefly noted inasmuch as it points to the need to continue to search for more accurate and reliable indicators and techniques for water examination. There is a need also for indicators of bacterial contamination of non-intestinal origin, particularly organisms responsible for eye, ear, and nose and throat infections (495, 496, 497), as well as for viruses, protozoa, and worms (330).

On the other hand, the status of the coliform test has been strengthened by analytical studies of the relationship between the coliform index and the probable number of *S. typhosa* of different supplies (489). It has appeared likely from these statistical analyses that the pathogen/coliform ratio remains reasonably stable throughout the purification process up to about 99.9 percent reductions from contaminated water, although after that it is possible that the coliforms die off more rapidly. Further studies are needed, especially to explain the occurrence of occasional outbreaks of typhoid caused by water supplies that meet the accepted standards but have already undergone over 99 percent purification and are in the doubtful ratio-value area.

Other potential indicator organisms have been suggested to supplant or supplement the coliform tests, but although better and more rapid procedures are constantly being sought, the coliform test still remains the most practicable (see Fecal Streptococci) (2552).

In a Louisiana survey, water samples with high total counts also had high counts of coliforms, *E. coli,* and enterococci. In polluted surface waters, numbers of coliforms increased in heavy rainfall. But in heavily polluted drainage ditches, numbers decreased. The difference between the most and the least polluted waters in this survey was better indicated by the enterococci than the coliforms (2555).

Connecticut River samples over a two-year period gave a coefficient of correlation of *E. coli* and enterococci of +0.84. The density of the enterococci was 7.6 times that of *E. coli* (2556). The work of Kenner, et al. (2557) shows that the densities of fecal streptococci in various polluted waters are of the same approximate order of magnitude as the confirmed MPN coliform density. In classifying the extent of fecal contamination of bottom deposits, the numbers of *E. coli* and *S. faecalis* together constitute the best criterion (2558).

Actually, an effort is made in practice not to give undue weight to the results of the bacterial tests alone, and it is required that the interpretation of quality of a water should be based on the combined findings of the bacterial examinations and a sanitary survey of the area in question, covering such items as potential sources of pollution, opportunities for self-purification, tides, and currents (334, 469, 481, 483). In studies of the ocean, for example, no coliforms have been found in open waters at places remote from possibilities of contamination from land, but they are often found in bays and estuaries, and in high densities near outfalls (484, 485, 486).

Coliform organisms may gain access to water from many sources, primary among which are excretions from human beings, animals (476), amphibians (477, 478),

and birds (479, 480); surface run-off; and the multiplication of non-fecal forms on fibrous and vegetable substances in the water. More coliforms are discharged by healthy individuals than by sufferers of diarrhea (2559). Inasmuch as the bacterial flora vary from one source of supply to another, standards must not be interpreted too rigidly. A recommended practice is to rate each individual supply on the basis of frequent examinations, and to suspect at once any deviations from the usual quality (469). Nevertheless, many state and interstate water-pollution-control agencies have established standards or criteria for coliform densities in surface waters. For details regarding such standards, See Chapter III and the appendices thereto.

Not only the variety of sources, but also the complexity of microbial interrelations is deserving of mention. Vinberg and Sivko (2560) note that the viability of *B. coli* is greately reduced by profuse growth of green plankton. Also, Neel and Hopkins (99) point out that coliform bacteria in sewage lagoons are markedly reduced during seasons of prolific algal growth. Indeed, the antagonistic relationships of some pathogens and coliforms (1504) has raised the question as to whether it is desirable to exclude coliforms from water entirely when promulgating standards (1383).

Studies of the survival of coliform organisms under a variety of environmental conditions continue to accumulate. Buck et al. (1506) studied survival in estuary water in the laboratory and reported results ranging from 293 to 450 days. Public Health Service laboratory studies with sea water, temperature 2.5-5°C, demonstrated significant survival after 15 days for both *E. coli* and *Salmonella* (1509). Carlucci and Pramer (2155) conclude that the reaction and salinity of natural sea water do not favor survival of *E. coli*.

Vaccaro et al. 486 report the viability of *E. coli* in sea water to be less in summer than winter. Sterilized sea water (boiled, autoclaved, pasteurized, or chlorinated) has less bactericidal activity than raw sea water. Addition of organic matter decreases bactericidal activity. See also De Balsac (1508).

Ketchum et al. (1507) state that dilution is the least important and bactericidal action the most important factor in the decrease of coliforms in sea water.

Coliform organisms were inoculated into sea water and incubated at 5°, 18°, and 30°C. The 18° and 30° samples behaved similarly, showing a rapid decrease after a stable one- to three-day period. The 5° samples showed insignificant changes for periods up to 7 days. In these studies, it was demonstrated that viability in fresh water was not appreciably greater than in sea water and at times it was even less (2561).

Viability studies in water from Great Salt Lake indicated 8-hour survival at 23°C was of the order of 1 to 2 percent, whereas at 6°C, 50 percent survived 24 hours (2562). *B. coli* is reported to be less viable in the pH range 6 to 7 than at 5 to 6 or 7 to 8 (2563). Carlucci and Pramer (2155) noted that pH 5.0 was more favorable than alkaline reactions for the survival of *E. coli* in both sea water and in 3 percent NaCl solutions. Studies of coliforms in sediment around marine sewage outfalls disclosed that generally they were found in areas beneath the path of movement of the effluent field in the surface water (2564).

The presumptive coliform count was found to be unsatisfactory for studies of pollution in harbor areas where old sewage was returned by wind and tidal action (2567). Two Sardinian waters of alkaline bicarbonate type were inoculated with *E. coli* suspensions and maintained at cold and room temperatures in the dark. Results showed 20-day survival at the cold temperature, 28 days at room temperature (2565). At 8°-10°C, *E. coli* in water survive 3 to 5 weeks, and at 20°-30°C they multiply and will still be present to an extent greater than their original numbers after 40 weeks (2566). Czechoslovakian work on the *coli-aerogenes* group in different waters showed survivals of 8 to 12 months (2568). An *E. coli* titer of 0.004 ml (25,000/100 ml) remained constant for 160 days in a water containing humus (2569). *B. coli* type I was reported to survive much longer in alkaline than in acid soils (2570).

Season is reported to be a major factor in the occurrence of coliforms in wells, higher counts reported for warmer months, lower counts for colder months (2571). Some evidence was obtained showing that percolation of rain increased numbers, none that rainfall decreased numbers by dilution.

The numbers of *E. coli* in foams were reported to be 100-1000 times the number present in the body of the water (2295).

Many papers have been concerned with the percolation of polluted water through soils and the likelihood that ground waters will subsequently become infected. Rudolphs et al. stated that the distance pollution travels from bored latrines to ground waters depends mainly on the velocity of the ground-water flow. This distance varies with soil structure, gradient of the water table, and other factors (1382). Subrahmanyan and Bhaskaran (1399) believe that the safe distance between a borehole latrine and a ground-water source is the distance represented by about 8 days of ground-water travel. Stiles et al. reported that *B. coli* have travelled 1 to 232 feet from the source of pollution, and Caldwell found that coliforms travelled only 10 feet from a pit privy (1511). For more complete information, see the references cited in Chapter II, section on Natural Purification in Ground Water.

In studies on water-saturated sand columns, coliforms did not penetrate much beyond six feet in 417 days (water velocity 0.4 ft/day). Doubling the velocity reduced coliform arrival time at four feet from 114 to 82 days (122). Underground travel of *E. coli* of 250 feet is reported from Washington (1467a).

The question of the time factor in sewage saturated ground is pointed up in Minnesota experience (97). Fifty percent of well samples in older communities and 10 to 20 percent in young communities contained coliform organisms.

Wyss (2572) reports that there is still no evidence of coliform organisms attaining any resistance to chlorine in spite of the large scale use of chlorine for over 40 years.

For an excellent discussion of the entire coliform group of organisms, see Senn (2573).

2. Cross References. Pathogenic Bacteria, Protozoa, Fecal Streptococci, *Mycobacterium tuberculosis, Pasteurella tularensis,* Viruses, and Chapters IV, V, VIII, IX, and X.

3. Effects on Beneficial Uses.

a. Domestic Water Supplies. The first bacteriological standard for water adopted by the U. S. Public Health Service, at that time in the Treasury Department, was devised for waters supplied to the public by common carriers in interstate commerce. It has been pointed out (498) that the standard was established for drinking water in specific conditions where judgment must be based on laboratory analysis alone and without the benefit of repeated tests and information about the sources of supply. As the application of this standard came to be extended to water supplies in general all over the country it was open to criticism as being too strict, arbitrary, and impractical for many municipal supplies. On the other hand, it has been held that the standard is not strict enough (499, 500, 501, 502, 503, 1744, 1745, 1746) and that the ultimate goal should be the elimination of all coliform organisms from drinking water. Orchard (504) has reviewed the early history of the Treasury Department Standard and made comparisons in effect in other proposed or tentative requirements in effect in various states. The most recent revision in the USPHS Drinking Water Standards, dated 1962, still bases bacteriological requirements solely on the coliform group (2574).

Reviews of the development, history, and significance of drinking-water standards in the United States have been presented by Buswell (1513) and by Gilcreas (1514). The limits of coliform bacteria used as guides in several American countries, as described by Caballero (1457), are given in Chapter V.

The incidence of water-borne disease attributable to public water supplies in the U.S. has dropped markedly since the beginning of this century and most of the few outbreaks in which public supplies have been incriminated have been traced to accidents or breaks in the sanitation (417, 507, 508). One exception that has gained wide attention is an epidemic of gastroenteritis in Minneapolis, unquestionably water-borne although the water conformed to the standard (334, 671). While it is possible that the outbreak was caused by some other factor than bacteria, it has been suggested that possibly a standard based on a presumptive coliform test only might be safer.

Although pathogenic coliforms were reported as far back as 1929 (520) and 1936 (711), studies of these forms seem not to have created interest until the past decade (see Pathogenic Bacteria). It is perhaps unfortunate that the pathogenic coliforms were overlooked for so long, since a decision such as that rendered by the Supreme Court of the State of Illinois could not possibly have been made (see Chapter IV, People v. Bowen (1941) 376 Ill. 317, 33 N. E. (2d) 587).

Pathogenic serological types of *Escherichia coli* reported from Russia include 0111, 0125, 026, 055, and 408 (2575); from Yugoslavia 025 and others (2305); from France 111:B4 (2576); and from Hungary 0124:K72 (B17): H 32 (2577). All varieties were associated with gastroenteritis in young children. The Russian paper reported the above strains to be quite resistant, surviving four minutes in water heated to 90°C, and from 10 days to 165 days under various environmental conditions. Burdin and Schmitt (2578) regard the pathogenic forms as further argument in favor of regarding water nonpotable if it contains *Escherichia coli.*

Nevertheless, H. A. Thomas, Jr. has estimated that as typhoid incidence and carriers decrease among the general population, the capacity of a water with a fixed coliform density for transmitting typhoid fever also decreases. It has been suggested, therefore, that the present U. S. standard could be relaxed without loss of safety to permit 35 percent of 10 ml tubes to be positive (503).

Although the foregoing discussion is of general interest to most sanitary engineers, it is relevant to this investigation of water-quality criteria only in that it provides background information for, and leads up to, a consideration of allowable coliform densities in raw water in streams, lakes, and ground-water basins. The U.S. Public Health Service has established a classification system for raw-water sources according to the kind and degree of treatment they will require in order to bring them up to the standard set for drinking water.

The first such raw-water standard was adopted prior to World War I by the International Joint Commission of Canada and the United States for sources of water located in the international boundary waters between the two countries. A yearly average *B. coli* index not exceeding 500 per 100 ml in the raw water was fixed as a limit on the advice of a board of expert sanitary engineers. This limit was predicated on the assumption that waters carrying such a bacterial load actually could be purified by methods in use at the time to meet the drinking-water standard, i.e., an index of two per 100 ml (334, 340, 509).

Shortly after this standard was promulgated, H. W. Streeter of the USPHS, began a comprehensive series of field and experimental investigations of the efficiency of current treatment systems under actual operating conditions in order to establish an empirical basis for raw-water criteria (156, 337, 509, 510). Using the information gathered over a period of years, the USPHS has made certain recommendations for limiting coliform densities in raw-water. By no means mandatory and without the effect of law, these recommendations are interpreted as a guide in the field of water sanitation. The 1946 recommended classification which is still in effect is quoted in Appendix Q.

It is of interest to note that in the Chanute, Kansas, drought experiment (2119), in which a treated sewage effluent was cycled through a domestic water reservoir, the number of coliform organisms in the raw water was less than that found when the river was flowing normally. The tap water during this emergency consistently met Drinking Water Standards for bacteriological quality.

b. Industrial Water Supplies. The bacterial requirements for industrial water vary widely depending on the purpose to which the water is put. In the food-handling industries, bacterial quality requirements may be more stringent than for domestic water supplies inasmuch as bacterial contamination may result in fermentation, taste changes, and/or spoilage. For further details relating to

specific industries, see Baking, Brewing, Carbonated Beverages, Confectionary, Fermentation Processes, Food Canning and Freezing, Food Equipment Washing, Food Processing—General, and Ice Manufacture, in Chapter V. In many such industries coliform organisms are used as indicators of contamination, although where fermentation and spoilage are involved, a total plate count would appear to be more indicative.

The California State Board of Public Health has prescribed standards for water used in fish canning operations. The *E. coli* standards for untreated waters is 7/ml, and for treated waters 3/ml. In the whole fish handling operation, 20 percent of the samples may equal or exceed the bacterial standard, whereas in the cut fish handling operations only 5 percent may equal or exceed the standard (2579).

c. Irrigation Water. Few studies have been made of the sanitary quality of irrigation water, and there are differences of opinion among health authorities as to the health hazards involved. There is a definite need for basic data (511).

Norman and Kabler (1515) made coliform and other bacterial counts in samples of river and ditch waters, and of soil and vegetable samples in the fields to which these waters were applied. They found that although the bacterial contents of both river and ditch waters were very high, both soil and vegetable washings had much lower counts. For example, where irrigation water had coliform counts of 230,000 per 100 ml, leafy vegetables had counts of 39,000 per 100 grams and smooth vegetables, such as tomatoes and peppers, only 1000 per 100 grams. High enterococcus counts accompanied high coliform counts in water samples; but enterococcus counts did not appear to be correlated in any way with coliform counts in soil and vegetable washings (1515). Dunlop et al. (2580) obtained ratios of 255,000 coliforms and 4,800 enterococci to one *Salmonella,* computed from the median values.

British workers filtered a gallon of type I coliforms in peptone water (150×10^6/ml) through 18 inches of uncultivated garden soil and found no reduction in numbers. Two days later, sterile distilled water was filtered through the same place and yielded a type I count of 1×10^6/ml. Three months later, a count of 100 per ml could still be obtained in this manner (2581).

Bringmann and Kuhn (2582) studied *B. coli* on plants from the sprinkler application of sewage under controlled conditions of relative humidity. They could not remove all the bacteria from leaf surfaces so they planted plant parts in a liquid medium, incubated for 24 hours, and then streaked Endo plates. Although their studies with plant extracts demonstrated that some were bactericidal or bacteriostatic, neither mechanism was observed when using the plant part. *B. coli* counts were stable at 100 percent humidity, lower humidities giving shorter survival. At 40 to 60 percent humidity, *B. coli* frequently did not survive the 6-day experimental period.

In later studies on clover, they found that Endo-positive clover leaves of sewage-sprinkled plants dropped in the first 12 to 14 days to the same level as controls and remained at the same value for up to 26 days. Sunshine was observed to have a restricting influence on bacteria survival, but rainfall had little effect (2583).

The work of Bringmann and Trolldenier (2584), however, states that although the percentage of coli-positive leaves reached a constant value after 14 days, this constant value was always higher than that of controls not watered with sewage. The later paper also discusses secondary infections of clover leaves which give higher counts and which depend on rainfall and relative moisture.

In a discussion of bacterial and chemical pollution of ground water (2585), bacterial build-up in the soil is reported to occur owing to the long survival time of *E. coli* in comparison with percolation rates. Coliforms are reported to have travelled from 10 to 232 feet with ground water.

In the absence of widely accepted bacterial standards for irrigation, health authorities in this country have recommended a high degree of treatment for sewage effluents to be used in irrigation. The California State Department of Health (512, 513) adopted in 1933 the following rules, in part, regarding the use of sewage effluents for crop irrigation purposes: Partially treated or disinfected effluents shall not be used to irrigate garden truck, berries, or low-growing fruit. Nursery stock, cotton and field crops (to be processed after harvesting) may be watered with such partially treated or disinfected effluents provided that no milk cows are pastured on land moist with sewage or have access to ditches carrying such sewage. These restrictions do not apply to well-oxidized and disinfected effluents which meet the following bacterial standards: "In any 20 consecutive samples from which five 10-cc. portions each are examined, not over 10 portions shall be positive for members of the coli-aerogenes group, and in no single sample shall over half the 0.1 cc. portions of the sample of effluent be positive for the above organisms." In addition the effluent must be inoffensive.

The Conference on Polluted Irrigation Waters at El Paso, Texas, in 1950 recommended that for use in cultivation of human foodstuff, especially that to be eaten raw, water reclaimed from sewage should be sterile (511).

Studies of the use of sewage-contaminated irrigation water indicate that bacteria, both pathogenic and coliform types, do not penetrate the whole unbroken skin of fruits and vegetables. However, superficial contamination of food may be caused by splashing or other accidental means, and slightly cracked or injured fruit may then be attacked by surface bacteria and become heavily infected internally (302, 511, 514, 515, 516). On crop surfaces and in sewage-irrigated soil, intestinal bacteria of many kinds can survive for periods varying from a few days to many months, long enough to render foods infectious to human beings. The work of Rudolfs et al. (515) has demonstrated the survival of *E. coli* for as long as 35 days. Outbreaks of enteric bacterial diseases among people have been linked with the consumption of raw foods which have been sewage irrigated (272, 511). On the other hand, it has been declared that over a period of one hundred years during which sewage farming has been carried on, in South Africa particularly, extremely few or no epidemics among humans or animals

have been traced directly to this practice (301, 517, 518, 1027).

It seems that still further studies, both laboratory and epidemiological, are needed in order to clarify and evaluate the relationship between the use of contaminated irrigation water and the health hazard of eating vegetables irrigated with such water.

d. Stock and Wildlife Watering. Limiting concentrations of coliform organisms in contaminated water used for stock watering have not been found in this survey. Few studies have been made on the degree of pollution safe for natural waters consumed by stock, and there is a difference of opinion as to the need for regulation. It is recommended that more work be done to determine whether cattle and other livestock can be infected by the bacteria found in sewage (301, 303), even though cows have been reported to remain free from disease when their only source of water was contaminated by sewage and pathogenic bacteria (299, 300, 301, 302). See Fecal Streptococci, *Mycobacterium tuberculosis, Pasteurella tularensis,* Pathogenic Bacteria, and Viruses.

e. Fish and Aquatic Life. Guelin (2321) compared the coliform flora of fish caught in the open sea in the Mediterranean with fish caught in coastal waters. In open-sea fish, he found no *Escherichia coli,* no coli bacteriophage, and no other bacteria that would ferment lactose. Among the coastal fish however, he found *E. coli,* coli phage, and other organisms from the human intestine. Several years later (2586) he studied the possibility of using fish as a means of testing the sanitary quality of the water in which they were found. He found that fish placed in contaminated (*E. coli*-containing) water became contaminated themselves and within a 60-minute exposure, organisms could even be isolated from the viscera. If the fish were transferred to fresh (presumably uncontaminated) water, they freed themselves of the organisms in a 6 to 7-day period.

f. Shellfish Culture. Outbreaks of typhoid fever and other intestinal diseases have been directly traceable to the consumption of contaminated oysters (334, 522, 523, 524). In 1925, following widespread shellfish-borne typhoid epidemics, the USPHS was requested by state and local health authorities and the shellfish producers to exercise supervision over the sanitary quality of shellfish shipped in interstate commerce. The Surgeon General appointed a committee from a panel of health officials to formulate recommendations. With regard to shellfish-growing areas, the committee's report stated that the beds should be free from any justifiable suspicion of dangerous contamination with disease-producing microorganisms, as well as other deleterious contamination. Subsequently, many studies of the quality of growing areas were made by the USPHS and state personnel, and the sanitary status of all of the important shellfish-producing areas was generally determined (523, 525, 526).

On the assumption that evidence of sewage pollution in a shellfish-growing area must be looked upon as a potential hazard, limits have been prescribed for the bacterial concentration of the water overlying beds used for the production of marketable shellfish, including oysters, clams, and mussels (527). However, it is recognized that the bacterial counts of shellfish cannot always be correlated with those of the water, since the counts vary with the temperature, tides, and the physiological activities of the individuals (334, 528, 869). Also studies in Manila waters showed no correlation between seasonal variations in the coliform content of the waters and the coliform content of the oysters taken (1518).

E. coli appears, in some respects, to be a more suitable indicator for such waters than the coliform group (334, 472, 529, 2452); nevertheless, in the absence of practical routine methods for *E. coli* determinations, bacterial criteria applied to shellfish waters have been based on coliform concentrations and *E. coli* differentiations are optional. According to the Manual of Recommended Practice for Sanitary Control of the Shellfish Industry (481), the coliform group shall be considered as including all organisms which upon transfer from a positive presumptive test show fermentation in brilliant-green lactose bile broth, the confirming medium for coliforms, without proof of the presence of *E. coli;* although, if there is a possibility that the examination may become the basis for legal action, it is recommended that all tests for *E. coli* be completed. The results of the bacteriological examinations of water samples are not accepted alone as sufficient evidence for judging the quality of a water, but must be interpreted as an integral part of a thorough sanitary survey.

The first tentative standard suggested for controlling the bacterial contamination of shellfish waters provided that the waters should not ordinarily show the presence of coliforms in one-ml amounts when tests were made in 10-, 1.0-, and 0.1-ml volumes, a standard equivalent to a limiting coli index of 10 per 100 ml. A modification of this proposal, permitting waters to show coliforms in 50 percent or less of the one-ml volumes tested, or a maximum coli index of 50 per 100 ml, was for many years recognized as the "unwritten standard" of the U. S. Public Health Service (334, 509, 523, 530).

At the present time, on the basis of a survey plus bacterial results, waters are classified as: (a) approved; (b) conditionally approved; (c) restricted; (d) prohibited. Approved waters show a median coliform density (as MPN) not in excess of 70 per 100 ml when tests are made on a series of samples determined by the survey to be most probably exposed to fecal contamination. Restricted waters show contamination of probably human origin and the median coliform density does not exceed 700 per 100 ml. Prohibited waters are subject to continuous or intermittent pollution and have a median coliform density greater than 700 per 100 ml.

Shellfish from approved areas may be marketed directly; from restricted areas shellfish must be cleansed before marketing. Shellfish from prohibited waters must be relaid and thoroughly cleansed before marketing (2431). Water used for conditioning or cleansing shellfish from approved or moderately polluted areas must meet drinking-water requirements pertaining to bacteria.

A recent study (2452) investigated the potential of bottom deposits as contributors to the coliform content of water and oysters. Bottom materials consistently showed higher MPN values than overlying water. Hence, during periods of turbulence, resuspension can occur thereby effecting an increase in the coliform content of

the water and possibly an increase in the oyster coliform density.

After studying Virginia tidal waters and shellfish beds, the Virginia State Department of Health recommended that effluents from sewage plants located near shellfish waters should carry a MPN of not over 500 coliform bacteria per 100 ml. Plants farther from the shellfish areas need not meet so stringent a requirement (1517).

Studies of oysters in Galveston Bay, Texas (2587), indicated that coliform counts of oysters and water decreased in the Spring, remained low from July to December, and increased to high values in January. This finding is verified for the Gulf Coast area in a later report (2452).

This observation is in contrast to North Atlantic Coast findings where coliform counts (of oysters) decrease as water temperatures fall. The authors attribute this difference to the temperature of the water of the Gulf Coast area not dropping low enough to cause hibernation of the oyster. Nevertheless, ratios of coliform counts in water to counts in oysters did not correlate with other factors studied. The ratio of *E. coli* to total coliform content of the water over the oyster beds increased when stream flow into the bay was minimal, and decreased as stream flow increased.

In a study of mussels (2588), coli-aerogenes bacteria did not grow well in water from the mussel's shell cavity. Nor did the organisms survive in sea water that had been chlorinated, dechlorinated, and filtered by mussels during cleansing. *E. coli* survived in oysters between 7 and 17 days according to one report. In another report, there was a 458 percent increase in *E. coli* in shell liquor of shucked oysters in 12 days at 5°-8°C. In living shell oysters at 5°-8°C, *E. coli* is usually gone in 60 days. *E. coli* have survived 49 days of oyster storage at 5°C. (2448, 2589).

g. Recreational Use. While it is recognized that harmful contamination of outdoor bathing beaches may be caused by sewage from dwellings, hotels, sewer systems, and boats, by the dumping of refuse, and by the individual bathers themselves, the transmission of disease by bathing waters has not been established epidemiologically as a major public-health hazard. The condition of bathing waters varies from day to day and season to season, and it may be altered by permanent sources of pollution, the incidence of disease among the local population, the bathing load, wind, weather, temperature, and composition of the water. Before effectual bacterial standards can be developed for such waters, several questions remain to be answered, such as, how important is the small amount of water swallowed while bathing as an etiological factor, which diseases are transmissible by bathing waters, and to what degree are diseases contracted at beaches through exposure to infected water, infected people, and infected bathing appurtenances, respectively (531, 532, 2590).

The U. S. Public Health Service has recently supported statistical studies of illness in relation to natural bathing-water quality in an effort to secure epidemiological data adequate for the development of definitive and logical criteria. The first study on Great Lakes waters concluded that bathing in natural fresh water with a median coliform density of 180/100 ml appears to present no general hazard to public health. The second study on inland rivers concluded that waters with a median coliform density of 2300/100 ml may possibly cause an increase in gastro-intestinal illnesses, but outdoor pool swimming in chlorinated water with 93 percent of samples having MPN less than 3/100 ml resulted in more eye, ear, nose, throat, and skin infections than river swimmers. The third report on coastal waters shows no evidence of illness related to water quality (2149). These studies are discussed in Chapter V in the section on Swimming and Bathing Waters.

Among the diseases whose possible transmission by bathing needs further investigation are not only gastro-intestinal maladies, but also eye, ear, nose, and throat ailments (see Adenoviruses), skin irritations (see Pathogenic Bacteria), tuberculosis (see *Mycobacterium tuberculosis*), leptospirosis (or Weil's disease—see Leptospira), and others (495, 496, 497, 519, 533). For bathing waters especially, an indicator of contamination of other than intestinal origin is needed, one that will give warning of the potential presence of nasopharyngeal or skin organisms. Levine et al. (2316) suggest that *Staphylococcus pyogenes, Streptococcus salivarius,* and possibly *Neisseria catarrhalis,* and *N. sicca* are worthy of consideration.

Sea water samples collected in the vicinity of a sewage outfall on the Mediterranean demonstrated the presence of fecal coliforms within a 1200-foot radius, and of non-fecal coliforms only near the outfall. Although the authors rate swimming in polluted water as less hazardous to health than eating shellfish therefrom, they recommend that sea water at approved bathing beaches contain no fecal coli (2767).

A survey during the summer of 1951 of 25 beaches on Lake Erie and its tributary rivers revealed that at 11 beaches the average number of coliforms exceeded the maximum allowable count of 1000 per 100 ml. At 7 beaches near Cleveland the average number of coliforms was approximately 30,000 per 100 ml (1521).

In a 1953 summary of the results of the latest surveys of Connecticut beaches, Scott again pointed out the lack of evidence to incriminate natural bathing waters in the spread of intestinal or other ailments, even in states where coliform values of 2400 or more per 100 ml are permitted. He commented that although bathing in grossly polluted waters could not be recommended, on the other hand, the setting of unreasonably strict or arbitrary standards should be avoided because they might force the closing of many beaches, causing considerable hardship and financial loss, in the face of no evidence that they have ever been a source of disease (1522).

In addition to the USPHS epidemiological studies of bathing and illnesses, another highly significant study of the same type was concluded in England (2468). The Committee on Bathing Beach Contamination made a 5-year bacteriological and epidemiological study of more than 40 popular bathing beaches on the coasts of England and Wales, the waters of a great majority of which were subject to contamination with sewage. Surveys for the rough grading of beaches were generally planned to ensure representative sampling from the areas on the

beaches where bathing actually took place, and whether the grading was effected on the basis of results of the presumptive coliform test or on fecal coliform counts, a similar order of ranking was obtained. The median presumptive coliform counts varied from 40 to 25,000 per 100 ml. Meteorological and other factors played a part in the degree of pollution of bathing waters. The isolation of pathogenic organisms from sea water was restricted to the *Salmonella* group although *Staphylococcus aureus* was occasionally isolated (see Salmonella, Pathogenic Bacteria, Polioviruses).

The general conclusions of the committee were that bathing in sewage-polluted sea water carries only a negligible risk to health, and where the risk is present it is probably associated with the chance contact with intact aggregates containing infected fecal material. Unless the water is so fouled as to render the bathing beach esthetically revolting it would seem that public health requirements are reasonably well met by the present policy of preventing the pollution of waters with undisintegrated fecal matter. Coetze (2591) refutes the applicability of these conclusions to South African beaches.

It has been agreed by the Joint Committee on Bathing Places of the American Public Health Association and the Conference of State Sanitary Engineers, that on the basis of information available it is not possible to establish a precise standard to distinguish between bathing waters that are safe and those that are unsafe (335). This committee recommended that the following classification system of Connecticut be used as a basis for further study, with a view particularly to clarifying the position of the intermediate classes of water:

Class	*Description*	*Average Coliform MPN*
A	good	0-50 per 100 ml
B	doubtful	51-500 per 100 ml
C	poor	501-1000 per 100 ml
D	very poor	over 1000 per 100 ml

This classification (337), based on bacteriological analysis, was found to agree well with the results of concurrent sanitary surveys of the beaches.

At the present time there is still no uniform system of classification in effect. The quality of water for bathing is regarded as a local problem to be solved by state or local health agencies, and existing codes relative to bacterial concentrations vary so widely in their requirements that the limiting values in effect in various parts of the United States range from 50 to 3,000 coliforms per 100 ml. For a more complete discusson of the standards in effect throughout the U.S., see Swimming and Bathing Waters in Chapter V.

Bathing in Berlin is permitted in waters with a coli titer of 0.1, 1.0, or 10.0. The coli titer is used in some foreign countries to express coliform counts, and is defined as the minimum quantity of water that yields a positive result. It is not related to bacteriophage terminology. Hence, in American parlance, the above coli titers would amount to coli indices of 1000, 100, or 10 per 100 ml. When coli titers in Berlin rise to 0.01 or 0.001 (10,000 or 100,000 per 100 ml), bathers are warned of the risk (2461).

Two foreign papers express coliform standards in another manner. Kedrov (2592), in discussing indoor and outdoor pools in Russia, states that the coli titer must not be more than 100 per ml and the total bacterial count not more than 1000 per ml. It is interesting to note that the Russian standards also require that a sample volume of at least 100 ml must be used in testing for "pathogens and coli," and that the pool volume must be displaced each 45 days (2592). Dubois (2458), in a similar Belgian discussion, suggests that pools have a *B. coli* titer of not more than 100 per liter. From the manner in which "titer" is used in these cases, one suspects that the translators used this word in place of "count".

Levine et al. (2316) favor an "*E. coli* index," or ratio of *E. coli* to coliforms, as the most practical criterion of pollution of marine beaches. See the similar statement in the discussion on Fish and Aquatic Life immediately preceding this section whereby the same criterion is favored for oyster waters.

CLAMS

(see Mollusks)

COXSACKIE VIRUSES

(see also Viruses, Polioviruses, and Worms—Free-Living)

The Coxsackie virus is relatively small and spherical, measuring 25-30 mμ in diameter. It causes a number of diseases ranging from obscure fevers to an illness that is clinically indistinguishable from poliomyelitis. Two groups of Coxsackie virus are known. Group A with 28 serotypes causes extensive damage to the skeletal musculature of suckling mice. Group B with 6 serotypes affects the brain, pancreas, heart muscle, and subdermal embryonic fat pads of newborn mice (2159).

Although Coxsackie viruses have been isolated from treated sewage (2119, 2593), no outbreak of Coxsackie infections has been attributed to water. The virus resembles poliovirus in its size, ability to withstand a wide pH range, and method of spread (1722). All strains tested at room temperature survived pH 2.3 to 9.4 for one day, and 4.0 to 8.0 for 7 days. With respect to heat, most strains tolerated 49°C, but 30 minutes at 53°-55°C inactivated all (2594). They survive storage at 8°-10°C for up to 40 weeks, and at 20°-30°C for 30 weeks (2566).

Clarke, Stevenson, and Kabler (2595) performed an extensive series of tests on the survival of Group A Type 2 Coxsackie virus in sewage, distilled water, raw Ohio River water, and autoclaved Ohio River water, all at two temperatures, 8° and 20°C, on four different river waters at 20°C, and on samples of the same river water that were withdrawn from different points at 20°C. These results demonstrated that a water relatively free of microorganisms and dissolved organic materials (namely distilled water) permitted the longest survival (over 272 days at 8°C. Autoclaved river water also permitted lengthy survival (over 171 days at 8°C). However, at the same temperature of 8°C, all viruses were dead in 117 days in sewage and in 24 days in raw river water (1722). For further information on storage effect on several enteric viruses see Clarke and Chang's review (2596). Coxsackie A9 virus, when ingested by the nematode *Diplogaster nudicapitatus*, is completely protected from the action of chlorine (2597).

CRUSTACEA

(see also Chironomids, Rotifera, Insects, and Chapters IX and X)

The Crustacea compromise many groups of microscopic fauna, the majority of which are aquatic. Respiration is accomplished by means of gills or directly through the body surface (15). Ingram and Bartsch (2420) cite three groups of the Crustacea as organisms of interest in potable water supplies: Cladocera *(Daphnia, Bosmina)*, Copepoda *(Cyclops)*, and Isopoda *(Asellus)*. In polluted waters, crustaceans along with rotifers predominate in the latter stages of the zone of recovery. The Crustacea, as with the Rotifera, are not primary pollutants. As corollary pollutants, any detrimental effects appear to be rather insignificant compared to the probable good they do as fish food.

The only detrimental effects attributable to crustaceans have been: (1) shortened filter runs resulting from *Cyclops* and *Daphnia* (2420, 2598); (2) the esthetic problem of their swimming about in a glass of water about to be consumed (2599); (3) a report that attributes a depletion of dissolved oxygen in a lake to an enormous population of copepods, namely *Diaptomus ashlandi* (2600); and (4) heavy populations that choked and killed fish (389).

The oxygen consumption rate of *Asellus* has been measured (2601). It was found to be approximately proportional to the 0.7 power of the net weight. The work involved *A. aquaticus*, which is found in polluted streams, and *A. meridianus*, which is usually not found in polluted waters. Butcher reports that in polluted streams, *Asellus* replaces *Chironomus* with increasing distance from the source of pollution, populations of over 100,000 per square meter having been reported (2126, 2545). Although the "Asellus zone" in the recovery of polluted streams is recognized by European workers, Americans do not mention it. Hynes (2602) states that this is probably because the *Asellus* community in America consists primarily of mollusks and leeches.

In water made turbid with suspended yeast cells, *Mysidium gracile*, a sea-water crustacean, showed a significant preference for swimming perpendicular to the plane of admitted polarized light. In carefully clarified sea water, this preference is not shown (2603). The survival of marine crustacea in varied salinities has been studied (2604). The maximum percent salinity changes that allowed survival as long as controls were for Brachyuran larvae ±20 percent, for Amphipoda —15 to +5 percent, and for Copepoda —7 to +5 percent. In the Delaware River Estuary, *Neomysis americana* was not found above the 4-percent isohaline. That larger animals were located in the upstream area was attributed to their increased age (2605). Similarly, the brackish water tolerance of fresh-water crustaceans has been studied (2608). Among *Daphnia* species, *D. magna* could withstand a salinity of 5.8 parts per thousand, but *D. pulex* and *D. longispina* had high mortalities at 2.9 parts per thousand. *Asellus* also gave variable results. The existence of a limiting value of chlorides for smaller crustacea has been mentioned (2542).

In a lake that was "acid-trophied" by wastes from a powder factory, only two species of Cladocera *(Daphnia cristata* and *Bosmina coregoni)* could be found (2168). *Daphnia magna* was unable to survive for 24 hours in sewage in which NH_3 was demonstrable (2606). *Gammarus pulex* tolerates heavy pollution provided that the dissolved oxygen content of the water is not too low (2607).

The Crustacea are important also because of their value as tools of toxicity. Owing to its transparency and role as a fish food, *Daphnia* has been repeatedly utilized in toxicity studies. Crustacea can concentrate radioisotopes from water (see Chapter VIII, Radioactivity). *Daphnia* can be used as a specific indicator of radioactivity in reservoirs, according to Lebedeva (2609).

Crustacea are sensitive to many insecticides (2610), although Tarzwell cites an increased population of copepods in small ponds seasonally treated with DDT (2611). Turner used the insecticide pyrethrum to eliminate an infestation of *Asellus aquaticus* from a distribution system (2612). Only 0.005 mg/l of pyrethrum was necessary to kill the organism efficiently. DDT and DDD are also effective (74). Rotenone markedly reduced populations of *Daphnia* and *Diaptomus* (2613) (see also Chapter IX).

Various suspended materials were tested on *Daphnia magna* (2614). Charcoal and montmorillonite were toxic at 100 mg/l; ground glass at 98 mg/l; chlorite at 120 mg/l; illite at 264 mg/l. Pond sediments were not toxic at any level tested—presumably as high as 1,458 mg/l based upon the abstract available. Lackey et al. (2615) report that silt can be quite detrimental to *Daphnia* or ostracods.

Haynes (2602) states that the major controlling factor in the distribution of aquatic invertebrates is the nature of the river bed, with hardness (approximately 20 mg/l) a relatively unimportant second factor.

Experiments with phenol on *Planaria* (worm), *Daphnia, Gammarus,* and *Tubifex* (worm) showed *Gammarus* to be least resistant, *Tubifex* most resistant (2616).

Infestation of oysters in Puget Sound by *Mytilicola orientalis*, a copepod parasite, ranged from 0 to 62 percent. Heavy infestations can result in oyster death by secondary bacterial invaders (2415).

DIATOMS

(see also Algae, Protozoa, and Chapter IX)

Diatoms are unicellular algae characterized by a siliceous cell wall of varying thickness. Because of obvious morphological distinction provided by the siliceous cell wall, these algae are readily distinguished from other forms of algae. Indeed, the cell walls also provide means of differentiating individual species of diatoms.

Since all diatoms secrete a gelatinous material that covers the cell wall, they may grow singly, in colonies, or in strands. The jelly also provides a mechanism for attachment to substrate in fast-moving streams (15).

Patrick has suggested that diatoms can be useful as indicator organisms of pollution (2617) since (a) they need no special treatment for preservation; (b) they constitute a great variety of species which lends itself to statistical treatment; (c) a great deal is known of their

sensitivity to various ecological conditions; and (d) some species are sensitive to environmental changes whereas other species are not (2618). However, she emphasizes that great care should be exercised in interpretation of results (2617). In a study of the biological succession in a polluted river from which the pollution was markedly reduced, certain algal species and diatoms reappeared first (2544). Alfimov and Mironov (2619) found that the numbers of diatoms varied inversely with ammoniacal nitrogen content. A British report found it possible to estimate the maximum areal productivity of a lake by means of photosynthetic rate measurements, the rate of increase of *Asterionella* populations, and the rate of depletion of silica in the water (2623).

Diatoms appear to require silica (2202) in concentrations above 0.5 mg/l (1453), small quantities of iron (2620), and grow best at temperatures of 15°-30°C (2190, 2621). The brackish water diatom *Nitzschia filiformis* grows best at 26° and shows no growth at 34°C. The tolerant fresh-water diatom *Gomphonema parvulum* grows best at 22°C and still shows considerable growth at 34°C. The least tolerance to high temperatures is shown by the sensitive fresh-water diatom *Nitzschia linearis* which grows best at 22°C and shows little or no growth at 30°C. Although growth of *N. linearis* was inhibited at 4°C, the cells remained in good condition. For mixed populations from a normal healthy stream, a large number of diatom species were present at 20°C and dominated the culture. At 30°C, only two species of diatoms were numerous and blue-green algae predominated (2190). Investigations on a pollution-free river into which heated condenser water was discharged showed that diatoms of the family Fragillariaceae became more abundant. However, irregularities in the population not related to temperature were noted and were believed attributable to chlorination of condenser water (2622). Silicic acid has led to the development of diatoms (2171). During periods of maximum growth there was a marked decrease of silica in the water (2186). *Asterionella* blooms have been known to use up all the available silica in their environment, thereby creating conditions detrimental to their own survival (2126).

Diatoms discolor water (414, 2164, 2245), cause taste and odor problems (386, 409, 414, 422, 2166, 2213, 2221, 2236, 2242, 2244, 2246, 2247, 2252, 2624), and shorten filter runs (428, 738, 2166, 2221, 2625, 2626), *Synedra* being particularly important in both respects in the Chesapeake Bay area (2165). A heavy growth of diatoms was implicated in reducing normal filter runs from 50-110 hours to 5 hours (2627). Another report associates counts of 4800 cells of *S. delicatissima* per ml with reducing filter runs from a normal 50 hours to less than 1 hour. An upstream water treatment plant, with a maximum count of 20 *Synedra* per ml, had no difficulty (2628). However, Baltimore reports no filter-clogging difficulty despite (what was termed) high counts of diatoms (2629). Also, in the Loch Raven Reservoir, pH changed from an average of 7.0 to 9.0 in an area where diatoms were abundant. pH was normal where diatoms were scarce.

Gamet and Rademacher (2630) studied the relationship between lengths of filter runs on Lake Michigan water and diatom counts. In general, there was a relation between high counts and short runs, but exceptions occurred. Tabellaria appeared to have the greatest influence on shorter filter runs of all organisms studied (3256). They conclude that possible nannoplankton, excreta from micro-organisms, or siliceous gel also contribute to short filter runs. The diatoms *Asterionella* and *Fragilaria* are identified as taste- and odor-producing forms (2196, 2246, 2624), *Tabellaria, Synedra,* and *Melosira* as filter-clogging forms (2166).

Diatoms have developed in "very low" concentrations of phosphate, but higher concentrations stimulated growth. Numbers of diatoms were inversely related to the concentration of nitrate nitrogen and unrelated to ammonia nitrogen (2186). Bamforth (2185) reported that diatoms grew at 0.02 mg/l of phosphate (see also 2202). Diatoms are reported to occur in larger numbers in alkaline ponds than in acid ponds (2202). Diatoms have been reported to grow when doses of 3 mg/l copper sulfate removed *Oscillaria* (389). Seasonally, they appear in the Spring, are replaced by green or blue-green algae in the Summer, and reappear in the Autumn (371). An Israeli study of fishpond phytoplankton showed that although blooms of blue-green algae occurred in all ponds, diatoms predominated in ponds of high salinity, green algae in ponds of low salinity (2186). Diatoms have also been identified as cooling water contaminants (208). They have also been reported to flourish during blooms of the Florida "Red Tide" (420). Whereas certain flagellates may be important food sources for shellfish *(Mya arenaria),* diatoms are probably of little importance (2631). Nevertheless, concentrations of 75,000 *Nitzschia* per ml markedly reduce the pumping rate of oysters (1448).

In a study on the antibiotic effects of *Nitzschia palea,* it was found that the effect was greatest in cultures 4-8 days old, and was pronounced against *B. coli, Proteus vulgaris,* and *Pseudomonas fluorescens,* but not against other organisms tested (2632).

When the biological balance of a Delaware reservoir was upset by DDT spray from an airplane, the diatom *Synedra* multiplied until the high count of 10,000 per ml was reached. At this level the color was 15 mg/l and turbidity 20 mg/l (2245).

Palmer (2213) states that sometimes certain diatoms in small or moderate numbers are responsible for tastes and odors, citing *Melosira* and *Synedra* in Alabama. The Metropolitan Water Commission of Boston sets allowable limits in Standard Units per ml for *Asterionella*-3000, *Tabellaria*-2500, and *Synedra*-3000, at which treatment is begun (2255). Fox (2250) reports diatoms to be most prominent in the Spring and Fall, and that concentrations of 2000 units/ml of *Asterionella* and *Tabellaria* are troublesome (see also 384, 385, 386).

ECHO VIRUSES

(see Enteroviruses)

EELS

The presence of eels in cistern water supplies on the island of Pantellaria, Italy, are reported to have no effect on the hygienic quality of the water (2633).

ENTEROCOCCI

(see Fecal Streptococci)

ENTEROVIRUSES

(see also Viruses, Coxsackie Viruses, Polioviruses, and Infectious Hepatitus Viruses)

Enteric viruses are found in the gastrointestinal tract and feces of man and many lower animals. Some of the enteroviruses invade beyond the confines of the alimentary canal, and most have a higher incidence in children than in adults. The incidence is seasonal, being highest in the summer months, the period when enteroviruses are most readily isolated from raw sewage and polluted waters. This group of agents is transmitted generally by the oral-fecal route; hence, presumably all viruses of this group have the potential for transmission in polluted waters (2159).

In the past 15 years over 70 newly discovered viruses have been added to the original list of enteric viruses known to be present in human wastes (125).

Enteric viruses, other than those mentioned separately (see Coxsackie Viruses, Infectious Hepatitis Viruses, and Polioviruses) include the ECHO viruses and reoviruses. These latter two have not been incriminated in waterborne illness.

The ECHO (enteric-cytopathogenic-human-orphan) viruses constitute 28 serotypes of 30 mμ-diameter spheres that have been isolated from human feces. The first ECHO viruses were discovered in the feces of healthy persons during studies of poliovirus. At the time no illness was associated with these ECHO types. However, later studies found a number of clinical syndromes associated with these types. An array of similar such agents is found in other animal species such as swine, cattle, chickens, monkeys, dogs, etc. But the relationship of these latter viruses to human disease is poorly understood, although some are pathogenic to man. The ECHO viruses are readily isolated from sewage (2119, 2159, 2634).

The reoviruses constitute a small group (four serotypes) of viruses possessing certain characteristics common to both ECHO and Coxsackie viruses. The reovirus is a large spherical particle, about 72 mμ in diameter and has a very broad host range. It is responsible for enteric and respiratory infections in children and has been encountered in chimpanzees, monkeys, and calves. The role of water in the transmission of reoviruses is not known (2159).

ESCHERICHIA COLI

(see Coliform Bacteria)

FECAL STREPTOCOCCI

(see also Protozoa, Coliform Bacteria, and Pathogenic Bacteria)

The fecal streptococci include, in addition to the enterococci, the *Streptococcus mitis—S. salivarius* group, *S. bovis, S. equinus,* and an atypical group resembling the enterococci. Interest in the use of the fecal streptococci as an indicator of potential pathogen contamination by intestinal wastes (2635) stems from the appearance that this group is characteristic of fecal pollution, that they do not multiply in surface waters, and that they rarely occur in surface soil or on vegetation not contaminated by sewage (1501, 2636).

The enterococcus group, suggested by Sherman in 1937 as indicator organisms of fecal pollution, includes *Streptococcus faecalis, S. faecalis* var. *liquefaciens, S. faecalis* var. *zymogenes,* and *S. durans* (2637). The enterococci have also been suggested as indicator organisms for soil and vegetables contaminated by sewage, whereas *E. coli* was unreliable (1502). Recently, Kabler (2636) stated that undue emphasis has been placed on the enterococci (as an indicator group) with little or no consideration for other streptococcal strains present in the intestinal tract of warm-blooded animals. He quotes studies in the Cincinnati area that revealed the enterococcus group represented only 77 percent of the total streptococci present in humans, 25 percent in sheep, 12 percent in cows, and 10 percent in swine. Bartley and Slanetz (2635) conclude that the "membrane filter tests for fecal streptococci are more practical, efficient, and accurate than the coliform tests for evaluating the sanitary quality of almost all types of water."

Ritter (2638), in comparing enterococci with coliforms in well-water samples, stated that the test for the entire enterococcus group—including atypical strains—was to be preferred to tests for *Streptococcus faecalis* only. Morris and Weaver (2639) claim a good correlation between coliforms and streptococci on both freshly collected and 24-hour stored samples (room temperature). Leininger and McCleskey (1501, 2555) reported that pollution of water from drainage ditches was more readily detected by enterococcus counts than by coliform counts. A South African paper by Keller (2553) reports that enterococci survived longer than *Escherichia coli* and therefore can be regarded as "delayed indicators" of pollution when *E. coli* is absent or present in small numbers. In a two-year series of samples on the Connecticut River, the density of enterococci to *E. coli* was approximately 7.6:1. But water samples from the harbor of New Haven, Connecticut, indicated a ratio of 63:1 of *E. coli* to enterococci (2556). The Water Pollution Research Board in England reported laboratory studies on settled sewage from three different sewage-treatment plants. The samples were maintained at different temperatures between 5° and 25°C and counts of *B. coli* and *S. faecalis* determined at intervals. At temperatures above 10°C, *B. coli* multiplied whereas *S. faecalis* did not (2640). In a study to determine the extent of fecal pollution of mud from lakes and estuaries, British investigators (2558) state that counts of *B. coli* and *S. faecalis* together probably constitute the best index (1505), although few such organisms are present at depths of more than two inches.

British workers also report that *S. faecalis* is less viable in the pH range 6 to 7 than in other ranges studied (pH 5.0 to 8.0) (2563), and also that *S. faecalis* persisted for a much longer period in alkaline soils than in acid soils (as did *B. coli* type I). This explanation was suggested for the greater fecal pollution found in surface water supplies in limestone areas than occurs in waters from acid areas (2570). *B. coli* and *S. faecalis* survived longer in Thames River water having a salinity of 20.8 per thousand than at 30.8 per thousand. Also,

S. faecalis survived appreciably longer than did *B. coli.*

Kenner et al. (2641), in quantitating fecal streptococci from man and various animals, recorded the following totals per animal per day:

HUMAN	450,000,000
CHICKEN	619,000,000
COW	30,680,000,000
SHEEP	42,900,000,000
PIG	226,800,000,000

Hence, surface drainage from agricultural land, feeding pens, and grazing areas can be expected to contribute substantially to the bacterial contamination of streams. Kenner et al. (2557) also shows that densities of fecal streptococci in various waters is of the same order of magnitude as the confirmed MPN coliform density.

Mallman (1503) suggested the enterococci as a supplementary indicator group to *E. coli* in rural areas, since the absence of *E. coli* indicates a satisfactory water but presence does not necessarily indicate an unsafe supply.

High enterococcus counts in irrigation water samples accompanied high coliform counts in water samples; but enterococcus counts did not appear to be correlated in any way with coliform counts in soil and vegetable washings (1515).

FUNGI

(see also Sulfur Bacteria, Iron Bacteria, Algae, Yeasts, Slime Bacteria, and Chapter V)

The true fungi are the chlorophyll-free organisms of the phylum *Thallophyta.* The algae comprise the other division of this phylum and are the chlorophyll-containing forms. Although the fungi lack chlorophyll, they closely resemble green algae in their structure and mode of reproduction. Many species of the fungi are aquatic, many are terrestrial. The fungi include the yeasts which are discussed in this book separately. The term "molds" is applied to those fungi which are multicellular and filamentous (2289).

In the sanitary engineering field, the term "sewage fungus" is frequently used. This is a general term applied to a number of organisms which may include bacteria and even protozoa as well as fungi. *Sphaerotilus,* for example, is often termed a "slime fungus" because of its appearance (2642). Waldichuk describes a grey-green fungal slime, possibly *Sphaerotilus,* that is characteristic of sulfite-liquor pollution (1949). Among the more important organisms in this heterogeneous group are *Sphaerotilus, Carchesium, Leptomitus,* and *Fusarium,* only the latter two of which are fungi.

Naturally occurring water fungi are generally not primary pollutants although some such as *Penicillium* may be present in waste discharges from the manufacture of penicillin (661). However, the most important water-pollution problem presented by fungi is that of corollary pollution, namely the heavy growth of "sewage fungus" as referred to above, which is the result of wastes rich in organic matter (see Slime Bacteria) (457, 1191, 1192, 1193, 2542).

Hohnl's work (2643) on waste-water fungi notes that the bacteria *Sphaerotilus* was the primary organism at pH values of 5.8 to 9, but when the pH reached 5.2 to 5.6, the fungi *Fusarium* and *Mucor* predominated. *Fusarium aqueductum* and *Sporotrichum,* cultured in a medium containing glucose, hydrolyzed casein, and mineral salts, grew over a pH range of 3 to 10, optimum growth being over the wide range of 4 to 9. *Oospora fragrans* grew best at pH 3 to 9, but did not grow at pH 2. *Sepedonium* (not identified in Ward and Whipple) (15) had a narrow optimal range of pH 7 to 8 (1540). The growth of the fungus *Sepedonium* in the presence of small amounts of metals indicated that zinc, calcium, and manganese are essential elements, but that molybdenum is not. Results for iron and copper were inconclusive. For best growth, zinc of 0.5-1.0 mg/l and calcium of 5 to 12.5 mg/l were required (2640).

Sepedonium and *Ascoidea rubescens* require organic nitrogen, *Oospora fragrans* and *Sporotrichum* can utilize ammonium salts or organic nitrogen, and *Fusarium aqueductum* is capable of using nitrate as well as other forms of nitrogen (1540, 2644).

Cooke (2645) identified *Trichoderma viride, Myrothecium verrucaria, Aspergillus fumigatus,* and *Penicillium ochro-chloron* as very active among the organisms helpful in the decomposition of cellulose. *Gliomastix convoluta, Cladosporium cladosporiordes,* and *Geotrichum candidum* showed no significant activity in this respect. *Pullularia pullulans* was unable to use cellulose. A report of the mass development of *Achlya prolifero* downstream from a wood fiber plant has been reported in Sweden (2646). Some fungi can use rubber as a source of carbon (666).

Nutritional studies on the Peronosporales and Blastocladiales indicate a need for thiamine. All Saprolegniales investigated were capable of producing all the vitamins they need. The Chytridiales include both self-sufficient and thiamine-deficient species (2623).

DDT has been observed to accumulate on aquatic mosses as a result of aerial spraying and results in the death of aquatic organisms (2647).

Fungi are among the organisms that metabolize hexadecanol evaporation—retardant films (2293, 2648). Increased stream temperatures are mentioned as encouraging to the growth of "sewage fungus" (2649). Optimum temperature for "sewage fungus" is 20° to 30°C (2602, 2650). Lichens, a symbiotic relationship of fungi and algae, have been associated with concrete corrosion (see Algae) (2235). Species of *Fusarium* have caused extensive corrosion of turbine blades. Decomposition of the organisms liberated sulfur which combined with iron surfaces to form iron sulfide (400).

The indicator value of fungi towards pollution has been the subject of several papers. Heukelekian (2278) mentions a study that reports the absence of some fungi from heavily polluted waters, the presence of others in moderately polluted areas. *Aphanomyces* had an affinity to polluted matter. Cooke (2651) reports the isolation of 105 species of molds from a stream subject to primary effluent pollution. Of these, 6 were present in all samples collected in the 12-month survey, and 48 were present in 4 or more of the 8 stations. Little correlation was noted between the number of species and number of colonies produced (2651, 2652). Although fungi are commonly associated with decaying organic matter, Cooke concludes that they cannot be used as indicators of pollution largely because of their ubiquitous nature (2653, 2654).

Leptomitus lacteus is described as a fungus found in polluted water. Among problems associated with it are the blocking of weirs, pipes, and fishing nets, and objectionable odors upon decomposition (949). Fungus growths have been linked with problems of caking and short circuiting through filter sands (2304).

In a study of Lytle Creek in Ohio, fungi showed definite response to stream pollution. At the outfall of a primary treatment plant, fungal populations were suppressed. In the downstream clean water zone, they surpass the populations of the clean water zone above the plant. It is assumed that the fungi contribute to the purification of the stream (2655).

Fungi have been discussed as a pollutant of cooling waters (2263) and as a factor in the deterioration of wood in cooling towers (2656).

The increased use of sewage effluents for irrigation may lead to outbreaks of plant diseases from pathogenic fungi (2657). Crook root disease of watercress is caused by a water-borne fungus. Contamination by zinc from galvanized piping of a tap water resulted in 2.0 mg/l zinc in the water and completely suppressed the infection (2658). At least 18 species of fungal plant pathogens have been identified in sewages and sewage-polluted waters. See Cooke and Kabler (2659) and Cooke (2644) for the species and their diseases.

Labrynthula appears to cause disease in eelgrass which is resulting in the disappearance of eelgrass from both American and European shores and subsequent depletion of foodstuffs of both fish and wild fowl (667). Other fungi, *Saprolegnia* and *Ichthyosporidium hoferi* for example, cause diseases in fish (308, 384, 2260). The collective term "sewage fungus" has been applied to mats covering the bottom of streams where they destroy spawning beds, benthic organisms, and impede fish migration (see Slime Bacteria) (1575, 2661). A decline in the oyster industry of the Gulf Coast has been reported to be due to *Dermocystidium marinum,* a parasitic organism related to the fungi (2415).

GASTROENTERITIS ORGANISMS

(see also Copper, Pathogenic Bacteria, Salmonella, Shigella, and Algae)

Water-borne outbreaks of gastroenteritis of unknown etiology sometimes occur in communities whose drinking water appears to meet the bacteriological standards of the U. S. Public Health Service. Several possible causative agents have been suggested, including heavy growths of blue-green algae, anaerobic decomposition products of decaying algae, chemical toxins, or irritants of other biological origin, decomposing organic matter, toxic pollens or dusts, miscellaneous bacteria of non-sewage origin, viruses, various streptococci, staphylococci or other bacteria not detected by conventional techniques, or bacteria of the colon-typhoid-dysentery group present because of a temporary excessive raw-water contamination and inadequate purification (152, 393, 424, 492, 669, 670, 671, 672, 1272, 2124, 2429).

Petersen and Hines (2662) related the morbidity rates of summertime gastro-intestinal illnesses in Rocky Mountain communities to the sanitary quality of the drinking water. Contaminated water supplies were associated with an attack rate of 14 percent, whereas non-contaminated supplies were associated with a 9 percent attack rate. Among the users of contaminated supplies, those who had been exposed for over two years had only one-half the attack rate of those exposed for less than two years.

An outbreak of 8,000 to 10,000 cases of human gastroenteritis involving suspected water gave strong circumstantial evidence that algae blooms which existed at the time were responsible (2274).

INFECTIOUS HEPATITIS VIRUSES

The etiological agent of infectious hepatitis has been suspected as being of viral nature for several decades. Proof, however, has been only recently established. The incidence of this illness has increased dramatically in recent years. The mortality from infectious hepatitis, although low, is highest in older children and older adults. The incidence can be high in troops and also is not uncommon in overcrowded, understaffed, public institutions housing the sick. Hence, its incidence is generally related to unhygienic conditions. The major route of transmission is probably by direct contact (2159, 2663).

The virus of infectious hepatitis has resisted efforts at laboratory cultivation thus far. Hence, definitive studies are scarce. Nevertheless, some illuminating work has been done, particularly that by Neefe and Stokes (2664). Their work demonstrated that the virus of infectious hepatitis would survive storage in relatively clean (well) water for at least 4 weeks and possibly up to 10 weeks, and be virulent at the end of that time. A report from Europe indicates that the virus remains virulent in ground water for at least a month (2665). The virus is quite resistant to heat, especially when suspended in protein. Exposure to 56°C for 30 minutes failed to render the virus noninfective, since 3 of 4 men who ingested it contracted the illness (2666). Exceedingly minute amounts of infective excreta can cause the disease when taken orally. Human volunteers have developed it from as little as a 10^{-20} dilution of infected feces (2667). According to Clarke and Chang's literature review (2596), a 30-minute chlorine contact period protected all of 12 volunteers. The conditions of these findings were: fecal-borne infectious hepatitis virus suspended in distilled water, room temperature, pH range 6.7-6.8, initial and final free chlorine residuals of 3.25 and 0.4 mg/l. There is need for more information (2528).

1. Cross References. Viruses, Enteroviruses.
2. Effects on Beneficial Uses

a. Domestic Water Supplies. Many outbreaks of infectious hepatitis have been reported to have originated from contaminated water supplies (883, 885, 2573, 2667, 2668, 2669, 2670, 2671, 2672, 2673, 2674, 2675, 2676, 2677). The massive outbreak in Delhi, India, was reported to be the first of its kind in the world (2678). More probably, it was the first to be recognized and reported. The plant that treated the water at Delhi apparently turned out a safe supply when the water was prechlorinated to a free residual of 0.7 mg/l. When sewage backed up to the waterworks intake, however, sufficient ammonia was present to destroy the free chlorine and to produce a low, combined chlorine residual

(2676). The latter treatment, nevertheless, rendered the water safe according to incomplete coliform testing results, but it apparently did not destroy viruses of hepatitis. Records of a water plant in New York State, which supplies water for two communities in both of which a simultaneous epidemic of jaundice (infectious hepatitis) occurred, showed flash orthotolidine residuals of not less than 0.35 mg/l during a one-month period (2671). For more information on treatment, see Senn et al. (2573).

b. Fish and Other Aquatic Life. The virus of infectious hepatitis can also be transmitted by shellfish. Coetze (2591) quotes Roos' finding in Sweden (2453). See also Jensen (2454), and Chapter V—Shellfish.

c. Recreational Use. Coetze (2591) also quotes Buttiaux as concluding that of the virus diseases, especially poliomyelitis and infectious hepatitis, there is little convincing evidence for the part played by bathing places in the spread of the disease, and none at all relating to sea water.

INSECTS

(see also Chironomids, Mosquitoes, Chapter VI—Hardness, Chlorides, and Chapters VIII and IX)

Although most insects are terrestrial, a great number are aquatic, occurring almost entirely in fresh water (15).

Generally speaking, each group of aquatic insects is most abundant in certain fresh-water habitats. For example, damselflies and dragonflies are predominantly pond species, stoneflies and mayflies are running water forms, and caddis flies are abundant in well-aerated streams and lakes (15). Hynes generalizes aquatic fauna with respect to hardness (2602). In very soft water (presumably less than 20 mg/l of calcium) stoneflies and mayflies are dominant creatures; in slightly harder waters mayflies and chironomids become more abundant; and in hard waters, shrimps, snails, worms, and caddis flies dominate.

Aquatic insects are natural fauna and not primary pollutants. As consumers of minute plants and animals and as food organisms for fish, insects and their larvae and nymphs are an integral part of stream life, and their destruction can have far reaching consequences upon the biological nature of streams. However, the presence of insects in excessive numbers can be highly obnoxious and render water undesirable for various uses. The multiplication of many types of insects such as flies, midges, mosquitoes, etc., is encouraged by heavy algal growths and decaying vegetation (388). Certain insect larvae, such as rat-tail maggots and bloodworms, thrive best in sewage-polluted waters and have been useful as indicator organisms in studies of natural purification of streams.

Because of the large number of references to chironomids and to mosquitoes, these groups of aquatic insects are discussed separately.

Among the insects of interest in potable water supplies, Ingram and Bartsch (2420) list *Chironomus* (see Chironomids), the predacious diving beetle, *Dyticus*, the whirling beetle, *Gyrinus*, and the water strider, *Gerris*. Other than the clogging of traps in water lines by large numbers of *Dyticus*, they are apparently fairly innocuous creatures. Some insect larvae that flourish in treated water reservoirs may reach the consumer in a state of partial decay (388). An increase in the number of midges has been reported to result in an increase in the number of bacteria (682).

Although mosquitoes predominate as an insect problem associated with irrigation waters, biting black flies are also mentioned (2679, 2680). A German organization called the Association of Beekeepers has protested against the spray irrigation of crops by sewage in areas where bees are numerous. Their argument is that if bees become contaminated with pathogenic bacteria, the organisms might be transferred to humans by their stings (2681). In a study of aquatic fauna associated with several species of submerged aquatic plants, midge larvae and freshwater annelids together made up 59 to 93 percent of the population (2682). Aquatic insects concentrate radioactive elements. Concentrations of P-32 in *Hydropsyche* of 370,000 times that in the surrounding water have been reported. See Chapter VIII, Radioactivity.

The application of monomolecular films for retarding evaporation from reservoirs results in a lowering of the surface tension of the water. This action may have an effect on emergent insect populations such as mayflies of up to 70 percent (2683, 2684). The storage of waters behind dams with subsequent change of temperature of the released water is reflected in changes in the insect population. The most abundant in one study were Tendipedidae, Simulidae, and Hydropsychidae. However, another study of mayfly nymphs reports that temperature and dissolved oxygen have little, if any, effect on their distribution in a lake (2685). Sediment may cause a marked change in the insect fauna (2615, 2686, 2687). The bottom tube-building forms may increase (2686).

The midge *Cricotopus bicinctus* was found to be particularly resistant to electroplating wastes containing hexavalent chromium (25mg/l), cyanide (3.2mg/l), and copper (2.2 mg/l), all accompanied by a low dissolved oxygen level. Another midge, probably *Pentapedilum*, was also present (2688). In general, a population containing numerous gill-breathing forms such as mayflies, stoneflies, and caddis flies indicates clean water and their absence denotes organic pollution and/or low dissolved oxygen (2542, 2689). Insect larvae are among fourteen indicator forms of the saprobic classification system used by Stammer in tests of hydrogen sulfide (2690). The once abundant mayfly nymphs of Lake Erie (which averaged about 400 per square meter of lake bottom) have declined today to about 40 per square meter (2136). In Europe, Hynes remarks that organic matter causes a decline in Ephemeroptera but only slight sewage pollution may increase the caddis worms (2607). Caddis fly larvae can apparently adapt to wide levels of dissolved oxygen (2691). Mayfly nymphs showed a tolerance to chlorides less than that of fish (see Chapter VI, Chlorides) (2692). British streams polluted with 3 to 6 mg/l of lead have an animal fauna restricted to the midge *Tanypus nebulosus* and the mayfly *Cloeon simile* (2693).

The rat-tailed maggot, *Eristalis tenax*, is not dependent on oxygen in the water and is one of the few survivors in heavily polluted waters from which most other animals have disappeared (2143). In the recovery of

streams, the tendipedids, dragon flies, and damsel flies together with the beetles make their appearance sooner than the mayflies and stoneflies. The caddis flies seem to be a more variable group (2544). Because individual species of water beetles are extremely variable in their tolerance of pollution, these predaceous organisms have been suggested as an indicator group (2697).

Pesticide effects have been extensively studied. For more information see Chapter IX, Pesticides. Forest spraying of insecticides can have disastrous results on aquatic insects. Since they are a major fish food, spraying is reflected in a declining fish population (2694). The immediate effect is a drastic reduction in the emergence of all insect forms for several weeks. Chironomids show the first recovery but represent only minor fish-food volume. One to two years later, total numbers of emerging insects exceed prespray figures, but they are much smaller in size and represent only ½ to ⅓ of prespray volume. After the chironomids come the mayflies and stoneflies. Caddis flies are virtually eliminated and do not recover until 2 to 4 years later. Other insects severely reduced include blackflies and Dobson flies (2533, 2534, 2695).

The use of DDT to control *Simulium damnosum* in Africa resulted in most species of Ephemeroptera and Trichoptera being eliminated (2696). The fish that fed on these insects and were able to change their diet to other forms of plant and animal matter survived, and those that couldn't moved away or died.

In Saskatchewan, *Simulium arcticum* feed on suspended solids and the single application of DDT at rates as low at 0.09 mg/l destroyed the larvae for distances of 40-98 miles downstream. This mortality was much higher than that suffered by other aquatic insects (2698).

The seasonal effects of routine DDT applications to small ponds resulted in decreases of the insects, viz., chironomids, Hemiptera, Coleoptera, and Ephemeroptera, but increases in nematodes, viz., Oligochaetes, copepods, and gastropods (2611).

Gaufin (2699) found that there is a considerable difference in resistance of aquatic flies to insecticides, the larger being more resistant than the smaller to the insecticides studied.

IRON BACTERIA

(see also Iron, Manganese, Sulfur Bacteria, Bacteria-General, and Slime Bacteria)

Detrimental or inimical growths of iron bacteria in natural waters that result from man's activities can classify such organisms as "corollary" pollutants. Such growths may occur in surface streams that receive acid mine drainage, spent pickling liquor, or sugar wastes. They may also be found in ground waters that leach soluble ferrous compounds from the soil (346).

The principal iron bacteria are *Leptothrix ochracea, Gallionella ferruginea,* and *Crenothrix polyspora.* These are also the most common microorganisms causing trouble in water supplies (2189). *Leptothrix* and *Gallionella* appear to require ferrous iron for proper nutrition and utilize it for sheath and cell structures (2700). *Crenothrix* derives energy from the oxidation of iron, but does not embody iron in its sheath (1036). These organisms can thrive in water containing less than 0.1 mg/l of iron (1219, 1220). Wolfe has isolated a large, sheathed bacterium, tentatively described as *Clonothrix putealis,* that concentrates iron and manganese in its sheath and which flourishes in a water supply containing by chemical analysis no detectable manganese and less than 0.02 mg/l iron (2701). Leathen, et al. (2702) reports that ferrous sulfate in water is rapidly oxidized by *Ferrobacillus* to ferric sulfate, which then hydrolyzes (2703) to insoluble ferric hydroxide. The iron compounds deposit as a sludge, leaving the stream acid.

Detriment to natural waters is caused when these organisms form slime growths on rocks, bottoms, pipes, and other surfaces. Frequently the growths are red, and at times they die or slough off to form red or brown sludges or deposits. These bacteria, therefore, are indicators of iron in the water and foretell its unsuitability for use in boilers, laundries, tanneries, paper mills, beverage plants, and other processes where iron and/or the iron bacteria may be inimical (239, 665).

Well screens and gravel packing around wells in iron-bearing ground waters become clogged with growths of iron bacteria even when the water contains as little as 0.2 mg/l of iron (1050). Hem (2704) has rendered an excellent discussion of this subject. Most sedimentary rock contains iron in the form of pyrite. The rapid increase in solubility of pyrite with an increase in redox potential suggests that pyrite is likely to be relatively unstable in most ground-water environments. Colonies of iron bacteria may become established in or near wells and they accelerate oxidation and precipitation of the iron from the water. Occasionally, the precipitated iron will break lose and be pumped from the well. Also see Oborn and Hem (2705) for more on microbiota and vegetation and their relationship to iron in waters.

The metabolism of iron bacteria has been associated with the corrosion of iron and steel (1037, 1222, 1223). These organisms remove soluble ferrous compounds or free ions from the water and deposit it in their sheaths or separately as ferric-oxide tubercles (2700). In so doing they may augment and accelerate the electrolytic phases of corrosion of iron and steel.

Another mechanism associated with iron bacteria and corrosion is reported by Wolfson (2700). These bacteria can build up many layers thick, clogging pipes and screens, and providing anerobic conditions underneath for corrosive bacteria to multiply (see Sulfur Bacteria).

A Russian report (2706) indicates that an oxygen deficiency prevents the growth of *Gallionella.* Earlier, Suzuki (2707) reported that *Gallionella ferruginea* required more oxygen than *Leptothrix ochracea.* Both agreed that iron had to be present in order for growth to occur.

Carter (2238) mentions that the combination of chlorine with iron bacteria has resulted in chlorophenol tastes. *Crenothrix* and *Sphaerotilus natans* have been cited as causing tastes and odors (2226, 2242).

LEPTOSPIRA

(see also Pathogenic Bacteria)

Various *Leptospira* species distributed throughout the world have caused outbreaks of leptospirosis (also known

as Weil's disease or hemorrhagic jaundice) in man (520). When transmitted through water, the organism originates in the urine of infected animals, most frequently including dogs, rodents, foxes, skunks, raccoons, and opossums. Hence, it is most generally transmitted to swimmers exposed to contaminated waters, and is an occupational hazard to rice-field workers, sewer workers, miners, etc. In the United States, *L. icterohemorrhagiae, L. canicola, L. autumnalis,* and *L. pomona* have been recovered from human cases. At least 21 serogroups containing 59 serotypes are recognized (2663).

Leiguarda (2708) examined 348 samples of untreated surface and ground water and 25 samples of sewage in Argentina. He recovered *Leptospira* from 44 percent of the surface-water samples, 14 percent of the groundwater samples, and all of the sewage samples. Treated potable water did not contain *Leptospira.* Coagulation and sedimentation were ineffective, but chlorination destroyed them all.

A Yugoslavia epidemic is reported which originated with a poorly constructed well. Rats and even a cat had access to a chamber at the top of the well (2709). Leptospirosis among rice-field workers is described from Italy (2710). A report attributes one epidemic to harvest mice (2711).

L. icterohemorrhagiae survived in river water at 5°-6°C for 8-9 days, 25°-27°C for 5-6 days, and 31°-32°C for 3-4 days. In bacteria-free tap water at pH 7, they survived more than four weeks—with small amounts of nutrients present. High and low pH values were both detrimental. Survival in sewage was limited to 12-14 hours, but when diluted with 99 parts of tap water rose to 7-8 days. The organism survived 18-20 hours in sea water (710).

Russian workers have found that *Leptospira* persisted a maximum of 36 days after infection of river water. Freezing temperatures apparently are quite destructive to the organism in the water environment (2485). Other Russian work identified a water fever in an endemic area as leptospirosis. The main source is the field mouse, and water has played a leading role in the spread of the disease. Areas with acid soils are not associated with this so-called water fever (2712).

An Australian investigation demonstrated the presence of *L. australis* in drainage water from infected soil for up to 24 days. The pH of this soil ranged from 6.1 to 6.2, and of the infected water from 6.6 to 7.6 (2713).

Smith and Turner (2714) studied the relationship of pH to leptospiral survival. Survival in the laboratory under pH 7.0 ranged from 10 to 117 days, and over pH 7.0 from 21 to 152 days. In soil extracts, however, they found no such correlation. They observed that Bentonite clay absorbed about half the leptospires from a suspension and recommend further studies of this observation.

A probable connection between human cases and piggeries is reported from Israel. Bathing and drinking of lake water into which the pigsties drained is thought to be the route of transmission (2486, see also 2715).

In the United States, three additional papers have been published relating leptospirosis with swimming. These originated in the States of Alabama (2490), South Dakota (2489), and Idaho and Wyoming (2487). A fourth investigation found leptospires in raccoons near a military establishment in North Carolina, noting that human infections had occurred among the soldiers who had been quartered near a potentially infected stream (2716). According to Galton (2488) recognized cases of leptospirosis in the United States have appreciably increased, and the disease in cattle has spread rapidly. Leptospirosis associated with swimming has been reported by others (1627, 1628). Rat access to a closed swimming pool was found to be a source of *Leptospira* to swimmers in Czechoslovakia (2491).

Foxes at a fox farm have contacted leptospirosis from drinking water drawn from a rat-infested pond (1629). In Denmark (2305), 91 percent of cases of leptospirosis are thought to be due to direct contact with water (18 percent accidental contact, 60 percent bathing, and 13 percent working in water).

MARINE FLAGELLATES

(see also Algae, Diatoms, Bacteria-General, Worms—Free-Living, and Chapter V—Shellfish Culture)

Marine flagellates are responsible for two highly toxic situations, viz., they are the source of shellfish toxins that can cause illness and even death in man, and they are the agent of the so-called "poisonous tides," one of which is better known in the United States as the "Red Tide" off Florida's Gulf Coast (420, 539, 545, 749, 750, 2427, 2429, 2717, 2718).

Gonyaulax catenella has been reported present in Alaskan shellfish and is said to be preventing the further development of the shellfish industry in Alaska. The toxin can be present in certain clams during every month of the year (2429). Shellfish feed upon these organisms (751) and concentrate the alkaloid toxin which is not harmful to them (2428). *Gonyaulax catenella* toxin from shellfish was ingested by a man and his wife in Marin County, California. Each had consumed more than 15,000 mouse units of toxin (both recovered) (2719). See Chapter V, Shellfish Culture.

In a discussion of toxic shellfish in British Columbia, a suggestion has been made to investigate the use of clam types as plankton indicators (2433). Waldichuk's review of the British Columbian outbreak states that Pacific oysters, *Crassostrea gigas,* as well as various species of clams and mussels were involved. Toxicities of 200 mouse units or less are considered harmless. When toxicity exceeds 400 mouse units, a ban is usually imposed on collecting shellfish (2430). The toxin is described as ten times as potent for mice as strychnine (2427). Flagellates, nevertheless, constitute an important source of nutrition for some shellfish (*Mya arenaria*) (2631). For a thorough discussion of shellfish poison, see the work of McFarren et al. (2429).

Salinity has been studied as an ecological factor in marine flagellates and phytoplankton. *Syracosphaera carterel* had an optimum salinity of 30 parts per thousand; *Amphidinium* grows best at "low" salinities, while the optima for *Exuviella trochoideum, Prorocentrum micans,* and *Cryptomonas* are 15-20 per thousand (2720). *Gymnodinium brevis,* the "Red Tide" dinoflagellate, grew well throughout the salinity range of 27 to 37 per thousand. The organism could not survive in a

salinity of 13.7 per thousand, indicating that salinity may be a factor in limiting natural distribution (2721). A high light intensity, vitamin B-12, and sulfides are all essential for mass development of the red-tide organism (2722).

The toxin from *Gymnodinium veneficum* consists of a large molecule that cannot penetrate a dialysis bag, is soluble in water and lower alcohols, insoluble in ether and chloroform, unstable in acids, decomposed by hot alkali and thermostable in neutral solution (2723). It is toxic to a wide range of animals to a greater or lesser extent, but not to polychaetes. Death in fish is due to respiratory failure, and the action is on the nervous system (2724). Bacteria-free cultures of *G. brevis* were as toxic to fish as cultures containing bacteria. Concentrations of *G. brevis* used in these studies were 0.6 to 4.8×10^6/liter (2725).

A reduction in estuarine pollution, with a consequent reduction of nutrients in coastal water, "might help" control blooms of *G. brevis*. Plankton blooms are believed to be initiated by natural characteristics of near-shore waters, but once started the nutrients contributed by sewage may increase the bloom (2726). Another paper suggests that a high phosphorus content in Florida rivers draining into the Gulf may be related to *Gymnodinium* blooms (2718). Total phosphorus in the colored water of *Gymnodinium* blooms is 10 to 20 times as great as in adjacent waters (2727). Significant quantities of titanium and zirconium are also present in bloom waters (2722). Phosphorus rate of uptake was found to be 25 times greater in *Cladophora* than in *Fucus*. Both rate and productivity were porportional to algal surface per (water) volume ratio. Under conditions of high surface to volume ratio and favorable light conditions, uptake is related to potential productivity and not to existng metabolic level (2179). Copper sulfate at 20 lbs/acre reduced the count of *G. brevis* from several million per liter to none, but in two of five test areas, the organisms rose to lethal concentrations to fish 10 to 14 days later. The technique is recommended only for temporary use in relieving an area of the respiratory irritation caused by the airborne toxin (2728, 420).

Prymnesium is another fish killer (648, 2012). The toxin from *Prymnesium* is rapidly inactivated by the bacteria *Bacillus subtilis* and *Proteus vulgaris*. The toxin appears to be a substance of high molecular weight with some of the properties of protein that have a pronounced liability to oxidizing agents.

Collier (2297) writes that the sudden increase in multiplication rates of the organisms is caused by a complex of biological factors with physical factors playing a major role in effecting mechanical concentration. Naturally occurring organic factors are important, as may be succeeding concentrations of bacteria that produce vitamin B-12. Interaction between environmental copper and hydrogen sulfide may be a factor. Once started, the bloom may replenish the required nutrients through the decomposition of the fish that are killed. Particulate iron could not be related as a limiting growth factor for phytoplankton in inshore waters (2729).

The brown color of the Thames Estuary and other waters along the English coast during summer months is caused by great numbers of brown flagellates and dinoflagellates (752).

Davis observed no toxic effects upon oyster larvae when to larval cultures of *C. virginica* were added flagellates at concentrations up to 15,000 cells per ml per day. For some flagellates, concentrations of 25,000 cells per ml of water were harmless. Davis reported that although Korringa obtained good growth of oyster larvae in water containing 10,000-20,000 flagellates per ml, he later recommended "that water containing initially more than 5,000 flagellates or a commensurate great number of other phytoplankton, should be mistrusted" as it may contain toxic concentrations of phytoplankton excretory products (1647).

Hexamita, a marine flagellate, causes a winter disease in oysters of the Gulf Coast, and has been implicated in oyster mortalities in the Gulf of St. Lawrence (2415).

MOLDS

(see Fungi, Yeasts, and Actinomycetes)

MOLLUSKS

(see also Insects, Weeds, Worms—Parasitic, and Chapter IX)

Mollusk pollutants include both snails and clams, either of which can be important natural or corollary pollutants. Snails are primarily troublesome because they are the intermediate hosts of trematodes that are parasitic to man and animals. Snails and clams both are troublesome in potable water supplies because of their sheer physical presence in large numbers. Records of mollusk infestation of water supply systems in the U. S. concern mainly the faucet snail, *Bythinia tentaculata*. This organism accumulates in pumping station wells (also of irrigation systems), chokes service lines, and plugs up faucets. Other genera reported include the snails *Pleurocera, Helisoma, Physa,* and *Goniobasis,* and the Asiatic clam *Corbicula* (2418, 2420, 2421).

Mollusks have been included as pollution indicators. They are reported to be intolerant of heavy metals (2607), and in the succession of recovery of organically polluted streams appear after *Asellus* has replaced *Chironomus* (2545), i.e., they appear in a lesser polluted area than that occupied by the worm fauna (2540). The fingernail clam, *Pisidium,* was more prevalent in polluted areas than *Sphaerium* or *Musculum,* but none can survive the severe conditions of pollution tolerated by tubificids (2729, 2730). Ide (2541) explains how animals exhibit various degrees of tolerance to oxygen deficiency based on their respiratory mechanism. All clams and snails, except for pulmonate snails which are lung breathers, respire by means of blood gills or by the general body surface.

Temperature rise to 40°C has wrought disastrous effects on clams (2731). In French domestic and industrial water systems the warm water mussel *Dreissenia polymorpha* is a nuisance and can be controlled by flushing water at temperatures over 40°C through the systems for 15 minutes (2426).

Ecological studies of water infested by planorbid snails in Brazil show a wide variation in pH, 4.9 to 8.8, and also of sulfates and ammonia, but low total hardness,

iron, nitrites, nitrates, phosphates, and chlorides (2732). In studies of the distribution of *Australorbis glabratus,* it is suggested that large amounts of carbonates and bicarbonates relative to the amounts of chlorides and sulfates may account for its absence from some habitats, such as limestone streams, and that copper and zinc may be limiting quantities in those waters that are low in dissolved solids (2733). Other studies on *A. glabratus* in Puerto Rico showed water temperature to range from 21° to 35°C, and pH 6.0 to 9.1. The snail can be maintained about two weeks in redistilled water (2734). Hynes (2602) notes that shell-forming creatures require at least a minimum amount of calcium, about 20 mg/l. This hardness is the limiting value between hard and soft waters from the biological point of view. Extremely heavy pollution is unsuited to the snail, but light pollution provides an abundant food supply (2735). Organic pollution and lowered oxygen tension killed off two species of snails in Africa, one of which is an intermediate host of the liver fluke (2736). Streams treated with the molluscacide, sodium pentachlorophenate, (see Chapter IX, Pesticides) become repopulated with *A. glabratus* within four months (2737). For field tests of 33 compounds against the snail, see this latter reference. In stagnant water, 10 mg/l—sometimes as little as 1 mg/l—effecting 99-100 percent kills included pentabromophenol, pentachlorophenol, sodium and copper pentachlorophenates, bis (3, 5, 6-trichloro-2-hydroxyphenyl) methane and its sodium salt, and phenyl mercuric acetate.

The snail, *A. glabratus,* infected with cercariae of *Schistosoma mansoni,* has a lesser resistance to an anaerobic environment than normal snails. The shedding of cercariae is greatly reduced under anaerobic conditions. But infected snails, after six hours or longer in an anaerobic environment, shed normal cercariae when returned to an aerobic environment (2738). Most infected snails died if kept under anaerobic conditions for 16 hours.

The snail intermediate hosts of *Fasciola hepatica,* the common liver fluke, inhabits wet-weather springs, slowly moving fresh water, stagnant water, swampy and marshy lands, banks of ponds, streams, and ditches, and in mud (2739). Snail intermediate hosts of the schistosomes not specific for man can nevertheless release cercariae which penetrate man's skin causing swimmer's itch or cercarial dermatitis (2495, 2740).

Snails have been found in increasing numbers in Canadian streams that have been treated every year with aircraft-sprayed DDT (2534). Snails are reported to be quite adversely affected by silt (2615).

MOSQUITOES

(see also Insects and Chapter IX)

The problems caused by mosquitoes as a corollary pollutant appear to be on the increase. The larvae of *Culex pipiens,* also known as "sewage mosquitoes", feed on the organic compounds of sewage. When such mosquitoes emerge from their aquatic habitat, they will have contributed to BOD removal and therefore have been referred to as "flying BOD" since their emergence represents BOD taken out of the water (2143).

Heavy breeding of mosquitoes in areas of water impoundments and under irrigation has always been a difficult problem. A report from Russia mentions an increase in malaria among inhabitants along the shores of the Ribinsky impounding reservoir (2741). Encephalitis (2680) is the major U.S. illness associated with irrigation (2679). In both illnesses mosquitoes are the vector.

Although *Culex pipiens* is associated with polluted waters, most other mosquitoes prefer cleaner waters. Hence the environmental requirements of some mosquito larvae have been studied and some mosquito species have been considered as possible indicators of pollution (2543). The larvae of *Anopheles punctipennis, Culex tarsalis,* and *Aedes vexans* were found not to be affected by high concentrations of salts. Therefore it was concluded that oil-well brine pits would constitute suitable larval habitats (2742). Although mosquitoes vary considerably in their reaction to salinity, it is thought that the combination of salinity and organic matter better indicates optimum conditions than salinity alone. Also, the type of salinity may be an important factor (2736).

Surface tension studies revealed that *Culex pipiens* (as well as *C. fatigans* and *C. molestus*) developed normally in water of lowered surface tension. *Aedes aegypti,* the yellow fever vector that lives in close association with man (breeding in such artificial water retaining places as tin cans, tires, flower vases, etc.) prefers higher surface tensions. The malaria vector, *Anopheles quadrimaculatus,* demonstrated the most limited tolerance to surface tension change (53-73.5 dynes/cm) at which normal development occurs (2743). Therefore, changes in the surface tension of water because of pollutants may be responsible for changes in the type of mosquito life in the environment.

Mosquito larvae appear to be quite sensitive to temperature. Water temperatures of 40°-45°C for a five-minute exposure will kill many species of larvae. Shallow tanks in the tropics can reach 40°C and streams can reach 34°C. The rate of temperature change is also of importance.

In mosquito ecology, dissolved oxygen is not indicated as a factor of great importance and therefore has not been the subject of much study. Nevertheless, organic content is a factor of great importance to anopheline mosquitoes. Most anophelines prefer clean water. It is interesting to note that the larvae themselves are unaffected by degrees of pollution up to 20 or 30 times that in which the female refuses to oviposit. Further, although *Anopheles gambiae* will tolerate a considerable degree of fecal pollution, it is very sensitive to the organic pollution of decaying vegetation. An anopheline of India shows a preference for waters in freshly dug tanks or borrow pits, and as these waters eutrophy, larval density declines.

Odom and Sumerford (2744) demonstrated that mosquito (*Culex*) pupae are more resistant to DDT than are its larvae. Less than 0.001 mg/l effected 100% kill of the larvae, whereas 0.025 mg/l were required for an equivalent kill of pupae. See Chapter IX, Pesticides.

MYCOBACTERIUM TUBERCULOSIS

(see also Pathogenic Bacteria)

Although the transmission of bovine tuberculosis by drinking unpasteurized milk from infected cows has long been acknowledged, transmission through polluted waters is not mentioned in the American Public Health Association's official report of 1960 (2663). Nevertheless, the organism appears hardy enough and the potential real enough for researchers to continue to probe the dangers.

Klein (1756) cites a paper by Christiansen and Jepsen in which they report that cows drinking brook water polluted by waste waters from a slaughterhouse remained free from tuberculosis in spite of 8 of 15 water samples containing *Mycobacterium tuberculosis* (see also 519, 2492, 2745).

In 1954, Miller and Anderson (2493) reported two cases of primary tuberculosis in children following immersion in sewage-contaminated water. Hence, Greenberg and Kupka (2482), in reviewing the literature on tuberculosis transmission by waste waters, state that the danger is most marked with swimming or near-drowning.

In a series of laboratory investigations in which sewage was added to various pathogenic cultures, it was found that sewage had an inhibitory effect on tubercle bacilli (2746). However, Dedie (2403) reports that the tubercle bacillus can remain infective for three months in waste waters, and up to six months in soil. The Yearbook of Agriculture on "Animal Diseases" states that tubercle bacilli dropped in stagnant pools with manure may remain alive for a year or longer (2402). Haigo (2747) verifies the presence in feces of other organisms antagonistic to the tubercle bacillus, but estimate survival in natural waters and sewage as about one month. In a study of sewage sludge, Jensen (2748) reported that it took 11 to 15 months before the organism could no longer be demonstrated in sludge-drying beds.

Experiments with guinea pigs injected with milk from tubercular cattle show an inoculating dose to be 30 bacilli. The same paper gives an interesting account of the dangers associated with feeding cattle on land spray-irrigated with waste water from a German dairy, using actual figures obtained in a study. These figures show that cattle would be exposed to 111 virulent organisms in each square meter of soil (2404).

In terms of distance, tuberculosis bacteria have been found over one mile downstream from a sanitorium sewage outfall (1382, 2305).

PASTEURELLA TULARENSIS

(see also Pathogenic Bacteria)

Pasteurella tularensis is the etiologic agent of tularemia. This disease can be transmitted in a number of ways of which the drinking of water contaminated by infected animals is probably a lesser avenue. Nevertheless, a Russian paper notes a tularemia epidemic among muskrats that infected hunters and fishermen (2749); another associates the illness with washing and bathing in infected waters (2305, 2494); and a third discusses the nature of outbreaks associated with wells as compared to outbreaks associated with surface streams (2750). Outbreaks associated with wells generally involved a large number of people drinking the water in a short time. The source of difficulty, usually small rodents falling into wells through unsound casings, is easily corrected and eliminated. Outbreaks associated with streams, however, result in only a few patients at a time over a much longer period of time and greater geographical area. Also, elimination of the source is much more difficult, the only rational method being to provide the population with clean drinking water (see also 2492).

Banfield (2751) reports the first demonstration of *Pasteurella tularensis* in the natural waters of Canada. It was associated with an epizootic among beavers and muskrats that died in the water. However, no known human cases resulted.

Ten years earlier, Parker et al. (2752) had observed a tularemia epizootic among muskrats and beavers in the northwestern part of the U. S. This occurrence resulted in a number of human cases, but transmission through water was not involved. Nevertheless, Parker stated that water transmission was potentially possible and that under favorable conditions such contamination could persist for periods of at least seven months and at any season of the year. In 1942, Jellison (2753) published the results of an investigation of tularemia among beavers in Montana. At this time he indicated that tularemia could possibly be transmitted among animals via contaminated water and also suggested that *P. tularensis*-contaminated stream water may be the occasional source of human infection in the U. S.

In 1950, Jellison et al. (2754) described tularemia among humans that was traced to an infected domestic rural water supply. Although farm animals had been fenced out of the area of the spring, ferral rodents had access to it.

Pasteurella tularensis was isolated from 14 of 37 samples by workers who were studying tularemia infection in a population of voles (2755). Quan et al. (2756) calculated the LD_{50} of *P. tularensis* suspensions in drinking water of mice as 10^7 organisms. In a series of experiments by intubation into the stomach with a blunt-nosed hypodermic needle, the LD_{50} was calculated at 10^6 organisms.

PATHOGENIC BACTERIA

(see also Coliform Bacteria, Salmonella, Shigella, Mycobacterium tuberculosis, Leptospira, Pasteurella tularensis, Protozoa, and Insects)

Bacterial diseases that have been transmitted to human beings or livestock by contaminated water supplies include not only intestinal illnesses, such as dysentery, typhoid fever, paratyphoid fevers, cholera, and gastroenteritis (497, 520, 533, 702, 703, 704) but also, though less frequently, tuberculosis (302, 573, 1382, 1383, 1626), anthrax (346, 497, 520), brucellosis (497, 705), tularemia (671, 706, 707, 708), leptospirosis (330, 423, 520, 709, 710, 1382, 1627, 1628, 1629), and other infections. Occasionally strains of coliforms may be pathogenic, although they are ordinarily harmless (711, 2575, 2576, 2577, 2578). Some diseases, such as brucellosis, tuberculosis, tularemia, and salmonella infections are common to man and certain animal species. Conceivably, therefore, they may be transmitted in water polluted by animal discharges (301, 334).

Except for the intestinal pathogens, most disease organisms do not gain access to water in any great numbers and can survive in it for only a limited time, so that their spread by this route has been rare. In recent years, however, more attention is being directed towards an understanding of the factors involved in the spread of disease by water, because greater demands are being made upon limited water supplies in populated areas and it has become necessary to draw sometimes upon questionable sources for various uses.

The survival of disease organisms is dependent on many factors, both physiological and environmental. Among the more important physiologically are (a) their ability to form resistant spores, (b) reaction to heat, cold, and chemicals, and (c) competition with other organisms. Factors that are environmentally significant include oxygen supply, pH, temperature, sunlight, nutrient supply, sedimentation, dilution (619, 712, 713, 714), and the antibiotic and competitive activity of other organisms (301, 302, 435, 487, 715, 716, 718).

On the other hand, whether pathogenic organisms can cause infection depends on other factors, such as their virulence, chance of ingestion by proper hosts, and the resistance of the hosts (301).

Because of the operation of so many independent variables, public-health practice is based not on the probability but on the possibility of infection. It is not concerned with defining limiting or threshold concentrations of pathogenic organisms, because the limit is zero. It is concerned with providing "safe" water, which may be consumed internally or otherwise used without menace to the health of the consumer. It is hardly possible for water to be so safe that absolutely no risk is taken in using it; on the other hand, it is possible to provide water safe to such a degree that the danger involved is small enough properly to be disregarded (152, 334). Nevertheless, both France and Belgium have standards for drinking water that specify "no" pathogenic organisms (717).

According to Maxcy (719), to be considered waterborne a disease must meet four criteria: (a) it must be associated with insanitary conditions; (b) its incidence must be reduced when water-supply improvements are made; (c) it must increase with epidemic rapidity when water supplies are subject to temporary pollution; and (d) it must be of greater incidence among those using a suspected supply than among others using different supplies, alike in all other aspects. The isolation of pathogenic organisms from water does not constitute prooof that the diseases they cause are water-borne unless this evidence can be supported by epidemiological data. The intestinal diseases are commonly accepted as water-borne because they have met the conditions listed. At the present time, the water route of transmission of other diseases is still considered epidemiologically unimportant, even though they may occasionally be spread by water supplies.

Kruse (2122) discusses a "so-called water fever" that appears immediately after infection of a water supply and precedes a true typhoid epidemic. There has been some concern expressed for a reappraisal of water-borne ailments, for many aspects are not clearly understood (2123).

Vibrio comma was isolated from 12 of 89 samples of water taken from the River Hooghly and associated canals in Calcutta, India (2757). *Vibrio* survived 81 days in sterilized harbor water, 64 days in sterilized open-ocean water, and less than 32 days in polluted fresh water. Other reports state that the organism survived over 36 days in heat-sterilized sea water, 10 days in harbor water, 47 days in sea water (2589), 7 days in Yugoslav river water, and 1 day in badly polluted water (2305). Cholera bacteria in drinking water can be destroyed by adding HCl to give a concentration of 0.06 N (pH about 4.0) for 10 minutes (2758).

Infection by tetanus after a bathing accident led to the examination of water samples from the Elbe River. Of 30 samples, eight were positive (2759).

Brucellosis infection may occur by water (2305). So may listerosis (sheep), coccidiosis (sheep, goats, and poultry), and pullorum disease (2402). *Brucella* can survive up to 90 days in distilled water, and up to 72 days according to another author. Others have claimed up to 120 days in tap water. Frogs have been experimentally infected with *Brucella melitensis,* as were a tortoise and carp. Brucella were isolated from internal organs of the latter two in 15 to 40 days after placing in a contaminated aquarium. The strain lost virulence as measured in guinea pigs (2760). See Koser (2745) for *Brucella* in sewage-irrigation water.

In a review of anthrax infection, Gillissen (2761) concludes that infection by the water-borne route is rare, but that the potential is still sufficiently strong so that drainage from leather factories should not be permitted to enter meadows and fields (520).

An epidemic of granulomatous skin lesions caused by an acid-fast *Mycobacterium* which secondarily invaded abrasions in the skin of swimmers in Sweden was first reported in 1951 (2479). In 1954, the new bacterium was described as *Mycobacterium balnei* (2762), and the disease called "swimming pool disease" or mycobacteriosis balnearea (2480). Cases from Canada and Sweden were reported in 1957 (2481). In 1959, an epidemic of considerable proportions occurred in Colorado, involving 262 cases. *M. balnei* was found in both water and tissues (2476, 2477, 2478).

Staphylococcus aureus was found to be harmful to some ciliates (2313). This organism was occasionally isolated from sewage-contaminated sea bathing waters in a 5-year study of illness associated with bathing in such waters (2468). *S. aureus* reportedly succumbs rapidly in polluted sea water (2589). Less than 15 lbs. of a dried, purified *Staphylococcus* enterotoxin in one million gallons of drinking water would poison the greater part of the exposed population.

The toxin of *C. botulinum* is even more potent. Less than 10 lbs of partially purified toxin in one million gallons of drinking water would infect more than 90 percent of the exposed population (2763). *Clostridium botulinum* partially purified type A toxin, in the pH range 7.0-10.0, is inactivated to the extent of 99.90-99.99 percent in 15-30 seconds at 25°C by free available chlorine (see also 2764, 2765). Chloramines are relatively ineffective (2766). There have been undocumented statements that decaying algae can support the growth of *Clostridium botulinum,* which in turn can result in the

death of water fowl (2012, 2285). Chickens have contracted botulism from drinking pond water. The toxin was found in the water even though decomposing animal matter was not present (1629). Sea water samples collected in the vicinity of a sewage outfall in the Mediterranean showed *Clostridium welchii (perfringens)* to occur up to 1200 feet from the outfall (2767). Fish caught in coastal waters were reported to contain *C. perfringens,* those caught at sea did not (2321).

Erysipelas is a skin inflammation caused by hemolytic streptococci. This infection may arise from skin punctures obtained while handling fish (1630, 2768). *Listeria monocytogenes* and *Erysipelothrix rhusiopathiae (insidiosa)* were isolated from surface waters, sometimes along with *Pasteurella tularensis,* as well as from ferral rodents. This occurrence resulted in a recommendation that when combatting epidemics of illnesses caused by these organisms among either humans or animals, the water-borne route must be considered (2769). *E. rhusiopathiae* was found in 10 of 30 samples of sewage in Konigsberg, and in 5 of 52 samples in Munich. Slaughterhouses were believed to be the sources (2770).

Furunculosis of fish is caused by *Bacterium salmonicida (Aeromonas salmonicida),* an obligate parasite that survives longer in polluted than unpolluted waters—up to 67 days (2771).

An Association of Beekeepers (Germany) has protested against the application of sewage to ground as a spray in areas where bees are numerous. Bees flying through the spray may become contaminated with pathogens and these may in turn be transmitted to humans by stinging (2681).

Water free from disease-producing organisms may still be capable of harming milk. Such water should be treated to give a "low" colony count at 22°C and should contain no milk-souring organisms in 10 ml samples (2772).

POLIOVIRUSES

1. General. The polioviruses comprise a group of three primary types, Type I being the most prevalent. It is a relatively small sphere 25-30 mμ in diameter, indigenous to man but capable experimentally of infecting other primates and some rodents. The majority of infections caused by poliovirus are inapparent or abortive, the most common clinical manifestations being febrile illness and enteric disturbance. On occasions, however, the virus will affect nerve tissue, causing aseptic meningitis and paralysis of varying kind and severity (2159).

The British Medical Journal reports that a patient with poliomyelitis may excrete in one gram of feces enough virus to infect one million monkeys. Half the patients are still excreting virus after 3 weeks and some continue to excrete it up to 12 weeks (2773). It is, therefore, not surprising that the virus has been found in sewage, in the early stages of disposal processes at treatment plants, or even in treated effluent (533, 719, 721, 722, 723, 724, 727, 884, 2119, 2593, 2774). Earlier work (724, 725) indicated that the virus can be transported in sewage for at least ⅛ mile, and can persist in water or refrigerated feces for several weeks. Russian workers in 1956 (2775) reported that the poliovirus remains viable in human feces for over 6 months, in milk at room temperature for 31 days and at 10°C for over 150 days, and in experimentally polluted water for at least 118 days. A 1950 Canadian paper reported that a strain of poliovirus in stools survived at least 188 days after addition to river water, temperature 4°C. Dilution was 1:200, and the virus retained its ability to induce paralysis in monkeys (2776). German experiments with the Columbia-SK-virus reaffirmed Maxcy's statement that the virus is unable to grow and multiply while suspended in sewage (723). The same work showed that virulence of the virus in moderately or highly polluted river water continued for up to 6 days, and in sterile physiological salt solution for over 11 days (2496). The virus has been inactivated when inoculated into cultures of sewage protozoa (726).

Poliovirus reportedly resembles Coxsackie virus in its ability to withstand a wide range of pH. All strains tested survived pH 2.3-9.4 for one day and 4.0 to 8.0 for 7 days at room temporature (2594). With respect to heat, it is reported that the virus is destroyed by the higher pasteurization temperature when the virus is present in such concentrations as are "to be expected in milk" (2496). Lawson (2777) states that a rodent poliovirus is 95% reduced in infectivity when exposed to 45°C for 30 minutes, but requires 60°C for 30 minutes to be 99.9% or greater inactivated. Robinson (2594), using a Y-SK strain, reports no infectivity after 30 minutes at 53°-55°C. Also, an MM strain demonstrated more heat tolerance but still lost most infectivity at 62°-64°C for 30 minutes.

2. Cross References. Viruses, Coxsackie Viruses, and Infectious Hepatitis Viruses.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. There is still no positive evidence to prove that polio is spread by drinking water. Epidemiologically, polio does not behave like a water-borne disease (719, 722, 723, 724, 883, 2124). Nevertheless, two polio epidemics have been reported in which water-borne poliovirus is highly suspect. A Canadian outbreak is reported to have had all the characteristics of a water-borne epidemic postulated by Maxcy and Howe, namely incidence correlated with poor supplies, and explosive outbreaks occur in widely scattered areas of the municipal supply (2778). Also, the epidemiological investigation of a Nebraska epidemic strongly indicated that back siphonage from sewage was responsible. In this study, an absence of chlorine and presence of coliforms in the water at a date later than the epidemic were observed (2779). South African studies, as interpreted by Russian workers in the light of their own experience, were also highly incriminating of a water-borne source of virus in a polio epidemic (2774). Earlier papers that reported a possible association between lower fluorides and a higher polio incidence, and also between low nitrates and lower polio incidence remain unconfirmed (729, 730, 1033).

b. Irrigation Waters. The mode of transmission of polio and the role of contaminated food have not been established (302). Bingel and Engelhardt (2496) state that since the poliovirus is sensitive to temperatures of 85°-100°C, fruit and vegetables can be disinfected by

immersion in boiling water. Also, soil studies show that the virus can reach ground water, but it is so dilute that chlorination would be quite effective.

c. Fish and Other Aquatic Life. Crovari (2455) studied Type 2 poliovirus and mussels. His conclusions: (1) If mussels are left for some hours (he used 3 hours) in sea water containing poliovirus, they become contaminated. (2) Passage of the virus through the mollusk does not affect its pathogenicity. (3) Virus can be recovered from infected mussels even after 24 hours cleansing in running sea water, but not after 48 hours.

One investigator has pointed out similarities between the effects of poliomyelitis and mussel poisoning, relating both to poisoning by cyanogen compounds, possibly produced in water by cyanophore glucosides of plant origin. Sulfocyanates and cyanides are said to be capable of producing gastrointestinal and neurological symptoms identical with those of polio. It is suggested that the virus might be a cyanolytic enzyme in the human body, or an endogenous protein produced in the body by cyanophore compounds (421, 731, 732).

On the other hand, a detailed comparison of the symptoms of polio with those of cyanide poisoning has shown such clear-cut differences between the two symptom-complexes that it is hardly likely that the two could be related or should be confused (733).

d. Recreational Use. Certain studies of localized polio epidemics have indicated that swimming at polluted beaches has been second to direct contact (722, 734, 735). Moore (2468) states that a statistically controlled epidemiological study of the bathing histories of 150 poliomyelitis cases in children living permanently by the seaside gave no evidence that sea bathing played any part in causing the disease (also see 2469, 2470). Van Den Bossche concludes that swimming pools were not a factor in a Belgian epidemic (2498). An investigation of an epidemic in Germany concluded that swimming in untreated or in purified water had no effect on incidence (2497).

PROTOZOA

1. General. The occurrence of protozoa in a water can be either beneficial or a nuisance. Protozoa have been found to be factors in the elimination of viruses and pathogenic bacteria from water, and are an important part of the food chain for higher forms of animal life. They also assist bacteria in the processes of decomposition of organic matter (726, 736, 737, 740). Apparently not all bacteria affect protozoa in a similar manner, however (2313). *Escherichia coli* and *Salmonella typhimurium* were found to be harmful to *Paramecium caudatum, Chilodonella uncinata,* and *Colpidium colpoda,* as was *Staphylococcus aureus* although after a longer period. *Salmonella enteritidis* and *Bacillus subtillis* were not harmful. *B. subtilus* was soon preferred by the above three experimental ciliates. Protozoa may occur as part of a biological slime or sewage fungus (see Slime bacteria, Fungi). Heukelekian (2144) ascribes to the role of the protozoa the second order of importance in the biology of the self-purification of waters, because of their ingestion of small organic particulates, utilization of soluble organic material, ingestion of bacteria, and role as food for higher organisms. Common protozoans include *Epistilis, Vorticella, Colpidium,* and *Stentor* (2143).

A number of protozoa are intestinal parasites of man (2492), a few of which are pathogenic. They may be regarded as primary polluting substances, whereas the protozoa that proliferate because of high bacteria and algae counts in waste waters would be corollary pollutants.

Stammer (2690) tested fourteen indicator organisms of the saprobic classification system against hydrogen sulfide and ammonia. The protozoa of these tests all had a low resistance to these chemicals in the absence of oxygen, regardless of the position of the protozoa in the saprobic system. Nevertheless, some species of protozoa, such as *Stentor coeruleus*, become abundant in anaerobic situations (2780). The formation of slimes containing the methane-oxidizing bacteria *Methanomonas methania* and protozoa has caused the blocking of filters (2781). Prechlorination can sometimes lengthen filter runs by killing small, motile, gelatinous organisms such as *Monas* which otherwise would not settle out (2260).

Mohr (2782) has discussed the protozoa as indicators of pollution. Intestinal protozoa are fragile and distinct from the forms commonly found in water, except when encysted. Identification of protozoa is difficult (2503). Protozoa may be more sensitive to temperature changes than many other life forms. Trembley mentions that green algae and many protozoa became less abundant in heated waste waters (2622). Protozoan populations of swamp-drainage and sewage areas of a Pennsylvania lake were similar except that more species were associated with the area of sewage pollution (2783). Seasonally, protozoa have been reported in the fall (738), and reflect the size of the bacterial population. Acid waters show a great decrease in protozoa. Some of the *Ctenostomata* and *Metopus* have been found a few times at pH 6.5, but a bottom dwelling *Pelomyxa* is the sole acid water organism (2503). In a stream from which pollution was greatly reduced, protozoa and algae became diversified more quickly than the insect fauna (2544).

In a study of ciliate ecology, it is reported that growth is best in cultural conditions closest to that of the natural environment. In enriched cultures, most species die out. Most ciliates are sensitive to high bacterial concentrations. However, ciliates have a wide range of sensitivity to carbon dioxide and oxygen tensions. Species sensitive to acid conditions were likewise sensitive to high carbon dioxide tensions, whereas less sensitive forms tolerated higher carbon dioxide tensions (2784). Lackey (2230) reports that oxygen depletion of a stream tends to eliminate all but a few species of anaerobic ciliates and colorless flagellates. After many years of study the status of indicator species seems little near clarification (see also 739).

A Baltimore reservoir with an average pH of 7.0 rose to 9.0 in a portion where diatoms were abundant. Where the pH was normal, protozoa were abundant and diatoms scarce (2629). Another paper states that pH will increase if blue-green algae are present but if protozoa become abundant, the pH would go down (2628).

Studies on cellulose decomposition showed that a potassium deficiency hinders protozoan development (2139). The succession of organisms in the decomposition

of cellulose approximates that of digestion: small bacteria and small flagellates, followed by small ciliates, and finally larger ciliates, amoeba, and rotatoria. In a study of a small artificial pond, chrysomonads were not found when the water contained more than 0.02 mg/l phosphate but cryptomonads and diatoms increased at this concentration (2628). See Provasoli's work (2202) for further information on protozoan nutrition, and also see Johanna Resigs' work (2790) on marine protozoans of the order Foraminifera and their relation to pollution.

Cationic and nonionic surfactants in low concentration were toxic to *Pelomyxa carolinensis*, but low concentrations of nonionics were growth-stimulating. Protozoa have been associated with the metabolism of hexadecanol evaporation-retarding films (2293). In the harbor of Hamburg, Germany, ciliates predominate among the plankton during the winter (2785). In a German river, all aquatic life was snuffed out by a discharge of phenols. First protozoa appeared when the DO reached 5.3 mg/l and phenols dropped to 4.3 mg/l. In increasing from zero to 2.0 mg/l, phenols generally decreased the variety and quantity of protozoa with no relation to its saprobic classification (2786). Laboratory tests showed the protozoa could not utilize phenols directly.

Bartsch and Ingram show that counts of ciliates reach their greatest numbers in the zone of decomposition in polluted streams (2143).

Cysts of *Endamoeba histolytica* are well recognized for their durability (744, 745). Chang's work (2787) showed that they survive in feces and in distilled water at 0°C for 62 days. Below 0°C, the lower the temperature, the more rapidly the cysts are killed. The cysts die out quickly upon drying out, but survive months in water and sewage (720, 745). They pass quite readily through biological sewage treatment processes (743, 746, 2788). Emile and Tomasic (2305) relate survival not only to temperature but to the number of bacteria in the water. If "a lot of microbes" are present and the temperature is 27-30°C, cysts may disappear within nine days. At 22°C and with fewer bacteria, cysts may subsist for 29 days. In pure water without organic matter (no temperature mentioned) cysts may survive several months. In Denver, 46 percent of the *Endamoeba coli* cysts survived primary treatment and chlorination which removed 99 percent of coliforms and enterococci (2789).

Protozoa concentrate radioactive materials (2109). See Chapter VIII, Radioactivity.

2. Cross References. Bacteria—General, Slime Bacteria, Fungi, Algae, Polioviruses, and Chapter X.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Ingram and Bartsch (2420) have compiled a booklet of animals associated with potable water supplies. Among the protozoa most frequently mentioned are *Paramecium, Didinium, Arcella,* and *Difflugia.* Pathogenic protozoa include *Endamoeba histolytica, Giardia lamblia,* and *Balantidium coli* which may be transmitted by infected drinking water or sewage-irrigated foods that are eaten raw (497, 745).

As recently as 1949, the water transmission of amoebic dysentery was not universally acknowledged (742). However, a factor in this slow recognition may be explained by findings of the germ-free animal studies. They show that amoebic dysentery in animals does not occur unless other intestinal microflora are present (2791). Persons afflicted with acute diarrhea discharge trophozoite forms in their stools and few cysts. The trophozoites are fragile forms, not capable of lengthy survival under adverse environmental conditions (2305). Chronic amoebiasis sufferers discharge few trophozoites, but many of the hardy cyst forms (720).

The water treatment processes of coagulation, sedimentation, and filtration are partially effective as removal processes, but chlorination cannot be relied upon for destruction of the cysts as it can with pathogenic vegetative bacterial forms (741, 2305). The drought experience at Chanute, Kansas, in which treated sewage effluent was recycled through a water supply reservoir (2119) did not disclose any *E. histolytica* cysts in the treated water. However, cysts of free living amoebas comparable in size to *E. histolytica* were found regularly in the treated water.

Vorticella has been associated with bitter taste as well as odor in Valparaiso, Indiana (2247). *Bursaria* is another odor-producing form (2242).

A violent outbreak of amebiasis occurred among industrial workers in Indiana in 1953 (2792, 2793). Chlorination had successfully abolished bacteriological evidence of contamination, but did not influence the cysts. Rendtorff and Holt (2794) performed some interesting human studies on the transmission of *Endamoeba coli* and *Giardia lamblia* in drinking water. Cysts of both species were introduced into a bottled water dispenser from which 20 men drank. Four became infected with *Endamoeba,* none with *Giardia.* In another experiment, cysts were added to aerated tap water maintained at 8°C and fed to volunteers from 0 to 16 days after storage. *Endamoeba* infected 70.6 percent, *Giardia* 64.7 percent.

Ratios of amoebic cysts to coliform bacteria of 1:100,000, and of carriers to the general population of 1 or 2 to 20, have been reported (747). Cysts have been reported to be non-infective until they have matured for 24 to 48 hours (748).

b. Industrial Water Supplies. Protozoa are undesirable pollutants for various uses, such as the bottling of carbonated drinks, papermaking, dyeing, and photography (346, 403).

c. Irrigation Waters. The use of *Endamoeba*-polluted irrigation water may cause dysentery among consumers of uncooked produce. Waterborne epidemics occur in temperate climates only as a result of massive quantities of fresh pollution, and are rare in the United States. Rudolfs et al. (748) contend that in the temperate zone field-grown crops subject to contamination by cysts are safe, and can be eaten raw, one week after contamination has stopped. In wetter, tropical regions, however, they are safe only after two weeks (2795). This contention is likely to be disputed by many public-health authorities.

A study of the degree of intestinal parasitism of urban sewage workers with *Endamoeba histolytica* revealed a 14 percent rate; that for workers in irrigation fields, a 9 percent rate (2912).

d. Fish and Aquatic Life. Parasitic protozoa are known to cause disease in fish (308). Protozoa serve as food organisms for shellfish.

RICKETTSIA

The only rickettsial agent reported in connection with polluted water found in this survey is *Rickettsia burneti*. This rickettsia, also known as *Coxiella burneti*, is the causative agent of Q fever, a relatively mild illness of benign nature with a negligible fatality except for the aged. Its mode of transmission is commonly airborne in or near contaminated premises or infected animals (2663). Out of 19 water samples collected on 6 ranches in California (an endemic state), 6 samples contained *Rickettsia burneti* in detectable amounts (2796). However, Q fever is not a waterborne disease.

ROTIFERA (ROTATORIA)

(see also Crustacea)

The Rotifera are a group of small, usually microscopic animals, that have attracted much attention from microscopists because of their wide-spread distribution in waters of all kinds, and the great abundance in which they frequently occur (16). Among the small aquatic animals, the Rotifera and Crustacea comprise groups with which the water pollution specialist generally has a limited amount of familiarity. However, there exist many other groups of small aquatic fauna.

These aquatic fauna are not primary pollutants. They may become corollary pollutants owing to the development of ideal conditions for their growth in abundance by means of certain waste discharges. For example, Bick (2139) cites the succession of organisms in the decomposition of cellulose as corresponding approximately to that of digestion: small bacteria and small flagellates followed by small ciliates, and finally by larger ciliates, amoeba, and Rotifera. Also, Bartsch and Ingram (2143) cite the predominance of rotifers and crustacea in stream purification in the latter stages of the recovery zone. However, there was not uncovered in this survey a single reference as to any detrimental effects that large numbers or any particular species of Rotifera may have on any of the beneficial water uses. Rather, since they are a part of the aquatic food chain, one would suspect that large numbers of rotifers may eventually lead to an increasing fish productivity.

In a study of acid mine pollution, it was noted that the predominant aquatic population remaining after an excessive amount of acid had drastically reduced the plankton population consisted entirely of rotifers (2797). However Järnefelt (2168) reports only one species of Rotifera as being present in a lake that was "acid-trophied" by wastes from a powder factory.

Ingram and Bartsch (2420) cite two Rotifera as of interest in potable water supplies, viz, *Philodina* and *Brachionus*. Rotifers are commonly found in uncovered finished water reservoirs and have been reported to penetrate filters in England.

SALMONELLA

1. General. The genus *Salmonella* is responsible for some of the most severe diseases in man. *Salmonella typhosa* causes typhoid fever, and *S. paratyphi*, *S. schottmuelleri*, and *S. hirschfeldii* cause paratyphoid fever (2289). The genus *Salmonella* includes a large number of species. Bergey's Manual (2637) lists 343 serotypes that were recognized by the year 1955. The species *S. typhosa*, known also by the names of *Bacterium typhosum*, *Bacillus typhosus*, *Bacillus typhi*, *Bacillus typhi abdominalis*, *Bacterium typhi*, *Eberthella typhi*, *Salmonella typhi*, etc. (2637), has been studied most extensively. Although it differs in some respects from the others, being somewhat longer lived and more resistant to bactericides, it is fairly representative of the other intestinal pathogens as a group (302).

Considerable research has been directed toward determining the survival times of various salmonella species in a variety of media. Some of the data pertinent to water pollution are tabulated in Table 7-1. The bulk of the organisms, when thrust into unfavorable media, undergo an initial rapid die-off such that for most, survival is for only a few days (488, 716, 2492). A few, however, can survive for considerable periods of time, and on occasion have been known to multiply outside the human body (716, 720, 2467, 2798). One investigation (2799) reports survival in terms of distance; salmonella was recovered from a stream 15 to 21 miles below a sewage outfall. Unidentified factors in waters antagonistic toward salmonella have been reported. These waters include ground water (deep well) (2800) and a saline lake water (2801).

Salmonella were isolated from 83 percent of the water samples taken from a canal in Argentina. Recovered salmonella included 11 species, the more common of which were, respectively, *S. newport*, *S. typhimurium*, *S. muenster*, *S. bredeney*, *S. montevideo*, and *S. anatum*. These six species were also isolated from children suffering from enteric disorders with the same respective frequency (2802). *S. panama* was recovered from a harbor water in the neighborhood of a patient infected with the same organism (2803). *S. typhosa* has been isolated from gull feces (479). Sea birds excrete typhoid and paratyphoid bacteria for long periods after removal from access to polluted waters (2804).

2. Cross References. Pathogenic Bacteria, Algae, Protozoa, Worms—Free-Living, and Chapter V—Swimming and Bathing Waters.

3. Effects on Beneficial Uses

a. Domestic Water Supplies. Salmonella in humans was attributed to gulls that were contaminated while scavenging in the area of sewage outfalls and later polluted the community surface-water supply. Cultures from the gulls and two hospitalized patients were identical (2813). Kruse (2122) states that infection (with typhoid) is not conveyed by drinking polluted water but by contact of the water with some easily polluted food stuff under suitable conditions of temperature and growth of the bacteria. Nevertheless, improvements in the quality of a potable supply have been followed by a reduction in the typhoid fever rate among the population and thus the incidence of typhoid fever in a community has served as a measure of the sanitary quality of the water supply. The number of *S. typhosa* organisms in a supply is dependent, of course, on the past history of typhoid in the community; thus a reduction in incidence with a consequent reduction in the

TABLE 7-1

SURVIVAL DATA FOR *SALMONELLA*

Type Organism Reported	*Media and Conditions*	*Survival Time*	*Reference*
S. typhi, *S. enteritides*, *S. paratyphi*	Spring water	10% survived 44 hours	2805
S. typhi	Synthetic well water, pH 5.9, 5°-25° C	7 days	2806
S. typhi	Synthetic well water, pH 7 & 9, 5° & 21° C	77+ days	2806
S. typhi	Synthetic well water, pH 8, 5° & 21° C	196+ days	2806
Typhoid bacilli	Tap, well, and distilled	2 weeks to 80 days	2589
S. typhi	Tap and distilled water, innoculum 1×10^9/ml	211 days	2807
	Distilled water, innoculum 5×10^3/ml	59 days	2807
	Distilled water, innoculum 1×10^8/ml	494 days	2807
S. paratyphi B.	Surface water	3 months	2808
Typhoid bacteria	Water 0° C	9 weeks	2809
	Water 5° C	7 weeks	
	Water 10° C	5 weeks	
	Water 18° C	4 weeks	
S. typhi	Water without humus	58-69 days	2569
S. paratyphi A.	Tap water, aged 24 hrs.	29 days	2798
S. paratyphi B.	Tap water, aged 24 hrs.	25% remaining in 29 days	2798
S. typhiabdominalis	Tap water, aged 24 hrs. + 0.2 gm/l sterilized feces, 16°-18° C	Multiplication	2798
S. typhosa	Water or sewage, warm weather	Several days	533
S. typhosa	Water or sewage, cold weather	months	533
S. typhosa	Fresh water	4 days	488
S. typhi, *S. enteritides*, *S. paratyphi*	Sea water	50-60% survived 44 hours	2805
S. typhi	Autoclaved sea water	Several days	2588
S. paratyphi B.	Autoclaved sea water	Several days	2588
E. typhosa	Sea water, heat sterilized	25 days	2589
E. typhosa	Sea water, raw	9 days	2589
E. typhosa	San Francisco Bay, 10° C	12-28 days	2589
E. typhosa	San Francisco Bay, filtered, 10° C	14-34 days	2589
S. typhimurium	Sea water	Few died in 24 hours	2589
E. typhosa	Tidal water	>2-3 weeks	2589
E. typhosa	Sea water	14 days	2589
Typhoid organisms	Sea water	12-16 hours	2589
Paratyphoid bacteria	Sea water	21 days	2589
S. paratyphi B.	Sea water, innoculum 10×10^6/ml	2 months	2466
S. typhosa	Sea water	>30 days	487
S. typhosa	Sea water	>32 days	488
Salmonella group	Muddy waste water	180 days	2403
S. schottmuelleri	Sludge banks and ditch water	1.5 years	2810
Typhoid bacilli	Septic sewage	5 weeks	2589
S. typhi	Water with humus added	85-104 days	2569
S. typhosa	Polluted water	>4 days	488
E. typhosa	Imhoff tank sludge	11 days	2589
Typhoid bacilli	Oysters	9-42 days	2589
S. typhosa	Shell oysters	14-60 days	2589
S. schottmuelleri	Oysters, 5° C	49 days	2589
Typhoid bacteria	In gut of fish	7-9 weeks	1630
Typhoid bacteria	Carp	4-6 weeks	2768
Salmonella	Soil and potato (surface)	40+ days	2811
	Carrot (surface)	10+ days	2811
	Cabbage and gooseberries (surface)	5+ days	2811
Typhoid bacteria	Excreta	10-84 days	488
	Septic tanks	>27 days	488
S. typhosa	Within gut of Nematodes of Rhabditidae family	3-4 days	2812

number of carriers results in a corresponding reduction in the contamination of the water. Typhoid may be contracted from infected shellfish, milk, and other foods; nevertheless, over the years, the incidence of typhoid has dropped enormously in this country, so that under the present favorable conditions there is less likelihood that water pollution will result directly in serious epidemics of typhoid fever, although pollution can still cause widespread outbreaks of other enteric diseases.

Less than one pound of a preparation of *Salmonella anatum* added to one million gallons of drinking water is required to produce salmonellosis in 50 percent of the exposed population. HCl added to water to give a 0.06 N solution (pH about 4.0) for 10 minutes will destroy salmonella (2758).

b. Irrigation Waters. In studies lasting several years, salmonella including *S. typhosa*, were isolated from over 50 percent of sewage contaminated irrigation waters. However, only one isolation was made from vegetables to which the water had been applied (1516, 2788). Previous workers had essentially the same results, viz., they recovered salmonella from the water samples but none from vegetable washings (1515). Ratios of enteric organisms computed from irrigation water samples were 255,000 coliforms: 4,800 enterococci: 1 salmonella (2580, 2814). The median value of salmonellae in eleven irrigation waters was 0.9 per 100 ml (1383). The work of Rudolfs et al. (515) showed that salmonellae could not survive on the surfaces of vegetables more than one week under the prevaling atmospheric conditions (see also 2795, 2816). Salmonella organisms have survived for 15-46 days on the surface of sewage-irrigated soils, and for as long as 10 weeks in the lower layers (2817) (see also 2745).

c. Stock and Wildlife Watering. Fowl typhoid caused by *S. gallinarium* was successfully treated by the addition of 0.0375 percent sulfaquinoxaline in the drinking water (2815). Calf typhoid was traced to hay which had been grown on ground flooded in the spring by a stream polluted with sewage. The bacteria deposited in the grass were experimentally demonstrated to survive natural drying and remain virulent in the winter (2814).

d. Fish and Aquatic Life. *S. typhimurium* was found to be harmful to ciliates; *S. enteritides* not harmful (2313). Fish may act as carriers of typhoid and paratyphoid (1630).

Survival of *S. schottmuelleri* in shell oysters was similar to that of *S. typhosa,* which is about 15 to 60 days in shell stock. *S. schottmuelleri* persists at least as long as *E. coli,* but the rate of reduction during storage is not as great as that for *E. coli. S. schottmuelleri* seldom multiply in animals in marketable condition. However, increases do occur on prolonged storage (2448).

e. Recreational Use. Five different strains of *S. typhosa* were found in water at an Australian beach where 10 people contracted typhoid fever. A nearby sewage treatment plant effluent was implicated as the source (2475). Two cases of typhoid fever were attributed to swimming in a river (2474). Two other cases of typhoid fever in Spain were attributed to bathing in the sea (2473). None of the above cases were adequately documented (see Chapter V—Swimming and Bathing Waters).

In a 5-year study of sea-bathing waters and illness of bathers, the British Committee on Bathing Beach Contamination encountered small numbers of salmonella in a high proportion of samples; as many as 40 percent of those samples containing over 10,000 coliforms per 100 ml had salmonella. Although the most frequently isolated salmonella was *S. paratyphi B.* (see also 2818), a total of 33 different species was encountered (2468). Possibly a million or more salmonellae would have to be ingested before clinical infection results (2470). See Chapter V, Swimming and Bathing Waters, Coliform Bacteria—Recreational Use. A Polish study reported the same findings as the British study (2464).

According to Moore (2466), *S. typhi* could be recovered from sewage sediment in samples maintained at room temperature although the organism was not present in the supernatant. Later, Steiniger found salmonellae in North Sea water (again mostly *S. paratyphi B.)* and calculated that the numbers could not be explained in terms of numbers in sewage reaching the harbor area. Hence, he assumed the organisms were multiplying—a conclusion supported by Guelin (2467).

SEWAGE FUNGUS

(see Fungi)

SHIGELLA

(see also Pathogenic Bacteria, Salmonella, and Worms —Free-Living)

The genus *Shigella* is the chief cause of dysentery (bacillary) in man. Important species include *S. dysenteriae, S. flexneri,* and *S. sonnei* (2289). The *Shigella* group does not come within the definition of the coliform group in one respect—it does not produce gas in lactose. *Shigella* has been implicated in water-borne epidemics on several occasions (2492). The seventh edition of Bergey's Manual lists five species in addition to the above three (2637).

The *Shigella* species are not as hardy as are the salmonellae. Concentrations of 1×10^9/ml in tap water, distilled water, and normal saline of *S. sonnei, S. flexneri,* and *S. shigae* did not remain viable as long as *Salmonella typhi* (211 days) at room temperature, diffuse daylight, and aerobic conditions. Inoculations of 5×10^3/ml of the following species in distilled water gave viable results as indicated: *S. sonnei* 107 days; *S. flexneri* 238 days; and *S. shigae* 7 days (2807). Inoculation densities of 1×10^9/ml gave the following results: *S. sonnei* 197 days, *S. flexneri* 391 days, and *S. shigae* 7 days (2807).

In a synthetic well water, pH 5.9 and temperature 5°-25° C, *S. boydi* survived at least 469 days. At pH 7 or 9, and temperatures of 5° and 21°C, *S. sonnei, S. shigae,* and *S. flexneri* survived at least 77 days, and at pH 8, both temperatures, at least 196 days (2806). The viability of *S. flexneri* in water was increased from 98-111 days to 160 days by the addition of humus (2569).

In other viability reports, dysentery bacteria (presumably Shigella) were largely destroyed within 12 hours when placed in sea water, but a few persisted for up to 12 days (2819). In an unidentified water at room temperature, *S. flexneri* and *S. boydi* survived 52 days, and *S. sonnei* 105 days (2820). Studies of *S. flexneri* in Black Sea water, Lake Tekirghiol water (saline), well water, and a piped supply indicated survival was dependent upon size of inoculum, exposure to sunlight, and salt content. Survival in non-sterilized water usually did not exceed one or two days (2801).

The work of Rudolfs et al. (515) showed that *Shigella* could not survive on the surfaces of vegetables for more than one week under the atmospheric conditions that prevailed (see also 2795, 2816). *Shigella* survived 3-4 days within the gut of worms of the Rhabditidae family (2812).

SLIME BACTERIA

(see also Iron Bacteria, Sulfur Bacteria, and Fungi)

The collective term "slime bacteria" as used in this report includes a host of slime-forming organisms. As differentiated from chemical slime, biological slime refers to films of microorganisms that form slippery layers on wetted surfaces. This term is applied to the larger, often filamentous aggregates of sphaerotilus, zoogleae, and fungi that occur in polluted streams (2821). Most commonly reported in the literature of water pollution among the slime bacteria are the iron or higher bacteria and certain fungi.

Terminology among these corollary pollutants is quite confusing. In order to minimize this confusion, the nomenclature as set forth in the 1957 edition of Bergey's Manual (2637) will be followed. Where an investigator utilizes a name that differs from the above guide, the Bergey's terminology is presented immediately following within parentheses.

The literature on slime bacteria most frequently concerns *Sphaerotilus,* occasionally *Leptothrix.* These bacteria have one point in common, viz, they belong to the family Chlamydobacteriaciae. Bergey's Manual recognizes three genera in this family, *Sphaerotilus, Leptothrix,* and *Toxothrix.* The latter genus is a group of iron bacteria and has not been referred to specifically in the literature as being among the slime pollutants.

One of the main reasons for the confusion in the nomenclature is that the same organism can assume two or possibly three different physiological forms. Wurtz (2822) states that *Sphaerotilus natans* appears in the more polluted streams while the other physiological form known as *Cladothrix dichotoma* (*Sphaerotilus dichotomus*) is produced in less polluted streams. Bahr (2823, 2824) also concludes that *Cladothrix dichotoma* (*Sphaerotilus dichotomus*) is the form that *Sphaerotilus natans* takes during unfavorable environmental conditions. The National Council for Stream Improvement has sponsored a considerable amount of research on slime bacteria, and one of their bulletins (2825) states that *Sphaerotilus natans, Cladothrix dichotoma* (*Sphaerotilus dichotomus*), and *Leptothrix ochracea* are one and the same organism (2821). Also, that *Sphaerotilus discophorus* (not in Bergey's) and *Leptothrix discophorus* (*L. discophora*) are the same organism.

Eldridge (2826), in summarizing a conference held on the sphaerotilus problem, notes that the term "sphaerotilus" includes a large association of organisms and that these apparently take three forms; the slime form, a "pond" form that does not produce slime, and a third form that is associated with iron. Hohnl (2643) makes the same statements but also cites varying environmental conditions. Slime growths require, in addition to the usual organic and inorganic nutrients and suitable environmental conditions, 18 amino acids, and a sugar content of 20 to 50 µg/l. *S. natans* predominated at pH values of 5.8 to 9, *S. dichotoma* (*S. dichotomus*) at pH 6.4, and when the pH dropped to the range of 5.6 to 5.2, the fungi *Fusarium* and *Mucor* predominated. However, the fungi most frequently mentioned among the slimes is *Leptomitus* (2823), more specifically, *Leptomitus lacteus* (2825, 2827).

Sphaerotilus problems are frequently associated with sulfite waste liquors. Concentrations of 25 to 100 mg/l have been responsible for abnormal growths (2642). Jenkins (2828) notes that in streams receiving paper-mill effluents, conditions responsible for *S. natans* growth were a biological imbalance resulting from the addition of organic matter giving a high dilution, near neutral pH, and insufficient dissolved oxygen. It is an aerobic organism and its growth is inhibited by salt water and also by ammonia (2826). Nevertheless, a pH limit of 4.6 to 8.0 and E_h (redox potential) limit of 200 to 500 v for the growth of *Sphaerotilus* in estuary studies has been cited (2829).

The major nutrient requirements include mono- and disaccharides, organic acids (acetic and lactic), amino acids, nitrate nitrogen, organic nitrogen, and phosphates.

Hexose sugars appear to be more available to the organism than pentose sugars. There is some question as to its need for iron. Phosphate is a major factor controlling growth, extremely small amounts (0.01 mg/l) being required to implement growth. There is little growth below 3°C. The temperature range for growth is 5° to 24° C, with growth being more rapid at the higher temperatures. pH values below 5.0 and above 11.0 will prevent growth, optimum being between pH 6.0 and 9.0. Stream velocities influence growth (2826). Individual cells of *S. natans* are motile when set free (2830).

Among the statements cited by Eldridge in his summary of the sphaerotilus conference was that this organism is not associated with gross pollution. There appears to be an overwhelming amount of disagreement in the literature with this statement. Two examples among several include statements by Dondero (2821) and Butcher (2545). Dondero states that *Sphaerotilus* is a dominant organism in zones of heavy pollution resulting from sewage and wastes containing mono- and disaccharides, lower organic acids, alcohols, and amino acids. Butcher maintains that with gross organic pollution the flora of a river consists of sewage fungus, chiefly *Sphaerotilus natans*.

Hohnl (2831) found that dilutions of as little as 1:694,000 of spent sulfite liquor, 1:470,000 of spent yeast waste, or 1:350,000 of spent liquor from alcohol fermentation would support growth of *S. natans*.

The major problem involved with *Sphaerotilus* growths is interference with commercial and sports fishing by the large floating masses. The slime reduces benthic organisms, hence has an influence on the biology of the stream. If the growth occurs on salmon-spawning beds, it can smother the eggs (2826). Ingram and Towne (2183, 2197) cite additional problems, viz, a cause of tastes and odors upon decay, nuisance to swimming, water skiing, and boating, and clogging of rapid-sand filters. An unusual problem related to slime growths but not specifically to *Sphaerotilus* occurred in refinery wastes (2502). When the slimes deteriorated in the receiving waters, large amounts of entrained oil were liberated creating an objectionable appearance. The same paper states that spent and regenerated caustic wastes containing phenols effectively controlled slime growths in the refinery's cooling waters.

Secondary effects from *Sphaerotilus* may be even more serious than the primary effect of being esthetically offensive or a nuisance to fishermen. The detached growths can settle in sludge banks many miles from their origin, giving rise to anaerobic decomposition and the subsequent offensive effects (2500). The oxygen demand of dead *Sphaerotilus* sludge is reported to be 11 times that of living *Sphaerotilus* (2821).

One control method under study is the method of intermittent discharge (1920, 2832). It has been applied to sulfite waste liquors, which are stored for 5 days and then released to the river in a 24-hour period. It is estimated that such intermittent discharge has reduced slime growth by 80 to 90 percent (2833, 2834). Cawley (2835) succeeded in reducing growth by intermittent discharge but could not control the growth by this technique.

Another possible control method is reported by Waitz and Lackey (2836). In studying *Sphaerotilus natans*, they noted that the addition of 1 mg of copper sulfate per liter of medium completely inhibited growth. However, this was not studied in vivo. The same paper suggests that, on the basis of the characteristics exhibited in these experiments, *Sphaerotilus* could be more accurately classified as a sulfur than as an iron bacterium.

Wolfson (2700) reports that in waterflood systems, the primary slime formers are *Pseudomonas, Flavobacterium, Escherichia, Aerobacter*, and *Bacillus*, many of which are capable of growing in a 10 percent NaCl en-

vironment. Such slimes allow anaerobic organisms to thrive underneath the layers where sulfate-reducing bacteria can thrive.

SNAILS

(see Mollusks)

SPHAEROTILUS

(see Slime Bacteria)

STREPTOCOCCUS FAECALIS

(see Fecal Streptococci)

SULFUR BACTERIA

(see also Slime Bacteria, Fungi, Iron Bacteria, and Chapter VI—Sulfur)

The sulfur bacteria constitute a heterogeneous group of autotrophic bacteria of varying forms. They are found in soil containing large amounts of sulfur and in the mud of lakes, rivers, estuaries, and harbors. Some species live only in aquatic habitats (2289).

When excessive growths of sulfur bacteria occur in surface or ground waters as a result of man's activities, such growths are considered to be corollary pollutants. Sulfur bacteria may occur as thick slimy growths in streams that are subject to direct pollution of sulfides from tanneries or paper mills, or to the indirect formation of sulfides resulting from anaerobic decomposition of organic matter.

Sulfur bacteria include sulfate-reducing forms (*Desulfovibrio*) and sulfide-oxidizing forms (*Thiobacillus, Beggiatoa*), and others. The sulfate reducers are strict anaerobes and are found in large numbers only in places where sulfate is present in abundance. The oxidative forms, also called colorless or white, filamentous, sulfur bacteria, are restricted to places where H_2S is present but can flourish only where oxygen is available. Hence, they are generally found in areas where one body of water with an adequate oxygen content borders on another which supplies the H_2S (15). An example is furnished by the Aitolikon Lagoon in Greece. Here a saline bottom water devoid of oxygen content underlies a fresh water of high oxygen content. Periodically, H_2S rises in large masses to the surface waters, resulting in patchy blooms of sulfur bacteria (2837).

Another group among the sulfur bacteria are the photosynthetic forms. They include the sulfur purple and green bacteria and they differ from the colorless forms in that they require light and an absence of oxygen. They utilize sulfide in much the same way, but being anaerobes do not occur frequently in natural waters (1036).

Filamentous white sulfur bacteria, such as *Beggiatoa alba*, obtain the energy necessary for their growth by oxidizing sulfide ion to colloidal sulfur, which is then stored in the cells. This stored sulfur, however, eventually disappears, being itself oxidized to sulfate to yield more energy (1036).

Lackey (2838) published studies on certain sulfur bacterial, viz., *Beggiatoa gigantea, B. arachnoidea,* and *Thiodendron* sp. He reports that they tolerate a wide range of most environmental conditions but that all require H_2S—as little as 0.2 mg/l or less, and also at least a trace of oxygen—0.1 mg/l or less. The above three require a "high-salt" water of approximately one-half strength sea water or more, and all prefer alkaline waters of pH 7.0-8.4—the latter not necessarily limiting. *Beggiatoa,* generally presumed to indicate presence of pollution and anaerobiases, actually simply demonstrates the presence of H_2S (2503).

Mutze and Engel (2839) added thiosulfate to samples of water from the Elbe River. The rate at which thiosulfate was oxidized was found to be a function of the number of sulfur-oxidizing organisms present. *Thiobacillus thiooxidans* and *T. dentrificans* were isolated from these samples. Another report (2703) mentions the accelerating effect that *T. thiooxidans* and *T. thioparus* have on the hydrolysis of sulfur compounds, partly simultaneously and partly alternately.

The corrosion or deterioration of concrete exposed to waters or atmospheres rich in hydrogen sulfide appears to be attributable in part to sulfide- and sulfur-oxidizing bacteria such as *Thiobacillus thiooxidans* (1039, 2358) or *Thiobacillus concretivorous* (198, 1040). Where such organisms are introduced or fostered in natural waters, concrete corrosion may be accelerated. CO_2 and H_2S react on the surface of fresh concrete lowering the pH from 11 to 7.5 and producing sulfate. Then *T. thiooxidans, T. thioparus,* and similar sulfur-oxidizing organisms can develop. These organisms oxidize the sulfur compounds to H_2SO_4 (2358, 2840).

Iron and steel are corroded by sulfate-reducing bacteria. This type of corrosion is rapid and is not self-stifling, as is rusting, where the adherent oxide film partially protects underlying metal (2358). Harbors and estuaries are considered as offering ideal conditions for this type of corrosion because the bottom mud is rich in organic matter and anaerobic conditions develop quite readily (2841, 2842). A study of harbor mud from 46 different areas of the world indicated that the amount of sulfur increases with the amount of organic matter—as determined by loss on ignition.

The addition of ferrous iron causes an increase in the rate of corrosion of cast iron based on studies with *Desulfovibrio desulfuricans* (2843). Mortensen (2844) states that corrosion of steel in soil where no oxygen is available is due mainly to sulfate-reducing bacteria, but in harbors, it is mainly an electrolytic form of corrosion.

The sulfate-reducing *Desulfovibrio* bacteria are most common and important in water flood systems (2700). They are obligate anaerobic (facultative) autotrophic organisms with an optimum growth pH of 7, but a growth range of 5.5 to 8.5. Optimum temperature range is 24°-43°C, but they are found to exist at 0°-100°C. They can grow in fresh water or brines of up to 30 percent NaCl. Such organisms in pipe lines cause corrosion in several ways: (1) The H_2S released is corrosive and this action is accelerated in the presence of O_2. (2) Electrochemical reactions are propogated by the organisms. (3) Chemical corrosion itself enhances the growth of these organisms, and leads to more corrosion.

Sulfides in the Thames Estuary are believed to be formed mainly by sulfate-reducing bacteria rather than by the breakdown of sulfur-containing organic compounds by sulfhydryl-splitting bacteria. The loss of re-

duced sulfate is a small factor in the over-all oxygen balance of the estuary (2845).

Sulfate-reducing bacteria liberate H_2S in anaerobic environments. This gas not only corrodes various surfaces, but is also ordorous and highly toxic. Such organisms are of the *Desulfovibrio desulfuricans* type (1037, 1038). Springer reports that heavy growths of water weeds may lead to anaerobic conditions, which in turn permit the growth of sulfate-reducing organisms, the H_2S from which can kill oysters (2424).

In H_2S-bearing ground water, sulfur bacteria may occur on well screens or gravel packings, or in pipes and reservoirs receiving such water. Sulfate-reducing bacteria are reported to be the most common organism found in the slimes that plug up tubes of cooling systems, or line the tube walls thereby acting as insulators and decreasing efficiency (2846).

Drummond and Postgate (2847), in an attempt to control hydrogen sulfide odors emanating from a waterlogged sanitary landfill, found that concentrations of chromate acted bacteriostatically on sulfate-reducing bacteria thereby controlling odors. Furness (2848) introduced cultures of sulfide oxidizers (*Chlorobium* and *Chromatium*) in an unsuccessful effort to reduce odors.

A colored sulfur bacterium, *Rhodocapsa*, caused taste and odors and a purplish-pink color in a service reservoir. Gulls feeding at a sewage works, and resting on the reservoir at night were believed to be responsible (2849). *Beggiatoa* has also been responsible for tastes and odors (2226, 2242).

TREES

(see also Phenols)

Alder trees are reported to be undesirable growths when adjacent to water reservoirs. The leaves discolor the water and impart an unpleasant taste. Leaf worms attack the tree in great number, covering the ground with offal. Hence, it was recommended that they be cleared from areas 100 to 150 feet around impoundments and conifers planted in their place (2314, 2850).

A blue-violet color of a pond in Poland was found to be a complex of tannins and iron, the tannins originating from fallen alder leaves (2851).

Maple leaves were reported to have an oxygen demand equivalent to 75 percent of their weight, whereas oak leaves and pine needles exerted only a 50 percent demand (2852, 2853).

Some trees, such as cottonwood and willow, transpire enormous quantities of water and when located along irrigation canals the water loss is substantial. Such plants, whose root systems extend into the ground water, are known as "phreatophytes." The reader is referred to a USGS publication of that name (2854).

Investigations were made on the influence of vegetation on the quality of drinking water from dunes in the Netherlands to ascertain whether afforestation would have a beneficial or a harmful effect. Water from pine and oak forests showed higher values of color, oxygen consumed, bicarbonate ion, hardness, and chloride content than water from soil with a natural low vegetation. Pine gave lower values for color and oxygen consumed than oak or birch, but higher values for chloride and hardness. An oak forest with elder undergrowth showed high nitrate values for the retained water (2855).

The latest study of the effects on water quality of trees and other vegetation left intact on the ground that was to form the bed of a reservoir again substantiates earlier work that no adverse effects are observed (2856).

Afforestation with spruce and red cedar are said to affect trout streams adversely (2857). Lackey (2503) reports that cypress leaves so soften and color waters as to reduce their recreational, fishing, and domestic-use attractiveness. Tupelo gum leaves blacken swamp waters and inhibit the development of certain plants and animals. He also mentions Hoaks' work that demonstrated the production of phenolic compounds by decaying oak leaves.

TUBERCULOSIS BACTERIA

(see *Mycobacterium tuberculosis*)

TULAREMIA BACTERIA

(see *Pasteurella tularensis*)

VIRUSES

(see Enteroviruses, Adenoviruses, Coxsackie Viruses, Infectious Hepatitis Viruses, and Polioviruses)

Collectively, the viruses are infectious agents of both plant and animal cells. They are ultramiscroscopic, obligate intro-cellular parasites that manifest their presence by destruction or impairment of the host cells. Most viruses fall in the size range of 10 to 500 mμ. Morphologically they vary, some being rod-shaped, some spherical, and some appear as crystals. Whether viruses are organisms in the strict sense is still a point of controversy. However, it appears that they are devoid of any intrinsic metabolism, depending entirely upon the host cell for development and replication. All viruses contain a core of ribonucleic (RNA) and/or deoxyribonucleic acid (DNA) surrounded by a coat of protein. Some viruses contain additional substances. The vital portion of the virus particle is the nucleic acid core which in some cases can alone establish infection. Among the characteristics of virsuses is their high degree of host specificity, their affinity to certain tissues, and their ability to elicit the formation of antibodies in suitable hosts.

The viruses tend to fall in groups or "families," each member of the family sharing certain basic properties with the others but having certain individual properties. Hence, there usually exists a number of types within each family. The type is identified by its antigenic specificity and is referred to properly as a "serotype." In addition to serological tests, identification requires several other tests—host range, nature of infection in an experimental animal, type of cytopathic effect, ability to clump red blood cells, etc. (2159).

The problems associated with viruses in waters are of increasing concern. Seventy-six viruses are now known to be pathogenic to man (2062). Viruses found in polluted water include the adenoviruses, Coxsackie viruses, ECHO viruses, viruses of infectious hepatitis, polioviruses, and reoviruses. Of the illnesses caused by these organisms, only infectious hepatitis is known sometimes to be water-borne. However, the implication that polio-

virus and adenovirus infections can at times be transmitted by polluted water is quite strong. These findings are of considerable importance to the water industry and have been responsible for a great deal of speculation as to the adequacy of water treatment processes and of the coliform tests as a measure of the water's sanitary quality.

The control of pathogenic viruses appears to require greater chlorine residuals than do vegatative bacteria. Also, viruses require that the chlorine be of the free form (2858), the combined form being substantially less effective. Hence, the treatment generally afforded water in the control of enteric bacterial infection is, at times, apparently not sufficient for the control of viruses (1772, 2859). For more information on the effects of chlorine and other disinfectants, see the reviews by Kabler, Clarke, Berg, and Chang (2860), and by Clarke and Chang (2596). No further information has developed with respect to the association of viruses with goiter (887, 1368).

Viruses causing pretibial fever and inclusion conjunctivitis have been associated with swimming (883).

WEEDS

1. General. Weeds can be defined as any plants growing in an unwanted place. However, water weeds are those aquatic growths that have a root system and are attached, thus, in main, differentiating them from free-floating growths such as algae. There are a few free-floating species, however. They include duckweed (*Lemna*), bladderwort (*Utricularia vulgaris*), Canadian pondweed (*Elodea canadensis*), and sometimes normally rooted plants that are found as free-floating plants, such as *Myriophyllum* species (2861). Plants and animals in an aquatic environment exist in complex communities in a state of dynamic balance. It is not a state of stable equilibrium, for change is inherent and is due to the many varying factors that affect the abundance of any one member of the community which in turn reflects upon all others (2693).

Water weeds can be grouped into two types, emergent and submerged (2861). Up to a certain point, either or both types can serve a worthwhile purpose. Beyond this point they have a nuisance value. In a polluted river, lush growths of *Elodea* and *Potamogeton* are likely to occur in the recovery zone (2143, 2541). The heavy growths of aquatic plants in the Potomac River below Washington, D.C., are due partly to the sewage of that city (2012).

Heavy growths of plants are indirectly related to such human diseases as malaria and schistosomiasis since these allow protected breeding sites for the *Anopheles* mosquitoes and feeding areas for snail intermediate hosts of the trematode. Water weeds may be directly associated with human illness, such as a dermatitis from the smartweed (*Polygonum hydropiperoides*) or pollinosis from aquatic grasses (2495).

Plants such as the water hyacinth (*Eichormia crassipes*), parrot-feather (*Myriophyllum brasilliense*) sometimes grow so thick that the backwater buildup floods marginal land. In one situation, sewage backed up into a populated area (2495).

Such dense growths have an effect on the water also. *E. crassipes* can cut off light, exhaust nitrates and phosphates, and exclude oxygen (2012). Water lettuce (*Pistia*) can accumulate sulfates thereby limiting plankton production (2503). Dense mats of *Trapa natans* can cause the water underneath to become stagnant (2165, 2505).

A study of macrophytes in polluted and unpolluted reaches of a river suggested that the pollution that prevailed was not responsible for the changes in the community, enrichment by sewage discharge having no obvious effect. In this study, growth is believed to be primarily determined by the amount of available solar radiation. Net primary productivities of 0.31, 0.47, and 0.85 grams of organic carbon per square meter per day and efficiencies of utilization of solar energy of 0.19, 0.24 and 0.25 percent were recorded. The average increase in dissolved oxygen due to the plant growth (March to June) in the area of maximum growth was about 1.2 mg/l/mile (2862). Another report by the same authors quotes 1.65 mg/l/mile (2863).

Aquatic vegetation contains large amounts of iron and is quite important in adding or removing iron in water (2705).

The use of aniline dyes to inhibit aquatic weeds by excluding light led to the finding that the following year normally semi-emergent weeds failed to reach the surface (2195).

An edible seaweed has been used as one of several criteria in limiting discharge of radioactive effluents (2864). See Chapter VIII, Radioactivity, for further information on this item and on concentration factors for isotopes of several weeds. Kelp is a commercially important seaweed off the California coast. Apparent indications that kelp beds were being damaged by sewage pollution led to a series of investgiations on kelp ecology and effects of various pollutants. The effects are given in Chapters VI, IX, and X under the name of the polluting substance. Of pertinence to this chapter however, is the discovery that effects on photosynthesis of kelp is one of the most sensitive parameters used in biological assays that has yet been found. Thus far in these studies, however, sewage pollution of sea waters does not appear to be an important contributing factor to the decline of kelp in those areas of the Pacific coast that were studied (2106, 2107, 2108, 2503, 2865, 2866, 2867, 2868, 2869, 2870). The presence of water lilies and bladderwort serves as an excellent indication of the presence of desmids (freshwater algae) according to Palmer (2213). Such waters tend to be brownish in color, low in nutrients, and neutral to acid in reaction.

Pest plants reported in the south-central states include *Myriophyllum*, American lotus, cattails, sawgrass, water hyacinth, pond lily, duckweed, floating evening primrose, watermilfoils, sedges, and water fern (2213).

An added risk to the over-abundance of plant growth is the danger of secondary pollution from dead plants (2610). Lush vegetation on exposed flats during a dry year presents a considerable organic loading for the following year when the flats are submerged (2514). This condition has been suggested as an explanation of high manganese content in the waters.

The effects of water weeds are usually much greater in rivers than in lakes (2872). In addition to the chemical effects exerted in both bodies of water, the physical effects of weeds in restricting water movement which are reflected in water level, velocity, silting, and flooding, all have an additional indirect influence on pollution.

Turbidity of water exerts a considerable influence on aquatic vegetation (1355, 2873). *Potamogeton pectinatus* is said to be somewhat unaffected by excessive sedimentation because of its thin leaves (2286, 2546). Nevertheless, fine solids render water opaque and plant growth nil (2546, 2615, 2874). On the other hand, large-sized grit settles and leads to a decline in number of weed beds (2875).

The application of hydrated lime to increase alkalinity of an unproductive lake led to a marked increase in *Myriophyllum tenellem* over a previous barren bottom. An incidental observation was the increased bottom life underneath *Myriophyllum* (2174). Alkaline waters destroy the effectiveness of copper sulfate for the removal of water weeds (2876) (see Chapter IX, Pesticides).

2. Cross References. Insects and Chapter IX.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Weeds, living or decomposing, may impart bad tastes and odors to water supplies (152, 858, 889, 2213, 2424, 2877, 2878). Significant reduction of water quantity may be caused by transpiration (2525). Other problems include the clogging of water intake pipes, and imparting of color to water (2424, 2878).

b. Industrial Water Supplies. There is no information available relative to any specific industrial use. However, the change in water quality, such as decrease of carbon dioxide, increase of dissolved oxygen, increase of pH and chlorine demand, and associated effects of heavy weed growths can cause problems to various industrial users (858).

c. Irrigation Waters. Heavy weed growths can choke irrigation channels (858, 889, 891, 1355, 2226, 2262, 2265, 2525, 2879), impede metering devices and piping (2265, 2525, 2880), and cause water loss though transpiration (890, 2525, 2880). Losses from aquatic and ditch bank weeds in 17 western states have been estimated at 1,272,-480 acre-feet annually (2881). The reduced flow causes increased sedimentation (2525). Water losses from aquatic weeds were more than three times those caused by ditchbank weeds (2525). Flow reduction from floating weeds such as water hyacinth can be as much as 40 percent; that from submerged weed such as naiad and pondweeds as much as 97 percent (2880). Grass-type weeds are among the more troublesome problems in the more humid states (2882).

d. Stock and Wildlife Watering. Decomposing aquatic plants washed up in windrows on shores serve as breeding areas for the stable fly, *Stomoxys calcitrans,* which attacks man and domestic animals quite viciously. Cattle and sheep are sometimes poisoned by eating leaves of arrowgrass, *Triglochin maritima.* Weeds also offer protected breeding areas for mosquitoes including types that transmit encephalitis (2495). Eurasian watermilfoil (*Myriophyllum spicatum, M. exalbescens*) is reported to be increasing in one area such that it could have a serious effect on the distribution of the total canvasback population (2883).

e. Fish and Aquatic Life. As shelter for fish-food organisms and small fish, weeds are beneficial to fish production. In addition, they help to maintain favorable stream conditions for aquatic life by consuming carbon dioxide and producing oxygen during photosynthesis (667, 589, 858, 889, 892, 2174, 2682). However, there is some question as to just how thick vegetation should be to make conditions ideal for fish life (2884).

Decaying weeds reduce dissolved oxygen, resulting at times in fish kills (2495), or otherwise ruin waters for fish (2012, 2495). They make it difficult to harvest fish (891, 2424, 2495, 2878, 2884). In a study of the animal population on seven species of submerged plants, it was found that *Myriophyllum spicatum* and *Potamogeton crispus* were more densely populated than the rest. Midge larvae and freshwater annelids together made up 59 to 93 percent of the population on most plants. *Myriophyllum* averaged a greater population than *Potamogeton* (2682). The growth of the alga *Anabaena* has been reported to be suppressed by the growth of water weeds (2885). This condition can be expected when the waters are shaded by the plants, and has led Krecker (2682) to remark that a beneficial effect of watermilfoil is that it may occupy areas that might otherwise be occupied by objectionable algae.

Heavy stands of Eurasian watermilfoil seriously affect oyster growth by smothering oyster grounds or reducing water movement, which affects food supply, circulation, and temperature, and may lead to anaerobic conditions, the growth of sulfur bacteria, and release of toxic hydrogen sulfide (2424, 2883). The presence of aquatic plants has been reported to reduce the effect of copper salts used to control the snail *Australorbis glabratus*, intermediate host of *Schistosoma mansoni* (2886).

f. Recreational Use. Both living and dead weeds may be objectionable because of appearance or odors. Massive growths can impede fishing, boating, swimming, and other recreational uses (858, 889, 2012, 2262, 2495, 2878, 2883). Bathing sites may be made unpleasant by the spiny caltrops of the water chestnut, *Trapa natans*, or by the cercariae of swimmers itch which comes from snails dependent upon aquatic vegetation (2495). Swimmers have become entangled in weeds and drowned (2262). Surber has published an excellent booklet pertaining to improved fishing by means of weed control (2887).

g. Navigation and Drainage. Heavy growths of water weeds impede the movements of boats and the flow of drainage waters through ditches (589, 858, 889, 891, 893, 2495).

WORMS, FREE-LIVING

(see also Phenol, Insects, Chironomids, Crustacea, and Chapter X)

The recognition of free-living nematodes associated with public water supplies is of long standing. Whereas the pathogenic worms are all primary pollutants, the free-living forms are all either corollary pollutants in that they develop in waters as a result of some direct or indirect facet of man's activities, or are just naturally occurring. Even in this latter case, however, it is difficult

for an individual, who is about to consume a glass of water and notes the presence of small worms, to believe that this is not pollution of the most vile form.

Interest in the free-living worm has been increasing in the past decade, probably for two reasons. First is the interest that is being expressed in their pollution-indicating value, and second, the papers that have been published about their occurrence in finished-water supplies.

Tubificid populations have been linked with heavy organic pollution in a number of articles (2126, 2143, 2540, 2542, 2545, 2688, 2888). Tubificids can reach enormous populations, 1.7 million per square meter being reported found in the River Elbe. The respiration of such masses is comparable with that of the microflora of the water (2126). Surber (2888) reports that an abundance of tubificids in excess of 100 per square foot apparently represents polluted habits, but cautions against non-judicious use of this figure especially since severe pollution can destroy even these resistant life forms. Phenol kills *Tubifex* worms at a minimum concentration of 200 mg/l although 150 mg/l was not lethal in five days (2889). Tests with *Planaria, Daphnia, Gammarus* and *Tubifex* showed *Tubifex* to be the most resistant to phenol (2616). *Tubifex* were reported to be common among benthic invertebrates in river water containing concentrations of chromium as great as 25 mg/l, copper 2.2 mg/l, and cyanides 3.2 mg/l, accompanied by a low dissolved-oxygen content (2688).

Goodnight discussed the entire group of oligochaetes as indicators of pollution (2890). Results of this study are not yet conclusive. Sludge formed on river bottom from wood fiber is said to influence the benthic fauna, favoring the oligochaetes (2891, 2892). When a stream becomes organically polluted, clean water Insecta such as mayflies, caddisflies, and stoneflies become replaced by oligochaetes and others (2533) providing that the former clean, firm stream bottom also changes. Sediment deprives cryptic animals of hiding places, and covers surfaces of rocks and stones rendering ineffectual various hold-fast mechanisms. Organisms that survive such conditions include the tube-building oligochaetes and chironomids (Insecta). Sodium arsenite, however, is reported to be directly toxic to oligochaetes (2893). On the other hand, results of aerial forest spraying with DDT in Canada indicate that oligochaetes (and snails) are found in considerably larger numbers in a stream sprayed every year than in any other stream including the control (2534). Results from England state that 2 lb/acre of DDT and DDD are not toxic to tubificid worms (74).

Tubifex, also referred to as "sludge worms," although dependent upon dissolved oxygen in the water, adjust well to levels as low as one-half mg/l. The Tubificidae protrude their tails above the mud into the water and wave them rhythmically. The lower the dissolved oxygen content, the further the tails protrude. Their blood contains hemoglobin which is an efficient collector of oxygen and the oxygen collected in this manner is pumped down to their front ends buried in the mud (2602). They feed directly on the sludge, removing some organic matter from it and reducing the BOD. Sludge worms one and one-half inches long and the diameter of a needle have been observed to pass fecal pellets totaling five feet nine inches in a 24-hour period. These pellets have been found to have one-half the BOD of the surrounding sludge (2143). Hence, the sludge worms aid the self-purification process of polluted streams (2894).

A report on the degradation of Lake Erie water states that pollution-tolerant worms have increased from 12 to 551 per square meter from 1929 to 1961 (2136). The effects of siltation from logging operations on bottom fauna have been reported showing fewer organisms in the silted areas (2895). Annelids and turbellaria are among the indicator organisms of the saprobic classification system (2690). Turbellaria are intolerant of heavy metal pollution (2607). A bad taste from mineral oils can be transmitted from oil-containing muds to fish meat when the fish ingest mud-dwelling tubificids (2904).

Marine forms of free-living worms are also being studied. In the Los Angeles-Long Beach Harbor, *Capitella capitata* is being investigated (2896, 2897, 2898) as an indicator of pollution as well as other forms (2506). Reish (2898) reports that in this area a healthy bottom is characterized by a large number of polychaetes with *Tharyx parvus, Cossura,* and *Nereis procera* predominating; a semi-healthy area by *Polydora* (Carazzia) *paucibranchiata* and *Dorvillea articulata*; a second semi-healthy area by *Cirriformia luxuriosa,* and a polluted area by *C. capitata.* A very polluted bottom area has no animal life.

Mass development of marine forms also occurs where sedimentation of nutrient matter occurs and current is sufficient (2785). Newell reports that the lugworm in estuaries lives as well in water one-fifth saturated with dissolved oxygen as in water fully saturated, and that another polychaete *Scoloplos armiger* grows faster and lives longer at one-fifth saturation (2899).

Marine *Bankia* species bore holes in wood. Logs sprayed with sodium arsenite have been tested as a temporary means of protection from teredo (2900). However, another means of controlling borers has been suggested (2506). Since borers are more prevalent in clearer waters and since large early populations of fouling organisms (amphipods and polychaetes) appear to restrict borers, it may be possible to admit small continuous supplies of organic waste particles that would favor growth of the fouling organisms.

Felice has studied the bottom fauna of San Francisco Bay quite extensively. In 1954, he stated neither pH nor dissolved oxygen correlated with the presence of waste waters (2901). In 1958 (2902), he adds that neither pH nor dissolved oxygen correlated with any species distribution of bottom fauna, and also that salinity strongly influences species distribution. However, even in the latter case, there were some unusual responses, such as *Capitella capitata* being found in two widely separated areas at opposite ends of the salinity gradient. Felice's 1959 paper (2903) remarks that there is little doubt but that *C. capitata* displays an unusual tolerance for pollution. Since Felice's work involves much more than worms, the reader is referred to his papers. Also, see Olga Hartman's work in the Southern California area (3383).

Among the more prominent worms associated with potable water supplies according to Ingram and Bartsch (2420) are the roundworm, *Diplogaster nudicapitatus,* and the segmented worm, *Nais.* For descriptions of these

animals, see Ingram and Bartsch's work. *Diplogaster,* as well as *Monhystera* and *Seinura,* have been found in filter influent, effluent, and tap water. *Tribolus gracilis* has been reported from English slow-sand-filter effluent. Other worms reported from England include *Dorylaimus* and *Rhabdolaimus. Nais* has been found in unfiltered, chlorinated supplies in California, but, although they are reported to pass through slow-sand filters, they have not been reported to pass through rapid-sand filters.

Chang, in reporting on a nematode in a water supply, identified it as *Diplogaster nudicapitatus.* Pathogens, such as *Salmonella, Shigella,* and Coxsackie A9 virus, ingested by the worm are protected from the effects of chlorine (2597). Later work reported the presence of nematodes in 16 of 22 water supplies investigated in both raw and finished water. Further, three species, *D. nudicapitatus, Rhabditis limicola,* and *Cheilobus quadrilabiatus* (latter species uncertain), liberated an odorous, oily substance that produced a distinct earthy or musty odor when only one drop was diluted with one liter of water (2905).

D. nudicapitatus and *C. quadrilabiatus* are extremely resistant to the action of chlorine, such that 50-60 percent survived a 5-minute contact with 95-100 mg/l chlorine. Pathogens ingested by the worms were completely protected even when as many as 90 percent of the worms were immobilized by chlorine. About 5 to 6 percent of ingested *Salmonella typhosa* or *Shigella sonnei* and 12 to 16 percent of ingested *Salmonella paratyphi* or Coxsackie A9 virus survived for 24 hours (2812).

In a study of the animal populations associated with various water plants, midge larvae and annelids together made up 59 to 93 percent of the population on most of the plants (2682). Polychaetes were among the only aquatic fauna tested that were not affected to some extent by toxins from the marine dinoflagellate *Gymnodinium veneficum* (2724).

WORMS, PARASITIC

(see also Worms—Free-Living, and Chapter IX—Acrolein)

The more important worm parasites of man include the ascarids, hookworms, flukes (schistosomes), and tapeworms. The worm parasites have many individual remarkable properties including host specificity (*Enterobius*), multiple-host requirements (schistosomes), durable ova (*Ascaris*), enormous size (tapeworms, up to 10 meters long), and many others.

The parasitic worms are primary pollutants, even though some species have free-swimming secondary stages in which form they were not introduced, such as the cercarial stage of the schistosomes. Although water may be essential to the completion of the life cycle of many worms, infection by drinking is generally one of the less hazardous methods of acquiring infection. The ova of parasitic worms are quite resistant to sewage-treatment processes (743, 745, 902, 2589) as well as to chlorination (741) and have been studied quite extensively in the application of sewage to various crops (2745, 2906, 2907).

The life cycle of the schistosomes, briefly, commences with the infected individual or animal passing ova in urine or feces, and subsequent hatching of the free-swimming miricidia. The miricidia must locate and penetrate a suitable snail host (such as *Australorbis glabratus* for *Schistosoma mansoni*), in which further change occurs and from which the cercariae evolve. Free-swimming cercariae must then locate a suitable animal host and penetrate its skin in order to complete its life cycle. Oxygen studies of cercariae of *S. mansoni* and *Australorbis* snails show that the cercariae cannot survive outside the snail in an anaerobic environment. Those within the snail were unaffected although the snail itself cannot tolerate more than 16 hours exposure to a lack of oxygen. As little as five percent of oxygen in the environment permitted normal shed and survival of cercariae (2738). The schistosome egg is not likely to be harmed by usual chlorination processes, although the miracidia and cercariae are quite susceptible (585, 908). Since the ova may not hatch until it has passed through the sewage treatment plant, control of the snail intermediate host has been studied and utilized as a method of controlling schistosomiasis (907). Humans contact the illness by bathing, wading, or working in infested streams. Drinking water is of secondary importance (497) since the cercariae cannot survive stomach acid. They must penetrate the mucous membranes of the mouth before being swallowed in order to complete their life cycle (2305). Filtration is an effective screening device for ova (840) but miracidia and cercariae can penetrate filters.

Schistosomes of other animals, especially of aquatic birds (2908), are the source of considerable annoyance to humans since the organism manifests itself as an itch or a cercarial dermatitis, commonly known as "swimmers' itch." The syndrome is spreading (906) southward from the major foci of the U.S.-Canadian Great Lakes area into the Tennessee Valley area (2484), and has since been reported in Washington, Oregon, and southern California. In spite of a decreasing waterfowl population, cases of swimmers' itch are increasing. Marine forms of cercarial dermatitis also occur on California's coast (2483).

The common liver fluke *Fasciola hepatica* is the most widespread of cattle liver parasites in the U.S. (2739). Sheep are extremely susceptible to infection (2910). The snail intermediate host lives in wet-weather springs, slow-moving, stagnant, swampy waters, on the banks of ponds, streams, and ditches. After development in the snail, these flukes form tiny cysts on grass or in the water. Mammals are then infected by eating this grass or drinking the water. The cysts can remain infective for several months in or out of water. Destruction of snail hosts is a primary method of control.

The heavy-walled *Ascaris* ova is deposited with feces from an infected individual. Under suitable environmental conditions, namely of temperature, moisture, and oxygen, the enclosed larva will mature, and after its first molt becomes infective. If it is consumed at this time with drinking water, on vegetables, or in any other manner, it will hatch in the duodenum and complete its life cycle. The ova of *Ascaris* are extremely resistant and long-lived (741, 1728, 2278, 2305, 2589, 2911) and are often used as criteria (1728, 1729) of effectiveness of various treatment processes or in investigations (1729, 2789, 2912) of the disease transmission potential of sew-

age-irrigated crops (2278, 2788, 2795). A study of ascarid eggs and their fate in streams has been reported (1727). Fresh cultures of ova were incubated under conditions similar to those occurring in streams. The development of the ova varied with the region of the stream, and under the same temperature conditions, varied with the dissolved-oxygen content. At 7.0-9.0 mg/l, larva developed in two months. At 5.0-5.6 mg/l, ova did not develop larva in two months. In the absence of dissolved oxygen, no development at all occurred. Percentage of eggs in water and bottom deposits remaining viable after 15 months were 11.8 and 17.1 respectively. Ascarid ova have been reported to survive for two years in irrigated soil and have been found on irrigated vegetables even when chlorinated effluent was used for irrigation (1728, 1729). According to one report, the effect of chlorine in doses up to 223 mg/l only accelerates the development of the ova (2909).

Studies of the survival of suspensions of ova on vegetable surfaces have been made (1729). Under hot, dry climatic conditions, the number of eggs decreased to 10 percent of the initial value in 27 days. Plants cannot free themselves of contamination in a month's time or even longer under more humid conditions. In a rural area of Germany, the population had a 39 percent rate of infection by *Ascaris*. Within a district where sewage was applied for irrigation, the rate was 90 percent (904). A later German study investigated the degree of parasitism by *Ascaris lumbricoides* of urban sewerage workers and workers of sewage-irrigated fields. The respective percentages for the two groups were 2 and 16 (2912). The ova of *Toxocara canis*, ascarid of the dog and fox, was capable of surviving the winter months of 1927-1928 in Minneapolis, Minnesota (2913). Exposure to —30°C or to desiccation in sunlight was reported to be lethal to this species.

The tapeworms include *Taenia saginata* (beef tapeworm), *Taenia solium* (pork tapeworm), *Echinococcus granulosus* (dog tapeworm), and *Diphyllobothrium* (fish tapeworm). *Taenia, Echinococcus* and *Diphyllobothrium* differ somewhat in their life cycles and therefore in their water transmission roles. *Taenia* species ova are passed in the feces of an infested individual. Domestic sewage applied to grazing lands is one mechanism whereby cattle or hogs are exposed and ingest the ovum (511, 746, 903, 905, 784, 2912). Within the animal host the organism penetrates the intestine and migrates to any of various tissues, forming a cyst. In order for the life cycle to be completed, man must then ingest the cyst, which may happen when meat is not properly cooked. Ordinary sewage treatment is not effective in removing tapeworm eggs (2589, 2914), the ova being quite resistant (746). Dog tapeworm ova are passed in the feces of an infected dog, and if man subsequently ingests the ova, hydated cysts will form. They may form in any area of the human body and can be quite serious, depending upon the numbers and location. Yugoslav investigations have disclosed a higher rate of *Echinococcus* infections where water sources and courses are not so abundant and where people depend a great deal upon cisterns as a main source of water supply. The highest rate of infection exists where people obtain their water directly from open pools (2305).

The fish tapeworm commences its life cycle with the discharge of an ovum in the feces of an infected individual into water. The coracidium, or free-swimming form, hatches and must be found by a suitable copepod host (*Cyclops* or *Diaptomus*) which ingests it. Within the copepod the organism undergoes further change and then a suitable species of fresh-water fish must ingest the copepod. Within the fish, the procercoid larva migrates to various tissues and develops into an encysted plerocercoid larva. In order for the life cycle to be completed, this larva must then be consumed (improperly cooked fish flesh) by a susceptible host (308, 720). Diphyllobothriasis in a population in Russia is attributed to discharge of untreated sewage to an impounding reservoir (2741), and the main sources of infection are the pike, eel, and river bass(2749).

Hookworm has been reported to enter the body through drinking water (909), but this is a very rare occurrence (2305). The ova are quite resistant but not nearly so resistant as the ascarid egg (746, 2305). Hynes remarks that helminth ova can be dispersed from sewage treatment plants in wind-carried detergent foams, and quotes an ova density in sewage of one to six eggs per gallon (2602).

YEASTS

(see also Bacteria—General, Fungi, and Chapters V and VIII)

The yeasts comprise a large group of fungi that are characteristically unicellular. Ordinarily they do not form a mycelium, although a few under certain conditions produce a rudimentary mycelium. Along with the bacteria and other fungi, the yeasts perform an indispensible function in the decomposition of organic materials in water (2289) although Cooke (2644) notes that this role of yeasts has not been sufficiently studied.

The pathogenic yeast, *Histoplasma capsulatum*, is of the type that has both mycelial and yeast phases. It is responsible for histoplasmosis in human beings, an illness involving the reticuloendothelial system, the primary lesion generally (if not always) being in the lung. It is transmitted through inhalation of air-borne spores of saprophytic origin, rarely by ingestion of contaminated food (2663). Since surveys of skin test sensitivities to histoplasmin indicate that water may be a factor, studies on the survival of *H. capsulatum* have been conducted (2915, 2916). Both mycelial and yeast phases persisted in sterilized Ohio River water at 50°C and 20°C for at least 180 days, at 37°C, generally, for 140 days. The yeast phase converted to the mycelial at both 5°C and 20°C, and the mycelial phase, at least in part, converted to the yeast at 37°C (2916).

In a sterilized tap water, yeast-phase survival was demonstrated for 621 days at 4°C, 306 days at 25°C, and 62 days at 37°C. No viable cells were recovered after 3 days at 45°C. The mycelial-phase survival was 421 days at 4°C, 245 days at 25°C and 34°C, and 269 days at 37°C. Again, there was no survival after 3 days at 45°C. *H. capsulatum* survived alternate freezing and thawing for 198 days and survived in triple-distilled water for 28 days (2915).

Cooke (2917) identified 30 species of yeast from 136 isolates obtained from various sewages and polluted waters. No pathogenic yeasts were found. He suggests that the habitats sampled were adequate for growth and reproduction of the yeasts, the organisms thereby contributing to sewage purification.

The interrelation between *Candida albicans* and normal intestinal bacteria has been studied. This yeast is the etiologic agent of candidiasis (moniliasis, thrush), which is generally characterized by localized skin lesions. When systemic infection is present, the illness is extremely grave. Long or repeated exposure of the skin to water may lower resistance to this organism, although host susceptibility is considered a more important factor in clinical disease than communicability (2663). Intestinal bacteria may be inhibitory, stimulatory, or indifferent to the yeast's growth. The atmospheric environment is important in determining the degree of its activity (2918).

Yeasts have been found more active than bacteria in taking up uranium (2919) (see Chapter VIII, Radioactivity).

CHAPTER VIII

RADIOACTIVITY

Criteria of water quality are governed not only by potential chemical and biological pollutants but also by the physical properties of extraneous substances in water. Foremost among these physical properties is radioactivity, which is sufficiently unique to warrant special consideration in this separate chapter. Abnormal levels of radioactivity may be deleterious to several beneficial uses of water. Radioactivity in water is especially significant in relation to human health, first through the direct consumption of water and second through the ingestion of agricultural products, stock, and aquatic or marine life that have accumulated radioactivity.

Surface and ground waters may acquire radioactivity in several ways. First to be considered are natural sources such as mineral deposits. Indeed, many springs and deep well waters have high levels of radioactivity, as described later in this chapter.

Radioactivity of natural waters may be increased by atmospheric nuclear detonations and the resulting fallout products, especially Sr-90, Cs-137, and I-131. There is no evidence to date, however, that underground nuclear explosions have raised significantly the level of radioactivity in ground water (1977). A third major source of radioactivity in water is the direct action of the atomic-energy industry in mining and separating uranium, in the manufacture of atomic weapons, and in the production of radioisotopes in piles and reactors. Finally, the radioactivity of water may be raised by the utilization of radioisotopes in medical therapy, scientific research, and industrial processes. Radioisotopes are used, for example, to detect flaws in metal bodies or welds, to note changes of grade of petroleum oil in pipelines, to locate leaks in pipes, to determine the levels of liquids in tanks, and to find the density of soil (1484, 1611, 1661, 1662, 1663, 3646, 3647, 3771, 3772).

In general, the wastes from nuclear reactor operations have a high level of radioactivity and are carefully controlled and monitored by the Atomic Energy Commission before discharge to streams. Low-level radioactive wastes, however, originate in laundries, hospitals, research laboratories, plant wash waters, and myriads of small operations. Many of these wastes are discharged to municipal sewerage systems and subsequently reach natural waters (3773).

Radioactivity is an indestructible property; that is, it cannot be neutralized or cancelled by known chemical or physical methods. Radioactive substances must be disposed of by dilution with water or stable isotopes, or by concentration and storage, according to the hazards involved. Natural decay will then reduce the radioactvity.

All radioactive substances are in a state of decay or disintegration; hence, they give off energy in the form of particles or rays. The rate of decay is expressed as "half-life," i.e., the time required for a radioactive substance to lose one half of its original radio energy. One half of the remainder will be lost in another half-life and the process continues until the end product is substantially inert. Thus, the radioactivity remaining after n half-lives equal $(\frac{1}{2})^n$ times the original value. Decay curves have been determined for most radioisotopes (760, 3774). Some substances give up their energy in the form of radiation and become inert quickly; others, such as radium, plutonium, and uranium, are radioactive for thousands of years. Half-lives of some important radioisotopes are shown in Table 8-1.

TYPES OF RADIATION

Radiation has been divided into four general categories, all of which may be encountered in polluted water.

1. *Alpha particles* are large, highly damaging, and positively charged; in fact, each alpha particle is a helium nucleus with a high velocity, often as high as 10^9 cm/sec. Although alpha particles cannot penetrate skin, they are particularly dangerous when ingested and deposited within the body.

2. *Beta particles* are high-velocity negative electrons. They are moderately damaging and moderately penetrating. Some beta particles have velocities close to that of light.

3. *Gamma rays* are a form of electro-magnetic radiation, rather than particulate matter. They are deeply penetrating but relatively less damaging than alpha or beta particles. The wavelength of gamma rays is in the range of 10^{-7} to 10^{-10} millimeters. X-rays are similar to gamma rays but with a shorter wavelength (10^{-5} to 10^{-8} millimeters).

4. *Neutrons* are uncharged, high-speed particles, deeply penetrating and highly damaging. They make dissolved salts radioactive and consequently they can make cooling water from reactors very dangerous.

Alpha, and to a less extent beta, particles are particularly hazardous in water and food because they can be ingested and can become concentrated in specific tissues, bones, or organs where they can act for a long time. Beta particles, neutrons, x-rays, and gamma rays from radioisotopes in water outside the body can also be hazardous because of their power to penetrate skin and flesh. Table 8-1 shows which type of radiation is given up by the common radioisotopes.

UNITS AND DEFINITIONS

Before discussing the biological effects of radiation it is well to review the terms and units used in this chapter. The two basic units of measurement of radioactivity are the curie and the roentgen.

The curie (c) was originally intended to represent the rate of disintegration of one gram of radium. It is now defined as 3.70×10^{10} disintegrations per second, whereas

TABLE 8-1

SIGNIFICANT PROPERTIES OF SOME RADIONUCLIDES

Radionuclide	Type of decay	Specific activity (curies/gram)	Critical body part	Radioactive half-life	Biological half-life (days)	Effective half-life (days)	Maximum permissible concentration in water for general population (pc/l)
Hydrogen-3	β	9.78×10^{3}	whole body and body tissue	12.46 years	19	18.9	3×10^{6}
Carbon-14	β	4.61	fat	5570 years	35	35	8×10^{5}
Sodium-24	β, γ	8.74×10^{6}	whole body and GI tract	15.06 hours	19	0.61	2×10^{5}
Phosphorus-32	β	2.86×10^{5}	bone	14.3 days	1,200	14.3	2×10^{4}
Potassium-42	β, γ	5.96×10^{6}	GI tract	12.44 hours	37	0.51	3×10^{5}
Calcium-45	β	1.77×10^{4}	bone	164 days	18,000	164	9×10^{3}
Iron-59	β, γ	4.92×10^{4}	GI tract and spleen	45.1 days	65	26.7	6×10^{4}
Cobalt-60	β, γ	1.14×10^{3}	GI tract and total body	5.27 years	9	9	5×10^{4}
Zinc-65	β, γ	8.21×10^{3}	total body, liver and prostate	250 days	23	21	1×10^{5}
Strontium-90 and Yttrium-90	β	1.44×10^{2}	bone	28 years	3,900	2,820	1×10^{2}
Ruthenium-106	β, γ	3.39×10^{3}	GI tract	365 days	20	19	1×10^{4}
Iodine-131	β, γ	1.24×10^{5}	thyroid	8.08 days	180	7.7	2×10^{3}
Cesium-137	β, γ	98.5	total body, liver, spleen, and muscle	30 years	17	17	2×10^{4}
Barium-137	β, γ	98.5	GI tract	30 years	200	196	2×10^{5}
Cerium-144	α, β, γ	3.18×10^{3}	GI tract and bone	282 days	500	180	1×10^{4}
Radium-226 and daughters	α, β, γ	0.98	bone	1622 years	20,000	19,400	10
Thorium-232	α, β, γ	1.11×10^{-7}	bone	1.39×10^{10} years	40,000	40,000	2×10^{3}
Uranium-238	α, β, γ	3.34×10^{-7}	GI tract and kidneys	4.51×10^{9} years	30	30	4×10^{4}

one gram of radium has 3.61×10^{10} disintegrations per second. The term *specific activity* is used to express curies per gram of a solid or curies per ml of a liquid or solution. In water quality control, levels of radiation are such that they can be expressed better as one-thousandth of a curie, or millicuries (mc); as one millionth of a curie, or microcuries (μc); and as 10^{-12} curies, or micromicrocuries, or picocuries (pc). In this report, the term picocurie is used insofar as possible.

The roentgen (r) is defined as that amount of gamma or x-radiation that produces in 1 cc of air (at standard temperature and pressure) ions carrying one electrostatic unit of charge of either sign. One roentgen is equivalent to 83.6 ergs per gram of air. The original definition has been extended by common usage to include the effects of alpha, beta, and neutron particles as well as gamma and x-rays as described below.

Strictly speaking, the term "roentgen equivalent physical" (rep) is used to describe the quantity of alpha, beta, and neutron radiation producing the same amount of ionization or energy change in tissue as does one r of x-rays. To be even more specific, the term "radiation absorbed dose" (rad) was created to quantitate the absorption of radiation by tissues. It is defined as the energy imparted to matter by ionizing particles per unit mass of irradiated material at the place of interest. Thus, the rad is the unit of dose and is equal to the absorption of 100 ergs per gram of tissue. For most considerations of radioactivity and its effect on life processes, the number of roentgens is substantially equal to the number of reps and rads. Indeed, the three terms are used interchangeably and roentgen is used for all three (3688, 3772).

Although the roentgen has been broadened in definition to cover particles as well as rays, it must be recognized that the same absorbed dose of different kinds of radiation does not, in general, produce the same biological effect. The quantitative effect of a given physical dose of radiation depends on the tissue or animal irradiated, the characteristics of the radiation, and the particular effect being studied. Alpha radiation, for example, is much more damaging than most other types. The relationship among radiation classifications is expressed as the "relative biological effectiveness" (RBE). The present accepted values for RBE of different kinds of radiation are given as follows (3688):

Radiation	*RBE*
Gamma and x-rays	1
Beta particles	1
Alpha particles	10
Neutrons and protons (up to 10 Mev)	10
Heavy recoil nuclei	20

The term "roentgen equivalent man" (rem) is the quantity of radiation producing, when absorbed by man, a biological effect equivalent to that resulting from the absorption of one r of x-radiation. Thus the unit of RBE dose is the rem, considered to be that dose which is biologically equivalent to one roentgen of gamma or x-radiation. For example, one rad of neutrons is considered to be equivalent to 10 roentgens of gamma radiation, or its RBE dose is 10 rem.

Experimental results, however, have shown that the same RBE dose of different radiation in bone does not always produce the same effect. Hence, a numerical relative damage factor has been introduced to provide for this difference, the biological effect being represented by the product of the RBE and the relative damage factor (3688).

When the high-energy particles strike the individual atoms and molecules that make up tissue, electrons are knocked out of their orbits and these stray electrons may themselves cause further damage. The process of ejecting electrons from their atomic orbits is known as ionization. The number of ionizations caused by a radiation per unit of path length is known as "specific ionization". Specific ionization is the major factor in judging the relative biologic effect of various types of nuclear radiation (3775, 3776).

BIOLOGICAL EFFECTS OF RADIATION

Estimates of probable radiation injury to human beings are based largely on experimentation with lower animals; clinical studies of human beings exposed to radiation by medical treatment, accident, or war (e.g. Hiroshima); and observations made on populations living in areas of high background radiation. The 1956 report of the National Academy of Sciences-National Research Council (3777) notes that radiation injury is a complex matter and that it is not possible to extrapolate with confidence from one condition of radiation exposure to another, nor from animal data to man.

Exposure to radiation from cosmic rays and concentrations of radioactive minerals in the earth's surface has always been a part of life. Man has reached his present state of development amid radiation of the order of 80-170 millirems per year (3688). It is not known whether exposure to this level of radiation is essential to the development of biological systems or whether it is simply another deleterious force that such systems have learned to tolerate (3826).

The intensity of radiation in both air and water varies from place to place. The radioactivity of natural waters generally lies in the range of 1 to 1000 pc/l. In some radioactive springs it may reach 100,000 pc/l (3778, 3779). The radium (long-lived) content of some New York community water supplies is 0.5 pc/l (425) and the radon (short-lived) content of mineral springs has been found to be as high at 750,000 pc/l (761). The radon content of springs and wells in certain regions of France exceeded 4000 pc/l in more than two-thirds of the samples (3780). Smith et al. (3642) found the radon activity of well waters in Maine and New Hampshire to be as high as 1,130,000 pc/l. During and immediately following the 1953 spring weapons tests, the radioactivity of rainfall in Cincinnati varied from 280 to 319,000 pc/l as compared with a pre-bomb test background of 50 pc/l (3781). In general, it has been estimated that the radioactivity of surface waters amounts to 1 to 10 percent of that of the rainwater (3782), or for the Cincinnati area about 0.5 to 5.0 pc/l before bomb testing. Such naturally occurring radioactivity results in an average background radiation of 0.0005 to 0.002 reps per day, or about 0.2 to 0.73 reps per year (762).

The therapeutic value of naturally radioactive waters is open to question. On the other hand, many authorities maintain that the continuous consumption of such waters is dangerous, although no cases are known of radioactive poisoning from natural waters (624, 761). The problem of interest in water pollution control is how to avoid additional exposure to artificial radiation that may cause damage.

The biological effects of radiation are classified as somatic (directly affecting the individual cell and organism) and genetic (which do not apparently influence the irradiated individual but do affect his descendants, perhaps after several generations). Somatic effects may be acute or chronic. A dose of radiation will be acute if it damages a critical number of cells such that the organism cannot recover by normal cell mitosis. The same total dose delivered over a longer period may have much less adverse effect because the repair mechanisms of the organism enable it to recover. Yet, there may be chronic long-term damage. Acute exposure can result in both immediate and delayed biological effects; but chronic exposure is usually considered to produce only delayed effects (3688).

The following table summarizes the effects resulting from acute whole-body external exposure of radiation to man (3688, 3775):

Acute Whole-Body External Exposure in Rads	*Biological Effects in Man*
0–25	No detectable clinical effects, but delayed effects may occur.
25–100	Slight transient reductions in leucocytes and neutrophils; disabling sickness not common; delayed effects possible but serious effects very improbable.
100–200	Nausea and fatigue with possible vomiting; reduction in leucocytes and neutrophils, delayed effects may shorten life expectancy.
200–300	Nausea and vomiting followed by two-week latent period, then loss of appetite, general malaise, sore throat, pallor, diarrhea, and moderate emaciation; recovery likely in about three months unless complications develop.
300–600	Nausea and vomiting in first few hours followed by a latent period of 3 to 7 days, then epilation, loss of appetite, general malaise, and fever during second week; hemorrhage, purpura, petecheae, inflammation of mouth and throat, diarrhea, and emaciation in third week; some deaths in 2 to 6 weeks; possible eventual death to half of individuals exposed to 450 r.
over 600	Nausea and vomiting in first few hours, short latent period, then toward end of first week diarrhea, hemorrhage, purpura, inflammation of mouth and throat, fever; rapid emaciation and death as early as second week, and eventual early death of up to 100 percent of exposed individuals.

The effects of acute radiation, as described above, are quite well understood. They are not of primary concern, however, in water pollution control. Chronic effects that arise from frequent small doses, year after year, are much more important, but difficult to evaluate. They may include the production of eye cataracts, the induction of leukemia from irradiation of the bone marrow, the development of bone cancer from ingestion of radium, the production of skin cancer, and the reduction of life span owing to the imperceptible impairment of many bodily functions. It is difficult, if not impossible, to determine the threshold levels of radioactivity that give rise to these manifestations.

The relationship between chronic dose and effect has not been established for somatic action. Three possibilities have been considered in the literature: (a) a linear, no-threshold concept; (b) a non-linear, no-threshold concept, and (c) a non-linear, threshold concept. Authorities are not in agreement as to whether or not a threshold can be established for somatic effects. The evidence is insufficient to prove either the hypothesis of a damage threshold or the hypothesis of no threshold in man at low doses. It is prudent, therefore, to keep radiation exposure to the lowest practical level (3688).

For genetic effects, however, there appears to be no possible threshold dose below which some effect may not occur. Any amount of radiation, no matter how small, is bound to produce some mutations. Once completed, mu-

tations are irreversible and almost all mutations are deleterious. Irradiation of a population from any source is bound to have some genetic consequences (3688, 3772, 3783, 3784).

There is an increasing reluctance on the part of knowledgeable scientists to establish radiation protection standards on the basis of the existence of a threshold for radiation damage. On the premise that there is no threshold, every type and amount of radiation involves some biological risk either to the individual or to his descendants. In contrast, however, radiation can bring numerous benefits to man in medicine, industry, and research. It is necessary, therefore, to weigh the risks against the benefits. Maximum benefits cannot be obtained without some biological risk, and the risk cannot be eliminated without loss of benefits (3688). As a consequence, radiological standards have been recommended by several scientific agencies.

Among the oldest of these organizations are the International Commission on Radiological Protection (ICRP) and the National (USA) Committee on Radiation Protection and Measurement (NCRP). Formed in 1928 by the International Congress of Radiology, ICRP is now a commission of the International Society of Radiology. The NCRP started as the Advisory Committee on X-ray and Radium Protection and adopted its present name after a reorganization in 1946. Its recommendations are generally published in handbooks of the National Bureau of Standards, one of the agencies represented on NCRP. Reports have also been published in 1956 and 1960 by the National Academy of Sciences—National Research Council for its Committees on the Biological Effects of Atomic Radiation. In general, the recommendations of all three bodies (ICRP, NCRP, and NAS-NRC) are in close agreement. The United Nations also has a Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) which published a report in 1958.

As a result of congressional hearings, Public Law 86-373 was enacted in 1959 to form the Federal Radiation Council. It is the purpose of the Council to provide a federal policy on human radiation exposure, to advise the President with respect to radiation matters, and to guide all federal agencies in the formulation of radiation standards. The 1962 Drinking Water Standards of the U. S. Public Health Service (2036), for example, are tied into the Federal Radiation Council. As of September 1961, the Council had issued two reports of background material for the development of radiation protection standards (3688, 3785). Much of the information in this chapter was drawn from these reports.

Insofar as ICRP is concerned, the major recommendations are contained in ICRP Publication 2, "Report of Committee II on Permissible Dose for Internal Radiation" (3786). This report has been abridged by NCRP and published as Handbook 69 of the National Bureau of Standards (3784). Table 1 of Handbook 69 presents the maximum permissible concentrations of radionuclides in air and in water for occupational exposure, both for a 40-hour week and a 168-hour week. Outside of a controlled area of occupational exposure the concentrations should not exceed one-tenth of that for radiation workers. The values presented in Table 8-1 of this chapter were taken from Handbook 69 but they have been corrected by this factor of one-tenth and hence may be considered to apply to the general population. ICRP and NCRP also specify that radiation "outside a controlled area, but attributable to normal operations within a controlled area, shall be such that it is improbable that any individual will receive a dose of more than 0.5 rem in any one year from external radiation" (3784).

It is also a basic recommendation of the Federal Radiation Council that "the yearly radiation exposure to the whole body of individuals in the general population (exclusive of natural background and the deliberate exposure of patients by practitioners of the healing arts) should not exceed 0.5 rem" (3688). However, on the arbitrary assumption that the majority of individuals do not vary from the average by a factor greater than three, and since no one should be exposed to more than 0.5 rem per year, the Council recommends the use of 0.17 rem for yearly whole-body exposure of average population groups. Insofar as genetic dose is concerned, the Federal Radiation Council recommends that the exposure to the gonads be limited to 5 rems over a period of 30 years for the average population (3688). For most individual organs of the body, the permissible body burden is taken as 15 rems and for the thyroid or skin it is 30 rems.

Once the permissible body burden has been established, the daily intake which would not result in an accumulation over a 50-year period of more than the permissible body or organ burden is calculated. To determine the permissible intake by ingestion it is also necessary to consider the fraction of ingested material that is absorbed into the blood from the gastro-intestinal tract, the fraction of material in the blood that will be deposited in the critical organ, the rate of uptake, and the time of retention of the material in the critical organ. This last factor is expressed as the "biological half-life", or the time required for half of the material to be removed by normal metabolic processes. The "effective half-life" is a combination of radiological and biological half-lives such that

$$\frac{1}{T_e} = \frac{1}{T_r} + \frac{1}{T_b}$$

Values of these half-lives are shown in Table 8-1.

Once the permissible daily intake by ingestion has been determined, the maximum permissible concentration (MPC) in water can be derived by assuming that the total daily intake of water is 2.2 liters. The MPC values for several important radioisotopes, adjusted downward by a factor of 10 to allow for the general population, are shown in Table 8-1.

A study of Table 8-1 shows that strontium, radium, and thorium are most dangerous because they are bone seekers and have very long effective half-lives. Strontium is a serious problem also because it is produced in high yield by fission reactions (3787). Radium is especially harmful because it occurs in natural waters and it is also an alpha emitter. Calcium and phosphorus are bone-seekers with long biological half-lives, but they are not serious health problems because their radiological half-lives are short.

The Federal Radiation Council has also established the terms Radiation Protection Guide (RPG) and Radioactivity Concentration Guide (RCG). The RPG is "the radiation dose which should not be exceeded without careful consideration of the reasons for doing so; every effort should be made to encourage the maintenance of radiation doses as far below this guide as possible". The RCG is "the concentration of radioactivity in the environment which is determined to result in whole body or organ doses equal to the Radiation Protection Guide".

The Radiation Protection Guides shown below are considered by the Federal Radiation Council to represent an appropriate balance between the requirements of health protection and of the beneficial uses of radiation and atomic energy. It is recommended (3687) that the following RPG values be adopted for normal peacetime operation:

Organ	*RPG for Individuals*	*RPG for Average of Suitable Sample of Exposed Population Group*
Thyroid	1.5 rem per year	0.5 rem per year
Bone marrow	0.5 rem per year	0.17 rem per year
Bone	1.5 rem per year	0.5 rem per year
Bone (alternate guide)	0.003 micrograms of Ra-226 in the adult skeleton or the biological equivalent of this amount of Ra-226	0.001 micrograms of Ra-226 in the adult skeleton or the biological equivalent of this amount of Ra-226

By September 1961 the Federal Radiation Council had established RPG values for iodine, radium, and strontium in three ranges. Range I covers intakes that would not under normal conditions be expected to result in any appreciable number of individuals in the population reaching a large fraction of the RPG. Range II involves intakes expected to result in average exposures to populations not exceeding the RPG, but involving surveillance and routine control. Range III deals with intakes that would be presumed to result in exposures exceeding the RPG if continued for a sufficient period of time. The following values have been given for the three ranges (3687, 3785):

Radionuclides	*Ranges of Transient Rates of Intake (in picocuries per day)*		
	Range I	*Range II*	*Range III*
Radium-226	0-2	2-20	20-200
Iodine-131	0-10	10-100	100-1,000
Strontium-90	0-20	20-200	200-2,000
Strontium-89	0-200	200-2,000	2,000-20,000

Inasmuch as different environmental media contribute to the total daily intake, the MPC values for water will be only a fraction of the values given above. For example, the MPC of Ra-226 for drinking water (see Domestic Water Supplies hereinafter) is 3 pc/1. Assuming 2.2 liters per day per person, the intake from this source is limited to 6.6 pc/day, or about one-third of the total daily intake to remain within Range II.

The Atomic Energy Commission (3788) has established standards for protection against radiation hazards arising out of activities of licensees, including the disposal by release into sanitary sewerage systems. These standards limit the average daily concentration of radionuclides, the total daily discharge, and the average monthly concentration. Furthermore the gross quantity of licensed and other radioactive material released to the sewer cannot exceed one curie per year. For the same radionuclides shown in Table 8-1, the AEC limits for discharges to sewers are given in Table 8-2. The quantity of any radioactive material released into the sewer system by a licensee in any one day shall not exceed the larger of columns 1 or 2 and the monthly average shall not exceed column 2. For comparison, the MPC for water is given again in column 3.

TABLE 8-2

AEC STANDARDS FOR DISCHARGE TO SEWERS

Radionuclide	*Maximum Daily Discharge in Microcuries* (1)	*Maximum Concentration, pc/1* (2)	*MPC for Water, pc/1* (3)
Hydrogen - 3	2500	1×10^8	3×10^6
Carbon - 14	500	2×10^7	8×10^5
Sodium - 24	100	6×10^6	2×10^5
Phosphorus - 32	100	5×10^5	2×10^4
Potassium - 42	100	9×10^6	3×10^5
Calcium - 45	100	3×10^5	9×10^3
Iron - 59	10	2×10^6	6×10^4
Cobalt - 60	10	1×10^6	5×10^4
Zinc - 65	100	3×10^6	1×10^5
Strontium - 90 + Yttrium - 90	1	4×10^8	1×10^2
Ruthenium - 106	10	4×10^5	1×10^4
Iodine - 131	100	6×10^4	2×10^3
Cesium - 137 + Barium - 137	10	7×10^7	2×10^4
Cerium - 144	10	3×10^5	1×10^4
Radium - 226	1	4×10^2	10
Thorium - 232	500	5×10^4	2×10^3
Uranium - 238	500	1×10^6	4×10^4

DOMESTIC WATER SUPPLY

The 1962 Drinking Water Standards of the U. S. Public Health Service (2036) contain limits for Ra-226, Sr-90, and gross beta activity, based on the recommendations of the Federal Radiation Council. A full statement of these limits is reproduced below.

"(1) The effects of human radiation exposure are viewed as harmful and any unnecessary exposure to ionizing radiation should be avoided. Approval of water supplies containing radioactive materials shall be based upon the judgment that the radioactivity intake from such water supplies when added to that from all other sources is not likely to result in an intake greater than the radiation protection guidance recommended by the Federal Radiation Council and approved by the President". (i.e. 20 pc/day of Ra-226 and 200 pc/day of Sr-90). "Water supplies shall be approved without further consideration of other sources of radioactivity intake of Radium-226 and Strontium-90 when the water contains these substances in amounts not exceeding 3 and 10μμc/liter respectively. When these concentrations are exceeded, a water supply shall be approved by the certifying authority if surveillance of total intakes of radioactivity from all sources indicates that such intakes are within the limits recommended by the Federal Radiation Council for control action.

"(2) In the known absence of Strontium-90 and alpha emitters, the water supply is acceptable when the gross beta concentrations do not exceed 1,000μμc/l. Gross beta concentrations in excess of 1,000μμc/l shall be grounds for rejection of supply except when more complete analyses indicate that concentrations of nuclides are not

likely to cause exposures greater than the Radiation Protection Guides as approved by the President on recommendation of the Federal Radiation Council."

The radiological requirements contained in the WHO Drinking Water Standards, both International (2328) and European (2329), are based on the recommendations of the International Commission on Radiological Protection (3786), but they contain an additional safety factor of 10 proposed by the Commission for water which is to be supplied to large communities. Thus, these standards are only one-tenth of the MPC values for Ra-226 and Sr-90 as given in Table 8-1. If the levels of radioactivity are lower than the following values, the water is considered to be safe for use without further investigation:

alpha emitters	1 pc/l
beta emitters	10 pc/l

"Higher figures than those given above are safe for many radionuclides and a water with a total radioactivity exceeding the above figures may be safe for use if the absence of the more dangerous radionuclides can be confirmed by radiochemistry" (2328).

The standard for alpha emitters (1 pc/l) was set this low to allow for the possibility that all alpha activity might be due to radium and its daughter elements—otherwise, it too might be 10 pc/l. Until it is found to be practicable to analyze all water supplies specifically for Ra-226, it appears prudent to retain the lower level for all alpha emitters (2328).

Many natural waters used for water supply throughout the world fail to meet the WHO standard for radium, or even the USPHS standard. A tabulation of activities of natural waters, presented by Smith et al. (3642), shows a variation in radium activity from as low as 0.01 pc/l in the River Thames to 709,800 pc/l for spring waters in Japan. The average for all river waters was given as 0.07 pc/l and for river waters in North America as 0.03 pc/l (3642).

Ground waters, however, tend to be higher in radium content. Lucas (3643) reported that the Ra-226 activity of certain midwest water supplies ranged from less than 0.03 to 37 pc/l. Surface supplies and wells in glacial deposits or limestone formations were with rare exceptions found to contain less than 1.0 pc/l, whereas wells penetrating deep sandstones had Ra-226 contents of 1.0 to 37 pc/l. A population of 1,000,000 was identified as consuming water in excess of 1.0 pc/l and 480,000 consume water having Ra-226 activity in excess of 4 pc/l. Smith et al. (3642) measured the natural radioactivity of ground-water supplies in coastal areas of Maine and New Hampshire, finding the average Ra-226 activity of drilled and dug wells in Maine to be 66 and 73 pc/l respectively and in New Hampshire 6.9 and 4.1 pc/l respectively. It is apparent, therefore, that many existing water supplies fail to meet the WHO or USPHS standards for Ra-226 and alpha emitters.

Insofar as Strontium-90 and total beta emitters are concerned, the situation is not so serious. As a result of nuclear testing, rain water has been reported to contain from 2 to 8 pc/l of Sr-90, or close to the USPHS limit. Surface waters in the U. S., however, generally contain less than 1.0 pc/l although activities in excess of 2 pc/l have been reported (3778). Ground waters generally contain less than 0.01 pc/l of Sr-90 (3680). Water, however, is not considered to be the major avenue by which humans ingest radiostrontium, for food is the principal source. It is estimated that less than 10 percent of the Sr-90 ingestion comes from consumption of water (3682).

Gross beta activity in all surface waters of the U. S. in 1958 averaged less than 100 pc/l (3789) and only a few rivers exceeded 500 pc/l in 1961 (3778). It is unlikely, therefore, that the USPHS limit of 1000 pc/l for gross beta activity will be exceeded by natural waters.

The first edition of WATER QUALITY CRITERIA and the 1954 Addendum contained extensive discussions of radioactivity in relation to domestic water supply. Much of that material, however, is not repeated here because it has been rendered obsolete by the recent publications of ICRP, NCRP, and the Federal Radiation Council.

INDUSTRIAL WATER SUPPLIES

Excessive radioactivity in water supplies may be harmful to certain industrial processes or to manufactured products such as film (762, 3790). Further details of such effects were not uncovered in this literature survey. Where water is used for cooling reactors and thus may be exposed to irradiation by neutrons, it should have a minimum of dissolved and suspended matter, especially iron, calcium, lithium, sodium, boron, and sulfur (3395, 3791).

IRRIGATION

Vegetation, in general, is more resistant to radiation than is animal life (767); however, plants can concentrate radionuclides and thereby raise the potential hazard to human beings and animals consuming such vegetation. Two questions, then, are of interest in relation to water-quality criteria, viz, what levels of radiation in irrigation water will damage crops, and to what extent will crops concentrate various radionuclides?

Without a doubt, extremely high levels of radiation will cause somatic damage to vegetation; but no attempt was made in this survey to study the literature on plant physiology to ascertain these levels. It is also probable that lower concentrations of radiation will have a genetic effect on plants, but this factor was not uncovered in this survey. Foster et al. (3762) maintain that somatic effects of radiation are not likely to be a problem insofar as irrigation water is concerned. According to Krumholz (3797) the only plant that has concentrated enough radioactive material to damage itself has been the American elm. No significant effects were observed in plants grown in soil that contained concentrations of radionuclides equivalent to 25 years of accumulation from the Hanford effluent (3762). Barley seed plots irrigated with five percent reactor effluent from Hanford showed no adverse effects, but the seeds contained less nitrogen when plots were irrigated with undiluted effluent (3792). In normal irrigation practice, genetic effects should not be evident because modern farmers almost always use seed from special seed crops rather than from the products of the irrigated soil. Kaufman (3794) noted that the maximum permissible levels of radiation for irrigation waters may well become more stringent than those for domestic water supplies; but undoubtedly he was

referring to the concentration factor rather than to direct somatic or genetic damage.

The only important radionuclides that are absorbed and possibly concentrated by plants appear to be strontium, cesium, barium, iodine, calcium, potassium, ruthenium, zirconium, zinc, and chromium (2382, 3795, 3796, 3797, 3798, 3799, 3800). The uptake by plants of various elements from the soil solution is affected by several factors, including plant species, stage of growth of the plant, the part of the plant studied, the acidity and chemical composition of the nutrient medium, temperature, light, and soil structure (3793).

At Hanford, barley irrigated with five percent reactor effluent demonstrated no increased beta activity over the controls. Plants irrigated solely with effluent demonstrated higher activity, but only by a factor of two. It appears, therefore, that little long-lived beta-emitting material is taken up by barley. Of 16 radionuclides identified in the effluent, only Zn-65 and Cr-51 were found in the barley plants (3800).

The accumulation of Zn-65 on pasture grass irrigated with Columbia River water below Hanford and its subsequent transfer to grazing animals and milk is an important biological pathway for this radionuclide to man (3762). Cesium-137 is readily adsorbed by soil, but plants can take up Cs-137 directly from the water before ions are fixed to the soil; as a consequence, animals grazing on wet lands can ingest 400 times as much Cs-137 as those on dry lands, and crops grown in standing water may accumulate high Cs-137 levels (3798).

Samples of crops irrigated with Columbia River water 30 miles below Hanford were assayed for Zn-65 to determine concentration factors. The activity of the irrigation water averaged 188 pc/l of Zn-65. The following concentration factors were observed (3763): pasture grass, 440; black-eyed peas, 2.9; tomatoes, 2.4; okra, 2.1; string beans, 1.5; corn, 0.83, and grapes, 0.47. Kaufman (3794) observed that radishes concentrate strontium 84 times.

Tsivoglou et al. (3644) studied the effects of wastes from uranium ore processing on the Animas River and its beneficial uses, especially with respect to radium. They found that the average radium content of truck vegetables and field crops (excluding alfalfa) was 2.0 pc/kg of live material for farms upstream of the mill and 3.6 pc/kg for downstream farms irrigated with Animas River water. Yet the average dissolved radium content of downstream river water varied from 12.6 pc/l immediately below the mill to 2.9 pc/l 59 miles downstream, in contrast with 0.6 pc/l upstream. Thus, while the dissolved radium content of the river increased by a factor of 4.8 to 21, the radium content of crops increased by a factor of only 1.8, thus indicating that the concentration factor was less than 1.0.

The uptake of Sr-89 by attached aquatic plants, and in particular by watercress (*Nasturtium officinale*) was studied by Owens et al. (3689). For five aquatic macrophytes they found concentration factors varying from 30.4 to 74.1 when the dosed hard river water had a Sr-89 activity of about 10^7 pc/l. In dosed soft water, however, concentration factors were about 200. The concentration factor was not influenced significantly by the level of Sr-89 activity in the water between the limits of 10^5 pc/l and 2×10^7 pc/l (3689).

From pond water to which 6×10^6 pc/l of Cs-137 had been added, Pendleton (3387) found the concentration factors for aquatic macrophytes to be as follows:

water weed	1000
coontail	400
sago pondweed	700
water fern	250
duck weed	500
American bullrush (culms)	50
hardstem bullrush (culms)	90
cattail (leaves)	200
spotted persicaria (leaves)	600

Several authorities have reported on the uptake by plants of specific radionuclides in relation to other substances in the soil moisture (3684, 3795, 3801, 3802), especially the effect of calcium on the concentration of strontium and the inhibiting action of potassium with respect to cesium uptake. A thorough review of these relationships is beyond the scope of this report.

STOCK AND WILDLIFE WATERING

Extensive experiments have been conducted with laboratory animals to determine the biological half-lives of various radionuclides and to find the levels of somatic and genetic effects. It is not possible, however, to transfer these results from one animal to another species, nor from any animal to man (3777). Although this survey of literature on the subject of the effects on animals of radionuclides in water was far from exhaustive, the following observations may be of interest.

Insofar as somatic effects are concerned, Dowdy and Charles (763) report that the acute LD_{50} among most warm-blooded animals is 200 to 790 r. The chronic LD_{50} for dogs, at a dosage rate of 10 r per day for 6 days per week was 1440 r, but three dogs survived 5000 r. At 6 r per day, the LD_{50} was 2940 r but five dogs survived 3000 r. At 3 r per day, seven out of nine dogs survived 1500 r. For rats, 10 r per day for six days per week resulted in an LD_{50} of 3500 r. At 8.8 r per day to mice the LD_{50} was at 4400 r. At 8.8 r per day to guinea pigs, the LD_{50} was at 2300 r (763). Lower forms of life are resistant to much higher levels of radiation, e.g., 200,000 r for viruses and bacterial cultures, 300,000 r for *Paramecium,* and 150,000 r for some insect pupae (767).

In relation to dose, the tentative LD_{50} dose of uranium for mice was given at 27,000 to 31,000 pc per gram of weight (1681). When plutonium-239 was administered to rats by stomach tube, a dose of 88 mc/kg of body weight caused deaths but 56 mc/kg caused no deaths among six rats. The lethal dose of 88 mc/kg is equivalent to 650,000 rem over the surface of the small intestine. Rats were fed 40 mc of the alpha-emitting radionuclide Pu-239 (equivalent to 80,000 rads to the surface of the large intestine) without discernible effect (3804). On the other hand, when 3 mc of beta-emitting Y-91 were fed to rats (about 4000 rads to the large intestine), one-half of the animals were killed (3804). It is apparent, therefore, that the alpha particles from plutonium-239 do not reach the sensitive tissues in the gastro-intestinal tract. The LD_{50} in rats for Y-91 after oral administration was

17 mc/kg of body weight. The average survival time was 9 days (3805). Sheep fed 5 microcuries/day of I-131 for five years developed thyroid tumors. The total thyroidal dose was approximately 30,000 rads (3804). A muskrat that fed on vegetation in and around a stream receiving radioactive discharges developed cancer as a result of Sr-90 accumulation (2126).

On the other side of the ledger, a daily intake of 0.15 microcurie of I-131 appears to be a safe level for sheep. This corresponds to a thyroidal dose rate of roughly 3 rads/week (3804). The thyroid glands of sows fed 45 microcuries of I-131 per day were smaller than the thyroids of normal animals but even at this level two out of eight experimental animals showed no definite histological damage (3806). The thyroids of two sheep fed 1.5 microcuries per day of I-131 for four years did not show definite evidence of damage. The total radiation dose probably exceeded 5000 rads (3803). Rats fed concentrated reactor effluent for up to one year concentrated Zn-65 more than any other radioisotope (3764), but their growth rate was not affected and no histological changes were observed (1682, 3804).

Ballou (3807) studied the metabolism of Zn-65 in the rat. Using bone as the critical organ and an estimated effective half-life of 21 days, the MPC for Zn-65 in water was calculated at 6×10^7 pc/l. Ballou's studies, however, indicate that the effective half-life is about 100 days and hence the MPC should be decreased to 1×10^7 pc/l.

Relatively little information was uncovered to show the direct concentration factors between animal flesh and drinking water. Most concentration factors in animals arise from ingestion of vegetation that had previously concentrated the radionuclide from irrigation water. For example, while the concentration factor of 440 existed for Zn-65 with respect to pasture grass, the factors for beef bone, flesh, and fat were only 13.4, 10.7, and 2.2 respectively in relation to the irrigation water. For cow's milk, however, the overall concentration factor for Zn-65 was 26 as compared with 440 for the pasture grass on which she fed (3763). Waterfowl have been shown to concentrate P-32 in muscle by a factor of 75,000 over the water from which they fed (3702).

FISH AND OTHER AQUATIC LIFE

The effects of radioactivity on fish and other aquatic life should be considered with respect to direct somatic damage or death, possible long-term genetic effects, and the accumulation of radionuclides in aquatic or marine life used as food by humans. On the basis of limited data, it appears that concentration is by far the most serious effect, i.e., levels of accumulated radionuclides that make fish and other aquatic or marine life hazardous for human consumption do not appear to be likely to release sufficient ionizing radiations to damage the organisms themselves (2109).

Radionuclides in the aquatic or marine environment may affect organisms by (a) direct radiation from the water or accumulated bottom sediments, (b) adsorption of radioactive material on the body surfaces, (c) absorption through cell membranes of soluble substances, and (d) ingestion of radionuclides along with food and water (3273, 3808). For algae, for other lower forms of life, and for higher aquatic vegetation, adsorption and absorption appear to be the principal mechanisms of accumulation. For herbivores and carnivores (e.g., crustacea, insects, worms, fish), ingestion of radionuclides concentrated by lower forms of life appears to be the major route of accumulation.

Although direct somatic damage is not likely to be a serious factor for aquatic life, it is well to establish general levels of lethal action and damage. In general, organisms of the lower phyla are more resistant to ionizing radiations than are the higher vertebrates (2439). The following comparison of susceptibility to radiation was presented by Donaldson and Foster (2439):

	Acute Dose in Roentgens Causing	
Organism	*50% Mortality*	*100% Mortality*
Algae	8,000-100,000	25,000- 600,000
Protozoa	10,000-300,000	18,000-1,250,000
Mollusks	5,000- 20,000	10,000- 50,000
Crustacea	500- 90,000	5,000- 80,000
Fish	600- 3,000	370- 20,000

By way of comparison, an acute dose of 450 roentgen would probably cause 50 percent mortality to a group of humans. Lowe et al. (3809) reported that ionizing radiations from a cobalt-60 source required a dosage of 2,000,000 rad to give complete destruction of microorganisms in sewage, although 90 percent kills of most organisms were achieved at a dose of 70,000 rads. Ridenaur and Armbruster (3810) found that a dosage of 100,000 reps was sufficient to kill 99 percent of the bacteria in contaminated water and sewage.

When nearly mature rainbow trout were exposed to x-rays at the rate of 8.25 r per minute for as long as 64 weeks, growth was retarded by total dosages as low as 50 r; radiation injuries were caused by 500 r; more than 1000 r caused significantly high mortalities as compared with the controls; and 2500 r killed all fish in 8 to 13 weeks (771).

In White Oak Lake, which receives the radioactive waste effluent from the Oak Ridge National Laboratory, white crappies *(Pomoxis sparoides)* and redhorse *(Moxastoma erythrurum)* gradually disappeared from the fish population. The total radiation dose received by the fish in White Oak Lake was estimated to be at least 57 rep per year from external radiation and was probably several times that from internal radiation. As a result of this irradiation, it is believed that the fish population of this lake may have suffered deleterious effects, as manifested by shortened life spans, slower rates of growth, and possibly decreased fertility of the breeding stock of the redhorse (3692, 3797).

Silver salmon fry held in cooled effluent from the pile area at Hanford suffered 100 percent mortality in eight weeks. Even at dilutions of 1:50 increased mortalities were evident. For eggs or fingerlings, dilutions of 1:25 or greater gave no increase in mortality (3811, 3812). On the other hand, no somatic damage was observed in fish that had accumulated P-32 to the extent of 9×10^7 pc/kg of fish flesh after 9 months of exposure and only slight damage was evident in trout having 42×10^7 pc/kg after 6 months of exposure. None of the fish sampled from the Columbia River itself had as much as 1/100 of the P-32 dosage that caused no damage. It can

be concluded, therefore, that present radioactivity in the Columbia River poses no direct somatic hazard toward fish (3811).

Blinks (1683) found that marine algae surviving at the most radioactive spot accessible on the Bikini Island reefs one year after the atomic explosions appeared to be normal in a number of physiological functions, except for one: catalase activity was considerably increased. No injurious genetic effects were observed, but this does not rule out the possibility that some occurred but were overlooked in the course of rapid sampling.

Genetic damage to fish appears to occur at lower levels of radioactivity than somatic effects. If genetic effects are considered in the strict sense of damage to chromosomes or genes, to the extent that modified characteristics are passed from one generation to the next, there is little to be found in published literature describing work on marine or fresh-water forms (2439).

Studies of the eggs and young produced by trout that were irradiated at the rate of 8.25 r per minute indicated that the number of abnormalities and the mortality rate among the spawn of parents receiving a total of 50 r was higher than among control eggs and young. A total exposure of 500 r to parents caused significantly higher frequency of death, slower rates of growth, and abnormality (772). Gonadal hemorrhage was observed in fish exposed to 500 r of total body irradiation (2439). Both sexes of fishes of all ages kept for periods of up to 150 days in water containing initially 200 microcuries per liter of P-32 were rendered sterile.

With respect to lower forms of life, higher levels of radiation are required to produce genetic effects. A sewage worm, *Aeolosoma hemprichi*, showed reproductive effects at 20,000 r and an occasional mutation (2442). Colonies of a colorless flagellate, *Entosiphon sulcatum*, were exposed to 500,000 r of gamma radiation from Co-60. In 72 lines with isolations every second day and each line carried for 50 generations, no effects on morphology or reproduction could be found (2442).

The concentration of radionuclides in aquatic and marine organisms is a complex phenomenon. The rate of uptake of any element or compound, and the extent of its retention in the organism, are dependent on many factors. It must be remembered that an organism does not distinguish among the isotopes of any element; hence the uptake depends on the ratio of a radionuclide to the non-radioactive form of the same element. The extent of concentration of any radionuclide is governed by the following factors (1936, 2436, 2437, 3273, 3813):

1. The particular element involved and its physiological importance to the organism. In the Columbia River below Hanford, for example, P-32 is the radionuclide most strongly concentrated in aquatic life, not because of any radioactive property but merely because phosphorus is a limiting element in the nutrition of aquatic organisms. Consequently any extra phosphorus in water is quickly assimilated into plant and animal life (1936, 2535, 3814, 3815).

2. The physical and chemical state of the element and its acceptability to the specific organism. Sulfide, for example, may be utilized and oxidized by one form of bacteria to which sulfate would be unacceptable. Also, particulate cerium-144 became associated with the cells of *Nitzschia* at a faster rate than ionic cerium-144 (3273).

3. The concentration of the element in the environment and the presence of other elements that may inhibit or enhance its uptake. The accumulation of one radioelement by an organism may be altered by the relative abundance of another in the environment by one of three ways: (a) elements of similar properties may substitute for one another, (b) some elements may have an inhibitory effect on others, and (c) one element may have a synergistic effect on others (3273). The uptake of strontium, for example, has been shown to be inversely proportional to the concentration of calcium in the water (3816).

4. The morphology of the organism, its life history, its condition and age, and its particular role in the food web. Closely related species may behave differently and organisms of the same species will concentrate radionuclids at widely varying rates depending upon other environmental conditions. Young and active organisms tend to accumulate most rapidly.

5. The physical and chemical characteristics of the environment. Temperature is especially important, for in cold-blooded animals it controls the rate of uptake, the animal's metabolism, and the rate of excretion of radionuclides. The pH of the water is also an important factor.

As a result of the interaction of these many factors, it is difficult to forecast the extent to which any radionuclide will be concentrated in a given organism. Some of the concentration factors that have been observed for aquatic and marine organisms are presented in Table 8-3, with each radionuclide being shown separately. A hasty perusal of this table will show that sodium, phosphorus, sulfur, and calcium are not likely to be accumulated significantly. After all, the non-radioactive forms are normally abundant in the water environment. The addition of phosphorus, iron, cesium, and zinc radionuclides, on the other hand, generally increases significantly the concentrations of these elements in the environment and this change is reflected in the organism, especially if the element is essential for nutrition and generally a limiting factor, such as phosphorus. In Table 8-3 attention is invited especially to the wide variation in reported concentration between species and even the wide range of values for the same species. This spread reflects the factors listed above.

Despite the variability of data and the fact that most information is of recent origin, Krumholz and Foster (2436) present the following generalizations:

1. Radioactive materials are taken into the body of an organism either through physiological processes and incorporated directly into the tissues, or they are adsorbed onto the surfaces of the organism. In general, adsorption and absorption are governing mechanisms for the lower forms of life while ingestion is the principal route for predators.

2. The concentration of certain radioelements reaches a higher level in the lower plant and animal forms, such as bacteria, protozoa, and phytoplankton than in higher forms such as vertebrates. In such instances there is an inverse correlation between the complexity of body struc-

ture and the concentration of the radioelement in question. This point was brought out clearly by the Columbia River studies made by the U. S. Public Health Service (3814, 3815).

3. Certain plants and animals have a predilection for concentrating specific radionuclides in certain tissues or organs. Iodine, for example, is concentrated in the thyroid, silicon in the tests of diatoms, calcium in the shells of mussels, calcium and phosphorus in the bony skeletons of vertebrates, and cesium in soft tissues.

4. Although certain radioelements may occur in amounts acceptable for drinking water, many fresh-water organisms have the ability to concentrate them to levels that might be harmful.

How can the true hazard of concentration of radionuclides in fish be assessed? One way is to consider that the average person eats about 0.5 kilograms of fish per week and drinks about 15 liters of water. On that basis, the fish muscle could have a level of radioactivity per kilogram 30 times greater than the MPC per liter for drinking water before the hazards are comparable (2109). In the Columbia River surveys, the maximum radioactivity observed in plankton was 7.9×10^6 pc/kg of weight (1684). The maximum total activity of an adult game fish was 2.2×10^6 pc/kg of body weight (3817). Caddis flies emerging from the river area averaged 3×10^5 pc/kg of wet weight (1936). A large percentage of this activity was probably due to P-32.

TABLE 8-3.

CONCENTRATION FACTORS FOR AQUATIC AND MARINE ORGANISMS

Radionuclide	*Organism*	*Concentration Factor*	*Reference*
Sodium-24	Marine invertebrates	0.5	3375
	Fresh-water fish	100	2436
Phosphorus-32	*Spirogyra*	850,000	3273
	Caddis-fly larvae	370,000	2535
	Insect larvae	200,000	3804
	Shiners	165,000	2535
	Fish	30,000–100,000	2436
	Marine vertebrates	40,000	3375
	Algae (*Ochromonas*)	4,900–10,500	3386
	Algae (*Platymonas*)	6,300–13,600	3386
Sulfur-35	Marine invertebrates	5	3375
Potassium-42	Marine algae	25	2440
	Marine invertebrates	10	2440, 3375
	Marine vertebrates	5	2440
	Crabs (*Carcinus*)	4	3820
Calcium-45	Marine invertebrate, skeletons	1,000	2440
	Marine invertebrate, soft parts	10	2440, 3375
	Marine vertebrate, skeletons	200	2440
	Marine vertebrate, soft parts	1	2440
	Marine algae	10	2440
Iron-59	Algae (6 species)	720–7,500	3386
	Fresh-water fish	10,000	2436
	Marine algae	20,000	2440
	Marine invertebrates, skeletons	100,000	2440
	Marine invertebrates, soft parts	10,000	2440, 3375
	Marine vertebrates, skeletons	5,000	2440
	Marine vertebrates, soft parts	1,000	2440
Chromium-51	Brown Algae	100–500	2442
Cobalt 60	Algae (*Ochromonas*)	1,070–1,500	3386
	Diatom (*Navicula*)	271	3386
	Copepods	50	2444
	Marine plankton (maximum)	10,000	3375
Copper-64	Algae (*Ochromonas*)	1,840–3,040	3386
	Sphaerotilus	3,890	3386
	Marine bacteria	990	3386
	Marine invertebrates, soft parts	5,000	3375
	Fresh-water fish	50	2436
Zinc-65	Algae (6 species)	312–67,800	3386
	Brown algae	400–1,400	2442
	Marine bacteria	290	3386
	Marine invertebrates, soft parts	5,000	3375
	Oysters	200,000	2446
	Oyster meat	1,400	2442

TABLE 8-3.—Continued

CONCENTRATION FACTORS FOR AQUATIC AND MARINE ORGANISMS

Radionuclide	*Organism*	*Concentration Factor*	*Reference*
Strontium-89 & 90	Algae (6 species)	100–3,060	3386
	Brown algae	8–90	2442
	Algae	1,840–2,110	3385
	Diatom (*Nitzschia*)	17	3690
	Algae	1–40	3819
	Crustacea	3–8	3819
	Mussels (*Mytilus*)	6	3819
	Brine shrimp	40	3821
	Bacteria	10–100	3822
	Marine bacteria	100	3386
	Marine algae	20	2440
	Marine invertebrates, skeleton	1000	2440
	Marine invertebrates. soft parts	10	2440, 3375
	Marine vertebrates, skeleton	200	2440
	Marine vertebrates, soft parts	1	2440
	Rudd, flesh	2.6	3691
	Rudd, bone	71	3691
	Rudd, scales	209	3691
	Fresh-water fish	20,000–30,000	2436
	Goldfish	10–150	3823
	Goldfish	35–53	3824
	Mosquito-fish, live	1.6–3.1	3385
	Mosquito-fish, dead	2.7–48.8	3385
Ruthenium-103 & 106	Algae	104,000	3385
	Algae (*Ochromonas*)	4,000–6,500	3386
	Diatom (*Navicula*)	7,900	3386
Iodine-131	Copepods	200	2444
	Marine algae	10,000	2440
	Marine invertebrates, skeleton	50	2440
	Marine invertebrates, soft parts	100	2440, 3375
	Marine vertebrates, soft parts	10	2440
Cesium-134 & 137	Algae (6 species)	29–2,180	3386
	Green algae	400–4,000	3387
	Fresh-water algae	52–1,530	3388
	Algae	342,000	3385
	Green, red & brown algae	4–30	3819
	Crustacea	10	3819
	Marine algae	1	2440
	Marine invertebrates, soft parts	10	2440
	Marine vertebrates, soft parts	10	2440
	Oysters, soft tissue	50	3375
	Crabs (*Carcinus*)	8	3820
	Mussel (*Mytilus*), soft body	10	3819
	Flavobacterium aquatile	26	3386
	Sphaerotilus	116	3386
	Marine bacteria	15	3386
	Zooglea ramigera	558	3386
	Snail	600	3387
	Amphipods	11,000	3387
	Tadpoles	2,600–6,000	3387
	Damsel-fly & dragon-fly nymphs	800	3387
	Bullfrog, muscle	9,000	3387
	Carp, muscle	3,000	3387
	Sunfish, muscle	9,500	3387
	Atlantic prawn	25	2444
Cerium-141 & 144	Algae (4 species)	5,100–28,500	3386
	Algae (several species)	2,000–4,500	3272
	Algae	2,040,000	3385
	Algae	314–4,498	2442
	Red, green & brown algae	300–900	3819
	Mussel (*Mytilus*), soft body	360	3819
	Crustacea	230	3819
	Marine bacteria	280–1740	3386
Gross beta emitters	Algae	43,300	3385
	Plankton	4,210	3825
	Bottom animals	526	3387
	Shiners, juvenile	474	3825
	Suckers, bone	263	3825
	Suckers, muscle	58	3825
	Bass, bone	184	3825
	Bass, muscle	24	3825
Mixed fission products	Algae (6 species)	1,700–16,800	3386

Another approach is to consider the radionuclides for which limits have been set in the 1962 Drinking Water Standards of the USPHS (2036), namely radium-226, strontium-90, and total beta emitters. No information was uncovered in this survey on the concentration of radium by fish, but since radium is relatively scarce in surface waters its measurable uptake by fish seems improbable. Strontium-90, however, is present in typical surface waters of the U. S. at concentrations that frequently exceed 1.0 pc/l and sometimes 2.0 pc/l. The limit set by the new standards for drinking water is 10 pc/l. If fresh-water fish should concentrate Sr-90 by a factor of 30 in their flesh (which according to Table 8-3 is possible but not likely) and if the surface water contained 2.0 pc/l of Sr-90, the fish flesh would contain 60 pc/kilogram. A man would have to eat 2.5 kg of such fish per week to get a Sr-90 dosage equal to the permissible dosage from drinking water. Moreover, the 150 pc/week so consumed would be only $150/7 \times 200$ or 10.7 percent of the limit set by the Radiation Protection Guide for total intake of Sr-90. It seems unlikely, therefore, that accumulation of radiostrontium in fish is likely to become a serious problem, but it warrants surveillance.

SHELLFISH

Many of the factors and principles relating to the effect of radioactivity on shellfish are discussed in the foregoing section on aquatic and marine life. The following information is specific for shellfish.

Snails vary considerably in their resistance to gamma irradiation; one species was killed by 213 r from Co-60, another by 1200 r, two species by 1750 r, but a fifth species survived 7461 r (3818).

Concentration factors for shellfish (oysters, mussels, crabs) are shown in Table 8-3. Clams can rapidly concentrate Zn-65, Fe-59, and Co-60 in their shells and soft tissues (2438). Oysters and clams are especially adept at accumulating Zn-65. A survey of food in Cincinnati showed that marketed oysters contained 40 to 178 pc/kg of Zn-65 in their meat compared with 3 to 17 pc/kg for other common foods (2445). Cesium is concentrated slightly by mollusks (3273, 3827). Accumulation factors for the mussel *(Mytilus)* are given in Table 8-3 for strontium-90, cesium-137, and cerium-144 (3819). Lobsters show a fairly rapid uptake of Sr-90; indeed they were the critical animal in limiting the discharge from the reactor at Winfrith in England (2444).

RECREATIONAL WATER USE

Radiation in water may be harmful to swimmers and bathers, especially when the period of contact is long. Most types of radiation, i.e., alpha and beta, can be absorbed through the skin and all types through wounds. Ideally, the radioactivity of recreational waters should be as low as possible; yet one must rationalize that the hazard from occasional bathing or swimming is not nearly as severe as that from daily ingestion. No clearly defined criteria of radioactivity for recreational waters, based on the latest standards of ICRP and NCRP were uncovered in this survey. It is assumed, therefore, that the ICRP, NCRP, and Federal Radiation Council standards for water apply to recreational waters as well as drinking water.

WASTE DISPOSAL

The Atomic Energy Commission, upon advice from the Federal Radiation Council, has established regulations for the discharge of radionuclides to sewers. For a summary of these regulations, see Table 8-2 and the discussion associated with it.

CHAPTER IX

PESTICIDES

by Ralph Pressman, Ph.D.*

If the number of fish killed per year in the streams of the United States were the criterion of water quality problems, first place among the list of pollutants would be occupied by pesticides (2879, 3012, 3013). There is no question but that mankind has reaped bountiful awards in the form of greater productivity, improved comfort, and better health from the widespread use of pesticides (3014, 3015). It has been estimated that in the first 10 years of broad-scale use, pesticides saved 5 million lives and prevented 100 million illnesses. Yields of many crops increased 10 and 20 percent or more and were of higher quality. Nevertheless, it is apparent that pesticides will have to be used with increasing care in order to avoid tragic, secondary effects (3016, 3017).

CLASSIFICATION OF PESTICIDES

Webster's New Collegiate Dictionary, 2nd Edition, defines pesticide as "any substance used to kill rats, insects, bacteria, fungi, protozoans, minute forms of plant life, etc." Such a broad definition includes many thousands of organic and inorganic compounds that have pesticidal properties but are not ordinarily recognized as pesticides. A simple example would be the use of common table salt to control weed growth in a homeowner's crushed-rock patio. The Federal Housing Administration report on ground-water contamination (2015) cites the following definition in Van Nostrand's Scientific Encyclopedia, 1958 edition:

> "A legal definition for a pesticide is that it is any substance or mixture of substances used in the production, storage, or transportation of food which is intended for preventing, destroying, repelling, or mitigating any insects, rodents, fungi, or weeds, and other forms of plant or animal life or viruses, except viruses on or in living man or other animals."

The FHA report enlarges the definition "to include many substances used to control objectionable insects, weeds, and probably other undesirable life or organisms. (Example: Some types of fish in ponds or water reservoirs.)"

The latter definition is that which has been most nearly followed in the preparation of WATER QUALITY CRITERIA. However, only those compounds that are generally recognized as pesticides are included within this chapter. For those compounds that are primarily used or classified in other ways, see Chapters VI and X.

The common pesticidal chemicals can be categorized chemically into three general groups, (a) Inorganic, (b) Synthetic Organic, and (c) Natural Organic. Examples of each include: Inorganic—arsenicals, mercurials, borates, and fluorides; Synthetic Organic—chlorinated hydrocarbons, organic phosphates, and thiocarbamates; and Natural Organic—rotenone, pyrethrum, and nicotine (3015). They may also be classified by their biological usefulness, viz, algicides, acaricides, fungicides, herbicides, etc. Other practical means of classification are also available such as the form of use, attractant, fumigant, and repellant, etc.

In general, the inorganic pesticides are toxic to plants and humans as well as to insects. Some formulations (3018) are more toxic to mammals than to insects. Inorganic pesticides have been supplanted largely by organics which can be adapted to more specific use. The natural organics are somewhat limited in supply and popularity. Present-day trends are towards the use of powerful synthetic organic pesticides, principally the chlorinated hydrocarbons and organic phosphorus compounds (3015). Similarly, the trend in aquatic weed control is towards chemical methods (3880).

MAGNITUDE OF THE PROBLEM

Pesticides may gain access to ground and surface waters through direct application, through percolation and run-off from treated areas, and/or through drift during application. Gross over-application of pesticides is a common practice that augments the pollutional effects of pesticides to a considerable extent. Although knowledge is lacking regarding the long-range effects of pesticides on our water resources, it is clear that these substances with their accompanying diluents and solvents are entering our natural waters in ever-increasing amounts (3015). Fish kills occurred in at least 15 streams in the Tennessee River Valley following application of a synthetic insecticide to over 400,000 acres of cotton in 8 Alabama counties (3019, 3030). Concentrations of DDT as high as 5 to 20 μ/l have been reported in the Detroit, Missouri, Mississippi, and Columbia Rivers (2699). The biological significance of trace quantities of many of the complex organic substances is only partially understood; their persistence in natural waters largely unknown (3015, 3021, 3022).

Because of these uncertainties, official limits for organic pesticides in water in the U. S. have not been established (3015). Serious consideration was given by the U. S. Public Health Service to recommending limits for the organic phosphate and chlorinated hydrocarbon pesticides in the 1962 Drinking Water Standards. It was difficult, however, to establish acceptable concentrations. Moreover, there are no simple analytical techniques for identifying and quantitating these chemicals (2062). Bean (1745), in a discussion of limits for an ideal water, concurs with these conclusions. Nevertheless, the English

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Mersey River Board in 1953 proposed that industrial waste effluents entering the River Alt and other fishing streams contain no insecticides (2950).

In contrast with water, pesticide limits are in existence for foods. In preparing limits for pesticides on foods, several assumptions are made:

(1) Man is 10 times more prone to injury than other species of warm-blooded animals.
(2) The most sensitive individual is 10 times more susceptible to injury than is the average individual.
(3) Some individuals subsist for lengthy time intervals on diets consisting almost entirely of fresh fruits and vegetables.
(4) When two or more toxic substances are present, the allowable quantity of each is calculated as the percent of its applicable tolerance, the sum of such percentages being not more than 100.

The last assumption is said to be good for the chlorinated hydrocarbons, organic phosphates, and dinitro compounds (3023). However, the reader is referred to EPN or malathion in this chapter as examples of synergistic compounds that do not behave in this simple way.

Until a pesticide is exempt (copper compounds, pryethrum, rotenone) or given an official tolerance, no trace of the pesticide is allowed on any product (3015). A zero tolerance for any particular pesticide generally requires that the product not be used on the crop during the period when it might result in the contamination of the harvested portion of the crop (3024). With few exceptions, pesticides are not taken up from the soil by roots or absorbed through the foliage of fruit and vegetable plants and translocated (3023). However, the reader is referred to a study that reports such uptake (3025).

It must be remembered when dealing with pesticides as water pollutants that any specific pesticide will rarely, if ever, be encountered as the sole, solitary pollutant. Many solvents, diluents, or other carriers used with the pesticide also have toxic properties, e.g., the light petroleum oils used in household insect sprays. Solvents, such as xylene, alkylated naphthenes, fuel oil, or kerosene, have some toxicity. This effect is believed to be particularly evident in aquatic environments when solvents have an opportunity to be emulsified by riffles in streams (3026). Rudd and Genelly cite three important aspects of solvents or dilutents from the wildlife standpoint: (a) they may be toxic themselves; (b) they may enhance the toxicity of the pesticide directly (synergism); and/or (c) they may increase the toxicity of the pesticide by making the toxic agent more readily available. None of these effects are common, and only certain types of formulation will show any one of these effects. In general, solvents and additives reduce the available amount of toxic agent (3005).

When considering pesticidal pollution of water, toxicity is not the only aspect of concern. There are also difficult problems posed by tastes and odors. Some synthetic organics cause highly objectionable tastes and odors, as do many of their solvents also (3024).

The addition of synergists and/or various adjuvants to make a particular pesticide more effective has been practiced by the pesticide manufacturing industry for many years. A marked synergistic effect, or potentiation, of up to 50-fold in acute toxicity exists for EPN and malathion when administered simultaneously to dogs (3027). For more on potentiation and toxicities of mixtures the reader is referred to three significant references (3028, 3029, 3030). Adjuvants include wetting or spreading agents, stickers, penetrants, and emulsifiers (2998).

When, to the complexity of various formulations, one adds the differing environmental conditions and inherent biological variations, it is not surprising to find that there is a wide variation in the literature in toxicity results of these compounds to various aquatic animals. Although recognizing that it is important to duplicate test conditions such as dissolved oxygen, pH, temperature, time, hardness of water, etc., several authors (2953, 2985, 3031, 3032, 3033, 3034, 3035) have indicated that (a) not enough variables are included, (b) ecological factors are not considered sufficiently, and (c) chronic effects of sub-lethal dosages are not considered. They conclude, therefore, that a great deal of additional study is necessary. Among the more obscure areas of knowledge is that delineated by Hueper (3330) who states that, based upon animal studies, a number of pesticides and preservatives, such as DDT, aramite, carbon tetrachloride, acetamide, thioacetamide, thiourea, thiouracil, aminotriazole, and isopropyl-chlorophenyl-carbamate are capable of eliciting benign and/or malignant tumors in various organs.

There is no doubt but that more study is needed, particularly in view of the burgeoning aspects of pesticides. The 9072 commercial pesticide products of 1961 represented a 15-percent increase over the number listed in 1960, and over twice the number, 4360, listed in 1952 (2998). Although over 200 different kinds of synthetic organic pesticides are now used, fewer than 30 account for approximately 80-90 percent of the estimated 900 million pounds of finished pesticidal preparations sold annually (3015). Over 300 million acres of cultivated crop land in the U. S. are sprayed each year with over 500 million pounds of organic insecticides (2699). Woodward (3024) has made the interesting observation that division of the 1958 production of pesticides (460×10^6 lbs) by the annual U. S. runoff (1160 bgd) results in a concentration of 0.13 mg/l.

In the face of this enormous and expanding use of pesticides, suggestions have been offered to limit undesirable side effects. In addition to the usual plea for proper dosage and careful application, it has been suggested that consideration be given to select that insecticide most effective against the pest desired and least hazardous to other organisms (3014), and also that the least persistent types of pesticides are to be preferred (3024).

ACP-M-569

(see ATA)

ACROLEIN $CH_2{=}CHCHO$

1. General. Acrolein has been introduced as an aquatic weed killer under the commercial name Aqualin. Also known as acrylic aldehyde, 2-propenol, or F-98, it is

a clear colorless liquid at ordinary temperature, having a pungent irritating odor. It is severely irritating to the skin and mucous membranes, and is soluble in water and various organic solvents (364, 2599, 2991).

2. Cross References. Pentachlorophenol and Pentachlorophenates.

3. Effects Upon Beneficial Uses.

a. Stock and Wildlife Watering. The principal constituent of Aqualin, 2-propenol, has an acute oral LD_{50} for rats of 46 mg/kg of body weight and for rabbits of 7.1 mg/kg (2991). Another report cites 600 mg/kg in food being administered to laboratory animals without effect (3036).

b. Fish and Aquatic Life. Acrolein at 3 mg/l applied to a pond killed tadpoles, 500 small bluegills, and a few big walleyes, but no large bluegills or bass (2878). Other field work indicated that fish showed severe agitation when exposed to concentrations of 10 to 15 mg/l. Carp and thread-fin shad appeared quite sensitive, whereas largemouth black bass seemed more resistant (2988). Springer (3010) reports that acrolein repels fish, is toxic to rough fish at 3 mg/l, and to game fish at 5 mg/l.

For F-98, a trade formulation containing acrolein, the 24-hour TL_m value for chinook salmon at 20°C in aerated solutions was 0.08 mg/l (2988). This formulation is reported to kill carp and thread-fin shad at 1-2 mg/l (3036). All test fish exposed to acrolein at 2 and 4 mg/l were dead within one hour after their removal from the solution (2988). Chironomids, tubificids, and *Tanypus* were not affected at doses up to 12 mg/l (3036).

In Lake Huron water at 12° C, rainbow trout, bluegill sunfish, and sea lamprey survived a 5 mg/l concentration for 24 hours (2976).

Van Overbeek et al. (3037) reports that acrolein is twice as effective against snails as sodium pentachlorophenate, and promises to become a useful tool against the blood flukes (see Worms, Parasitic, in Chapter VII). The molluscacidal properties of acrolein were first observed during field trials in California coincident to the destruction of submerged weeds in irrigation canals. Concentrations of 10 mg/l for 3 hours killed 100 percent of snail eggs and for 24 hours killed 98 percent of adult snails. Numerous field trials in Puerto Rico against water weeds and snails are cited (3038).

With respect to plant life, concentrations of 5 mg/l for 5 hours, or 0.5 mg/l for 24 hours, are toxic to *Elodia densa* leaf cells (3309). A dosage range of 1.5 to 7.5 mg/l, depending upon water temperature (15°-27°C), killed *Hydrodictyon, Cladophora, Spirogyra, Potamogeton, Zannichellia, Elodea, Callitriche,* and *Ceratophyllum* (3040). Another reference cites doses of 3-6 mg/l of F-98 for the control of weeds in lakes and static reservoirs (3036).

ACTIDIONE

(See Antibiotics)

AEROSPORIN

(see Antibiotics)

AGROXONE

(see MCPA)

ALDRIN $C_{12}H_6Cl_6$

1. General. Aldrin is the common name of an insecticide containing not less than 95 percent of 1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 8, 8a-hexahydro-1, 4, 5, 8-dimethanonaphthalene. It is crystalline, insoluble in water, slightly soluble in oils, and soluble in most other organic solvents. It is similar to dieldrin which has an oxygen substituted for two hydrocarbons. Aldrin is used agriculturally at rates varying from 2 ounces to 6 pounds per acre ,and is usually used as a dust or emulsifiable concentrate (3005).

2. Cross References. Chlorinated Hydrocarbons—General, Dieldrin, Isodrin.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Aldrin has moderately high acute and chronic effects by all routes of administration. A number of cases of human illness have been recorded, most of which resulted from its manufacture (3005). The estimated fatal dose for a 70-kg man is 5 grams (2995).

b. Irrigation Waters. Aldrin from contaminated soils was absorbed by carrots, potatoes, peas, cabbage, cucumbers, beans, radishes, beets, and rutabaga. These crops contained both aldrin and dieldrin in their tissues (3025).

c. Stock and Wildlife Watering. For aldrin, the acute oral LD_{50} to rats has been cited at values ranging from 39 to 66.8 mg/kg of body weight (2992, 2999, 3002, 3042, 3043). Chronic oral doses of 100 mg/kg in the diet produced a gradual increase in esterase levels (3041). A level of 25 mg/kg for two years caused no harm (3043). However, lower dietary levels (10-12.5 mg/kg) had effects on reproduction. Other animals are more susceptible than rats, dogs being five times more susceptible in chronic feeding tests. Sheep and cattle fed alfalfa treated at 0.5 lb/acre showed no noticeable reactions (3005).

Poultry and birds appear to be quite susceptible to aldrin. Levels of 3 mg/kg in the diet retarded growth in turkeys and chickens. The LD_{50} value for seven-day-old chicks is 25.5 mg/kg. Ducks and other water fowl suffered significant losses from single applications of aldrin as low as 0.125 lb/acre, which also effected a heavy mortality of insects (3006). Feeding of 1 mg/kg in the diet of quail caused a decrease of 40-percent reproduction (2996), and when fed to growing quail caused high mortality rates. Young pheasants failed to survive on a diet of 5 mg/kg. Nearly all quail died when fed winter diets containing 0.5 mg/kg. Hatchability of eggs and viability of chicks are adversely affected by aldrin (3044, 3045). The oral LD_{50} values for bobwhite quail and ringneck pheasant, considering all stages of the life cycle, are 4 and 14 mg/kg of body weight respectively (2994, 2996). The acute oral LD_{50} value for mourning doves lies between 15 and 17 mg/kg (3005).

d. Fish and Other Aquatic Life. Aldrin is highly toxic to fish. In aquarium tests there was no significant difference in toxicity between dust suspensions and emulsions of equal aldrin equivalence (3005). Aldrin dust, 2.5 percent, applied weekly for 20 weeks at 1 lb/acre (0.025 lb aldrin per acre) caused insignificant fish mor-

tality. Five lb/acre of dust killed 3.3 percent of the fish, 10 lb/acre killed 60 percent (3046).

Doudoroff, Katz, and Tarzwell (1449) tested the toxicity of aldrin toward ten goldfish or minnows in a 15-liter aquarium at 20°C with water of 75 mg/l total alkalinity. Tests were made with a dust containing 2.3 percent aldrin, 1.79 percent related compounds, and the remainder inert; and also with an emulsion comprising 22.8 percent aldrin, 17.5 percent related compounds, 50 percent petroleum products, and the remainder inert. In terms of aldrin, the effects of dust suspensions and emulsions were about the same. The concentration of aldrin fatal to 50 percent of the goldfish in 10 days (i.e., the 10-day TL_m) was 0.02 mg/l. At 0.01 mg/l 90 percent survived, while at 0.032 mg/l only 20 percent lived 10 days. A concentration of 0.05 mg/l caused some goldfish to turn over within 24 hours, but others withstood 0.1 mg/l. For minnows, the 10-day TL_m was about 0.018 mg/l but some fish were killed by as little as 0.01 mg/l.

Although young fathead minnows do not differ in susceptibility from large goldfish, it appears that trout are more sensitive (3005). Exposure of 4-7 inch brown trout to an emulsified concentrate at 0.025 mg/l killed 33 percent in 48 hours, and 0.033 mg/l killed 100 percent in the same time interval (3005, 3007, 3049). The toxicity threshold of aldrin to trout is reported as 0.025 mg/l (3007, 3048). Later studies that report the fish toxicity of aldrin are tabulated below:

Concentration of Aldrin mg/l	Temperature °C	Type of Water	Type of Fish	Result	Reference
0.013	25	(3)	Bluegill	96-hour TL_m	3000
0.028	25	(2)	Fathead	96-hour TL_m	3000
0.028	25	(3)	Goldfish	96-hour TL_m	3000
0.033	25	(3)	Fathead	96-hour TL_m	3000
0.033	25	(3)	Guppies	96-hour TL_m	3000
0.05	20-22		Guppies	Abnormal behavior in 3 hours, death in 48 hours	2992
5.0	13	(1)	Rainbow trout	No effect in 24 hours	2976
5.0	13	(1)	Bluegill	No effect in 24 hours	2976

(1) Lake Huron water, pH 7.5-8.2, DO 8.6-13.7 mg/l, free CO_2 5.0-9.0 mg/l, 10-liter aquaria, 6 liters solution, one or two test specimens only.
(2) Hard water, pH 8.2, DO 8.0 mg/l, alkalinity 360 mg/l, hardness 400 mg/l.
(3) Soft water, pH 7.4, DO 8.0 mg/l, alkalinity 18 mg/l, hardness 20 mg/l.

Silvey (2531) cites the concentration of aldrin required to kill 50 percent *Chironomus* larvae in eight hours as 0.012 mg/l. Batte, et al. (2993) found that 4.8 mg/l of aldrin killed *Lymnaeid* snails in 24 hours. The estimated concentration of aldrin required to immobilize *Daphnia magna* in 50 hours at 20°C is 29.2 μg/l (3255).

ALLETHRIN

(see Pyrethrum)

AMERICAN CYANAMID (4124 and 12009)

(see Organic Phosphates—General)

AMMATE

(see Ammonium Sulfamate)

AMMONIUM SULFAMATE $NH_4SO_3NH_2$

This compound also known as Ammate, AMS, and ammonium amidosulfate, is used to control aquatic vegetation. It occurs as crystals and is extremely soluble in water. It has low mammalian toxicity, the acute oral LD_{50} to rats being 3.9 g/kg (364, 1724, 2997, 3009). Ammate is reported to be the best herbicide for use around water supply reservoirs. The lethal dose for an average 90 kg man is 180 grams (1723).

A formulation of 0.8 pound of the chemical in 1 gallon of water, with or without a wetting agent, was reported not to kill fish although frogs were blinded by the spray. A concentration of 10 mg/l was non-toxic to fish (1725, 3009). Rainbow trout, bluegill sunfish, and the sea lamprey survived a concentration of 5 mg/l for 24 hours in Lake Huron water (2976).

The 24-, 48-, and 96-hour LD_{50} values for Ammate to fingerling channel catfish at 19°C were reported as 259, 206, and 203 mg/l respectively (2981).

AMINOTRIAZOLE

(see ATA)

AMITROL

(see ATA)

ANTIBIOTICS

Antibiotics will not likely be found as stream pollutants. An increasing amount of research, however, is being conducted to determine their effectiveness against algae. Since they appear to show high specificity to certain types of algae, their use as algicides may very well increase in the coming years.

Actidione, which has an acute oral LD_{50} against laboratory rats of 1.0 mg/kg (3009), was found to be effective against certain green algae and diatoms (2041, 3050). Concentrations of 2.0 mg/l inhibited the growth of 50 percent of the test algae during a 21-day incubation period at 22°C. Streptomycin inhibited all cultures of blue-green algae and 18 percent of green algae at 1 mg/l equivalent of pure base. A concentration of 0.015 mg/l retarded the growth of 25 percent of the blue-green algae tested (2041, 3050). Streptomycin sulfate at a concentration of 2.0 mg/l inhibited the growth of 66 percent of the tested algae during a 21-day incubation period at 22°C (2041).

Neomycin and terramycin were found to affect blue-green algae (2041, 3050). Terramycin at a concentration of 2.0 mg/l inhibited the growth of 17 percent of the tested algae during 21 days of incubation at 22°C (2041).

Concentration of Aerosporin-Polymyxin B (sulfate) and penicillin G, potassium (crystalline), at 2.0 mg/l inhibited the growth of 83 and 17 percent of the tested algae respectively after 21 days incubation at 22°C (2041).

Chlortetracycline at 20 mg/l was used in oyster rinse water at near 0°C but it failed to depress bacterial growth significantly and did not prevent the development of sour odors associated with spoiled oysters. The oysters retained 3 mg/l, a concentration that decreased by 50-60 percent during 12 days of storage (3051).

AQUALIN

(see Acrolein)

AQUATHOL

(see Endothal)

AQUATIC

(see Aromatic Solvents)

ARAMITE $C_{15}H_{23}ClO_4S$

Aramite is the common name for a compound whose chemical name is 2 (*p-tert*-butylphenoxy) isopropyl 2-chloroethylsulfite. It is a chlorinated acaricide commonly used to control mites on fruits, vegetables, and ornamentals. Application rates normally range between 0.25 and 0.5 lb/acre. Residues persist for at least one week.

The acute oral LD_{50} to laboratory rats has been reported as 6300 mg/kg of body weight (3002) and 3900 mg/kg (3005). The LD_{50} for mice is 2300 mg/kg. Chronic toxicity studies showed that 500 mg/kg in the diet of rats and dogs for a period of 1-2 years had no effect. The lowest dietary level that produced gross effects was 1000 mg/kg (3005).

Testing fingerling channel catfish against a 15-percent Aramite solution at 20°C, Clemens and Sneed (2981) found the 24-hour TL_m to be over 15 mg/l as Aramite. In tests utilizing *Lymnaeid* snails, a 100-percent mortality was caused in 24 hours by 1.5 mg/l of Aramite (2993).

AROMATIC #80

(see Aromatic Solvents)

AROMATIC SOLVENTS

1. General. Aromatic solvents are used for the control of submerged aquatic weeds in irrigation channels in the United States. These petroleum or coal-tar derivatives are also used in industry as paint thinners. Bruns, et al. have published studies of the aromatic solvents as aquatic weed killers in a U.S. Department of Agriculture Circular (2873). In this report, the type of solvent is identified by a table of A.S.T.M. physical characteristics.

Because the aromatic solvents are not soluble in water, emulsifiers (surface-active agents) are added. Earlier work by the Department of Agriculture utilized the petroleum sulfonates at 5-10 percent by volume of the solvents. Experience showed that the performance of these emulsifiers was adversely affected by lower temperatures or higher salt content. Nonionic type emulsifiers are now used at about two-percent concentrations and give satisfactory results (2873). The aromatic solvents are usually injected under the water surface to give a concentration of 300-600 mg/l for 30-60 minutes (2861).

2. Cross References. Chapter X.—Surface Active Agents, Chlorinated Benzenes.

3. Effects Upon Beneficial Uses.

a. Irrigation Waters. A 30-minute introduction of aromatic solvent at up to 1200 mg/l caused no visible or measureable damage to spring wheat. However, 2200 mg/l caused a 22-percent reduction. Irrigation for 60 minutes with concentrations as high as 900 mg/l caused no injury to potatoes, and 1250 mg/l caused no damage to sweet corn. Later studies indicated no effect by 30-minute concentrations of 1200 mg/l applied to sweet corn, potatoes, sugar beets, and beans. Although concentrations of 2400 mg/l for 10 minutes caused no damage to sweet corn, a 12-percent reduction was obtained from 1600 mg/l for 30 minutes. Applications of 2400 mg/l for 30 minutes did not affect oats, beans, and probably not carrots, the decreased yield of carrots at concentrations over 800 mg/l being insignificant.

Although concentrations of 400 mg/l for 40 minutes appeared to damage seedling alfalfa, new shoots developed and the plants grew normally. Mature stands suffered only the burning of lower submerged leaves. Grain sorghum was unaffected except for those plants completely submersed, which died. Cotton exhibited the same response. Other studies of 40-minute applications of up to 1600 mg/l to alfalfa, grain sorghum, and cotton exhibited temporary inhibition in growth but no decreased yields. Applications of 2400 mg/l for 30 minutes did not affect ammonifying or nitrifying bacteria (2873).

Further experiments are discussed by Bruns, et al., but the general conclusion is that the usual concentrations of aromatic solvents, when present in flood irrigation waters, will not harm crops provided that they are not in the seedling stage; i.e., provided that they are not submerged (890, 2873).

b. Stock and Wildlife Watering. Farm animals were observed to taste aromatic-solvent-treated water and then refuse to drink it. No subsequent ill effects were noted, nor are species or concentrations given. However, guinea pigs were confined with water treated with 800 mg/l and refused to drink it until the third day. Although they drank a substantial quantity (amount not specified) on this third day, no subsequent ill effects were noted (2873).

c. Fish and Aquatic Life. The aromatic solvents are toxic to aquatic life (2861). Fish, crayfish, snails, mosquito larvae, and other insects are killed when they come in contact with treated water of 150-740 mg/l (3009).

In aquaria, 4.2 mg/l of Aromatic #80 and Socal #3 killed 40-60 percent of the white crappies tested. Dead-X, when applied in a drainage ditch at 185-200 mg/l, killed most caged fish within 1 mile of the point of introduction, but unconfined fish were observed to flee before the chemical and certain insects were little affected. An Esso weedkiller, WS-1492, with an aromatic content of 99.5 percent applied at 250-350 mg/l killed gar, mudfish, and a few minnows. Within several hours of application, the emulsion broke, rose, and evaporated, leaving the water relatively unpolluted. Fish later returned (3009, 3011).

Ortho Aquatic Weed Killer, which contains 95 percent aromatic petroleum distillate, at 50 mg/l effected a 50-percent kill of 3-inch silver salmon in 72 hours. Aerated, fresh water was used, and all survivors were obviously distressed. The level recommended for use in weed control is 370 mg/l (2091). The 96-hour TL_m values of Aquatic to shiners in soft and hard waters at 20-22°C was 75 and 78 mg/l respectively. Field studies reported that water striders (Gerridae) were the only water fauna that survived (3011).

ARSENIC

(see Chapter VI)

ARSENICALS

(see Arsenic and the specific arsenic compounds in Chapter VI: Arsenic Trioxide, Calcium Arsenate, Lead Arsenate, Sodium Arsenate, and Sodium Arsenite)

The arsenicals have been in use for a long time as herbicides, rodenticides, algicides, and insecticides. A number of compounds are still available for use, but at least as insecticides, they have been largely supplanted by synthetic chemicals. For this reason and for convenience of presentation all arsenic-containing compounds except paris green are included in Chapter VI. Arsenicals comprise chemicals with widely different compositions and uses.

The best known arsenical insecticides are calcium and lead arsenate. Until recent years they were more widely applied than any other insecticides. They are still used commonly, particularly in Europe and in the southern United States. Sodium arsenite has been used in grasshopper and ant baits. It is better known in recent years as a herbicide to control submerged vegetation and as a debarking agent in the wood pulp industry. Dust and aqueous solutions of arsenic trioxide continue in limited use in rodent baits. Paris green (copper acetoarsenite), one of the first insecticides to be widely employed, is discussed in this chapter.

ARSENIC TRIOXIDE

(see Chapter VI)

ASUNTOL

(see Co-Ral)

ATA $C_2H_4N_4 \cdot HCl$

ATA, also known as amitrol or aminotriazole, is a water-soluble crystalline compound for which the chemical name is 3-amino-1-H-1,2,4-triazole. The chemical formula above is that of the hydrochloride. ATA is a weed killer that is not considered to be very toxic to animal life. The LD_{50} to rats is reported to be 14.7 g/kg of body weight (364) and also 25 g/kg (3009). ATA is used for the control of all species of cattails (3010), and is less hazardous than 2, 4-D to sensitive crops when sprays drift (2880).

Tests with bluegills indicated that all fish would be expected to survive a concentration of 1470 mg/l. A concentration of 10,000 mg/l was necessary to kill 50 percent of the bluegills during a 48-hour exposure (3009).

Toxicity studies were made with red-sided shiners. The 96-hour TL_m values at 20°-22°C for soft and hard water respectively were 983.3 and 1370 mg/l (3011).

According to Bond (2988) the 24- and 48-hour TL_m values at 20°C for coho salmon were both 325 mg/l. Bond notes that aminotriazole failed to kill largemouth bass in 96-hour standing-water bioassays at concentratitons up to 1,000 mg/l but in a constant-flow apparatus this concentration killed all test fish in six days. Largemouth bass survived 62.5 mg/l for a 14-day period in the constant-flow test. Bond also quotes other studies on the toxicity of aminotriazole to bluegills, indicating that the 24-hour TL_m is 14,300 mg/l and the 48-hour TL_m is 10,000 mg/l. Bond mentitons another report that indicates that the concentration safe to fish is only 1470 mg/l. He also studied a formulation called ACP-M-569 using chinook salmon and found the 24-hour TL_m value to be 185 mg/l and the 48-hour TL_m value 155 mg/l. However, this formulation contained other undisclosed ingredients as well as aminotriazole (2988).

BARON $C_{11}H_9Cl_5O_3$

(see also Dalapon, 2, 4, 5-T)

Baron is a trade name given to the herbicide erbon, whose chemical name is 2-(2, 4, 5-trichlorophenoxy) ethyl 2, 2-dichloropropionate. This molecule was designed to combine the selectivities of the phenoxy compounds with those of dalapon. Baron is applied to the soil as a residual nonselective systemic herbicide. It requires rainfall or irrigation to leach the compound into the soil of the root zone. It persists in the soil for a considerable period. Baron contains 41.3 percent technical grade erbon.

According to Bond (2988), the 24-hour TL_m value against bass at 20°C was 4.6 mg/l and the 24- and 48-hour values against chinook salmon were 2.62 and 2.3 mg/l respectively. Utilizing coho salmon, no mortalities occurred in 24 hours following 15- and 30-minute exposures to 5 mg/l. Bond quotes Lawrence as citing the safe concentration to fishes at 5 mg/l. Clemens and Sneed (2981) tested Baron against fingerling channel catfish at 19°C. The 24-, 48-, and 96-hour TL_m values were 7.2, 6.9, and 4.5 mg/l respectively (2981).

BAYER 13/59

(see Dipterex)

BAYER 17147

(see Guthion)

BAYER 21/199

(see Co-Ral)

BAYER 29493

(see Organic Phosphates—General)

BENOCLOR

(see Chlorinated Benzene)

BENZENE HEXACHLORIDE $C_6H_6Cl_6$

1. General. Benzene hexachloride is a widely used compound whose correct chemical name is 1,2,3,4,5,6-hexachlorocyclohexane. Of its nine possible stereoisomers five are known, the gamma isomer being the powerful insecticidal principle. It is also known under the names lindane, BHC, and Gammexane (677). The name lindane is applied to commercial preparations containing 99 percent or more of the gamma isomer. Originally the wettable powder formulation of benzene hexachloride contained 6-12 percent of the gamma isomer. Newer formulations contain as much as 36 percent. BHC has a residual life in the soil approaching that of DDT (3005).

An example of stream pollution by an industrial waste containing BHC has appeared in the German lit-

erature. In this report, concentrations of BHC were not given, but varying dilutions of the waste waters were made and tested against different organisms. The report notes that the treatment plant efficiency was diminished, stream organisms disappeared, and the odor was noticeable 15 kilometers downstream as a result of the pollution (3052).

2. Cross References. DDT, Chlorinated Hydrocarbons—General.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The A.M.A. Committee On Pesticides reports that an estimated 600 mg/kg of body weight, or about an ounce, of technical grade BHC containing 15 percent gamma isomer would be fatal for man (3053). Other reports (2995) estimate the fatal dose of lindane for a 70 kg man as 15 grams and of BHC as 28 grams. BHC imparts a disagreeable taste to drinking water at a level of 20 μg/l (3054). Still another report (2058) notes that as a source of taste and odor in water a mild effect is still noticeable up to two years after spraying.

b. Irrigation Waters. A most important hazard involved in irrigation water containing BHC is the tainting of the flavor of crops with a characteristic musty odor and taste. BHC has no effect on the pH of the soil or on its bacterial content (677). Crops grown in soils containing lindane absorb the insecticide into its tissues. Crops tested were carrots, potatoes, peas, cabbage, cucumbers, beans, radishes, beets, and rutabaga. Carrots accumulated lindane in higher concentrations than it was present in the soil (3025).

c. Stock and Wildlife Watering. The acute oral LD_{50} of technical grade BHC for the rat is reported as 600 mg/kg (2992, 3002, 3003), and 200 mg/kg (3055, 3056). Another report cites the acute oral toxicity of lindane against male rats as 88 mg/kg and against female rats as 91 mg/kg (2999). The beta isomer is reported to be toxic at 6000 mg/kg (3005). The chronic oral toxicity of lindane to rats fed daily for two years was found to be 5.0 mg/kg (3003).

The amount of BHC that will produce at least 50 percent mortality in bobwhite quail and ringneck pheasant considering all stages of the life cycle are 250 and 450 mg/kg respectively. The amount of lindane required for the same results with the same two birds is 200 and 400 mg/kg respectively (2994, 2996). Rudd and Genelly cite LD_{50} values of lindane for bobwhite quail as 120-130 mg/kg for males, 190-210 mg/kg for females. Yet, their own work indicated an LD_{50} for female ringneck pheasants as being between 60 and 100 mg/kg. Mourning doves proved to be more resistant to lindane with an approximate LD_{50} of 350-400 mg/kg (3005, 3058).

d. Fish and Other Aquatic Life. Gjulian, et al. (919), report that concentrations of 1.0 to 10.0 mg/l of gamma BHC were toxic to fish. The pure gamma isomer of BHC dissolved in acetone was highly toxic to one-inch-long rainbow and brown trout at 0.02 mg/l. Rainbow trout were killed by 0.025 mg/l but brown trout were not affected (3007). A summary of the toxicities of alpha, beta, gamma, and delta isomers follows:

BHC Isomer and Concentration mg/l	Temperature °C	Type of Fish	Results	Reference
Alpha 5.0	21	Guppies	Abnormal behavior in 18 hours, death in 36-48 hours	2992
Beta 0.5	--	Trout	Toxicity threshold to fingerlings	3007
2.0	--	Fish	Toxic	677, 679
Gamma 0.01	--	Trout	Toxicity threshold to fingerlings	3007
0.03	--	Bluegills	Toxicity threshold to fingerlings	3007
0.05	--	Fish	Toxic	677, 679
0.5	21	Guppies	Abnormal behavior in 5 minutes, death in 20 hours	2992
Delta 0.05	--	Trout	Toxicity threshold to fingerlings	3007
0.2	--	Fish	Toxic	677, 679
2.0	21	Guppies	Abnormal behavior in 45 minutes, death in 48 hours	2992

Linduska and Surber (679) state that bluegills were not killed by 0.45 mg/l of BHC. The concentration of Gammexane that will kill half a batch of trout in a day is about 5 mg/l and in 30 days it is about 1.8 mg/l (3057). However, the threshold of toxicity for rainbow trout has been reported as being in the neighborhood of 0.0125 mg/l (3058). BHC in aquarium tests at concentrations of 0.1 mg/l killed 50 percent of bluegill and bass fingerlings (681).

Mixtures of BHC and DDT show LD_{50} concentrations in 10 days of 3.0 mg/l for goldfish (680). Cope et al. (755) claim that BHC was more toxic than DDT, chlordane, or toxaphene to fish. Trout fingerlings were disabled by 1.0 mg/l of BHC in emulsion and by 10.0 mg/l in acetone solution (755). On the other hand, Warrick (920) quotes literature to the effect that BHC is less toxic than DDT to fish.

Doudoroff, Katz, and Tarzwell (1449) studied the toxicities of BHC-DDT mixtures to ten goldfish at 20°C. A dust containing 3 percent gamma BHC, 5 percent other BHC isomers, 5 percent DDT, 40 percent sulfur, and 47 percent inert compounds showed that 0.3 mg/l as dust was the 10-day median tolerance limit. This dose is equivalent to 0.24 mg/l of total BHC or 0.09 mg/l of gamma BHC, and 0.15 mg/l of DDT. Approximately 25 mg/l as dust caused the fish to turn over within 24 hours. For further information on the toxicities to fish of mixtures of various isomers with DDT, the reader is referred to Fielding (3059). Rudd and Genelly (3005) cite the statement that fish are less sensitive to BHC than to DDT, but add that toxaphene, dieldrin, and the other newer chlorinated hydrocarbons are many times more toxic to fish.

Later studies reporting the toxicity of benzene hexachloride and the gamma isomer to fish are summarized in the following table:

Concentration mg/l	Temperature °C	Type of Water	Type of Fish	Results	Reference
Lindane (gamma isomer)					
0.05	6–10	(2)	Trout	Less than 50% died in 81 days	2640
0.056	25	(3)	Fatheads	96-hour TL_m	3000
0.062	25	(4)	Fatheads	96-hour TL_m	3000
0.077	25	(4)	Bluegills	96-hour TL_m	3000
0.138	25	(4)	Guppies	96-hour TL_m	3000
0.152	25	(4)	Goldfish	96-hour TL_m	3000

Concentration mg/l	Temperature °C	Type of Water	Type of Fish	Results	Reference
25% Gamma Isomer					
0.032-0.056	14	(1)	Salmon	96-hour TL_m	2091
0.056-0.10	14	(1)	Trout	96-hour TL_m	2091
BHC					
0.790	25	(4)	Bluegills	96-hour TL_m	3000
2.000	25	(3)	Fatheads	96-hour TL_m	3000
2.170	25	(4)	Guppies	96-hour TL_m	3000
2.30	25	(4)	Fatheads	96-hour TL_m	3000
2.30	25	(4)	Goldfish	96-hour TL_m	3000

(1) Fresh water, pH 7.8, DO 9.0 mg/l, total alkalinity 73.0 mg/l, 300-liter aquaria, 16 fish per aquarium, chinook salmon 77 mm long, rainbow trout 92 mm long.
(2) Fixed volume aerated aquaria, fresh solutions added daily, rainbow, yearlings.
(3) Hard water, pH 8.2, DO 8.0 mg/l, alkalinity 360 mg/l, hardness 400 mg/l.
(4) Soft water, pH 7.4, DO 8.0 mg/l, alkalinity 18 mg/l, hardness 20 mg/l.

Five treatments per year with technical BHC applied each summer for three consecutive years at a rate of one pound per acre each time (i.e., about 0.1 mg/l) resulted in no fish injury (1475).

Dusts of 3 percent gamma BHC applied at 1 lb/acre/week for 20 consecutive weeks caused insignificant fish mortality (3046, 3060). Technical grade BHC (12 percent gamma isomer) can be applied safely as emulsions at a rate of one lb/acre at five-week intervals without damage to fish (3057). One lb/acre of wettable powder BHC was reported to have killed many fish in a small pond (3061). An aerial application of 0.2 lb BHC/acre caused heavy losses of fiddler and marsh crabs (3061). An application of an emulsified mixture of BHC at 12.3 lbs/34,848 square feet was lethal to coho salmon fry if they were within one foot of the surface, but not if they were below the one-foot depth (3062). In the study utilizing crude BHC and crabs, 65 mg/l caused death in four hours, 6.5 mg/l caused death in 24 hours, 0.65 mg/l caused no death in 72 hours, and 0.065 mg/l had no effect (3063). The 24-hour TL_m to shrimp (*P. setiferus*) was 35 µg/l; for *P. aztecus* it was 400 µg/l (3064).

Silvey (2531) cites the concentration of lindane necessary to kill 50 percent of *Chironomus* larvae in eight hours as 0.006 mg/l. Saturated aqueous solutions (10^{-4} g/100 ml H_2O or 1 mg/l) were tested against various aquatic organisms. Bacteria decreased in numbers during the first 24 hours then increased to former levels. Protozoa and algae were killed almost immediately, fish were injured in one to four minutes (3065).

Losses of rainbow trout due to parasitism by the copepods *Lernaea cyprinacea* Linn or *L.carassii* Tidd were controlled by a concentration of 5 mg/l of benzene hexachloride in rearing ponds. As little as 0.1 mg/l applied for 72-96 hours caused a 55-percent kill of the female copepods (3066).

Batte et al. (2993), tested the toxicity of benzene hexachloride isomers to *Lymnaeid* snails. Concentrations of the technical grade isomers of 10 mg/l, including alpha-beta, delta, beta, alpha, alpha-delta, beta-delta, and alpha-beta-delta failed to give any mortalities in 24-hour exposures. A similar concentration of 99.8 percent gamma isomer affected 100-percent kill, but only a 1.7 mg/l concentration of crude benzene hexachloride (five percent gamma isomer) was required for the same mortality.

Toward plankton, BHC may not be so toxic. Use of a concentration of 3.0 mg/l of the gamma isomer in rivers of the Belgian Congo (1476) to control blackfly larvae resulted in no significant change in the behavior or composition of river plankton. There is a report, however, that 5 mg/l inhibited growth of plankton presumably in sea water (2423), which plankton serve as fish food for oysters and clams. White shrimp, two inches long, suffered 100 percent mortality within 24 hours to a concentration of 25 µg/l. In laboratory tests it was demonstrated (1476) that concentrations could be raised to 30 mg/l before a marked affect on plankton occurred. Ciliates and flagellates showed the most resistance, becoming sluggish at concentrations of 40 mg/l. Tests of various isomers of benzene hexachloride against algae at concentrations of 2.0 mg/l for 21 days of incubation at 22°C showed that the alpha, beta, and gamma isomers did not inhibit any of the test algae, the delta isomer inhibited 17 percent, and the technical gamma isomer 33 percent (2041).

BHC

(see Benzene Hexachloride)

BSM-11

BUFFEN 30

BUTROL

These three compounds are grouped because they come together alphabetically and because the only water quality information found on them was published by Horn and Balch in the same paper (2986). These compounds are used in slime control in pulp and paper mills. The many variables, reported in the paper by the investigators, are not included here. In summary, the inhibiting concentration for each compound against the organism indicated was as follows:

	Inhibiting Concentration, mg/l		
Organism	*BSM-11*	*Buffen 30*	*Butrol*
Aerobacter aerogenes	25.0	6.0	20.0
Bacillus mycoides	3.0	0.8	2.0
Aspergillis niger	25.0	20.0	45.0
Penicillium expansum	25.0	30.0	30.0
Notropis atherinoides (Emerald shiners)	0.8(MLD)	0.02(MLD)	0.5(MLD)

CALCIUM ARSENATE

See Chapter VI.

CAPTAN $C_9H_8Cl_3NO_2S$

Captan is an approved name for the fungicide N-trichloromethylmercapto-4-cyclo-hexene-1, 2-dicarboximide (N-trichloromethylithiotetrahydrophthalimide). It is the active ingredient in the proprietary product Captan 50-W and was formerly known as SR-406, Vancide 89, and Orthocide. Captan is an insoluble crystal product that is partially soluble in some organic solvents. Captan 50-W is a wettable powder comprising 50 percent captan, and is used as a suspension for spraying or dipping (364, 2091).

Captan is described as practically non-toxic (3067), having an actute oral LD_{50} to laboratory rats of 15,000 mg/kg of body weight (3009) and 9,000 mg/kg (3005). The acute oral LD_{50} for rabbits is reported as 3,160 mg/kg. Rats fed 1,000 mg/kg in the diet for one year showed no differences from controls. Captan is readily hydrolyzed, the effective residual life being two weeks (3005).

In experiments wtih rainbow trout in lake water at 10°-13°C, a 50-percent kill occurred among the larger fish in 72 hours at 0.32 mg Captan 50-W per liter. The smaller fish suffered a 61-percent kill in 9 hours at 0.56 mg/l, and there were no deaths in the 0.32 mg/l concentration in 72 hours. Neither size was affected by 0.18 mg/l in 72 hours (2091).

CD - 68

(see Chlordane)

CHLORDANE $C_{10}H_6Cl_8$

1. General. Chlordane or chlordan is a common name for an insecticide containing 60-75 percent of octochlorohexahydromethanoindene. It is an amber-colored viscous liquid that is insoluble in water, but miscible with aliphatic and aromatic hydrocarbon solvents. It is also marketed under the trade names of Velsicol 1068, CD-68, Toxichlor, and Octa-Klor (364).

Technical grade chlordane is a mixture of toxic compounds that have not been separated in manufacture. The material is available as a wettable powder, an emulsifiable concentrate, a dust, or a solution. High chronic toxicity prevents its use on edible crops. Customary applicable application rates are below one lb/acre (3005).

2. Cross References. Chlorinated Hydrocarbons—General, Heptachlor.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The estimated fatal dose of chlordane for a 70 kg man is estimated as varying from 6 to 60 grams (2995). However a 100 mg/kg dose has been reported as fatal (364).

b. Irrigation Waters. Like BHC and other chlorinated hydrocarbons, chlordane can accumulate in the soil and conceivably be contained in return water from the drainage of treated areas. Chlordane is stable in the soil (677) and it is fungicidal.

c. Stock and Wildlife Watering.—The acute oral LD_{50} of chlordane to rats has been reported as 335-590 mg/kg of body weight (2999, 3002, 3003). Although daily doses of 50 mg/kg for 15 days resulted in toxic symptoms and death (1485), the chronic oral toxicity has been reported as 0.125 mg/kg fed on a daily basis for two years (3003). One sheep fed 1000 mg/kg died in 48 hours. A concentration of 250 mg/kg in the diet for 12 weeks produced toxic effects in rats. Concentrations of 660 mg/kg killed dogs in four weeks. Cattle studies indicated 91 mg/kg to be toxic, 129 mg/kg fatal (3070). Chlordane causes poorer reproduction among birds (3069). Ducks and waterfowl had suffered significant losses from single sprayings of chlordane at concentrations of one pound/acre (3006). A concentration of 25 mg/kg in the diet will cause a 40 percent or more decrease in the reproduction of quail (2996). The amount of chlordane that will produce a 50 percent mortality in bobwhite quail and ringneck pheasant considering all stages of the life cycle are 250 and 500 mg/kg of body weight respectively (2994, 2996).

d. Fish and Other Aquatic Life. Chlordane dust was toxic to fingerlings of bass and bluegills at concentrations of 0.2 mg/l (681). The minimum disabling doses against trout larvae were reported to vary from 5.0 mg/l in an emulsion to 30 mg/l in acetone solution (755). Chlordane at 0.125 mg/l killed 100 percent and 0.05 mg/l killed 50 percent of test goldfish in four days. Corresponding figures for DDT in these tests were 0.25 and 0.125 mg/l respectively (3005). The approximate toxicity threshold of bluegills and trout to chlordane has been reported as 0.03 mg/l for both fishes (3007, 3048).

Tests made on 32 species of fish in ponds gave complete mortality of black bass, adults and fingerlings, bluegill adults but not fingerlings, and of bream at concentrations of 0.2 mg/l (3005). Chlordane at 0.2 mg/l killed all bluegill and bass fingerlings in aquarium tests. In earthen ponds, 0.2 mg/l killed bass fingerlings (681). According to Surber, Stock reported that 0.041 mg/l killed 33 percent of 4-7 inch trout within 48 hours, and 0.025 mg/l killed 20 percent within 48 hours (3049). The toxicity threshold for bluegill sunfish 1.7 inches long with pure chlordane was found to be about 0.03 mg/l (3007).

In Alaskan streams chlordane emulsions proved more toxic than DDT to trout in 15-minute applications. As a suspension chlordane was not toxic at any level tested below 15 mg/l and in a few oil concentrations up to 6 mg/l no damage was observed. Chlordane appears to be damaging to some fish at one lb/acre (3005). Application at one lb/acre (10% in fuel oil) to outdoor ponds killed 87 percent of bluegill sunfish (3058). Most of the bluegills as well as other species survived an application of 0.5 lb/acre. Tarzwell reports that the fish population of ponds will be significantly reduced at a routine dosage of 0.1 lb/acre. Chlordane apparently has no significant effect on fish at a dosage of 0.025 lb/acre (3005). Most fish survived aerial applications of 0.5 lb chlordane/acre but succumbed at 1.0 lb/acre (679).

More recent studies are summarized in the following tabulation:

Concentration Chlordane mg/l	*Temperature °C*	*Type of Water*	*Type of Fish*	*Results*	*Reference*
0.022	25	(1)	Bluegills	96-hour TL_m	3000
0.052	25	(1)	Fatheads	96-hour TL_m	3000
0.069	25	(2)	Fatheads	96-hour TL_m	3000
0.082	25	(1)	Goldfish	96-hour TL_m	3000
0.1	13	(3)	Bluegills	no effect in 24 hours	2976
0.19	25	(1)	Guppies	96-hour TL_m	3000
0.5	25	(4)	Catfish	96-hour TL_m	2981
1.0	13	(3)	Trout	Death in 3 hours	2976
1.0	13	(3)	Bluegills	Death in 5 hours	2976
1.0	13	(3)	Sea lamprey	Death in 14 hours	2976
5.0	13	(3)	Trout	Death in 1 hour	2976
5.0	13	(3)	Bluegills	Death in 2 hours	2976

(1) Soft water, pH 7.4, DO 8.0 mg/l, alkalinity 18 mg/l, hardness 20 mg/l.
(2) Hard water, pH 8.2, DO 8.0 mg/l, alkalinity 360 mg/l, hardness 400 mg/l.
(3) Lake Huron water, pH 7.5-8.2, DO 8.6-13.7 mg/l, free CO_2 5.0-9.0 mg/l, only one or two test animals exposed for 24-hour period, technical grade chlordane.
(4) University of Oklahoma tap water, 4-gallon aquaria, 10 fingerlings per aquarium. 100 or 200 fish per experiment.

For another summary of the effects of chlordane on fish, see Fielding and Baldwin (3059). Silvey (2531) gives the concentration of chlordane necessary to kill 50 percent *Chironomus* larvae in eight hours as 0.015 mg/l.

CHLORINATED BENZENES

1. General. Chlorinated benzenes are used for the control of weeds in lakes and ditches. They are characterized by high specific gravities and settle to the bottom in quiet water unless emulsified. Benoclor, a trade product con-

sisting of a mixture of the non-emulsifiable forms of mono-, di-, and trichlorobenzene, is used in high-pressure subsurface sprays to control aquatic vegetation (897). Metadichlorobenzene is a colorless liquid, insoluble in water, and seldom used commercially. Orthodichlorobenzene, also a liquid and insoluble in water, is used as a solvent for waxes, for preserving plants, and for destroying insects such as termites. Paradichlorobenzene, a white crystalline solid with a characteristic odor, is used largely for killing moths and other insects. It is soluble in water to the extent of 79 mg/l at 25°C (364).

2. Cross References. Chlorinated Hydrocarbons—General, Aromatic Solvents.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. According to Rohlich and Sarles (759), the halogenation of an aromatic series markedly influences tastes and often a change from sweet to bitter is effected. Goudey (897) indicates that Benoclor has a pungent odor but no physiological effects on humans at odoriferous concentrations.

b. Irrigation Waters. The U. S. Bureau of Reclamation (890) reports that more than 500 mg/l of Benoclor in water is required to injure crops.

c. Stock and Wildlife Watering. Livestock were not harmed when they drank water treated with Benoclor. Mild symptoms of poisoning of sheep and cattle occurred at a concentration in excess of 2700 mg/l while a level of about 17,000 mg/l was considered to be fatal. Ordinarily these animals do not drink contaminated water because of the objectionable taste (890, 3009).

d. Fish and Other Aquatic Life. Paradichlorobenzene was reported to be lethal at 50 mg/l to fish (313, 431). Springer notes that the chlorinated benzenes are poisonous to fish and fish-food organisms in the concentrations necessary to kill submerged plants (150-600 mg/l), and their toxicities are of long duration (3009). Orthodichlorobenzene has properties similar to trichlorobenzene but is reported to be less dangerous to fish since it diffuses less readily. A concentration of 3.2 mg/l killed 40-60 percent of fish in aquaria studies (3009). Trichlorobenzene gives fish a very disagreeable taste (3068).

No mortalities occurred in coho salmon for 24 hours following 15- and 30-minute exposures to a mixture of trichlorobenzene (50 mg/l) with five percent Colloidal X-77 emulsifier. After a 30-minute exposure to 100 mg/l, 20 percent of the salmon died within 12 hours. However a 15-minute exposure to 100 mg/l caused no death in 24 hours (2988).

Three chlorinated benzenes differentiated only by number (Nos. 1, 2, and 3) were tested in the laboratory at 2 mg/l for 21 days at 22°C against various algae. Inhibition of growth of 50 to 100 percent of the cultures was observed (2041). Trichlorobenzene at a concentration of 5 mg/l reportedly killed both fish and weeds (3068).

CHLORINATED CAMPHENE

(see Toxaphene)

CHLORINATED HYDROCARBONS—GENERAL

(see also DDT, Dieldrin, Chlordane, Toxaphene, Aldrin, Heptachlor, Benzene Hexachloride, Chlorinated Benzenes)

The chlorinated hydrocarbons include a large number of chemicals with high insecticidal activities. They are especially resistant to degradation to non-toxic end products, and many persist for months or years following application (2528). Chisholm and Koblitsky (3071) applied aldrin, heptachlor, and toxaphene to soil plots. The following day, about one-half of the amounts applied to the soil could be found. After 42 days, no heptachlor could be found and after 84 days only eight percent of the aldrin and 4 percent of the toxaphene could be found. Although most chlorinated hydrocarbons are sparingly soluble in water, their solubility is still greater than the 96-hour TL_m values (3015).

The toxicity of a mixture of DDT and BHC to goldfish has been studied. The dust mixture contained 5 percent DDT, 3 percent gamma BHC, 5 percent other BHC isomers, 40 percent sulfur, and 47 percent inert compounds. The 10-day median tolerance limit was about 3.0 mg/l as dust, or 0.15 mg/l DDT and 0.24 mg/l BHC. A dust suspension caused fish to turn over within 24 hours (1449).

Shell Oil Company has investigated the feasibility of deep sea disposal of mixed chlorinated hydrocarbon wastes. The effects of wastes on organisms indigenous to the disposal area were studied. Those organisms that came into immediate contact with the waste were killed or seriously impaired, but within 3-8 hours the area had again assumed natural features. For further information, which includes specific effects, diffusion studies, etc., the reader is referred to the original paper (3072). Hexachloran is a Russian insecticide that is reported to be used in reservoirs. It can be detected by smell in cold water at 0.04 mg/l, and in warm water (60°C) at 0.01 mg/l. It does not affect BOD at concentrations up to 2.5 mg/l, nor the dissolved oxygen up to 50 mg/l, nor nitrification up to 12.5 mg/l. The maximum permissible concentration in reservoir waters should be limited to 0.01 mg/l, the concentration at which it can be detected by smell (3257).

CHLORO-IPC

(see IPC)

CHLORTETRACYCLINE

(see Antibiotics)

CHLORTHION

1. General. Chlorthion, also known as chlorothion, is a trade name for 0, 0-dimethyl 0 (3-chloro-4-nitrophenyl) phosphorothioate, a phosphoric acid ester related to parathion and containing chlorine (364). Chlorthion is a yellow oil miscible with benzene, alcohol, and ether, and is insoluble in water. It is used especially for combating boll weevils.

2. Cross References. Parathion, Organic Phosphates.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The estimated fatal dose for a 70 kg man is 60 grams (2995).

b. Stock and Wildlife Watering. The acute oral LD_{50} to rats has been reported at 550 to 1510 mg/kg of body weight (2997, 2999, 3002, 3073, 3074). Against

cattle 50 mg/kg has been reported as non-toxic, and against calves 25 mg/kg is non-toxic and 50 mg/kg lethal (3075). The amount of Chlorthion that will produce at least 50 percent mortality in bobwhite quail considering all stages of its life cycle is 700 mg/kg of body weight (2994, 2996). Fed to dogs, 15 mg/kg in the diet produced a questionable red-cell inhibition, 2 mg/kg had no effect (3076). Although no mortality occurred in rats with daily dosages of 50 mg/kg of body weight, mortality was 40 percent at 100 mg/kg (3005).

c. Fish and Other Aquatic Life. Chlorthion at 5 mg/l caused injury to both guppies and goldfish in two hours and resulted in death in 18 hours (3073). Levels of Chlorthion of 5.0 mg/l caused abnormal behavior in 2 hours and death in 18 hours to guppies and goldfish in tests conducted at 19-22°C (3073). The 96-hour TL_m value of Chlorthion to fathead minnows at 25°C in both hard and soft water was 3.2 mg/l of active ingredient (3000).

Laboratory tests at rates of 0.0125-0.25 mg/l killed 100 percent of *Psorophora* larvae when exposed 24 hours. In the field, applications up to 0.074 mg/l failed to give satisfactory control. Applied in a granular formulation to test plots as a pre-flood application, applications as high as 0.5 lb/acre failed to give satisfactory control. The same formulation at 0.2 lb/acre as a post-flood treatment, however, gave 100 percent control (3082).

CINERINS

(see Pyrethrum)

CMU $C_9H_{11}ClN_2O$

1. General. CMU 3-(p-chlorophenyl)-1,1-dimethylurea, is also known by the tradenames Karmex and Monuron. It is stable toward oxygen and moisture under ordinary conditions at neutral pH, very slightly soluble in water and in number 3 diesel oil, moderately soluble in methanol, ethanol, and acetone, but practically insoluble in hydrocarbon solvents (364).

CMU is a soil sterilent when used at 10 to 80 lbs/acre, and a pre-emergence herbicide in crops when used at 1 to 3 lbs/acre (3005).

2. Cross References. 2,4-D, Diuron.

3. Effects on Beneficial Uses.

a. Domestic Water Supplies. Chancellor (2861) states that CMU at concentrations up to 180 mg/l is non-poisonous to man. Another report cites the lethal dose of CMU for the average 90 kg man at 326 grams (1723). CMU is reported to be one of the best herbicides to use in water supply reservoir areas (1723). It is 5 times less toxic to humans than 2, 4-D (3083).

b. Irrigation Waters. CMU at concentrations of 7.4 and 18.7 mg/l in irrigation water killed tomatoes after 10 days and injured cotton plants after 41 days (3084).

c. Stock and Wildlife watering. The acute oral LD_{50} of CMU for rats is 3,500 mg/kg of body weight (3005, 3009). Chancellor (2861) states that CMU at concentrations up to 180 mg/l is non-poisonous to livestock. Rudd and Genelly (3005) state that apparently CMU will kill water weeds at concentrations safe for aquatic life; however, there is no predictable hazard to terrestrial animals from other use.

d. Fish and Other Aquatic Life. The initial toxicity of CMU to small golden shiners in aquaria occurred at 20 mg/l (3009). Two goldfish tolerated 14.9 mg/l of CMU for 35 days, but both fish died within a week after the concentration was increased to 59.5 mg/l (3005). The tolerance limit of 3-month-old rainbow trout is 100 mg/l over a 48-hour period (2861).

Preliminary studies with the bluegill indicate that this fish has a tolerance for CMU similar to that of coho salman. Treatment of two ponds with up to 10 mg/l caused no mortality of frogs, tadpoles, or fishes. Another report indicates that several warm water fish species can withstand 40 mg/l for 10 days. Bond notes that aeration partially detoxifies solutions of Monuron, and that Lawrence cited a safe concentration for CMU of less than 1.2 mg/l. Applications of 25-100 lbs/acre to vegetated ponds did not cause excessive mortality of largemouth bass, bluegills, and brown bullheads, but bluegills did fail to spawn. However, these effects are thought to have been due to low oxygen content resulting from plant decomposition (3009). Later work that reports various variables used in the testing procedures is presented in the following tabulation:

Concentration of CMU mg/l	Temperature °C	Type of Water	Type of Fish	Results	Reference
40	23–24	(5)	Bluegills Green sunfish Crappie Bluntnose minnow	No effects in 23 days	2211
40.1	21	(4)	Shiners	96-hour TL_m	3011
41.5	21	(3)	Red-sided shiners	96-hour TL_m	3011
63.1	20	(2)	Fingerling catfish	96-hour LD_{50}	2981
110	20	(1)	Coho salmon	48-hour TL_m	2988
230	20	(1)	Largemouth bass	10% dead in 48 hours	2988

(1) Aerated, pH 7.4-7.7, methyl orange alkalinity 41-71 mg/l, 15 liter aquaria, 10 fingerlings per aquarium.
(2) Tap water University of Oklahoma, 4-gallon aquaria, 10 fish per aquarium 100 or 200 fish per experiment.
(3) Soft water, pH 7.6, DO 9.0 mg/l, alkalinity 36 mg/l, hardness 18 mg/l, 10 liters solution, 5 fingerlings per jar.
(4) Hard water, pH 8.2, DO 9.0 mg/l, alkalinity 116 mg/l, hardness 156 mg/l, 10 liters solution, 5 fingerlings (6-7 cm) per jar.
(5) Aerated 10 to 20 liter aquaria.

No mortality to fish-food organisms (amphipods and isopods) was noted at concentrations of CMU up to 100 mg/l (3005). CMU at a few mg/l is a good general algicide (3050). A concentration of 2 mg/l of CMU prevented the growth of all blue-green algae and diatoms and of 65 percent of the green algae species tested. At this concentration, CMU is not toxic to fish (2041, 3083). Another article notes that concentrations of CMU of 0.5 to 1.0 mg/l prevented algal growth in aquaria (2211).

COPPER ACETOARSENITE

(see Paris Green)

COPPER SULFATE

(see Chapter VI)

CO-RAL $C_{12}H_{13}ClPSO_5$

Co-Ral is a trade name for O, O-diethyl 0-(3-cloro-4-methyl-coumarinyl-7) phosphorothioate (3085). Also known as Muscatox, Asuntol, and Bayer 21/199, its solubility in water is less than 18 mg/l, but it is soluble in aromatic solvents. The acute oral LD_{50} to rats is 56-230 mg/kg of body weight depending on the method of application (2997). The amount of Co-Ral that will produce at least 50 percent mortality in bobwhite quail con-

sidering all stages of the life cycle is 400 mg/kg (2994, 2996).

The 96-hour TL_m values of technical grade Co-Ral, 100 percent active in acetone, to fathead minnows in both hard (400 mg/l) and soft (20 mg/l) waters at 25°C was greater than 18 mg/l. In contrast, the 96-hour TL_m value for bluegills in soft water was reported as 0.18 mg/l (3000).

CUBE ROOT

(see Rotenone)

2,4-D $C_8H_6Cl_2O_3$

1. General. 2,4-D (dichlorophenoxyacetic acid) is the best known of present herbicides. Actually, it is a plant hormone that stimulates growth, often to the extent of causing a plant to destroy itself. There are many products of 2,4-D available commercially. Various salts, amines, and esters of 2,4-D are formulated. Usually the acid equivalent is given on the label to standardize comparisons (3005). The amine salts are soluble in water and the sodium salt is soluble to the extent of 3.5 percent (364). 2,4-D is also used in combination with TCA (trichloroacetic acid). Dichlorophenol and ortho-monochlorophenol sometimes occur as impurities in the manufacture of 2,4-D (901).

2. Cross References. TCA, 2,4,5-T, MCPA, Silvex, Chapter VI—Phenols.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Traces of weed-killing chemicals may reach domestic water supplies as a result of eradication work along streams or railroad embankments, or from aerial spraying of fields. A most serious case of pollution of domestic water occurred, however, through an industrial waste reaching an underground stream in the so-called Montebello incident (894, 895, 896, 897). A chemical corporation in Alhambra, California, was manufacturing 2,4-D weed killer from 2,4-dichlorophenol, monochloroacetic acid, caustic soda, hydrochloric acid, diethanolamine, and acetone. It appears that a batch of the raw materials did not react properly and the chemicals were dumped into the sewer, from which they passed through the Tri-Cities sewage treatment plant (activated sludge), and were discharged to the San Gabriel River. They traveled above ground some three to five miles then seeped into the underground stream from which Montebello drew its water supply. There was relatively little combined 2,4-D in the wastes and hence no damage to plants that were watered, despite rumors to the contrary; but the unreacted dichlorophenol caused tastes and odors.

It was estimated that the original chemicals were diluted 1:10,000,000 and still produced unpleasant tastes. The taste threshold for 2,4-D is 0.01 mg/l, whereas the same concentration of dichlorophenol causes noticeable tastes (897). The period of contamination lasted 4-5 years (3086). It is reported that 2,4-D waste waters containing dichlorophenol are considerably more difficult to treat than phenolic wastes (3087).

According to Chancellor (2861), 2,4-D is non-poisonous but may give water an unpleasant taste. The lethal dose for an average 90 kg man is 62 grams (1723).

b. Irrigation Waters. Tomato plants irrigated for 22 to 44 days with tap water containing up to approximately 20 mg/l of 2,4-D were, in most instances, more vigorous than the control which, in each case, received the same amount of untreated tap water. Cotton plants irrigated for 24 days with water containing concentrations up to 11 mg/l of 2,4-D were more vigorous than the untreated controls. Such stimulation is not surprising considering the fact that 2,4-D is a plant hormone.

Flame Tokay and Concord Grape plants irrigated with tap water containing up to approximately 11 mg/l of 2, 4-D showed variable leaf abnormalities at all concentrations, probably from vaporization of 2,4-D from water containing the isopropyl ester. The check also was affected, indicating the effects noted were not due to 2, 4-D entering the roots from irrigation water. The sodium salt of 2,4-D in the concentration 16.7 mg/l did not injure tomatoes but caused some injury to cotton after 41 irrigation days (3084).

A combination of industrial wastes discharged to a lagoon in Colorado generated 2,4-D. It was first noticed in 1951 when crop damage resulted from use of well water for irrigation, and some 60 square miles of ground water were said to be contaminated (3086, 3088).

Concentrations of 2,4-D of 100 mg/l in sterile solution inhibited callus and root formation of snapbeans (3089). Muzik et al. (3090) failed to observe movement of sodium 2,4-D applied to calcareous soil with high organic content beyond one inch. Great care should be taken to prevent treated water reaching cultivated land (2861).

c. Stock and Wildlife Watering. The acute oral toxicity of 2,4-D to laboratory rats is 500 mg/kg of body weight (3009). Another report cites the acute oral LD_{50} of 2,4-D and 2, 4, 5-T and their derivatives as ranging from 300 to 1000 mg/kg for rats, mice, guinea pigs, and rabbits (3091). Rudd and Genelly (3005) note the more specific LD_{50} values of 375 mg/kg for mice, 666 mg/kg for rats, 800 mg/kg for rabbits, and 100 mg/kg for dogs. An oral dose of 380 mg/kg is lethal to chicks. Chronic studies showed that 20 mg/kg of food fed daily to dogs was toxic. On a weight basis, the toxicity to cattle seems to be similar to laboratory animals. The hazard to livestock and wildlife appears negligible (3091).

d. Fish and Aquatic Life. Harrison and Rees (901) found that the safe concentration to minnows was 1500 mg/l and for sunfish and catfish 500 mg/l. Some mortality of bream and bass occurs at 100 mg/l and of carp at 65 mg/l (3005). Marini (1726) reports that a concentration of the sodium salt of 2,4-D of 260 mg/l was not toxic to carp. Surber (898) described an incident at Leetown, W. Va., where water from a pond treated with 2, 4-D seeped into an adjacent pond and made the fish therein unpalatable. The chemical imparts a disagreeable phenolic flavor to fish flesh making it unpleasant to eat (898, 2861).

The 24-hour TL_m value to bluegills and largemouth bass was 350 mg/l (3092). A mixture of neutral aromatic oils (57 percent), 2,4-D (12.5 percent), emulsifiers (8 percent), and water (to 100 percent) was toxic to three-month-old rainbow trout at a concentration of 3.0 mg/l over a 24-hour period, and at 2.2 mg/l over a 48-hour period (2861).

In laboratory tests, the lowest concentration of 2,4-D to cause mortality of fish was 100 mg/l, and von Bandt notes a threshold value of toxicity to perch and bleak of 75 mg/l (2977). However, certain esters and amines of 2,4-D have been found to be more toxic and, particularly in still, shallow water, may harm fish at dosages used for weed control. Fingerling bluegills suffered losses of up to 40 and 100 percent from concentrations of 1 and 5 mg/l, respectively, of the butyl ester. The isopropyl ester was somewhat less toxic but caused complete mortality of bluegills at 10 mg/l, as did the alkanolamine at 40 mg/l. A few fish also died during a 4-day exposure to 4 mg/l of the latter material. The sodium salt was not observed to kill small rainbow trout below a concentration of 112 mg/l (3009).

The Fish and Wildlife Service (2976) tested a large number of phenoxyacetic acids and related compounds in rough screening studies in Lake Huron water at 12°C. Trout and bluegill were killed but sea lamprey were unaffected by 2,4-dichlorophenoxyacetic acid, butyl ester during a 24-hour exposure to five mg/l.

Fish-food organisms vary in sensitivity to the derivatives of 2,4-D. Tests with the isopropyl ester showed that losses of over 25 percent were sustained by crustaceans at 0.1-0.4 mg/l, insects at 0.4-2.0 mg/l, and snails at 2.4-3.3 mg/l. These animals were more resistant to poisoning by the mixed propylene glycol and butyl esters of 2,4-D, and certain species of insects and snails were not killed at 6.6 mg/l (3009).

Worth and McCabe (434) report that 2 mg/l of 2,4-D inhibited the growth of aerobic bacteria but not facultative or anaerobic bacteria. Organisms that require free oxygen for respiration appear to be smothered by 2,4-D. The synergistic and antagonistic effects of various substances dissolved in water are not yet understood well enough to permit the establishment of safe procedures for all kinds of waters and weeds. The concentration of a chemical that will kill weeds in one lake without harming fish life may be very toxic to fish in another lake, according to Brown (589).

The use of 2,4-D has proved to be especially successful in the destruction of water hyacinth and other emergent water weeds. Concentrations of 2,4-D of 1000 mg/l have been sprayed on exposed leaves with successful killing, and hence the concentrations in the adjacent water will be very much less than 1000 mg/l. Application of 8 lb/acre (free-acid equivalent) to weed infested areas of the Mississippi Delta destroyed water hyacinths. Stronger growths of alligator weed were only slightly affected, but weaker growths were controlled by two applications (3093). Application of 1 ounce of Dicotex (2,4-D) per 100 square feet will kill submerged plants such as *Hydrilla* and *Naias* and rooted plants such as *Vallisneria* in about 20 days but will not harm fish (3096). A 6 mg/l concentration of 2,4-D controlled heavy growth of watermilfoil (3097). Application of 4-6 lbs/acre in an oil-water emulsion spray has been effective against cattails (2880).

DALAPON $C_3H_3Cl_2NaO_2$

Dalapon, also known as Dowpon, is a herbicidal chemical containing 2,2-dichloropropionic acid. It is crystalline and has a characteristic salty taste (364). Control of cattails and a variety of grasses is possible with dalapon. It is applied in spray to noncrop lands at 20 to 40 lbs/acre (2861). It is also used in combination with amitrol (3010), and is less hazardous than 2,4-D to sensitive crops when sprays drift (2880).

The acute oral toxicity of dalapon to laboratory rats is 6590-8120 mg/kg of body weight (3009). Rats receiving 1.08 percent dalapon in the diet showed slight histopathological changes and some changes in organ weights, but there were no effects at lower concentrations. Cattle given 1 g/kg daily for 10 days showed no serious irreversible effects. Feeding of dalapon to cows at 300 mg/kg of dry feed intake had no detrimental effects to milk production, butterfat percentage, body weight, feed intake, or animal behavior (3098).

The Lake Emerald shiner was not adversely affected when kept for 3 days in water containing 3,000 mg/l. However, all fish died when the concentration was increased to 5,000 mg/l (3005). Tests with Dowpon on red-sided shiners in both hard and soft aerated waters demonstrated very little difference in toxicity, the 96-hour TL_m values at 21°C for the two waters being 390 and 395 mg/l respectively (3011).

Dowpon was tested against largemouth bass and coho salmon at 20°C in aerated solutions. The 48-hour TL_m values were greater than 1,000 mg/l for bass and 340 mg/l for coho salmon. In constant-flow experiments, however, no bass survived 48-hour exposure to 1000 mg/l. Survival in 250 mg/l was complete over a period of seven days. Lawrence reports 3000 mg/l as being safe for fish (2988).

DBD

(see Guthion)

DDD $C_{14}H_{10}Cl_4$

1. General. DDD (dichlorodiphenyldichloroethane) is the same as TDE (tetrachlorodiphenylethane), and is also known as Rhothane. It is insoluble in water, has low solubility in oils, and is freely soluble in most other organic solvents (2997). DDD has much the same general properties and is used similarly to DDT. Chemically, it has one less chlorine atom than DDT. Its insecticidal activity approximates that of DDT but its mammalian toxicity is only about one-fifth that of DDT. It is formulated as dusts, wettable powders, and emulsifiable concentrates that are applied at slightly higher rates than DDT (3005).

2. Cross References. DDT, Chlorinated Hydrocarbons—General.

3. Effects Upon Beneficial Uses.

a. Stock and Wildlife Watering. The acute oral LD_{50} to rats is 3400 mg/kg of body weight (364, 3002, 3003). Chronic oral toxicity to rats fed daily for 2 years was 5 mg/kg (3003). Chickens fed 0.1 percent (1000 mg/kg) DDD in mash showed some mortality within a month. Domestic animals may be treated with DDD with comparative safety although calves are particularly sensitive (3005).

Repeated treatment of Clear Lake, California, with DDD for the control of gnats may be the indirect cause of death of hundreds of grebes (3014). The grebes dis-

appeared in 1950 shortly after the lake was treated with 0.01-0.02 lb DDD/acre. Treatment was repeated in 1953, 1956, and 1957. In November 1957, hundreds of grebes died, poisoned by fish they ate. DDE in fish visceral fat assayed 40 to over 2000 mg/kg (3099, 3100).

b. Fish and Other Aquatic Life. All bluegill sunfish died in aquaria receiving 0.2 mg/l of pure DDD. The approximate toxicity threshold to fingerling bluegills of DDD is 0.03 mg/l (3007). Using fingerling channel catfish, Clemens and Sneed (2981) found that the 96-hour TL_m was less than 2.6 mg/l at 20°C. Rainbow trout were not killed by immersion for 7 days in water that had been left in contact with sludge to which 2 lbs/acre DDD had been applied. Gudgeon fed on dying larvae (from a dose of 1 lb DDD/acre) at a rate of 20 larvae per day for four days were not killed (74).

Lindquist et al. (1585) reported that a concentration of 0.014 mg/l of an emulsion of DDD controlled gnats in Clear Lake, California, with no harmful effects on fish and other aquatic organisms (but see 3a above). A concentration of 0.013 mg/l controlled *Chaoborus* larvae in 35 days and temporarily eliminated chironomid larvae without affecting fish. These larvae, however, make up 10-20 percent of the fish diet and consequently the long-term effect may be harmful. DDD at 2 lbs/acre was not toxic to tubificids nor to *Aelosoma* (74).

In the Clear Lake gnat control program, concentrations of 0.013 to 0.022 mg/l affected an appreciable fish loss among black crappie. There was drastic temporary reduction of some fish-food organisms but recovery was rapid and no permanent reduction occurred (3005). However, subsequent analyses showed that all fish, bird, and frog samples contained DDD, the amount found in all flesh samples exceeded the application rate on an mg/kg basis, and flesh samples of largemouth bass and Sacramento blackfish hatched 7-9 months after the last DDD application contained 22-25 mg/kg and 7-9 mg/kg of DDD respectively. All areas of the lake contained DDD-contaminated fish (3101).

Rudd and Genelly (3005) cite work reporting the TL_m of DDD to goldfish as being 1.0 mg/l, and the following work by Tarzwell based on lb/acre applications:

Application Rate in lbs/acre	*Form*	*Effect on Fish*
0.025	Spray	Insignificant loss
0.05	Spray	Some mortality
0.1	Dust	Insignificant loss
0.1	Spray	Destroyed all fish

DDT $C_{14}H_9Cl_5$

1. General. Dichlorodiphenyltrichloroethane, commonly known as DDT, is a white amorphous powder that is used as an insecticide. Practically insoluble in water, dilute acids, and alkalies, it is readily soluble in many organic solvents. It has a low volatility and is not normally decomposed by sunlight; hence, it has high residual powers. It is used in a 5-percent solution with kerosene and xylene as the solvent, as a 25-percent emulsion in several types of vehicles, or as a dust diluted with talc or other inert substances. It is also used in paints and wall washes. The diluent or vehicle is very important in determining the toxicity of DDT (919).

DDT has been used so widely that it is stored to some extent in the body of almost everyone in the U.S. (3001). Its safety has been proven by a large body of experimental work and by extensive experience and it is the best insecticide we have (3258). DDT has been recovered from five major river-water sources in concentrations of 5 to 20 μg/l (3004, 3102).

2. Cross References. Chlorinated Hydrocarbons—General, Benzene Hexachloride.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Men who have been exposed to DDT for as long as 6.5 years, and consequently absorbed an average of 200 times as much DDT as the general population does in its food, have evidenced no chronic poisoning (3103). The oral ingestion of 0.7 gm DDT will produce a sensation of burning or itching of the tongue, lips, and part of the face. At 1.0 gm, tremors and convulsions may commence. However, men have recovered from swallowing as much as 20 grams. DDT in the diet at a level of 35 mg/day in human volunteers for 18 months caused no toxic symptoms (3104). The estimated fatal dose for a 70 kg man is about 30 grams (677, 2995). Water that has been treated with technical grade DDT can be used for domestic supply if the concentration does not exceed 2 mg/l. If an organic solution is used, the concentration should not exceed 0.25 mg/l (678, 1131, 3055, 3105). However, DDT has been cited as a source of taste and odor in water (2058, 3106). The Soviet Union established maximum permissible concentrations (MPC) for polluants based upon a most sensitive parameter. For DDT, the parameter was odor and the MPC set was 0.2 mg/l (3107).

b. Irrigation Waters. In general, plants are not harmed by DDT applied to kill insects (1132). The breeding of anopheline mosquitoes in water used for irrigating rice fields was controlled by the addition of 1.0 mg/l of DDT as an emulsion, without damage to rice plants (1154). DDT has no effect on soil pH or bacterial content, and it is not toxic to soil fungi or nitrifying organisms (677, 678).

Crops grown in soils treated with DDT absorb the chemical into their tissues. Crops tested were carrots, potatoes, peas, cabbage, cucumbers, beans, radishes, beets, and rutabaga (3025).

DDT is very stable in soil, decomposing at a rate of about 5 percent per year (dependent upon soil type). The growth of squashes, cucumbers, tomatoes, strawberries, and some peas and beans may be retarded by DDT at levels of 25 lbs/acre (3006).

c. Stock and Wildlife Watering. The acute oral LD_{50} of DDT to rats has been reported as 250 mg/kg of body weight (2992, 3002, 3003) and against males and females as 113 and 118 mg/kg respectively (2999). Other papers cite values from as low as 150 mg/kg for mice to over 1300 mg/kg for chickens (1132, 2994, 2996, 3108). Drinking water containing 10 mg/l of DDT, 30 mg/l of xylene, and a triton emulsifier had no effect on 10 mice for 75 days. Another 10 mice received 50 mg/l of DDT, 150 mg/l of xylene, and triton emulsifier for 58 days without deleterious effect (1131). Nevertheless, DDT is stored in the body and daily ingestion of sub-lethal doses may be accumulative and fatal. When fed a dietary level of 5

mg/kg, rats developed a detectable amount of liver damage (677, 1132, 3109).

A considerable literature on the toxic effects of DDT applied on an areal basis (lbs/acre) exists. A dosage of 1 lb/acre to water 5.0 ft deep, equally distributed, gives a concentration of 0.0735 mg/l. Hence, an assumption of 1 lb/acre approximating 0.1 mg/l is sometimes made. It cannot be positively said, however, that effects to land animals are due only to the contamination of the animal's drinking water sources.

An areal application of DDT at 5 lbs/acre is required in order to produce direct mortality in mammals. Indirect effects, however, are noted at applications as low as 0.5 lb/acre. Such an application, for example, kills most crayfish and forces raccoons subsisting on them to change their diet (to acorns and mussels). Four annual aerial applications of 2 lbs/acre and one of 1.1 lbs/acre caused reductions of 28-44 percent in treetop bird species. Five lbs/acre caused a reduction of 80 percent. Although the daily consumption by adult birds of as little as 8.5 mg for two months may have no overt effect, it produces significant reductions in reproduction and in fertility of eggs and survival of young (3110). The effect of 5 lbs/acre on birds, however, is not gradual since this level reduced the Maryland yellow-throat by 63 percent in 24 hours and the prairie warbler by 93 percent in 48 hours (3111).

It has been reported that a concentration of DDT of 100 mg/kg in the diet of quail will cause a 40 percent or more decrease in reproduction (2996, 3044, 3045). The amount of DDT that will produce at least 50-percent mortality in bobwhite quail and ringneck pheasant considering all stages of the life cycle are 500 and 300 mg/kg of body weight respectively (2994, 2996).

Terrestrial reptiles are reported to suffer losses at 2-3 lbs/acre (3110). Levels of 0.5 lb/acre were observed to have no ill effects on mammals, birds, reptiles, and amphibians when applied as a mosquito larvacide (3112). Adult birds have been reported to eat insects poisoned by DDT without harm (3069). See Von Oettingen for a detailed discourse of the toxicity of DDT (3292).

d. Fish and Other Aquatic Life. Most aquatic organisms are more susceptible to DDT than are terrestrial forms (3006). It is reported that fresh water animals on the Island of Cyprus have been devastated by DDT spraying for mosquito eradication (3113). The effects of DDT are persistent and widespread when employed in flowing streams. As a control operation against *Simulium arcticum*, a single application of 0.09 mg/l for 16 minutes was effective for 40-98 miles downstream (2698). Cope (3114) reports finding DDT in fish 85 miles downstream from sprayed areas, as well as in all control fish, in fish from an untreated stream, from a hatchery, and from a fish market. In standing waters, its effects persist for months. Earthen ponds treated with 0.4 mg/l wettable powders remained toxic to bluegills for several months. Emulsifiable applications at 0.1 mg/l remained toxic to fingerlings for a month, longer at higher concentrations (681). However, it is to be noted that rainbow trout were not killed by immersion for 7 days in water that had been left in contact with sludge to which 2 lbs DDT per acre had been applied (74). The accumulation of DDT on plants or in benthal deposits downstream may continue to cause insect and fish mortality. One month after spraying, moss contained as much as 128 mg/l six miles downstream from the area sprayed (2647).

The toxicity of DDT to fishes has been subjected to considerable study. Among the variables that have been cited are the type of water course and bottom, depth, vegetation, silt, turbidity, hardness, temperature, dissolved oxygen, organic content, species and age of fish (1133, 3006, 3015, 3058, 3115), formulation (emulsions are most toxic, water wettable forms least toxic) (678, 918, 3048, 3110, 3116), size of molecule (for DDT analogs) (2744), and volume of water and shape of receptacle (3005, 3117).

The effect of differing DDT formulations on rainbow trout have been cited as (3005):

DDT in acetone solution—not lethal at 30 mg/l

DDT in fuel oil—not lethal at 20 mg/l

DDT in xylene—toxic at 5 mg/l

DDT in emulsion—toxic at 3 mg/l

DDT in kerosene—toxic at 0.3 mg/l

Owing to these many variables, it is not surprising to find that there is a variation in concentrations of DDT lethal to fish as reported in the literature. The effects of various concentrations of DDT on different fishes encountered in this survey have been tabulated in order of increasing dosage:

Concentration of DDT mg/l	Type of fish	Results	Reference
0.001 {alcohol suspension}	Common sucker	Killed	3118
0.001 {alcohol suspension}	Speckled trout	Killed	3118
0.005	Common sucker	MLC less than this level	3005
0.005 {alcohol suspension}	Speckled trout yearlings	Killed	3118
0.01 {alcohol suspension}	Creek chub	Killed	3118
0.01	Bluegill	Toxicity threshold	3007, 3048
0.01	Dace	All killed	3005
0.01	*Gambusia*	Median lethal dose	2744
0.01	Guppies	Toxicity threshold	3007
0.01	Bluegill fingerlings	Killed	681
0.01	Bass yearlings	Killed	681
0.01-1.0	Fishes	Killed	1144
0.025-0.04	Bass and shiners, fry	Killed	3058
0.0237-0.074	Rainbow trout	96-hour TL_m	2199
0.04	Bluegills	Killed	3007, 3058
0.047	Salmon, young	24-hour TL_m	3119
0.05	Bass fingerlings	Killed all	681
0.1 {alcohol solution}	All fishes	Killed	3118
0.1	Goldfish	Toxic limit	2962
0.1	Goldfish	Median lethal dose	2744
0.1	Bluegill adults	Killed	681
0.1 {ponds emulsion in fuel oil}	Goldfish	Killed	681
0.14 {powder powder powder}	Bluegills	Killed	3058
	Darters	Killed	3058
	Sculpins	Killed	3058
	Trout	Killed	3058, 3007
0.15	Bluegill fingerlings	Killed all	681
0.18	Bluegills and crappies	Killed	3058
0.2	Goldfish	Killed	681
0.4 {ponds powder}	Fatheads	Killed	681
0.5	Golden shiners	Die	3058
1.6 {ponds powder}	Goldfish	Killed	681

It will be noted that the concentrations range from 0.001+ mg/l, an alcohol suspension, to 1.6 mg/l, in a wettable powder formulation. With respect to suspended DDT, Rudd and Genelly (3005) conclude that at 0.1 mg/l all fish will die within 12 hours, at 0.01 mg/l only a few will survive, and at 0.005 mg/l many but not most fish will be killed.

Later work that reports a number of variables is also tabulated in an order of increasing concentration:

Concentration of DDT mg/l	Temperature °C	Type of Water	Type of Fish	Results	Reference
0.016	25	(4)	Bluegills	96-hour TL_m	3000
0.027	25	(4)	Goldfish	96-hour TL_m	3000
0.032	25	(4)	Fatheads	96-hour TL_m	3000
0.034	25	(3)	Fatheads	96-hour TL_m	3000
0.043	25	(4)	Guppies	96-hour TL_m	3000
0.06	--	(2)	Brook trout	Tolerance limit	3122
0.1	21	--	Guppies	Distress in 30 min., death in 24 hours	2992
0.12	10–16	--	Bluegills	Killed all	3123
0.12	10–16	--	Darters	Killed 22%	3123
0.388	13	(5)	Rainbow trout	96-hour TL_m	3124
1.0	21–26	(1)	Mummichog killifish	Toxic symptoms in 4-6 hours, death in 6-12 hours	3014
10.0 (powder)	--	(2)	Brook trout	Tolerance limit	3122
17.8 (powder)	17	(6)	Chinook salmon	Critical level	2091
50.0 (powder)	17	(6)	Rainbow trout	Critical level	2091

(1) Brackish water, acetone suspension, 6 liters solution, 5 fish per test.

(2) pH 7.8-8.2, alkalinity 188-200 mg/l, DDT in kerosene, fish 2.5-5 inches long.

(3) Hard water, pH 8.2, DO 8.0 mg/l, alkalinity 360 mg/l, hardness 400 mg/l.

(4) Soft water, pH 7.4, DO 8.0 mg/l, alkalinity 18 mg/l, hardness 20 mg/l.

(5) Total dissolved solids 113 mg/l, pH 7.1, DO 8 mg/l, total alkalinity and hardness 102.6 mg/l, 10 liters solution, 5 fingerlings per jar.

(6) Fresh water, aerated, pH 7.7-7.9, DO 6.2-7.4 mg/l, total alkalinity 75.0 mg/l, average size of fish: salmon 90 mm, trout 120 mm, 3 days exposure, 300 liter aquaria, 16 fish per aquaria, concentration given is for the commercial powder, 50 percent wettable. The critical level is defined as the lowest concentration in which obvious deleterious effects were noted.

Other forms of aquatic life are affected in varying degree by DDT. Tarzwell (918) states that crabs, crayfish, and amphipods are more sensitive than fishes for DDT. Aquatic insects and larvae are particularly susceptive to DDT; in fact, it is to control such larvae as those of the anopheline mosquito that DDT is often employed. Amphipods, sowbugs, and bait shrimp are highly susceptible, whereas red mites, snails, and mussels are highly resistant (3125). High sensitivities of many of these forms, however, can reflect upon the fish population since fish have been reported to have starved to death because of the killing of fish-food organisms (3005, 3126). Although DDT is toxic to fish and many fish-food organisms, it does not follow that fish which consume insects killed by DDT will be harmed. Ginsburg (1152) reports that gold fish were not poisoned when they were fed for four consecutive days with larvae of *A.egypti* which had been killed by 0.05 to 1.0 mg/l DDT. Each fish consumed about 100 larvae during the test. However, snakes and turtles have died from eating DDT-killed fish (3005). The toxicity of DDT to various water organisms other than fish has been reported as follows:

Concentration of DDT mg/l	Temperature °C.	Type of water	Type of animal	Results	Reference
0.001	--	--	*Culex* larvae	Median lethal dose	2744
0.001	--	--	*Daphnia*	64-hour tolerance limit	2699
0.0014	20	(1)	*Daphnia*	Immobilized in 50 hours	3255
0.001-0.0015	--	--	*A. egypti* larvae	Kills 70-100% in 48 hours	1131
0.001-0.1	--	(2)	*Daphnia*	Immobilized 16-32 hours	1150
0.01	11.5	(3)	Stonefly (c)	96-hour TL_m	2699
0.01	--	--	*Chironomus*	Infestation controlled	3127
0.012	27.5	--	Brine shrimp larvae	TL_m in 5 hours	3128
0.025	--	--	Oyster larvae	Did not grow well	2515
0.03	--	--	*A. quadrimaculatus* larvae	Kills all in 48 hours	1131
0.04	--	--	*Chironomus*	Kill 50% in 8 hours	2531
0.1 (for 30 min.)	--	--	*Simulium*	Kills	1148
0.1	11.5	(3)	Caddis fly (d)	96-hour TL_m	3055
0.1	11.5	(3)	Stonefly (b)	96-hour TL_m	2699
0.1	--	--	*Daphnia*	All killed (laboratory)	1144
0.1	--	--	Tadpoles	All killed (laboratory)	1144
0.142	27.5	--	Brine shrimp adults	TL_m in 5 hours	3128
0.18	11.5	(3)	Stonefly (a)	96-hour TL_m	2699
0.25	--	--	*Daphnia*	Some survived (field)	1144
0.25	--	--	Tadpoles	All killed (field)	1144
0.25-0.5	--	--	Crayfish	Died	3129, 1144
0.5	--	--	Snails	Unaffected	1144
1.0	--	--	Oyster larvae	Death in 4 days	2515
20	--	--	Blue crabs	Survived	3061

The aerial application of DDT has been studied quite intensively. Studies have varied from a single, local application, such as spraying of the Blue Nile to control a green chironomid (at 0.09 mg/l which resulted in a massive fish kill) (3130) to repeated extensive forest applications, such as to the Miramichi Water Shed to control spruce budworm (3005). Reports made from these studies generally cite dosages on an areal basis. Some of the effects of this type of spraying on various water animals has been tabulated as follows:

Application of DDT lbs/acre	*Type of Animal*	*Results*	*Reference*
0.01–1.0	Fish	Toxic	1136, 918, 1137
0.025–1.0	Fish	Harmless	1146, 920
0.1	Frogs	Repeated application. Killed	3005
0.1	Crayfish	Eliminated	3131
0.2	Snakes	Repeated applications. Killed	3005
0.25	Fiddler crabs	10-20% mortality	3125
0.3	Snakes	Killed	3005
0.5	Toothed herring	Killed in agitated waters	3129
0.5	Shiners	Killed in agitated waters	3129
0.5	Dace	Killed in agitated waters	3129
0.5	Salmon	Reduction of 80%	3132
0.5	Killifish	Light kills at lesser dosage	3125
0.8	Crabs	Nearly complete kill	3125
1.0	Frogs	Killed	3110
1.0	Snakes	Killed	3110
1.0	Mayfly	90% reduction	2647, 3133
1.0	Caddisfly	90% reduction	2647, 3133
1.0	Trichoptera	Destroyed for 16 months	2647
1.0	Megaloptera	Resistant	2647
1.0	Odonata	Resistant	2647
1.0	Frogs	Killed	1137
1.0	Tadpoles	Killed	1137
1.0	Snakes	Killed	1137
1.0	Fish	Weekly for 20 weeks—caused insignificant mortality	3046
1.0	Hemipterans	Killed	3005
1.0	Coleopterans	Killed	3005
1.0	Fish-food insects	99% killed	3005
1.0	Amphibians and reptiles	Usually not affected	3005
1.0 (suspension)	Surface insects	No appreciable effect	1147
1.0 (suspension)	Bottom insects (caddis-fly larvae, mayfly nymphs)	Many killed	1147
2.0	Tubificids, *Aelosoma*	Not killed	74
3.0	Fish	Totally destructive	3135
7.5	Aquatic organisms	Virtually wiped out	3005

For further details of aerial spraying of DDT, the reader is referred to Rudd and Genelly's summary (3005) as well as to the following references (918, 1135, 1147, 2534, 2694, 2695, 3134, 3136, 3137, 3138, 3139, 3140).

Although the presence of aquatic plants reduces the toxicity of DDT toward fish, the plants themselves are unaffected. The alga *Scenedesmus* has been found thriving in high concentrations of DDT (2744). A laboratory study for 21 days at 22°C showed a 2.0 mg/l concentration to have no effect on any of the tested algae (2041). Applied as a dust or as a solution in fuel oil at concentrations of 0.05 to 0.2 lbs/acre at weekly intervals, DDT had little effect upon the plankton (1151).

It appears that DDT dusts can be applied routinely at 0.1 lb/acre with little or no significant harm to wildlife. Routine treatments with fuel oil solutions at 0.1 lb/acre generally kill large numbers of fish after the 10th treatment. For adequate mosquito control, dusts at 0.1 lb/acre or oil solutions at 0.05 lb/acre are recommended (2611). Rudd and Genelly remark that 0.1 lb/acre as used in mosquito control and 1.0 lb/acre as used in forest insect control are relatively safe levels of application from the standpoint of fish (3005). Another report states that the safe concentration of a formulation of DDT used in budworm control is less than 0.05 mg/l (3141). Some fish and shellfish mortality can be expected after a single application of 0.2 lb DDT/acre to water surfaces (3006).

Oysters that had been kept for 5-8 days in an aquarium treated with DDT at the rate of 5 lbs/acre survived and their shell movements were not affected. Spraying the shells directly with a solution of DDT in kerosene at a rate of 3-5 lb/acre caused a reduction in the setting of spat (1153).

DEAD - X

(see Aromatic Solvents)

DEHYDROABIETYLAMINE ACETATE

(see Rosin Amine Compounds)

DELNAB

(see Organic Phosphates—General)

DELRAD

(see Rosin Amine Compounds)

DEMETON

(see Systox)

DERRIS ROOT

(see Rotenone)

DIAZINON $C_{12}H_{21}N_2O_3PS$

(see also Organic Phosphates—General)

Diazinon is a registered trade name of 0, 0-diethyl 0-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothioate. Diazinon is a liquid that has a faint ester-like odor. It is miscible with a number of hydrocarbon solvents. Diazinon apparently has very high insecticidal and acaricidal properties (364, 3005).

The estimated fatal dose for a 70 kg man of Diazinon is 25.0 grams (2995). Reports of toxicities to laboratory rats have varied considerably. These reports include an oral LD_{50} to rats of a 95 percent technical grade formulation as 100 to 150 mg/kg of body weight (3005), 125 mg/kg (3002), and 235 mg/kg (2992). Against male albino rats Diazinon has an LD_{50} of 435 mg/kg (3142), 408 and 293 mg/kg (3143), and against males and females of 108 and 76 mg/kg (2999). A 23-percent wettable powder had an LD_{50} of 264.5 mg/kg (in terms of active ingredient). No gross effects were observed in rats fed 100 to 1,000 mg/kg of the wettable powder in the diet for 4 weeks (3005). The reported LD_{50} to mice of technical grade Diazinon is 82 mg/kg in the diet. Levels of 6.5 mg/kg/day of active product fed to dogs inhibited cholinesterase activity, 9.3 mg/kg/day resulted in the usual toxicity signs associated with cholinesterase activity (3144). Concentrations of 0.75 mg/kg fed to dogs produced significant lowering of plasma cholinesterase, but there was no effect at 0.25 mg/kg concentration. Levels of 75.0 mg/kg in the diet produced significant depression of red-cell cholinesterase, but 0.75 ppm produced no effect (3076).

Radeleff et al. (3075) investigated the oral toxicity of Diazinon to livestock. With calves 10.0 mg/kg was lethal, 1.0 mg/kg was toxic, and 0.5 mg/kg non-toxic; with steers 25.0 mg/kg was toxic, and 10.0 mg/kg was non-toxic; and with sheep 30.0 mg/kg was toxic.

Adlung (1992) reports that at temperatures of 20-22°C, Diazinon at a concentration of 4 mg/l caused abnormal behavior in guppies in one hour, death in 24-48 hours. The estimated concentration required to immobilize *Daphnia magna* in 50 hours at 20°C is 4.3 μg/l (3255).

DICAPTHON

(see also Organic Phosphates—General)

Dicapthon is a trade name for 0, 0-dimethyl 0-(2-chloro-4-nitrophenyl) phosphorothioate (2998). The acute oral toxicity of Dicapthon to male rats has been reported as 460 mg/kg of body weight (3142), and against males and females of 400 and 330 mg/kg respectively (2999). The lethal concentration to bivalve larvae is cited as about 2.0 mg/l (2989).

DICHLONE

(see Dichloronaphthoquinone)

DICHLOROBENZENE

(see Chlorinated Benzenes)

DICHLORONAPHTHOQUINONE $C_{10}H_4Cl_2O_2$

1. General. Also known as Dichlone or Phygon, this is a yellow crystalline substance, soluble to 0.1 mg/l, soluble in xylene and *o*-dichlorobenzene and moderately soluble in other organic solvents. Its full name is 2,3-dichloro-1,4-naphthoquinone. It is used as a fungicide for seeds, vegetables, and textiles, the application for the former being about one-quarter ounce per 100 lbs of seed (364, 3005, 3067), and as an algacide for ponds and lakes.

2. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Phygon has a low order of toxicity to man but may cause skin irritation to some persons (2262).

b. Irrigation Waters. Phygon is reported to be noninjurious to crop plants (2262).

c. Stock and Wildlife Watering. The acute oral LD_{50} to rats has been noted as 1500 mg/kg of body weight (3005, 3009, 3067). The highest level fed to rats without producing gross effects was 1000 mg/kg of food. Growth was retarded over 1580 mg/kg in food and death occurred at 2500 mg/kg (3005).

d. Fish and Aquatic Life. The 96-hour TL_m values to rainbow trout fingerlings have been reported to range from 0.0237 to 0.074 mg/l (2199). Bond, et al. (2988) report no mortality to coho salmon at concentrations of 0.042 mg/l and less. They mention having a great deal of difficulty in working with Phygon XL because it lost toxicity rapidly. Short exposures had the following effects on coho salmon:

Concentration of Phygon XL mg/l	*Exposure Time*	*Results*
0.1	30 minutes	no mortalities
0.5	15 minutes	20% dead one hour after removal 60% dead 24 hours after removal
0.5	30 minutes	80% dead one hour after removal 100% dead three hours after removal

Studies of the toxicity of Phygon to fish have been summarized as follows:

Concentration of Phygon mg/l	Temperature °C	Type of Water	Type of Fish	Result	Reference
0.07	20	(4)	Largemouth bass	48-hour TL_m	2988
0.074	13	(3)	Rainbow trout	96-hour TL_m	3124
0.11	21	(1)	Red-sided shiners	96-hour TL_m	3011
0.11	21	(2)	Red-sided shiners	96-hour TL_m	3011
0.14	19	(5)	Catfish	96-hour TL_m	2981
0.15	--	(6)	Fatheads	96-hour TL_m	2987
0.42	20	(4)	Coho salmon	48-hour TL_m	2988

(1) Soft water, pH 7.2, DO 9 mg/l, alkalinity 36 mg/l, hardness 18 mg/l, 10 liters solution, 5 fish (6-7 cm) per jar.
(2) Hard water, pH 8.2, DO 9 mg/l, alkalinity 116 mg/l, hardness 156 mg/l, 10 liters solution, 5 fish (6-7 cm) per jar.
(3) Water had total dissolved solids content of 113 mg/l, pH 7.1, DO 8 mg/l, alkalinity and hardness 102.6 mg/l, 10 liters solution, 5 fingerlings per aquarium.
(4) Aerated 15 liter solutions, pH 7.4-7.7, methyl orange alkalinity 41-71 mg/l, 10 fingerlings per aquarium.
(5) University of Oklahoma tap water, 4-gallon aquaria, 10 fingerlings per aquarium, 100 or 200 fish per experiment.
(6) Soft water, pH 7.2, DO 8.0 mg/l, alkalinity 36 mg/l, hardness 38 mg/l, *Pimephales promelas*.

Concentrations of 0.3-0.055 mg/l had no visible effects on fish and zooplankton when applied to a lake for control of algae. Other tests showed 0.1-1.0 mg/l to be toxic to various species of fish (3009). A safe concentration range of 0.05 to 0.6 mg/l has been reported (2988), while another paper mentions that 0.1 mg/l had no injurious effect toward fish (1542).

Observed effects of field applications of Phygon XL to various ponds on fish, algae, and other aquatic plants have been described (3094). Snails, *Daphnia*, and rotifers seemed to be unaffected by concentrations up to 100 mg/l (3005).

Laboratory tests conducted for 21 days at 22°C with a concentration of 2.0 mg/l showed that 2,3 dichloronaphthoquinone inhibited the growth of 33 percent, and a related compound, beta naphthoquinoline inhibited 50 percent, of the algae tested (2041). Other studies indicated it was lethal to blue-green algae in concentrations as low as 0.002 mg/l. It is selectively toxic to the bloom-producing blue-green algae and nontoxic to most green algae and higher aquatic plants even at concentrations of 0.1 mg/l. In the dark it is ineffective, but on exposure to sunlight it is highly toxic (1542). A concentration of 0.5 mg/l controlled 28 percent of blue-green algae, and no diatoms or green algae. Even 16 mg/l had little or no effect on 50 percent of the green algae (3050).

DICHLOROPHENOXYACETIC ACID

(see 2,4-D)

DICHLOROPROPIONIC ACID

(see Dalapon)

DIELDRIN $C_{12}H_4OCl_6$

1. General. Dieldrin is the assigned common name for an insecticide containing not less than 85 percent of the endo-exo isomer of 1,2,3,4,10,10-hexachloro-6-7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-dimethanonaphthalene. Dieldrin is insoluble in water and moderately soluble in most organic solvents except aliphatic petroleum solvents and methanol. It is stable in the organic and inorganic alkalies and acids commonly used in agriculture. It is affected by strong mineral acids but is compatible with most agricultural chemicals, fertilizers, and economic poisons. It may be formulated as a wettable powder, xylene solutions, or as a dust (364). Dieldrin is similar to aldrin as described hereinbefore.

2. Cross References. Chlorinated Hydrocarbons, Endrin, Aldrin, DDT, Benzene Hexachloride.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The estimated fatal dose of dieldrin for a man of 70 kg has been estimated as 5 grams (2995). In describing several cases of dieldrin intoxication, it was concluded that contamination of water even with small quantities is very dangerous (3145).

b. Stock and Wildlife Watering. The acute oral LD_{50} value of dieldrin for rats has been reported as ranging from 37-87 mg/kg of body weight (3005), 81.3 mg/kg (3002), 87 mg/kg (2992, 3073), 60 mg/kg (3042, 3043), technical grade against males and females of 46 mg/kg (2999). No harm was demonstrated to laboratory rats after feeding on technical grade dieldrin at a level of 25 mg/kg of food for two years (3042, 3146). Rats may be maintained for more than a year without injury on water containing 0.2 mg/l of dieldrin which concentration kills fish in a few hours (3001). Differences in dieldrin toxicities are prescribed to the grade of dieldrin employed and to the nature of the solvents (3005). Ground tobacco stems impregnated with dieldrin and applied at a rate of 0.2 lbs of dieldrin per acre had no immediate detrimental effect on meadow mice or rice rats (3147). Dieldrin ground sprays at 2 lbs/acre, on the other hand, gave complete kills of meadow mice (3148).

The application of dieldrin at 3 lbs/acre caused most losses in wildlife the week following application. Ground squirrels, muskrats, and rabbits were virtually eliminated, short-tailed shrews, fox squirrels, woodchucks, and meadow mice sustained heavy losses, but white-footed mice appeared resistant (2402). Another report cites that aerial applications of 1.5 and 3 lbs of dieldrin in attaclay granules per acre caused no appreciable change in the field mouse population (3149).

Ducks, pheasants, and wading birds were killed when dieldrin was applied at 0.5 lb/acre (3150). Granular dieldrin applied to marshes at 0.2 lbs/acre killed some birds within 3 days (3152). Application of dieldrin at 0.3 pound per acre in granular form to tidal marshes resulted in no apparent injury to birds (3151). Ground tobacco stems impregnated with dieldrin and applied at a rate of 0.2 lb of dieldrin per acre had no observable effect upon adult birds (3147). However, the application of dieldrin at 3 lbs/acre virtually eliminated meadow larks, robins, brown thrashers, starlings, common grackles, and ringneck pheasants. Horned larks sustained heavy losses, although house sparrows and mourning doves appeared resistant (3154, 3155). Dieldrin applied at 4 lbs/acre killed or otherwise affected songbirds and mammals (3153). An application of 0.5 lb/acre killed large numbers of water birds and a smaller number of up-land game birds (3005). In feeding experiments, 10 mg/kg in the diet of breeding pheasants demonstrated no effects on the adult birds but did result in decreased hatchability of eggs and viability of chicks (2945). Inclusion of 1 mg/kg in diet of growing quail resulted in higher mortality rates. Young pheasants failed to survive on a diet containing dieldrin in concentration of 5 mg/kg (3044, 3045). No ill effects were noted when quail were fed winter diets containing one mg/kg (3045). The amount of reproduction decrease in quails caused by 1 mg/kg of dieldrin in the diet has been noted as 40 percent (2996). The amount of dieldrin that will produce at least 50 percent mortality in bobwhite quail and ringneck pheasant considering all stages of the life cycle in mg/kg respectively are 35 and 50 (2994, 2996). Grasshoppers as well as four nestling marsh hens have been killed by 0.2 lb per acre (3147). Earth worms were decimated immediately after application of 1.5 and 3 lbs of dieldrin in attaclay granules per acre but six months later appeared to have recovered (3149).

c. Fish and Other Aquatic Life. Although dieldrin has been said not to persist in soil (3042, 3146), the report that applications of 1 lb of dieldrin per acre continued to kill aquatic beetles for at least ten months (3156) would seem to indicate rather lengthy persistance. A large number of reported lethal figures have been found in the literature with respect to dieldrin toxicity to fish. These are summarized as follows:

Concentration of Dieldrin mg/l	Type of Fish	Result	Reference
0.005-0.042	—	96-hour TL_m	2340
0.006	Bass	Approximate TL_m	3005
0.006	Goldfish	Approximate TL_m	3005
0.01	Bluegill	Approximate TL_m	3005
0.012	Goldfish	17% kill in 96 hours	3047
0.012	Goldfish	Toxicity threshold	3048
0.016	Trout	Toxicity threshold	3007
0.016	4-7" brown trout	40% dead in 48 hours	3049
0.016	Brown trout	Approximate TL_m	3005
0.02	4-7" brown trout	Death in 48 hours	3007
0.02	Goldfish	Toxicity threshold	3007
0.0237-0.074	Rainbow trout fingerlings	96-hour TL_m	2199
0.025	Goldfish	60% kill in 96 hours	3047
0.025	Trout	100% dead in 48 hours	3049
0.04	Golden shiners	Approximate TL_m	3005
0.050	Mullet, small	100% dead in 5 hours	2423
0.050	Mullet, large	100% dead in 24 hours	2423
0.125	All fish tested	Lethal	1383
0.2	Goldfish	Distress in one hour, death in 12 hours	3073
0.2	Guppies	Distress in one hour, death in 12 hours	3073
0.25	Goldfish	Approximate TL_m	3005

Later studies that report numerous variables associated with the toxicity tests are summarized in the following table:

Concentration of Dieldrin mg/l	Temperature °C	Type of Water	Type of Fish	Result	Reference
0.0079	25	(2)	Bluegills	96-hour TL_m	3000
0.016	25	(2) & (4)	Fathead minnows	96-hour TL_m	3000
0.022	25	(2)	Guppies	96-hour TL_m	3000
0.0237	13	(3)	Rainbow trout	96-hour TL_m	3124
0.037	25	(2)	Goldfish	96-hour TL_m	3000
less than 2.5	19	(1)	Catfish	96-hour TL_m	2981

(1) University of Oklahoma tap water, 4-gallon aquaria, 10 fingerlings per aquarium, 100 or 200 fish per experiment.
(2) Soft water, pH 7.4, DO 8 mg/l, alkalinity 18 mg/l, hardness 20 mg/l, acetone solution.
(3) Total dissolved solids 113 mg/l, pH 7.1, DO 8 mg/l, total alkalinity and hardness 102.6 as $CaCO_3$, 10 liters solution, five fingerlings per jar.
(4) Hard water, pH 8.2, DO 8 mg/l, alkalinity 360 mg/l, hardness 400 mg/l.

In landlocked ponds treatments with dieldrin emulsion at a rate of 1 lb of dieldrin per acre is an efficient larvicide but is totally destructive to fish (3135). Granular dieldrin applied to marshes at 0.2 pounds per acre killed many fish (3152). Fish died from 0.1 pound dieldrin per acre applied 20 days previously (3157). From an application of 0.2 pounds dieldrin per acre, there was observed only one dead minnow, no dying fish (3158). An application of 1 pound per acre controlled mosquito larvae for 62-78 weeks, but this concentration was decidedly harmful to fish and other aquatic organisms (1475). Dieldrin was applied as a spray at 0.3 lb/acre. One week later a heavy rain occurred. Two days after the rain, a large but not complete kill of fish occurred (3159). The same rate of application in granule form to tidal marshes killed some killifish and large numbers of small green and blue crabs. Horseshoe crabs, mussels, and snails suffered no apparent injury (3151). Ground tobacco stems impregnated with dieldrin and applied at a rate of 0.2 lb of dieldrin per acre had no immediate detrimental effect on turtles or frogs but blue crabs, fiddler crabs, beetles, flies, and caged killifish were killed. No mortality of killifish under natural conditions was observed (3147). When applied at 1 lb/acre, a fish kill was virtually complete. Mollusks were unharmed, but all crabs except fiddlers were annihilated. The fiddler crabs were apparently spared by uneven coverage (3160). Using two percent granules at the rate of five to ten pounds per acre, three species of *Tilapia* were killed. At 0.2 lb/acre in ponds one to three feet deep, there was a 16 to 40 percent mortality among *Tilapia* fingerlings over a three-week period (3060). After an area was treated with one pound of dieldrin per acre, repopulation with fish occurred in six weeks. Crab populations were still at a low level 13 weeks after treatment (3161). A concentration of 0.1 mg/l, which had no immediate effect, after two weeks decreased the activity of oysters, reduced the growth of small oysters by one-third to one-half of normal, and doubled their mortality rate (2423). Application of one pound of dieldrin per acre greatly reduced aquatic insects (3156).

An aerially applied dose of 0.5 pounds per acre was found to be concentrated in the water surface at 0.64 mg/l (3162). Runoff from an area treated with 4.66 pounds per acre was found by fish bioassay to contain 0.13 mg/l; by chemical determination 0.10 mg/l (3024, 3163). Weekly dusting for 20 weeks with 2.5 percent

dieldrin dust or 2 percent dieldrin granules caused insignificant fish mortality (3046). An application of 5 lbs/acre of 2.5 percent dust caused a 6.6 percent fish mortality, and 10 lbs/acre a 66.6 percent fish mortality (3046).

The concentration of dieldrin necessary to effect a 50 percent kill in *Chironomus* larvae in eight hours is 0.007 mg/l (2531). The TL_m of larval brine shrimp after 5 hours exposure was 0.595 mg/l, for adults 1.172 mg/l (3128). A dilution of 1:400,000 of 25 percent dieldrin (i.e., about 2.5 mg/l) killed 100 percent of *Lymnaeid* snails in a 24-hour exposure (2993). The estimated concentration of dieldrin required to immobilize *Daphnia magna* in 50 hours at 20°C is 330 µg/l (3255).

Bioassays with different formulations of dieldrin showed the 96-hour TL_m values to range from 0.0056 to 0.042 mg/l, depending on the formulation, species of fish, and water hardness (3163).

DILAN

The limits of tolerance of fingerling channel catfish to Dilan have been investigated by Clemens and Sneed (2981) for the Fish and Wildlife Service. At a temperature of 19°C, the 96-hour TL_m was less than 0.5 mg/l.

DINITRO COMPOUNDS

Dinitro compounds, originally introduced as insecticides, are now widely used as herbicides and as preharvest desiccants. As a crop spray for the control of aphids or mites, they are not used during periods of active plant growth because of their high phytotoxicity. These compounds are toxic to a wide variety of life including microorganisms, plants, arthropods, and higher animals, and as a group are more toxic to mammals than most other herbicides in common use (3005, 3009). Such compounds include 2,4-dinitro-6-sec-butylphenol (DNBP), 2,4-dinitro-6-methylcresol (DNC), 2,4-dinitro-6-cyclohexylphenol (DNCHP), dinitro-ortho-cresol (DNOC), and dinitro carbazole. They are discussed in Chapter VI.

DIPTEREX $C_4H_8Cl_3O_4P$

1. General. The chemical 0,0-dimethyl-(1-hydroxy-2, 2,2-trichloroethylphosphonate is known by the trade name Dipterex. This crystalline is soluble in water, benzene, chloroform, and ether, and insoluble in oils. Bayer 13/59 is a similar compound the chemical name being 0, 0-dimethyl-2, 2, 2-trichloro-1-hydroxyethyl phosphate (364).

2. Cross References. Organic Phosphates—General.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The estimated fatal dose for a 70 kg man of Dipterex is 25.0 grams (2995).

b. Stock and Wildlife Watering. The acute oral LD_{50} of Dipterex to rats has been quoted at values of 400 to 1100 mg/kg of body weight (364, 2999, 3002, 3005, 3073, 3143). More than 112 mg/kg fed in the daily diet for a 60-day period was required to produce mortality (3005). Fed to dogs in diet, 500 mg/kg produced significant and 200 mg/kg borderline depression on both plasma and red-cell cholinesterase. There was no effect at 50 mg/kg (3076). Dipterex at 10.0 mg/kg was toxic and at 5.0 mg/kg was non-toxic against calves (3075). The same authors reported the effects of Bayer 13/59 on sheep, 200 mg/kg being toxic and 100 mg/kg being non-toxic. With cattle, 100 mg/kg was toxic and 50 mg/kg was non-toxic.

c. Fish and Other Aquatic Life. For brook and rainbow trout, 0.01 mg/l of Dipterex was not toxic (3164). Concentrations of 1.0 and 10.0 mg/l caused no damage to fish (3165). Concentrations of Dipterex of 50 mg/l at a temperature of 19-22°C caused goldfish to demonstrate abnormal behavior within two hours and killed them in 30-60 hours (3073). The 96-hour TL_m values to fathead minnows at 25°C were 51.0 mg/l of active ingredient in hard water and 180 mg/l in soft water (3000).

When used to control mosquito larvae at concentrations of 1 and 10 mg/l, no effect on fish was noted (3165). Dipterex has been reported to be effective against *Psorophora* at 0.5 mg/l (2931). Although laboratory tests at rates of 0.0125 to 0.25 mg/l for 24 hours gave 100 percent mortality to *Psorophora*, preflood applications in granular formulation of up to 0.5 lb/acre failed to give satisfactory control of this organism (3082).

DI-SYSTON

(see also Organic Phosphates—General)

Di-syston is the name by which the chemical 0,0-diethyl S-2-(ethylthio) ethyl phosphorodithioate is known. It is a clear, oily, liquid slightly soluble (1:40,000) in water, but soluble in most organic solvents. The acute oral LD_{50} to rats has been reported from 2.6 to 12.5 mg/kg of body weight (2997, 3000, 3331), and to guinea pigs of 10.8 mg/kg (3331). With fathead minnows at 25°C, the 96-hour TL_m was found to be 2.6 mg/l in hard water and 3.7 mg/l in soft water (3000). Technical grade di-syston 90-percent active in acetone was tested against fish at 25°C in soft water. These tests gave 96-hour TL_m values to fatheads of 2.9 and 4.1 mg/l and to bluegills of 0.07 mg/l (3000).

DIURON $C_9H_{10}Cl_2N_2O$

(see also CMU)

Diuron, 3-(3,4-dichlorophenyl)-1-dimethylurea, is a crystalline compound only slightly soluble in water and oils. Its solubility in water at 25°C is 42 mg/l. The acute oral LD_{50} to rats is 3.4 g/kg of body weight (364, 2997).

Bioassays of diuron conducted at 20°C in aerated water gave 48-hour TL_m values for coho salmon of 16 mg/l. Preliminary experiments indicate that bluegills are resistant but white crappies are killed by concentrations as low as 6 mg/l. Aeration slightly reduced the toxicity of Diuron. Treatment of ponds with 5 and 10 mg/l resulted in the death of bluegills and largemouth bass. The 10 mg/l treatment eradicated all fish and bullfrog tadpoles. However, reduction of dissolved oxygen is thought to have been a contributing factor (2988).

The lethal concentration to bivalve larvae is cited as 5.0 mg/l (2989).

DOW (ET-14, 15, or 57)

(see Organic Phosphates—General)

DOWICIDE (A, F, G, 31)

(see Organic Phosphates—General)

DOWPON

(see Dalapon)

ENDOTHAL $C_8H_8O_5Na_5$

1. General. Endothal is the generic name for 3,6-endoxohexahydrophthalic acid disodium salt (364). The common name is disodium endothal and the tradename Aquathol (3166). It is soluble in water and has a selective effect on a number of weed species (3009).

2. Effects Upon Beneficial Uses.

a. Irrigation Waters. Endothal in a concentration of 100 mg/l killed tomatoes after 15 days but appeared not to have seriously injured cotton. Endothal at 200 mg/l in irrigation water killed tomatoes after 10 days and injured cotton at the end of 41 days (3084).

b. Stock and Wildlife Watering. The acute oral LD_{50} of endothal to rats is 35 mg/kg of body weight (3009). Based upon a 19.2 percent solution of sodium endothal marketed as Aquathol, the LD_{50} is 182-198 mg/kg. No internal pathological symptoms developed during 2-year feeding tests with diets containing up to 2500 mg/kg of disodium Endothal (3166).

c. Fish and Other Aquatic Life. Walker (3167) found that 40 mg/l had no harmful effects on three species of minnows in 96 hours. Other work demonstrates no fish toxicity to endothal in 21 days at a level of 100 mg/l (3168). The lethal dose to bluegills is greater than 100 mg/l but less than 200 mg/l. No mortalities were observed at 10 mg/l on rainbow trout, bass, and fathead minnows (3166).

Bennet (3169) reported that 800 mg/l killed all bluegills in 24 hours. At 400 mg/l about 50 percent of the bluegills were dead after a 48-hour exposure. At 300 mg/l none were killed in 48 hours. Biossays (2988) against largemouth bass and chinook salmon at 20°C gave 24- and 48-hour TL_m values for chinook salmon of 155 and 136 mg/l respectively, whereas the 24- and 48-hour TL_m values for bass were both 200 mg/l. In a 96-hour exposure period in a constant flow apparatus, no bass mortalities were observed at concentrations as high as 135 mg/l. Experimental field studies indicate that endothal controlled a plant species, *Potamogeton pusillus,* at 0.3 mg/l with no loss of bass or bluegills that were present in the pond.

ENDRIN

1. General. Endrin is the common name for the chemical 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-endo-endo-dimethano-napthalene (364). It is insoluble in water, acetone, and benzene, and slightly soluble in alcohol (2997). Although endrin is said to have a strong residual toxicity as does its closely related compound, dieldrin (3005), endrin is reported to disappear "relatively rapidly" based upon analyses of water, mud, aquatic vegetation, and surviving fish tissues (3170). Also, another report states that endrin does not persist in soil and presents no accumulation problem, but this report advises not to contaminate areas frequented by wildlife (3171).

2. Cross References. Chlorinated Hydrocarbons, Dieldrin.

3. Effects Upon Beneficial Uses.

a. Stock and Wildlife Watering. The acute oral LD_{50} value for laboratory rats has been reported at values ranging from 7.3 to 48 mg/kg of body weight (2992, 2999, 3002, 3005, 3172, 3173). Chronic feeding of 100 mg/kg in the diet of males and 25 mg/kg for females gave significant mortality to the same degree. Endrin has been used as a broadcast spray at up to 2.5 lbs/acre to control field rodents (3005). Younger males and older females appear to be more susceptible than their counterparts (3173).

Dogs appear to be more sensitive than rats. Death occurred within six weeks at dietary levels as low as 10 mg/kg, abnormal effects at 4 mg/kg (3005). Oral LD_{50} values for male and female monkeys were 3 mg/kg of body weight, female rabbits 7-10 mg/kg, and male and female guinea pigs 36 and 16 mg/kg (3173).

Inclusion of endrin at 1 mg/kg in the diet of growing quail resulted in high mortality rates. Young pheasants failed to survive on a diet containing 5 mg/kg of endrin. However, no ill effects were noted when quail were fed winter diets containing 1 mg/kg. Hatchability of eggs and viability of chicks were adversely affected by the inclusion of endrin in the reproduction diet (3044, 3045). The inclusion of 1 mg/kg in the diet will cause a 40 percent or more decrease in the reproduction of quail. The amount of endrin that will produce at least 50 percent mortality in bobwhite quail and ringneck pheasant considering all stages of the life cycle are 5 and 14 mg/kg, respectively (2994, 2996).

The LD_{50} values of one-week old chicks is 3.5 mg/kg of body weight. Young chickens, two-months old, incurred a 10 percent mortality at 4.3 mg/kg. In chronic feeding tests with chicks up to seven weeks of age, 95 percent died at 12 mg/kg, 15 percent at 6 mg/kg (3005).

b. Fish and Aquatic Life. Fish are highly sensitive to endrin. Various figures noted in the literature are as follows:

Concentration of Endrin mg/l	*Type of Fish*	*Results*	*Reference*
0.0006	Bluegills	96-hour TL_m	3012
0.001	Guppies	Abnormal behavior in 5 hours (20°C), death in 24 hours	2992
0.0015	Golden shiners	TL_m	3005
0.003	Bass	TL_m	3005
0.003	Bluegill	TL_m	3005
0.003	Goldfish	TL_m	3005
0.005	Carp fingerlings	48-hour TL_m (28°C)	3012
0.005	Carp fingerlings	24-hour TL_m	3175
0.006	Carp fingerlings	48-hour TL_m (18°C)	3012
0.051	Salmon	96-hour TL_m (20°C)	3174
0.1	Fish	Death	3102
0.14	Carp fingerlings	48-hour TL_m (8°C)	3012
0.61	5-day carp fry	24-hour TL_m (20°C)	3175
4.2	4-day cary fry	24-hour TL_m (20°C)	3175
10.7	2-day carp larvae	24-hour TL_m (20°C)	3175
20	Carp eggs	24-hour TL_m (20°C)	3175

Field applications to rice paddies of one or two lbs/acre remained toxic to fish for over one month (3175). Although laboratory studies indicated a TL_m value for endrin of between 1.0 and 1.5 μg/l, a dose of 1.0 μg/l to a pond gave no results. A second dose of 1.0 μg/l was

administered, but was still without effect. Rapidly lowering temperatures were thought to be inhibiting the lethal effects (3176, 3178). Endrin, used in sugar cane fields to combat borers, has washed into streams during heavy rains, causing fish kills (3177).

The 96-hour TL_m values of endrin to fathead minnows in hard and soft water at 25°C were, respectively, 1.3 and 1.0 µg/l. Tests with bluegills, goldfish, and guppies in soft water at 25°C gave values of 0.60, 1.9, and 1.5 µg/l respectively (3000).

In a series of screening tests of a large number of compounds, rainbow trout, bluegill sunfish, and the sea lamprey all died within 14 hours at 12°C when exposed to a 5 mg/l concentration of an 18½ percent emulsion of endrin (2976). The estimated concentration of endrin required to immobilize *Daphnia magna* in 50 hours at 20°C is 352 µg/l (3255).

EPN $C_{14}H_5PSO_5$

1. General. EPN is an abbreviation for ethyl *p*-nitrophenyl thionobenzenephosphate (364). The pure compound occurs as a yellow crystal, the technical material as an amber liquid, insoluble in water, soluble in most organic solvents (2997). Emulsifiable concentrates and wettable powders appear to be preferred formulations. It is used for the control of crop pests and mosquitoes (3005).

2. Cross References. Organic Phosphates—General, Malathion.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The estimated fatal dose of EPN for a 70 kg man is 0.3 grams (2995).

b. Stock and Wildlife Watering. The acute oral LD_{50} to rats has been reported at values ranging from 7 to 50 mg/kg of body weight (364, 2999, 3002, 3005, 3142). A two-year study, with male rats given up to 450 mg/kg and females up to 225 mg/kg in the diet, showed that amounts of EPN up to 150 mg/kg (males) and 75 mg/kg (females) had no effect on growth; the 450 and 225 mg/kg levels retarded growth. No other symptoms were noticed. Dogs given up to 2 mg/kg/day for one year showed increased kidney weights with increasing dosage (3179). A marked synergistic effect of up to 50-fold exists for EPN and malathion when administered simultaneously to dogs (3027). Against sheep, a concentration of EPN of 20.0 mg/kg of body weight was lethal, 10 mg/kg was non-toxic; with steers, 10 mg/kg was non-toxic; and with calves, 1.0 mg/kg was toxic, and 0.5 mg/kg was non-toxic (3075).

c. Fish and Other Aquatic Life. EPN used as an insect larvacide at 0.1 lb/acre demonstrated little effect on fish (3048). Tests of the toxicity of EPN at 25°C using fathead minnows as the test animal showed 96-hour TL_m values of 0.25 and 0.20 mg/l of active ingredient in hard and soft water respectively (3000). A concentration of EPN of 5 mg/l for 24 hours killed rainbow trout in one hour, bluegill sunfish in 12 hours, and sea lamprey in 12 hours in Lake Huron water at 13°C.

The toxicity of EPN miticide 31.5 percent formulation was tested against *Lymnaeid* snails. A 24-hour exposure in a concentration of 10 mg/l gave a 100 percent kill (2993). The estimated concentration of EPN required to immobilize *Daphnia magna* at 20°C is 0.1 µg/l (3255).

ERBON

(see Baron)

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(see Acrolein)

FENURON

This compound (3-phenyl-1,1-dimethylurea) also called phenyldimethylurea, is a substituted urea. It is a crystalline compound of low solubility in water and oils. The acute oral LD_{50} to rats is 7500 mg/kg of body weight (2997).

Results of bioassays with Fenuron using coho salmon were extremely variable and no TL_m was estimated. All salmon exposed to 56 mg/l survived for the 96-hour duration of the test, whereas a concentration of 750 mg/l killed all test fish within 24 hours. Ten concentrations between failed to give any consistent trend (2988).

FERBAM $[(CH_3)_2NCS_2]_3Fe$

Ferbam is an organic sulfur compound used as a fungicide. Designated as ferric dimethyl dithiocarbamate, it is also known as Fermate. It is a black powder, insoluble in water, and soluble in chloroform, pyridine, and acetonitrile. The oral LD_{50} to rats has been reported as about 4 g/kg of body weight (2997) and 17 g/kg (3009, 3056).

In aquaria, fingerling brook trout were killed at concentrations of one and two mg/l, but Ferbam was not lethal to pike, bass, or sunfish in ponds at 0.5 to 4.0 mg/l. However, tissue damage was indicated since all fish had eroded fins and some were completely blind (3009, 3094). The 96-hour TL_m of fingerling channel catfish to Fermate at 19°C was 2.2 mg/l (2981).

FERMATE

(see Ferbam)

GAMMEXANE

(see Benzene Hexachloride)

GEIGY 25039

(see Organic Phosphates—General)

GUSATHION

(see Guthion)

GUTHION $C_{10}H_{12}N_3O_3PS_2$

1. General. Guthion is designated as 0,0-dimethyl-S-(4-oxobenzotriazino-3-methyl) phosphorodithioate. Other names for this compound are DBD, Gusathion, and Bayer 17147. Guthion is a brown waxy solid, insoluble in water, soluble in most organic solvents (364, 2997). The half-life of Guthion spray and dust on cotton leaves has been reported as 2-4 days (3180).

2. Cross References. Organic Phosphates—General.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The estimated fatal dose of Guthion for a 70 kg man is 0.2 grams (2995).

b. Stock and Wildlife Watering. The acute oral LD_{50} to rats is reported as 11 to 80 mg/kg of body weight (2997, 2999, 3002, 3142), and to mice 20 mg/kg (364). The oral toxicity to calves is cited as: 25 mg/kg of body weight lethal, 0.5 mg/kg toxic, and 0.1 mg/kg non-toxic (3075). The amounts of Guthion that will produce at least 50 percent mortality in bobwhite quail and ringneck pheasant considering all stages of the life cycle are 5000 and 6000 mg/kg respectively (2994, 2996). Oral administration of Bayer 17147 at 25 mg/kg to sheep was toxic, 12.5 mg/kg non-toxic (3075).

c. Fish and Other Aquatic Life. The toxicity of Guthion to fathead minnows in hard and soft waters has been investigated. The 96-hour TL_m values at 25°C for hard and soft waters, respectively, were 0.16 and 0.093 mg/l of active ingredient. These values were obtained with acetone solutions. The 96-hour TL_m of a similar solution to bluegills in soft water was 0.0052 mg/l. For an emulsifiable concentrate under the same conditions, the value was 0.0048 mg/l. The toxicity of a commercial preparation of Guthion, 18.3 percent emulsifiable concentrate, was investigated in both waters against several species of fish. The 96-hour TL_m values of this formulation were reported as follows (3000):

Fish	*Type of Water*	*96-hour TL_m, mg/l*
Fathead minnows	Hard	0.92
Fathead minnows	Soft	0.68
Bluegills	Hard	0.032
Bluegills	Soft	0.030
Guppies	Soft	0.63

HEPTACHLOR $C_{10}H_5Cl_7$

1. General. Heptachlor is a refined ingredient of the well-known insecticide chlordane. Its uses and chemical characteristics, therefore, parallel those of chlordane. Heptachlor is formulated in dusts, wettable powders, emulsifiable concentrates, and granules (2997). No heptachlor could be found 42 days after application to soil (3071).

2. Cross References. See Chlordane, Chlorinated Hydrocarbons.

3. Effects Upon Beneficial Uses.

a. Stock and Wildlife Watering. The acute oral LD_{50} value for rats is given as 90 mg/kg of body weight (2992, 3002). Against male and female rats, LD_{50} values were 100 and 162 mg/kg respectively (2999). Daily doses of heptachlor of 5 mg/kg killed dogs after about one year of exposure. The amount of heptachlor that will produce at least 50 percent mortality in bobwhite quail and ringneck pheasant considering all stages of the life cycle are 125 and 150-400 mg/kg respectively (2994, 2996). A concentration of 10 mg/kg in the diet will cause a 40 percent or more decrease in the reproduction of quail (2996). The use of 0.3 lb/acre for mosquito control resulted in no ill effects to mammals, birds, reptiles, or amphibians (3112). Airplane spraying of 0.25 lb/acre to a marsh had no effect on birds (3181). Cows fed hay from forage sprayed with heptachlor in amounts up to 8 oz/acre did not show toxic effects (3005).

b. Fish and Aquatic Life. Heptachlor concentrations of 7.9-19 µg/l were found lethal to bluegills (2996). In aquaria tests, 0.1 mg/l caused 80 percent mortality in 24 hours to rainbow trout (3182). Tests conducted at 21°C using guppies showed that a concentration of heptachlor of 0.5 mg/l caused abnormal behavior in 30 minutes, death in 24 hours (2992). Heptachlor is much less toxic to fish as a dust or wettable powder (3005).

Later toxicity studies reporting numerous variables are summarized in the following table:

Concentration of Heptachlor mg/l	Temperature °C	Type of Water	Type of Fish	Results	Reference
0.019	25	(5)	Bluegills	96-hour TL_m	3000
0.056	25	(4)	Fatheads	96-hour TL_m	3000
0.094	25	(5)	Fatheads	96-hour TL_m	3000
0.096	21	(1)	Red-sided shiners	96-hour TL_m	3011
0.107	25	(5)	Guppies	96-hour TL_m	3000
0.11	21	(2)	Red-sided shiners	96-hour TL_m	3011
0.175	19	(3)	Catfish	96-hour TL_m	2981
0.230	25	(5)	Goldfish	96-hour TL_m	3000

(1) Soft water, pH 7.6, DO 9 mg/l, alkalinity 36 mg/l, hardness 18 mg/l, 10 liters solution, five fish (6-7 cm) per jar.
(2) Hard water, pH 8.2, DO 9 mg/l, alkalinity 116 mg/l, hardness 156 mg/l, same remaining conditions as (1) above.
(3) Tap water from the University of Oklahoma, 4-gallon aquaria, 10 fingerling channel catfish per aquarium, 100 or 200 fish per concentration.
(4) Hard water, pH 8.2, DO 6 mg/l, alkalinity 360 mg/l, hardness 400 mg/l.
(5) Soft water, pH 7.4, DO 8 mg/l, alkalinity 18 mg/l, hardness 20 mg/l.

Airplane spraying of 0.25 lb/acre to a marsh killed many small crayfish and aquatic insects (3181). Heptachlor at 0.16 lb/acre killed 50 percent of bluegills. Where 0.25 lb/acre was used, both bluegills and blackbass were killed (2996). Post-larval brown shrimp about ½ inch long showed 100 percent mortality in less than 24 hours at concentrations of 25 µg/l (2423). A spraying of 0.25 lb of heptachlor per acre killed 131 small game fish (3158). Twenty pounds of 10 percent heptachlor per acre were applied to a farm near a lake. A fish kill resulted, the young fish dying first. The few survivors were large and, except for shad and mosquito fish, thin (3184). A concentration of 0.033 mg/l killed mosquito larvae but did not injure trout (3184). The estimated concentration of heptachlor required to immobilize *Daphnia magna* in 50 hours at 20°C is 57.7 µg/l (3255).

HETP

(see TEPP)

HEXACHLORAN

(see Chlorinated Hydrocarbons—General)

HEXACHLOROCYCLOHEXANE

(see Benzene Hexachloride)

IPC

IPC has the chemical name isopropyl *N*-phenylcarbamate. Chloro IPC is a closely related chemical, the full name being isopropyl *N*-(3-chlorophenyl) carbamate. IPC is a selective pre-emergence herbicide, used at 2 to 5 pounds per acre which is then washed into soil. Its effect in soil is particularly strong on germinating grass seedlings (3005).

The acute oral LD_{50} to rats is 1,000 mg/kg of body weight (3005, 3009). Chloro-IPC is less toxic, the acute oral LD_{50} being 1,500 mg/kg (3005). The 96-hour TL_m value of IPC at 20°C for fingerling channel catfish is reported at 86.5 mg/l (a 50% formulation) (2981). A concentration of 56 mg/l of a wettable formulation and 1.8 mg/l of an unnamed preparation of IPC caused mortalities of 40 percent among white crappies (3009).

ISODRIN

(see also Aldrin, Chlorinated Hydrocarbons—General)

Isodrin is a common name for the insecticide 1,2,3,4,10, 10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-endo-endo dimethanonaphthalene (364). It is a stereoisomer of aldrin and is formulated as dusts, wettable powders, and emulsifiable concentrates. This crystalline compound according to Frear (2997) is no longer manufactured. The acute oral LD_{50} values for rats have been reported as 7 to 42 mg/kg of body weight (2999, 3005, 3073). The acute oral LD_{50} to rabbits lies between 5 and 7 mg/kg. The oral LD_{50} for 7-day-old chicks is 2.7 mg/kg. Chronic feeding tests with chicks at 12 mg/kg in the diet produced 92.5 percent mortality in 7 weeks; but there was no mortality at a 6 mg/kg level for the same period (3005).

Adlung tested isodrin against guppies and goldfish at 19-22°C and found that 0.05 mg/l produced abnormal behavior in 4 hours, and killed the fish in 12 hours (3073). The TL_m values for several common species of fish has been given as 0.0025 mg/l for bass, 0.006 mg/l for bluegill and golden shiners, and 0.0015 mg/l for goldfish (3005).

ISOLAN

Isolan, a tradename of dimethyl 5-(1-isopropyl-3-methyl-pyrazolyl) carbamate, has an acute oral LD_{50} against male and female laboratory rats of 23 and 13 mg/kg respectively (2998, 2999).

Used against Mummichog killifish at 20-21°C, 1.0 mg/l caused loss of equilibrium within 30 minutes and 5 mg/l caused death in 15 minutes (3014).

KARMEX

(see CMU, Diuron)

KELTHANE

Kelthane is a tradename for the miticide 1,1-bis (*p*-chlorophenyl)-2,2,2-trichloroethanol. It is a solid with a melting point of 77-78°C. The acute oral toxicity to rats has been cited as 575 to 1100 mg/kg of body weight (364, 2992, 2997, 2999). Toxicity tests with guppies at temperatures of 21°C showed that a level of concentration of 0.5 mg/l caused abnormal behavior in 30 minutes, and killed the fish in 48 hours (2992).

KURON

The chemical name of Kuron is 2-(2,4,5-trichlorophenoxy) propionic acid, propylene glycol butyl ether ester. It is insoluble in water, and irritating to eyes and hands. It has an LD_{50} to rats of 500 mg/kg of body weight (364).

The 48-hour TL_m value for chinook salmon at 20°C was 1.23 mg/l; the 24-hour TL_m value for largemouth bass was 3.5 mg/l. In open plot experiments, bass fingerlings were killed by a concentration of about 5 mg/l. Lawrence cites a safe concentration of 3 mg/l. Short term immersion tests with coho salmon after 15 and 30 minutes exposure to Kuron concentrations of 5 and 10 mg/l demonstrated no mortalities in a 24-hour period following removal (2988).

LEAD ARSENATE

(see Chapter VI)

LINDANE

(see Benzene Hexachloride)

MALATHION $C_{10}H_{19}O_6PS_2$

1. General. Malathion (or malathon as it was originally called) is a generic name for S-(1,2-dicarbethoxyethyl)-0,0-dimethyldithiophosphate. It is a deep brown-yellow liquid soluble in water to the extent of 145 mg/l. As an insecticide, it appears to be about 100 times less toxic to warm-blooded animals than parathion and two to four times less toxic to insects (364).

2. Cross References. Organic Phosphates-General, Parathion, EPN.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The estimated fatal dose of malathion for a 70 kg man is 60 grams (2995).

b. Stock and Wildlife Watering. Acute oral LD_{50} values to rats have been reported as 1000 to 2830 mg/kg of body weight (2999, 3002, 3074, 3142). Acute oral MLD concentrations of the 90 percent technical compound administered in vegetable oil were 885 mg/kg for male mice and 430 mg/kg for male rats. Male and female rats on chronic toxicity studies have satisfactorily tolerated 1,000 mg/kg in diets for six months (1536). A 99-percent pure material in vegetable oil had an LD_{50} to male mice of 3321 mg/kg of body weight, and to male rats of 1845 mg/kg (3185). No effects were noted on rats fed 5000 mg/kg of diet for 63 consecutive days (3186).

The toxicity of malathion to livestock was investigated (3075). With sheep, 150 mg/kg of body weight was lethal, 100 mg/kg was toxic, and 50 mg/kg was non-toxic; with cattle 200 mg/kg was lethal, 100 mg/kg toxic, and 50 mg/kg non-toxic; and with calves, 20 mg/kg was toxic and 10 mg/kg was non-toxic. A marked synergistic effect of up to 50-fold exists for EPN and malathion when administered simultaneously to dogs (3027). The amounts of malathion that will produce at least a 50 percent mortality in bobwhite quail and ring-neck pheasant considering all stages of the life cycle are 400 and 1600 mg/kg of body weight respectively (2994, 2996).

The use of 0.1 lb/acre of malathion for mosquito larvaciding resulted in no ill effects to mammals, birds, reptiles, or amphibians (3112).

c. Fish and Other Aquatic Life. Toxicities of malathion to fish have been summarized in the following tabulation:

Concentration of Malathion mg/l	Temperature °C.	Type of water	Type of fish	Results	Reference
0.05	--	--	*Gambusia*	40 percent killed	3008
0.033	--	--	Young salmon	24-hour TL_m	3121
0.1	--	--	Salmon fingerlings	Toxic	3008
0.12	9	--	Salmon fingerlings	96-hour TL_m	3188
2.0	21	--	Guppies	Death in 24 hours	2992
5.0	--	--	Bluegill	Lethal	3008
5.0	--	--	Goldfish	Lethal	3008
5.0	--	--	Rainbow trout	Lethal	3008
5.0	--	--	Sunfish	Lethal	3008
5.0	--	--	Yellow perch	Lethal	3008
8.9	25	(5)	Red-sided shiners (6-7 cm long)	96-hour TL_m	3011
9.6	25	(4)	Red-sided shiners (6-7 cm long)	96-hour TL_m	3011
12.5	25	(2) & (3)	Fatheads	96-hour TL_m	3000
25.0	--	--	Fatheads	24-hour TL_m	3008
52.2 (25% Malathion)	20	(1)	Catfish	96-hour TL_m	2981

(1) Tap water from the University of Oklahoma, 4-gallon aquaria, 10 fingerling channel catfish per tank, 100 or 200 fish per experiment.
(2) Hard water, pH 8.2, DO 8.0 mg/l, alkalinity 360 mg/l, hardness 400 mg/l.
(3) Soft water, pH 7.4, DO 8.0 mg/l, alkalinity 18 mg/l, hardness 20 mg/l.
(4) Hard water, pH 8.2, DO 9 mg/l, alkalinity 116 mg/l, hardness 156 mg/l.
(5) Soft water, pH 7.6, DO 9 mg/l, alkalinity 36 mg/l, hardness 18 mg/l.

Malathion applications at the rate of 0.2-0.75 lb/acre in either granular or emulsion form gave an extensive kill of killifish. Rates of 0.2-0.25 lb/acre also gave complete kills (3156). At 0.5 lb/acre in tidal marshes 26 percent of killifish died in four hours, and 42 percent were sublethally poisoned (3008, 3189). Two lbs/acre resulted in a complete mortality of killifish (3048). Malathion at 21 pounds per acre allowed 95 percent survival of fathead minnows (3008). Gaufin (2699) quotes Anderson's report (3255) that the tolerance limits of *Daphnia magna* in 48 or 64 hours is less than 0.9 μg/l.

Laboratory tests at concentrations of 0.0125 to 0.25 mg/l for 24 hours gave 100 percent kill of *Psorophora*. Applied as a preflood application to soil test plots, rates of 0.2, 0.4, and 0.5 lb/acre of granular formulation failed to give satisfactory control (3082).

The toxicity of malathion in hard water at 11.5°C was tested against several aquatic insects the results being reported as 96-hour TL_m values in μg/l of active agent (2699). For *Acroneuria pacifica* the 96-hour TL_m was 5.6 μg/l, for *Pteronarcys californica* 100 μg/l, *Claassenia sabulosa* 56 μg/l (all stoneflies), and *Arctopsyche grandis* 32 μg/l (a caddis fly).

MCP

(see also TBA, 2,4-D, 2,4,5,-T)

This chemical, 2-methyl-4-chlorophenoxyacetic acid, is generally compounded along with other chemicals, the mixture resulting in a better herbicide than any one constituent would be by itself. Marini (1726) reports that pure MCP was not toxic to carp in a concentration of 130 mg/l; however, 650 mg/l of Agroxone (10 percent MCP) was lethel. Screening tests of a large number of compounds at a concentration of 5 mg/l in Lake Huron water at 12°C showed that MCP had no effect on trout, bluegill, or the sea lamprey in a 24-hour exposure (2976).

MERCURIC COMPOUNDS

(see Chapter VI)

METADICHLOROBENZENE

(see Chlorinated Benzenes)

METASYSTOX

(see Systox, Organic Phosphates—General)

Metasystox is a mixture of 0,0-dimethyl 0-(2-ethylthio) ethyl phosphorothioate (A) and 0,0-dimethyl S-(2-ethylthio) ethyl phosphorothioate (B). Both isomers are liquid in pure form, both are very slightly soluble in water but soluble in most organic solvents. The acute oral LD_{50} to rats of (A) is 180 mg/kg of body weight, and of (B) 40 mg/kg (2997). The oral LD_{50} to rats has also been cited as 138 mg/kg (2992).

By analogy with systox, it appears that the toxic action of metasystox would be due mainly to sulfoxide and sulfones of the thiolate produced metabolically in both plants and animals. Both isomers, however, also undergo reactions in water and on storage that yield small amounts of sulfonium compounds of very high intravenous toxicity. Oral toxicity is hardly affected (3190).

The toxicity of metasystox to guppies ot 21°C has been studied (2992). A concentration of 5 mg/l caused abnormal behavior in 24-30 hours, death in 8-12 days.

METHOXYCHLOR $C_{16}H_{15}Cl_3O_2$

1. General. This compound is slightly soluble in water, soluble in xylene, very soluble in alcohol. Sodium and dimethylamine salts are freely soluble in water (2997). Methoxychlor has lower toxicity to warm-blooded animals than DDT and little accumulation in animal tissue. Concentrations used for sprays or dips usually range from 0.5-1.5 percent. When sprayed on crops dosages of 1 to 3 lbs/acre are required. Dusts and wettable powders are the preferred formulation.

2. Cross References. DDT, Chlorinated Hydrocarbons.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The estimated fatal dose of methoxychlor for a 70 kg man is 350 grams (2995).

b. Stock and Wildlife Watering. The oral LD_{50} to rats has been reported as 5000-7000 mg/kg of body weight (3002, 3003, 3005, 3055, 3056, 3073). Chronic oral toxicity to rats fed daily for 2 years was 10 mg/kg of body weight (3003). The amount of methoxychlor that will produce at least 50 percent mortality in bobwhite quail and ringneck pheasant considering all stages of the life cycle, are 22,000 and 25,000 mg/kg of body weight respectively (2994, 2996). Oral feeding of 1000 mg/kg in the diet will cause a 40 percent or more decrease in the reproduction of quail (2996). Cows eating alfalfa hay with a residue of 14 mg/kg of methoxychlor showed no toxic effects (3005). Methoxychlor in oil applied at 2 lbs/acre did not adversely affect terrestrial vertebrates (3005).

c. Fish and Other Aquatic Life. The toxicities of methoxychlor to various fish as noted from the literature are summarized in the following table:

Concentration of Methoxychlor mg/l	Temperature °C	Type of Water	Type of Fish	Results	Reference
0.025	--	--	Bluegill fingerlings	A few survived	3007
0.03	--	--	Bluegill fingerlings	Toxicity threshold	3007
0.035	25	(1)	Fatheads	96-hour TL_m	3000
0.05	--	--	Bluegill fingerlings	All died	3007
0.056	25	(2)	Goldfish	96-hour TL_m	3000
0.062	25	(2)	Bluegills	96-hour TL_m	3000
0.063	--	--	Goldfish	TL_m	3005
0.064	25	(2)	Fatheads	96-hour TL_m	3000
0.120	25	(2)	Guppies	96-hour TL_m	3000
0.125	--	--	Goldfish	90% killed	3005
0.2	--	(3)	Large bluegills	Killed	681
			Bass	Killed	681
0.250	--	--	Goldfish	100% killed	3005
1.0	19-22	--	Guppies	Distress in 30 min.	3073
			Goldfish	Death in 14 hours	3073

(1) Hard water, pH 8.2, DO 8 mg/l, alkalinity 360 mg/l, hardness 400 mg/l.
(2) Soft water, pH 7.4, DO 8 mg/l, alkalinity 18 mg/l, hardness 20 mg/l.
(3) Outdoor ponds, some of loss may have been due to DDT (3005).

Methoxychlor in oil applied at 2 lbs/acre killed all sunfish in a small pool within 24 hours. Black bullheads although slightly more resistant were also killed. Some catfish were killed but the entire population was not removed (3005). Clemens and Sneed (2981) subjected a mixture of 3 percent lindane and 50 percent methoxychlor to a toxicity test using fingerling channel catfish at 19°C. The 48-hour TL_m to this formulation was 1.0 mg/l.

The estimated concentration of methoxychlor required to immobilize *Daphnia magna* in 50 hours at 20°C is 3.6 µg/l (3255).

METHYL PARATHION

(see Parathion, Phosdrin, TEPP)

Methyl parathion, 0,0-dimethyl-0-*p*-nitrophenyl thiophosphate, is an organo-phosphorus similar in physiological action to parathion, Phosdrin, and TEPP, and has about the same toxicity (3191, 3192). The half-life of methyl parathion on cotton leaves is less than one hour (3180). The estimated fatal dose for a 70 kg man is 0.15 grams (2995). Methyl parathion has an acute oral LD_{50} for rats of 9 to 25 mg/kg of body weight (2999, 3191). Sublethal doses of methyl parathion lower cholinesterase levels. Methyl parathion at 50 mg/kg in the diet of dogs induced significant depression in plasma and erythrocyte cholinesterase, and at 20 mg/kg induced significant depression of erythrocyte and questionable depression of plasma (3076, 3191).

The 96-hour TL_m values of methyl parathion to fathead minnows in hard and soft waters were at 25°C determined respectively as 7.5 and 8.3 mg/l of active ingredient (3000).

MONOCHLOROBENZENE

(see Chlorinated Benzenes)

MONURON

(see CMU)

MUSCATOX

(See Co-Ral)

NABAM $C_4H_6N_2Na_2S_4$

Nabam, disodium ethylene bis dithiocarbamate, is a fungicide used for vegetables, ornamentals, and tobacco. It is described as being moderately toxic, the acute oral LD_{50} for rats being 395 mg/kg of body weight (364, 3009, 3056, 3067). However, in a discussion of bivalve larvae as a bioassay tool, Woelke notes that the lethal concentration of Nabam was 0.50 mg/l (2989).

NEOMYCIN

(see Antibiotics)

NEOTRAN $C_{13}H_2Cl_2O_2$

Neotran, bis (*p*-chlorophenoxy) methane, is a proprietary product containing 40 percent of the active chemical. It is a solid, insoluble in water and oils but soluble in ether and acetone. Neotran is used as a persistent acaricide and ovicide. There is no record of harm stemming from the use of Neotran. The acute oral LD_{50} to rats is 5,800 mg/kg of body weight. Rats on a diet containing 1,000 mg/kg of the pure chemical in food showed no effects after 50 days; 3,000 mg/kg produced a pronounced histopathology (3005).

Surber (3007) reported that 0.2 mg/l killed all bluegills at 17.5°C. According to Clemens and Sneed (2981) the 96-hour TL_m for fingerling channel catfish at 19°C was 21.8 mg/l.

NICOTINE $C_{10}H_{14}N_2$

1. General. This agricultural insecticide is commonly used in the U. S. as a 40-percent solution of nicotine sulfate (Black Leaf 40). It is a pale-yellow oily liquid, highly miscible with water below 60°C (364). Nicotine is available in low-percentage sprays and dusts. Nicotine alkaloid presents no residue problem because it is highly volatile; the sulfate, however, offers some residue hazard (3005).

2. Cross References. Pyrethrum, Rotenone.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Rapidly poisonous to man and animal, as well as insects, nicotine has a minimum lethal oral dose for man of 40 mg (364) to 60 mg (677). According to Smith (922), adults may safely drink daily one-half gallon of water containing 10 mg/l of nicotine (about 19 mg) but over 1 gallon per day would be fatal. The lethal oral dose for most animals is about 10 mg/kg of body weight.

b. Irrigation Water. Being of plant origin, nicotine is not phytotoxic (677); consequently, its pollution of irrigation water should not be detrimental.

c. Stock and Wildlife Watering. The acute oral LD_{50} of nicotine to laboratory rats is 55.2 mg/kg of body weight (3002), of nicotine sulfate to female rats, 83 mg/kg (2999)

d. Fish and Other Aquatic Life. The toxicity of nicotine increases with the nervous complexity of the aquatic species. Since it is less toxic to mosquito larvae than to fish, it is not used as a larvicide. The toxicity of nicotine to fish is evident in concentrations of 3 to 29 mg/l and is greater in alkaline than acid solutions. Fish can endure weak solutions for many days (3005).

OCTA - KLOR

(see Chlordane)

OMAZENE

The toxicity of omazene (copper dihydrazinium sulfate) has been studied with chinook salmon. The 48-hour

TL_m value at 20°C was 0.83 mg/l. A concentration of 1.8 mg/l killed all salmon in 48 hours (2988).

OMPA

(see Schradan)

ORGANIC PHOSPHATES - GENERAL

(see also TEPP, Guthion, Parathion, Para-oxon, Malathion, EPN, Systox, Metasystox, Dipterex, Chlorthion, Co-Ral, Diazinon, Dicapthon, Di-Syston)

The organic phosphorus compounds vary greatly in toxicity to different species of organisms, e.g., Delnab is 940 times more toxic to bluegills than to goldfish, and malathion is 500 times more toxic to salmon and 250 times more toxic to bluegills than it is to fathead minnows (3259). The *in vivo* inactivation of fish brain acetylcholinesterase by organic phosphorus insecticides has been studied as a means of assaying water for previous or concurrent pollution by these compounds (3030). This test was remarkably demonstrative of the synergistic effect of malathion and EPN in combination. As a group, the organic phosphates have been rejected as fish poisons since they are not toxic enough (3260).

The Russians have published water quality studies on two organic phosphate insecticides, metaphos and thiophos. Although these compounds are not likely to be used in the U. S., some aspects of this work may be of interest to water pollution control workers. The recommended limit in water for metaphos is 0.02 mg/l. The lowest concentration detectable by odor is 0.02-0.05mg/l. A concentration of 1 mg/l hinders nitrification and 20 mg/l hinders BOD (3261). Thiophos (NIUIF-100) has an odor perception threshold of 1 to 5 μg/l, and a taste threshold of 20-125 μg/l. Feeding of 0.01 and 0.0005 mg/l/kg body weight to rabbits and rats did not result in toxic symptoms. Concentrations of 50 mg/l do not affect pH, oxygen content, or BOD, although some bacteriostatic activity is evident during the first days (3262).

The acute oral toxicities of various organic phosphate insecticides noted in the literature are summed in the following table:

Name of Chemical	Concentration mg/kg	Animal	Result	Reference
American Cyanamid 4124	25	Calves	Toxic	3075
	5	Calves	Non-toxic	3075
American Cyanamid 12009	10	Sheep	Lethal	3075
	5	Sheep	Toxic	3075
Bayer 29493	211	Male rats	LD_{50}	2999
	245	Female rats	LD_{50}	2999
DOW ET-14	100	Sheep	Non-toxic	3075
	100	Calves	Toxic	3075
	50	Calves	Non-toxic	3075
DOW ET-15	100	Sheep	Non-toxic	3075
	100	Cattle	Toxic	3075
	25	Calves	Toxic	3075
DOW ET-57 (Ronnel or Trolene)	400	Sheep	Toxic	3075
	100	Sheep	Non-toxic	3075
	125	Cattle	Toxic	3075
	100	Cattle	Non-toxic	3075
	310	Lamb	Injured	2809
	1250	Male rats	LD_{50}	2999
	2630	Female rats	LD_{50}	2999
	1750	Male rats	LD_{50}	3263
	3140	Male guinea pigs	LD_{50}	3263
	640	Rabbits	LD_{50}	3263
	2140	Female mice	LD_{50}	3263
	500	Dogs	LD_{50}	3263
	5000	Ducks	LD_{50}	3263
	5000	Chickens	LD_{50}	3263
	500	Turkeys	LD_{50}	3263
(For toxicities of mixtures of Ronnel with other organic phosphates, see 3263.)				
Geigy 25039	25	Sheep	Non-toxic	3075
	25	Calves	Non-toxic	3075

In chronic toxicity studies of Ronnel, no morphological changes resulted from long-term feeding of rats at 15 mg/kg/day or in dogs at 25 mg/kg/day (3263).

The effects of several Dow Chemical Company organic phosphate formulations on various aquatic life forms are summarized as follows:

Name of Chemical	*Concentration, mg/l*	*Organism*	*Result*	*Reference*
Dowicide A	10.0	Bivalve larvae	Lethal	2989
Dowicide F	2	*Lymnaeid* snails	100% kill in 24 hours	2993
Dowicide G	0.25	Bivalve larvae	Lethal	2989
	200.0	*A. aerogenes*	Inhibited	2986
	4.0	*B. mycoides*	Inhibited	2986
	25.0	*A. niger*	Inhibited	2986
	30.0	*P. expansum*	Inhibited	2986
	0.05	Shiners	MLD	2986
Dowcide 31 (tech.)	5	*Lymnaeid* snails	100% kill in 24 hours	2993

ORTHO AQUATIC WEED KILLER

(see Aromatic Solvents)

ORTHOCIDE

(see Captan)

ORTHODICHLOROBENZENE

(see Chlorinated Benzenes)

OVOTRAN $C_{12}H_8Cl_2O_3S$

Ovotran is a white solid, soluble in acetone and aromatic solvents, and moderately soluble in alcohol and oils. It is insoluble in water and is designated as *p*-chlorophenyl-*p*-chlorobenzene sulfonate (2997, 2998).

Ovotran has a pronounced residual ovicidal action. It may be considered nonhazardous to warm-blooded animals. The acute oral LD_{50} to rats of the active material is about 2,000 mg/kg (2992, 3005). Minimal liver damage appeared at 1,000 mg/kg in the diet, and at 10,000 mg/kg adverse growth and pronounced liver and kidney damage were manifest (3005).

Ovotran at 10 mg/l and 21°C caused injury to guppies in 2 hours and death in 5 hours (2992).

PARADICHLOROBENZENE

(see Chlorinated Benzenes)

PARA - OXON

(see also Parathion)

Para-oxon is an oxygen analog of parathion. It is designated 0,0-diethyl 0-*p*-nitrophenyl phosphate. This brown liquid is soluble in most organic solvents and of low solubility in water and oils. The acute oral LD_{50} to rats is 3.5 mg/kg of body weight (2997).

In soft water, the 96-hour TL_m to fathead minnows at 25°C was 0.33 mg/l; in hard water it was 0.25 mg/l (3000).

PARATHION $C_{10}H_{14}O_5$ NPS

1. General. Parathion, 0,0-diethyl-0-*p*-nitrophenyl thiophosphate, is a phosphate ester insecticide. It is a yellow liquid, freely soluble in alcohols and aromatic hydrocarbons, but insoluble (20 mg/l) in water or kerosene. Parathion is incompatible with any substance having a pH higher than 7.5 (364). Parathion is most commonly applied to row and orchard crops (3005).

It has been noted that the chronic hazard from parathion appears slight. Among other reasons, residues disappear quickly (3005). However, Hayes (3193) reports a case of parathion poisoning in a child that demonstrated the persistence of parathion in soil. The child had eaten some of the soil which still contained nearly one percent of parathion six months after the insecticide had been spilled. Another illustration of the persistence of parathion is mentioned by Nicholson (3086). Parathion contamination of pond water, 0.02 μg/l, and mud, 1.9 mg/l, was found in the spring before any use of parathion that year. The contamination probably occurred in March the previous year during a period of accelerated soil erosion when orchard soil apparently washed into the pond.

2. Cross References. Organic Phosphates—General, Para-Oxon, Methyl Parathion.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The estimated fatal dose of parathion for a 70 kg man is 0.1 gram (2995). Absorption of 5 mg of parathion will produce symptoms, and minor physiologic response can be expected from even smaller doses if repeated. Such absorption can possibly come from a water supply that received a strong spray load or waste water from the cleaning of parathion-contaminated equipment (3194).

b. Stock and Wildlife Watering. The acute oral LD_{50} of parathion to rats has been cited as 3 to 15 mg/kg of body weight (2992, 2999, 3002, 3142, 3143, 3195). There is considerable difference in species and sex susceptibility. The extreme LD_{50} range seems to be 2 to 30 mg/kg. Rats fed diets containing parathion up to 100 mg/kg in their diet showed normal weight gains, survival, and food consumption (3005). At 10 to 20 mg/kg, second generation rats produced many dead in their litters (3186).

Cattle fed alfalfa contaminated at the excessive value of 14 mg/kg of feed showed no adverse effects (3005). Studies of parathion oral toxicity to steers showed 75 mg/kg to be lethal, 50 mg/kg toxic, and 25 mg/kg non-toxic; with calves, 1.5 mg/kg was lethal, 0.5 mg/kg toxic, and 0.25 mg/kg non-toxic; and with sheep, 20 mg/kg was lethal, and 10 mg/kg non-toxic (3075).

As little parathion as 1 mg/kg in the diet of dogs causes significant plasma cholinesterase inhibition, 2 mg/kg causes erythrocyte enzyme inhibition (3196). The use of 0.05 lb/acre for mosquito larvaciding resulted in no ill effects to mammals or birds (3112).

c. Fish and Aquatic Life. Numerous reports of the toxic effects of parathion on fish have been summarized as follows:

Concentration of Parathion mg/l	Temperature °C	Type of Fish	Results	Reference
0.01	--	Trout	Not toxic	3164
0.04	--	Bluegills	Heavy mortality	3005
0.063	--	Bluegills	Killed	3058
0.19	18	Bluegills 2.7" long	75% died	3007
0.2 (powder)	18	Bluegills 1" long	33% died	3007
0.2	13	Bluegills	Several died	3007
0.20	--	Bluegills	Toxicity threshold	679, 3007, 3048
0.38	--	Rainbow trout	Survived	3005
0.38	--	Brook trout	Survived	3005
0.4	13	Bluegills 2.7" long	67% died	3007
0.5	21	Guppies	Distress in 1 hour, death in several days	2992
1.0	—	Brook trout	No damage	3165
1.0	--	Rainbow trout	No damage	3165
1.5	--	Goldfish	TL_m	3005
2.0	--	Goldfish	All died	3005

Tests of parathion no. 1 (99 percent active ingredient) and parathion no. 2 (96.5 percent active ingredient) were made with fathead minnows in hard and soft water at 25°C. For parathion no. 1, the 96-hour TL_m in hard water was 1.6 mg/l of active ingredient, and in soft water 1.4 mg/l. For parathion no. 2, the corresponding values were 3.6 and 2.7 mg/l (3000).

The tolerance limits of *Daphnia magna* to parathion for 48 to 64 hours is less than 0.8 μg/l (2699, 3255). Concentrations of 0.5 and 1.0 mg/l applied for 5 minutes eliminated all black-fly larvae in 1.6 miles of slowly moving water. A 24-minute application failed to control an area 2-3 miles downstream (3197). Parathion at concentrations of 0.01 to 0.1 mg/l controlled *Aedes* spp., and 0.5 mg/l controlled *Psorophora* (2931).

The concentration of parathion necessary to kill 50 percent of *Chironomus* larvae in eight hours is 0.008 mg/l (2531). The 96-hour TL_m values at 11.5°C, based on active ingredient, were *Acroneuria pacifica* 0.1 μg/l, and *Pteronarcys californica* 3.2 μg/l; and for the caddis fly, 1 μg/l (2699).

The use of 0.05 lb/acre of parathion for mosquito larvaciding killed frogs (3112). A concentration of 0.325 mg/l of parathion killed all *Lymnaeid* snails in a 24-hour exposure (2993). The TL_m values for brine shrimp at a 5-hour exposure was 0.320 mg/l for the larva and 0.430 mg/l for the adult (3128).

A concluding test that expresses no finite values may be worth citing. Goldfish and Japanese killifish kept one month in a concentration of one-thirtieth of the 48-hour TL_m experienced retarded growth and consumed less food. The study concludes that the arbitrary concentration of one-tenth the 48-hour TL_m sometimes used as a limiting value is too toxic (3198).

PARIS GREEN $3Cu(AsO_2)_2 \cdot Cu(C_2H_3O_2)_2$

Paris green, copper aceto-arsenate, has been reported to have LD_{50} values to rats of 21.9 mg/kg of body weight (3002) and 100 mg/kg (females) (2999). A dose of 1.5 lb/acre is effective against the mosquito larvae *Aedes, Psorophora,* and *Culex.* A few dead tadpoles were also observed. Paris green does not persist in water (3199).

PENICILLIN

(see Antibiotics)

PENTACHLOROPHENOL AND PENTACHLOROPHENATES C_6Cl_5OH

1. General. Pentachlorophenol is a crystalline material that is almost insoluble in water but freely soluble in alcohol, ether, and benzene. Its sodium salt, however, is highly soluble in water. Pentachlorophenol, also known as Penta, possesses bactericidal, herbicidal, insecticidal,

fungicidal and molluscacidal properties (364). Its toxicity is strongly conditioned by the vehicle in which it is administered. The sodium salt is much less toxic than pentachlorophenol (3005).

2. Cross References. Acrolein, Phenol, Halogenated Phenols.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. Concentrated doses cause lung, liver, and kidney damage (364). Two fatal cases among foresters have been reported (3200). According to Gelfand (971), the smallest lethal dose of sodium pentachlorophenate taken internally for a 70-kg man is estimated to be 18 grams. Contact with solutions stronger than 1.0 percent produces dermatitis (3005).

b. Industrial Water Supplies. Turner et al. (976) report that a concentration of 1.0 mg/l of sodium pentachlorophenate in sea water prevented entirely the attachment of marine fouling organisms in pipe and conduit.

c. Stock and Wildlife Watering. The acute oral LD_{50} of undiluted Penta to laboratory rats has been cited as 78 mg/kg of body weight (3002, 3005, 3009). The oral LD_{50} of 5 percent Penta in fuel oil is 27.3 mg/kg, and of a 2 percent aqueous solution of sodium pentachlorophenate 210.6 mg/kg (3005). Chronic toxicity of Penta is not serious because it is rapidly eliminated from the body. Rats and dogs fed for 3 or 4 months on daily amounts up to 10 mg per animal showed no fatalities (3005).

To determine if these substances might harm livestock drinking treated waters, calves were fed drinking water as follows: calf A received water containing 60 mg/l of sodium pentachlorophenate for seven weeks, calf B received water with 51 mg/l of copper pentachlorophenate for five weeks, and calf C got 46.5 mg/l of pentabromophenol for six weeks. Postmortem examinations of all calves showed no significant changes except for slight enteritis. Such exposure did not affect the taste or quality of the meat. It was concluded that these chemicals have an adequate margin of safety toward livestock when used as molluscacides (1631).

A rhesus monkey was given 200 ml of water containing 20 mg/l of sodium pentachlorophenate without symptoms of toxicity, and a calf drank 40 gallons of such water over a period of four days without ill effect (1631).

Penta apparently is repellent to animals. Livestock avoided treated pasture and in general showed no effects from exposure to Penta (3005).

d. Fish and Other Aquatic Life. As a result of his studies of 19 varieties of fish in open jars, Goodknight (247, 973) showed that 0.2 to 0.6 mg/l of sodium pentachlorophenate and pentachlorophenol was lethal to fish. Between 9° and 24°C, temperature was not a factor but toxicity increased at pH values lower than 6.6. Another paper cites the 24-hour TL_m value for sodium pentachlorophenate to fathead minnows as being between 0.32 and 0.35 mg/l. All fish were killed at 0.4 mg/l. As the pH was lowered and as the temperature was raised the solution became more toxic (3201).

Sodium pentachlorophenate caused losses of white crappies at concentrations of 0.056-0.075 mg/l in laboratory tests and of guppies at 1 mg/l in field trials. A 9.5 mg/l concentration killed catfish, guppies, and eels in a small creek, but crayfish appeared unharmed. Guppies returned 24 hours after application (3009).

Van Horn (974) reports the survival times of several fish in solutions containing Santobrite, a commercial sodium pentachlorophenate. A concentration of 5 mg/l allowed fish to live from 16 to 21 minutes, 1 mg/l from 74 to 87 minutes, and 0.4 mg/l from 234 to 418 minutes. Fish used in his test are described as steel-colored minnows, blunt-nosed minnows, and Lake Emerald shiners. The limits of tolerance of fingerling channel catfish to sodium pentachlorophenate at 25° C were studied by Clemens and Sneed (2981). The 96-hour LD_{50} was reported as 0.46 ppm by volume.

Santobrite was tested against the bluegill in carbon-filtered, Philadelphia tap water at 20°C. The 48-hour TL_m value was 0.35 mg/l, and the safe concentration 0.10 mg/l (2093).

Fleming (210) reports that 0.2 mg/l of sodium pentachlorophenate is toxic to fish. Invertebrates such as *Daphnia* are less sensitive than fish and nearly all such organisms can survive concentrations of 5 mg/l. An 88-percent solution of sodium pentachlorophenate in a dilution of 1:400,000 affected a 100-percent kill in 24 hours to *Lymnaeid* snails (2993). According to Berry et al. (975), sodium and copper pentachlorophenates at concentrations of 10 mg/l killed 95 to 100 percent of snails (*Australorbis glabratus*). In a flowing stream the addition of 9.5 mg/l of sodium pentachlorophenate destroyed all snails for 1.5 miles downstream. In Egyptian irrigation waters, concentrations of 15 to 20 mg/l of sodium pentachlorophenate were necessary to control molluscan schistosome vectors. In these field studies, sodium pentachlorophenate was toxic to centrachid fishes at 6 mg/l. Violent reactions began in 10 to 20 minutes after application. Catfish were also affected but surfaced only after the majority of other fish had succumbed. No ill effects were reported by persons who collected and ate considerable quantities of the fish. Some aquatic insects were killed in water treated at 15 mg/l but on no occasion was the arthropod population depleted. There was no serious damage to aquatic plants (3005). Santobrite at 1 mg/l killed 94 percent of *Australorbis* snails in 48 hours, 2.5 mg/l killed 100 percent (2042).

According to Hirsch (972), a concentration of 5.0 mg/l of Santobrite retards the growth of water hyacinth without harming aquatic organisms, but a concentration of 80 mg/l is required for complete elimination of water hyacinth. Pentachlorophenol at 2.66 mg/l eliminated all photosynthesis by kelp (*Macrocystis pyrifera*) in 4 days and gave weak, flaccid blades. A concentration of 1.0 mg/l eliminated photosynthesis in 48 hours (2867). These studies also utilized the commercial product Santobrite. The concentration causing 50-percent inactivation of photosynthesis in bottom kelp fronds during a four-day exposure had been reported as 0.3 mg/l (2106).

A solution of 75 percent pentachlorophenate plus sodium salts of other phenols at a concentration of 2.0 mg/l in a 21-day laboratory incubation test at 22°C inhibited 50 percent of the test algae. Sodium pentachlorophenate at 75 percent plus sodium salts of other phenols at 13 percent at the same concentration inhibited 17 percent of the test algae (2041). The growth of algae in filtered

water from a spray pond was prevented by 15 mg/l of sodium pentachlorophenate, and in a pond containing living algae all growth was stopped in seven days (971). Algae stopped growing immediately in water containing a concentration of 20 mg/l. Horn and Balch (2986) studied the inhibiting concentration of Santobrite towards a number of organisms. Temperature, time, DO, pH, and alkalinity are given in the paper. The inhibiting concentration for *Aerobacter aerogenes* was 225.0 mg/l; *Bacillus mycoides* 4.0 mg/l; *Aspergillis niger* 35.0 mg/l; *Pencillium expansum* 30.0 mg/l; and for the fish *Notropis atherinoides* 0.2 mg/l was the MLD.

PERTHANE $C_{18}H_{20}Cl_2$

This crystalline compound, designated as 1,1-dichloro-2,2-bis (*p*-ethylphenyl)-ethane, is soluble in acetone and petroleum oils. The acute oral LD_{50} to rats has been cited as 8,200 mg/kg of body weight (364, 2997), and 8,500 mg/kg (2992). The amount of perthane that will produce at least 50 percent mortality in bobwhite quail and ringneck pheasant considering all stages of the life cycle is 9,000 and greater than 9,000 mg/kg respectively (2994, 2996). At a temperature of 21°C, a concentration of 0.1 mg/l distressed guppies in 4 days and killed them in 5 days (2992).

PHENYLDIMETHYLUREA

(see Fenuron)

PHOSDRIN

(see also Organic Phosphates—General, Parathion, Methyl Parathion, TEPP)

Phosdrin is designated as 2-carbomethoxy-1-methylvinyldimethyl phosphate. The commercial product is a liquid miscible in water, benzene, and acetone (364, 2997, 3202). The acute oral LD_{50} to laboratory rats has been cited as 6 to 13.1 mg/kg of body weight (2999, 3002, 3142, 3202). The amount of Phosdrin that will produce at least 50 percent mortality in bobwhite quail considering all stages of the life cycle is 90 mg/kg (2994, 2996). The physiological action of Phosdrin is similar to that of parathion and TEPP (3203). Chronic studies with rats show 100-200 mg/kg in the diet to be lethal (3202).

Concentrations of phosdrin of 0.25 mg/l controlled the mosquito larvae *Psorophora* (2931). Concentrations of 0.05 mg/l destroyed trout. Even 0.01 mg/l for 180 hours caused symptoms of poisoning (3165).

PHYGON

(see Dichloronaphthoquinone)

POLYMYXIN B

(see Antibiotics)

PRO - NOXFISH

(see Rotenone, Sulfox-Cide)

PYRETHRUM

1. General. This insecticide is a combination of pyrethrin compounds obtained from the dried flower heads of *Chrysanthemum cinerariaefolium* (364, 677). The ground pyrethrum flowers containing between 0.5 and 3.0 percent of active ingredients may be used directly as insect powder, or the pyrethrins and cinerins may be extracted in kerosene and concentrated. In commercial preparations, various synergists and activators are added to enhance the insecticidal activity of pyrethrum formulations (3005).

2. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The estimated fatal dose for a 70 kg man is 50 grams. Allethrin, the allyl homolgue of cinerin I, is even less toxic (2995). Pyrethrins have been used as anthelminthics.

b. Stock and Wildlife Watering. The acute oral LD_{50} of pyrethrum to rats has been cited as 200 mg/kg of body weight (2992, 3003). That of allethrin has been cited as 680 mg/kg (3002), and 920 mg/kg (3005). The chronic oral toxicity to rats fed daily for two years is cited as 250 mg/kg of body weight (3003). Neither rats nor their offspring suffered from intake of 0.2 percent in their diet for 24 weeks (3005). The acute oral LD_{50} of the cyclopentenyl analogue of allethrin to rats ranges from 1,400 to 2,800 mg/kg of body weight and is less toxic in dilutions (3005).

c. Fish and Other Aquatic Life. The toxic effects of pyrethrum against carp begin at 2 mg/l. The median lethal limit is between 5 and 10 mg/l (2977). In a test at 21°C, a level of 1 mg/l caused distress in guppies in 20 minutes and death in five hours (2992). Fish were poisoned at a water works in Germany by water containing up to 20 mg/l of pyrethrum (about 0.2 mg/l of pyrethrins). Thereafter, aquarium experiments showed that 2.0 mg/l affected movements of carp and 5 to 10 mg/l caused paralysis and death (313, 754). According to Ginsburg (756), pyrethrum in an insecticide spray does not remain toxic after 48 hours. Fish are more sensitive to pyrethrum in an emulsifiable formulation. At 0.33 mg/l, death occurred in 3 or 4 hours; at 10 to 20 mg/l death occurred in 15 to 20 minutes. Rainbow trout were distressed by concentrations of 0.1 mg/l in Alaskan streams (3005).

Turner (2612) notes that 0.005 mg/l of pyrethrum will destroy the crustacean *Asellus aquaticus* in water distribution systems. Another report notes that the concentration is not critical, anything between 0.0001 and 0.1 mg/l would be effective. This report cites the use of a concentration of pyrethrum of 0.01 mg/l as eliminating *Asellus* from water mains, and notes that this level was not injurious to humans (3204).

(see Rosin Amine Compounds)

RADS

(see Rosin Amine Compounds)

RHOTHANE

(see DDD)

RONNEL

(see Organic Phosphates—General)

ROSIN AMINE COMPOUNDS

1. General. Several rosin amine compounds have been used as algicides. Delrad is a proprietary name of

rosin amine D acetate, also known as RADA or dehydroabietylamine acetate. Other rosin amines include rosin amine D sulfate or RADS, rosin amine D pentachlorophenate, sodium carboxyethyl rosin amine, and N(3-aminopropyl) rosin amine D diacetate. Different formulations are available, i.e., Delrad 50 S is a liquid form, and Delrad 70 is a paste form.

2. Effects Upon Beneficial Uses.

a. Irrigation Waters. RADA at a concentration of 66.3 mg/l in irrigation water did not injure tomatoes but did injure cotton after 41 days (3084).

b. Stock and Wildlife Watering. The acute oral LD_{50} value of Delrad to laboratory rats is 850 mg/kg of body weight (3009).

c. Fish and Other Aquatic Life. Springer notes that Delrad is toxic to various fish species at 0.4-0.7 mg/l, although two to four applications at 0.25 mg/l during the bass and bluegill spawning seasons had no effect on production of young. Fish-food organisms, unaffected in the spring, were reduced in number during the summer (3009). As many as three applications at 0.3 mg/l in bass brood ponds did not affect spawning of bass, hatching of eggs, or survival of fry and fingerlings (3264). Short-term exposures of coho salmon, viz 15 and 30 minutes to 0.5 and 1.0 mg Delrad/liter, had no effect for 24 hours (2988).

Eipper (3094) has published the results of extensive field application of Delrad to various ponds on fish, algae, and other aquatic plants (3094). Results of other tests of rosin amine compounds on fish are as follows:

*Concentration mg/l**	*Test Conditions*	*Type of Fish*	*Results*	*Reference*
0.16 (RADS)	(3)	Fatheads	96-hour TL_m	2987
0.23	(2)	Fatheads	96-hour TL_m	2987
0.50	(4)	Trout	No deleterious effect	3095
0.65		Bluegills	MLC**	3264
0.65		Bass	MLC**	3264
0.67	(1)	Catfish	96-hour TL_m	2981

* Rosin amine D acetate unless otherwise indicated.
** Minimum Lethal Concentration—approximate value.
(1) University of Oklahoma tap water, temperature 25°C, 4-gallon aquaria, 10 fingerling channel catfish per aquarium, 100 or 200 fish per experiment, Delrad 70.
(2) Soft water, pH 7.7, DO 8.0 mg/l, alkalinity 34 mg/l, hardness 37 mg/l, fish *Pimephales promelas*.
(3) Soft water, pH 7.4, DO 7.5 mg/l, alkalinity 20 mg/l, hardness 23 mg/l, fish *Pimephales promelas*.
(4) Hatchery water, brook trout fingerlings, temperature 10°C, total alkalinity 127 mg/l.

Four rosin amine compounds were screened for effectiveness against the sea lamprey, rainbow trout, and bluegill sunfish at 13°C. The following results were noted (2976):

Compound	*Concentration mg/l*	*Animal*	*Result*
Rosin amine D	5	Trout	Killed in 4 hours
	5	Bluegill	Killed in 6 hours
	5	Lamprey	Killed in 9 hours
Rosin amine D acetate, 70 % paste	5	Trout	Killed in 2 hours
	5	Bluegill	Killed in 3 hours
	5	Lamprey	Killed in 12 hours
N(3-aminopropyl) rosin amine D diacetate	5	Trout	Killed in 2 hours
	5	Bluegill	Killed in 8 hours
	5	Lamprey	Killed in 12 hours
Rosin amine D pentachlorophenate	5	Trout	Killed in 1 hour
	5	Bluegill	Killed in 2 hours
	5	Lamprey	Killed in 4 hours
	1	Trout	Survived 24 hours
	1	Bluegill	Killed in 12 hours
	1	Lamprey	Survived 24 hours
	0.1	Trout	Survived 24 hours
	0.1	Bluegill	Survived 24 hours
	0.1	Lamprey	Survived 24 hours

Toxicity studies of a large number of rosin amine compounds against algae have been summarized in the following tabulation:

Compound	Concentration mg/l	Results	Reference
RADA	2.0	Controlled 90% algae species	2987
RADA	0.25	Controlled 20% algae species	2987
RADS	2.0	Controlled 70% algae species	2987
RADS	0.25	Controlled 13% algae species	2987
RADA	2.0	Inhibited 83% algae species	2041
RADS, powder	2.0	Inhibited 100% algae species	2041
Diethanol RADA	2.0	Inhibited 83% algae species	2041
Emulsifiable RAD pentachlorophenate	2.0	Inhibited 17% of algae species	2041
Sodium carboxyethyl rosin amine	2.0	Inhibited 0% of algae species	2041
N(3-aminopropyl) rosin amine D diacetate	2.0	Inhibited 100% of algae species	2041
Delrad 70	0.25	Killed *Ulothrix* in 6 hours	3095
Delrad	0.25	Killed *Pithophora*	3264

Springer (3009) notes that although Delrad may be used to control certain pond algae at less than 0.5 mg/l, a concentration of 1.0 mg/l is toxic to small crustaceans (copepods).

ROTENONE $C_{23}H_{22}O_6$

1. General. Normally obtained from the roots of *Derris elliptica,* rotenone is a white crystalline solid that is almost completely insoluble in water (0.17 mg/l at 18°C) but soluble in many organic solvents (364). Dried derris roots have an average rotenone content of about 5 percent. It is also found in some weeds in the eastern U. S. (362) and is widely used as a fish poison and insecticide. When exposed to air, rotenone undergoes decomposition to non-toxic substances. It is more effective than DDT against a few crop insects although historically rotenone is best known as a fish poison. Natives in all tropical regions of the world have employed this or similar plant poisons for centuries to obtain fish for food. As a tool in fish management, its use has expanded to such an extent that few fishery departments have failed to employ it in practical fish population control. The chief aim in such control is the improvement of sport fisheries (3005).

Since weathering breaks rotenone down rapidly, chronic effects appear to be unlikely. The main hazard concerns the contamination of water. Because of the extreme sensitivity of rotenone to fish and some small crustaceans, damage to these organisms will almost inevitably result from water pollution (3005).

2. Cross References. Toxaphene, Sulfoxide.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. For humans, the lethal oral dose of rotenone has been estimated as 0.2 grams (364). It has been used for animals and humans as an anthelminthic.

Bonn and Holbert (3205) have reported on the effects of rotenone products in water supplies. Turbidity decreased and plankton and bacterial counts increased. The most important change was in taste and odor. A temporary taste and odor problem can result from the decomposition of the fish killed. However, laboratory tests showed that these conditions can be easily controlled (see also 3206). Another report describes the use of rotenone to eliminate fish from a water supply reservoir.

A safe and palatable water was produced by the water treatment plant, the finished water demonstrating no fish toxicity and a threshold odor number of one (3207).

The threshold odor values of various rotenone formulations have been reported (2984). For rotenone, it was 13.8 mg/l; cube powder, 30.6 mg/l; and for two formulations comprising rotenone, rotenoids, solvent, and emulsifier, 0.018 and 0.023 mg/l.

b. Stock and Wildlife Watering. The acute oral LD_{50} to rats has been cited as 132 mg/kg of body weight (2992, 3208, 3209). The chronic oral toxicity to rats fed daily for two years is 0.25 mg/kg of body weight (3003). A level of 5 mg/kg in the diet produced necrosis of the liver (3215). Cattle were slightly affected by an acute dose of 18 mg/kg in the feed but pigs were killed by 3.7 mg/kg in four hours (3209). Livestock are ordinarily not harmed by drinking rotenone-treated water (3210).

Pro-Noxfish, a proprietary product containing 2.5 percent each of rotenone and sulfoxide (a synergist) was fed to rats in drinking water at a level of 100 mg/l for 70 weeks after which the average weight was less than that of controls. A similar formulation was exposed to light and air until no test for rotenone could be established, then was fed to rats in drinking water at a level of 100 mg/l for one year. A slight difference in weight was observed compared to controls (3211).

c. Fish and Aquatic Life. There is a great deal of information in the literature on the toxicity of rotenone and related products to various fish since rotenone has been used so extensively as a piscicide. It has been reported to persist for 5-6 days in the spring and 2-3 days in the summer. The rate of detoxification and hence the toxicity is proportional to the light energy (3215), alkalinity, temperature, ice cover (3068, 3215), pH, species of fish, time of exposure, depth of animals, volume of water (3212), turbidity, and vertical dissipation of the distribution of rotenone (2916). Other reports cite duration of toxicities of a maximum of five months but generally less than one month (606, 913, 3213, 3215).

Gersdorff's work of three decades ago compared the theoretical threshold of toxicity, i.e., the concentration just necessary to kill goldfish. For rotenone it was 0.125 mg/l, and for two derivatives, isorotenone-0.055 mg/l, and dihydrorotenone-0.005 mg/l (3214). Concentrations of 0.5 mg/l of powdered derris root (five-percent rotenone) killed bluegill, sunfish, sucker, shiners, stickleback, mud-minnow, and goldfish, but 0.25 mg/l was nontoxic (3212). Rotenone toxicity to small fish starts in the range 0.01 to 0.1 mg/l (2977). A summary of toxicities against different fish follows:

Concentration of Rotenone mg/l	Temperature °C	Type of Fish	Results	Reference
0.006	—	Fatheads	96-hour TL_m	3215
0.066 (cube root)		Fatheads	96-hour TL_m	3215
0.1	—	Trout	Toxicity limit	2977
0.2	21	Trout	Death in 22 minutes	2197
0.2	13	Trout	Death in 100 minutes	2197
0.25 (derris root)		Bluegills	Toxicity threshold	3048
0.4	21	Trout	Death in 15 minutes	2197
0.4	13	Trout	Death in 70 minutes	2197
0.47 *	25	Catfish	96-hour TL_m	2981
0.5	21	Guppies	Distress in 30 minutes, death in 5 hours	2992

* Cube powder, in tap water of the University of Oklahoma, 4-gallon aquaria, 10 fingerling channel catfish per aquarium, 100 or 200 fish per experiment.

For more complete information the reader is referred to the work of Burdick (3212, 3216) and others (3218).

Rotenone is non-injurious to humans when eating fish killed by it (3210). However, Cohen et al. (3215) note that because of the peculiar injury of rotenone to the gills of fish it is doubtful that any significant amount would enter the fleshy part of the fish.

A variety of insects, crustacea, and snails were not affected by 0.05 mg/l of rotenone but 1.0 mg/l killed *Chironomus* larvae in one hour and 0.25 mg/l killed *Daphnia* in 1.5 to 4.0 hours (913). Powdered derris root at 2 mg/l greatly reduced numbers of *Corethra* (insects), *Daphnia,* and diaptomus (copepoda). Tadpoles, leeches, and dragonflies were also greatly affected by a first poisoning, but less so by a second poisoning. Phytoplankton were not affected (2613).

A concentration of derris root of 5 mg/l killed *Daphnia* in 1.5 hours while 2 mg/l killed snails after several days and *Chironomus* and *Gammarus* in several hours. However, older fly larvae and Hemiptera of the Corixidae family can sustain concentrations of 10 mg/l for 48 hours (2977).

An application of rotenone of 1 mg/l to a reservoir did not affect the chironomid population but oligochaetes showed an initial increase that soon became stable (3219).

SANTOBRITE

(see Pentachlorophenol and Pentachlorophenates)

SARIN $(CH_3)_2CHOOPCH_3F$

(see also Parathion, TEPP)

Sarin is one of the nerve gases, used as an insecticide, described as isopropoxymethyl phosphoryl fluoride or isopropyl methylphosphonofluoridate. It is the most toxic of the three nerve gases, Tabun, Saman, and Sarin. It is a liquid that is miscible with and hydrolyzed by water. The lethal dose to man may be as low as 0.01 mg/kg (364). At concentrations of 0.01 mg/l and a temperature of 24°C, the time in minutes for the LD_{50} to minnows is 360, sunfish 320, and goldfish 2,000. At 1.0 mg/l, LD_{50} times are 3.25, 5.8, and 7.5 minutes respectively. At 12°C and 1.0 mg/l, the values are 11.0, 14.0, and 19.0 minutes respectively (3290).

SCHRADAN

1. General. Schradan is the accepted common name of a systemic insecticide also known as OMPA (octamethyl pyrophosphoramid). Applied to plants, it is absorbed and distributed to all parts, the plant sap thereby being rendered toxic. Normally it is used in water sprays at the rate of 1-2 lbs/acre (3005).

2. Cross References. Parathion.

3. Effects Upon Beneficial Uses.

a. Irrigation Waters. Concentrations of Schradan of 10 and 100 mg/l in nutrient solution stimulated cotton plant growth, but 1000 mg/l was phytotoxic. However, all levels were antagonistic to fruiting activity and reduced yields of seed. Concentrations of chlorophyll and carotenoids in the leaves correlated with the concentration of Schradan in the nutrient solution (3220).

b. Stock and Wildlife Watering. This compound is toxic in any form and by all routes of entry, being about

as toxic as parathion. The acute oral LD_{50} to rats has been cited as 9.1 to 42 mg/kg of body weight (2999, 3002, 3005). Male and female rats fed diets of 50 mg/kg of feed showed signs of poisoning and diminished growth in the males but not the females. Although cholinesterase was not affected greatly even in the rats with retarded growth, some inhibition was noted as low as the 1 mg/kg dietary level (3221).

c. Fish and Other Aquatic Life. The 96-hour LD_{50} value for Schradan against fingerling channel catfish at a temperature of 20°C has been reported to be 8150 mg/l (2981). Another study, utilizing OMPA in hard and soft waters, and fathead minnows at a temperature of 25°C reported 96-hour TL_m values of 135.0 and 121.0 mg/l respectively (3000).

SESONE

Sesone, a herbicide, is reported to have a single-dose LD_{50} to 90-120 gram rats of 640-1330 mg/kg of body weight when administered in drinking water. Capsular feeding to dogs equivalent to 360 mg/kg in diet for five days a week for one year was tolerated without ill effect. Rats tolerated 200 mg/kg for the same time but 600 mg/kg injured them slightly (3222).

SEVIN

1. General. Sevin, 1-naphthyl-N-methylcarbamate, is slightly soluble in water, sparingly soluble in most organic solvents, but freely soluble in amides such as dimethylformamide. It is used as an insecticide and has low mammalian toxicity (364, 2997, 3029, 3223, 3291).

2. Effects Upon Beneficial Uses.

a. Stock and Wildlife Watering. The acute oral LD_{50} of Sevin to laboratory rats has been reported as 500 to 2190 mg/kg of body weight (2999, 3002), and to cats, dogs, and rabbits as 125-250, 250-795, and 710 mg/kg respectively (3029). A paper that studied the toxicity of Sevin mixtures with other insecticides indicated oral LD_{50} values to rats varying from 390 to 750 mg/kg (3029). The amount of Sevin required to produce a 50 percent mortality in ringneck pheasant considering all stages of the life cycle is over 40 gms/kg (2994, 2996).

b. Fish and Other Aquatic Life. Toxicity values of Sevin toward various fish species are as follows:

Concentration of Sevin mg/l	Temperature °C	Type of Water	Type of Fish	Results	Reference
4.0 (d)	—	—	Goldfish	TL_m value (no time)	3291
5.0	21–26	(1)	Killifish	Loss of equilibrium in 5 hours but recovery in 10 hours	3014
5.6 (b)	25	(3)	Bluegills	96-hour TL_m	3000
6.7	25	(2)	Fatheads	96-hour TL_m	3000
7.0 (b)	25	(3)	Fatheads	96-hour TL_m	3000
11.0 (a)	25	(3)	Bluegills	96-hour TL_m	3000
12.0	25	(3)	Fatheads	96-hour TL_m	3000
13.0 (b)	25	(3)	Fatheads	96-hour TL_m	3000
28.0 (c)	—	—	Goldfish	TL_m value (no time)	3291
41.0 (a)	25	(3)	Fatheads	96-hour TL_m	3000

(1) Brackish water, 2-gallon aquaria, 5 Mummichog killifish per aquarium.
(2) Hard water, pH 8.2, DO 8.0 mg/l, alkalinity 360 mg/l, hardness 400 mg/l.
(3) Soft water, pH 7.4, DO 8.0 mg/l, alkalinity 18 mg/l, hardness 20 mg/l.
(a) Commercial Sevin, 50 percent wettable powder.
(b) Technical Sevin, 95 percent active in acetone.
(c) Ethanol solution.
(d) Wettable powder.

Sevin applied at a rate of 1.25 lb/acre in fuel oil with a paraffin oil sticker effected reductions in weight of invertebrate fish food of 48.9 to 97.2 percent. On one stream, little recovery was observed up to one month later. No fish mortality was noted (3223). In a discussion of bivalve larvae as a bioassay tool, the lethal concentration of Sevin was reported to be 5.0 mg/l (2989).

SILVEX

(see also 2,4-D, 2,4,5-T)

Silvex, 2 (2,4,5-trichlorophenoxy) propionic acid, is slightly soluble in water, freely soluble in acetone and methyl alcohol. It is used as a herbicide and is reported to be slightly less toxic than 2,4-D and 2,4,5-T type materials (3091). Its acute oral LD_{50} to rats is 650 mg/kg of body weight (2997) and to rats, guinea pigs, rabbits, mice, and chicks ranges from 500-2000 mg/kg (2424).

Concentrations of Silvex of 0.5-1.0 mg/l have proved satisfactory for control of some species of watermilfoils in still waters. A level of 2 mg/l added to a section of a lake reduced the number of plankton therein for two weeks (2424). It is reported that concentrations of 2.5 mg/l have failed to kill fish (3010) and also that concentrations of 4-5 mg/l water of the emulsifiable ester are safe for fish. However, 5 mg/l is reported to be a threshold of toxicity to emerald shiners, the TL_m value in 72 hours being 7 mg/l. Three fish kills at concentrations of 1.5 to 3 mg/l have been reported but it is not known if these were due to toxicological effects or to a temporary oxygen depletion caused by decomposition of the treated plants. In some instances, fish have acquired an unpleasant, oily taste following exposure to Silvex (2424, 3224).

SIMAZINE $C_7H_{12}Cl\,N_5$

Simazine, 2-chloro-4, 6-bis (ethylamino)-5-triazine, is a crystalline that is insoluble in water and usually formulated as a 50-percent wettable powder. The acute oral LD_{50} to mice is about 5 g/kg (364, 3038) of body weight. Toxicity studies at 20°C using chinook salmon showed that the 48-hour TL_m value was 6.6 mg/l. In a constant flow apparatus, the value was 7.7 mg/l (2988).

SINOX

The 96-hour TL_m value for Sinox using red-sided shiners in hard and soft waters at 21°C was 0.24 and 0.13 mg/l respectively (3011).

SOCAL #3

(see Aromatic Solvents)

SODIUM ARSENATE AND SODIUM ARSENITE

(see Chapter VI)

SODIUM BORATE

(see Chapter VI)

SODIUM CHLORATE

(see Chapter VI)

SODIUM CYANIDE

(see Chapter VI)

SODIUM PENTACHLOROPHENATE

(see Pentachlorophenol and Pentachlorophenates)

SR - 406

(see Captan)

STREPTOMYCIN

(see Antibiotics)

SULFOXIDE

(see also Rotenone)

Sulfoxide is said to have a synergistic effect on rotenone, and a formulation (Pro-Noxfish) of 2.5 percent rotenone, 2.5 percent sulfoxide, and 5 percent other cube extracts is designed to be as effective as preparations containing 5 percent rotenone. However, one investigator found it to be less effective against seven fish species than Noxfish which contains 5 percent rotenone (2685).

The threshold odor of sulfoxide has been reported as 0.091 mg/l. A formulation of sulfoxide, rotenone, rotenoids, solvent, and emulsifier had a threshold odor concentration of 0.007 mg/l (2984).

The 96-hour TL_m value for sulfoxide using fathead minnows is reported to be 0.75 mg/l (3215).

SYSTOX $C_8H_{19}O_3PS_2$

1. General. Systox, also known as Demeton, is designated as beta-ethylmercaptoethyl diethyl thionophosphate. Commercial systox is a mixture of isomers with varying toxicities. It is insoluble in water, soluble in alcohol (2997, 3005).

2. Cross References. Metasystox.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The estimated fatal dose of systox for a 70 kg man is 0.1 grams (2995).

b. Stock and Wildlife Watering. The acute oral LD_{50} to rats has been cited as 2.5 to 40 mg/kg of body weight (2992, 2997, 2999, 3142). No signs of intoxification were observed in rabbits fed 98 days on 1 mg/kg (3225). Significant plasma cholinesterase inhibition in dogs occurs with 2 mg/kg in the diet, and erythrocyte enzyme inhibition occurs with 5 mg/kg (3196). Technical grade demeton was fed in capsules to cows at a rate of 0.1 mg/kg for three consecutive days. The cows exhibited severe symptoms of organic phosphate poisoning, milk production dropped sharply, and fat percentage increased. Systox fed with hay at 51 mg/kg for 49 days caused mildly adverse effects on weight gains and red blood cell cholinesterase activity. At 41 mg/kg for 56 days, no adverse effects were observed, but red blood cell cholinesterase activity decreased gradually during the last 6 weeks of feeding (3226).

c. Fish and Other Aquatic Life. The toxicity of systox to guppies at 21°C was studied, demonstrating that a level of 0.5 mg/l caused distress in 90 minutes and death in 8-12 days (2992). The 96-hour TL_m value of systox to fathead minnows in hard and soft water at 25°C was 4.2 and 3.6 mg/l of active ingredient respectively.

2, 4, 5 - T $C_8H_5Cl_3O_3$

(see also 2,4-D, CMU, MCPA, Silvex)

This compound, 2,4,5-trichlorophenoxyacetic acid, consists of crystals that are almost insoluble in water, soluble in alcohol. It is a plant hormone with an estimated human toxicity for a 90 kg man of 54 grams (364, 1723). The acute oral LD_{50} to rats is given as 300 mg/kg of body weight (364, 3009). 2,4,5-T forms phenol as a breakdown product, similar to 2,4-D and CMU (1723). 2,4,5-T was ineffective against algae at 2 mg/l for the species and test conditions utilized in this screening study (2041).

A commercial weed killer that combines 6.25 percent 2,4-D and 6.25 percent 2,4,5-T with propylene glycol, butyl ether esters, and inert ingredients, in concentrations of 50 mg/l or more caused the test fish to become immediately distressed. In a 72-hour period, a 25-percent kill occurred at 10 mg/l, but no fish died at 5 mg/l (2091).

The threshold value of toxicity of 2,4,5-T for perch is 55 mg/l, and for bleak 60 mg/l (2977).

TBA (TRICHLOROBENZOIC ACID)

(see also MCPA)

TBA, a herbicide, is very effective when used with MCPA (2-methyl-4-chlorophenoxyacetic acid). It exists in several isomeric forms of varying toxicity. The most herbicidal isomers are least toxic to mammals. The acute oral LD_{50} values to laboratory animals range from 300-1500 mg/kg of body weight depending upon species. A diet containing 10,000 mg/kg caused no mortality or obvious toxic effect to rats, but there was histological evidence of cellular injury to the liver and kidneys (3227). The 24-hour TL_m for both largemouth bass and bluegill sunfish was 1500 mg/l for the isomer 2,3,6-trichlorobenzoic acid, and 150 mg/l for 2,3,5-trichlorobenzoic acid (3092).

TCA (TRICHLOROACETIC ACID) $CCl_3 \cdot COOH$

(see also 2,4-D, Dalapon)

Trichloroacetic acid (TCA) is a crystalline substance with a slight characteristic odor. It is soluble in 0.1 part water, and very soluble in alcohol (364). It is useful for the control of tough, deep-rooted emergent plants such as *Phragmites* and *Typha*. TCA is used in combination with 2,4-D (3010). The acute oral LD_{50} to laboratory rats has been cited as 3,300 to 3,370 mg/kg of body weight (364, 3005, 3009). Growth in rats was inhibited on consuming 0.3 percent TCA for four months but there was no mortality (3005). There appears to be no danger to livestock and man from normal application (2861). The lethal dose of sodium trichloroacetate for an average 90 kg man is 300 grams (1723).

Fish and the organisms on which they feed are reported to tolerate TCA at concentrations of 1.15 percent (2861). The 96-hour TL_m at 20°C to fingerling channel catfish is greater than 2,000 mg/l (2981, 3228). No mortalities of chinook salmon were sustained during 48-hour exposure to concentrations ranging up to 870 mg/l (2988). However, although the sodium salt of TCA was reported to have no effect on fingerling largemouth bass at 102 mg/l, a concentration of 56 mg/l caused a 40 percent loss of white crappies (3009).

TDE

(see DDD)

TEDION $C_{12}H_{16}Cl_4O_2S$

Tedion, designated as 2, 4, 5, 4′-tetrachlorodiphenyl sulfone, is insoluble in water, slightly soluble in polar organic solvents, soluble in chloroform, and aromatic hydrocarbons. The mammalian toxicity is low, there were no deaths among rats fed 14.7 g/kg. Tedion, at 1 mg/l and 21°C caused injury to guppies in 5 hours, after which recovery followed (2992).

TEPP $C_8H_{20}P_2O_7$

1. General. TEPP, tetraethyl pyrophosphate, is a hygroscopic, mobile liquid that hydrolizes in water; it is miscible with water and xylene but not with kerosene or other petroleum oils (364). TEPP was originally synthesized prior to 1938, being the much-publicized German nerve gas "bladan" of World War II. It was marketed as an insecticide after the war as HETP. This material contained varying amounts of TEPP and since 1948 has not been used (3005, 3229). Like the other organic phosphates, TEPP was particularly useful in the control of aphids, spider mites, and thrips. Although it possesses an initial high lethality, its toxicity declines very rapidly so that within one or two days residues may be considered nontoxic.

2. Cross References. Organic Phosphates-General, Methyl Parathion, Sarin.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The estimated fatal dose of TEPP for a 70 kg man is 0.05 grams (2995). Another source cites 100 mg of TEPP orally in a single dose as being lethal and 25 mg as causing severe nervous symptoms in man (3230, 3231).

b. Stock and Wildlife Watering. The acute oral LD_{50} of TEPP to laboratory rats has been cited as 1.2 to 2.0 mg/kg of body weight (3002, 3005, 3055, 3232). When food contaminated with TEPP was allowed to stand 24 hours before being fed to rats, the toxic LD_{50} value declined. Toxicity essentially disappeared in three days. There is no problem of residual toxicity for TEPP (3005).

c. Fish and Other Aquatic Life. TEPP is reported to be comparable to DDT in toxicity (3059). Toxicity figures noted in the literature for various fish have been summarized in the following table:

Concentration of TEPP mg/l	Temperature °C	Type of Water	Type of Fish	Results	Reference
0.25	17	--	Bluegill fingerlings	Killed	3007
0.25	--	--	Bluegills	Toxicity threshold	3007, 3048
1.0	25	(1)	Fatheads	96-hour TL_m	3000
1.2	22–26	--	Bluegills	Killed	3007
1.2	22–26	--	Bass	Killed	3007
1.6	19	(3)	Catfish	96-hour TL_m	2981
1.7	25	(2)	Fatheads	96-hour TL_m	3000

(1) Hard water, pH 8.2, DO 8.0 mg/l, alkalinity 360 mg/l, hardness 400 mg/l.
(2) Soft water, pH 7.4, DO 8.0 mg/l, alkalinity 18 mg/l, hardness 20 mg/l.
(3) Tap water of the University of Oklahoma, 4-gallon aquaria, 10 fingerling channel catfish per aquarium, 100 or 200 fish per experiment.

In aquarium tests, TEPP at an initial toxic concentration of 1.2 mg/l became safe for bluegills in 43 hours; 1.8 mg/l in 59 hours; and 3.5 mg/l in 70 hours. Temperature during this work averaged 17°C (3007). The concentration of TEPP required to kill 50 percent *Chironomus* larvae in eight hours is 0.008 mg/l (2531).

TERRAMYCIN

(see Antibiotics)

THIODAN $C_9H_6Cl_6O_3S$

Thiodan is an odorless crystalline compound that is stable toward water and dilute mineral acids. It is insoluble in water but soluble in alcohol and most organic solvents. The commercial product is a mixture of two isomers (364, 2997).

The acute oral LD_{50} to rats has been cited as 90 and 110 mg/kg of body weight (3002, 3073). Experiments on guppies and goldfish at 19°-22°C report that 0.01 mg/l caused distress in 30 minutes and death in 20 hours (3073).

THIRAM $C_6H_{12}N_2S_4$

Thiram (tetramethylthiuram disulfide) crystals are insoluble in water, soluble in alcohol, and partially soluble in acetone, benzene, and chloroform. It is used as a seed disinfectant, turf fungicide, and as a bacteriostat in soap (364, 3067). Thiram has an acute oral LD_{50} to laboratory rats of 350 mg/kg of body weight (3067) and 865 mg/kg (3009). The toxicity to rabbits is 350 mg/kg (364).

A toxicity test was made using fingerling channel catfish; at 19°C, the 96-hour TL_m was 0.79 mg/l (2981).

THURICIDE

(see also Chapter VII, Bacteria-General)

Thuricide is the world's first commercial biological insecticide. Although the bacterium, *Bacillus thuringiensis*, that produces the toxic component has been known for years (it was first isolated from a diseased moth in 1911), it was not until 1956 that industrial concerns began to show an interest in production (2527, 3085). The bacillus grown on an artificial media forms crystal-like inclusions upon sporulating. These crystals are harmless to mammals, fish, and plants as well as to insect predators and bees (3085). Eighteen human subjects ingested one gram of Thuricide capsules daily for five days with no toxic symptoms developing. A 33-percent suspension in water containing one percent carboxymethly cellulose was administered to rats by stomach tube at doses up to 24 grams of Thuricide per kilogram of body weight without any toxic symptoms developing.

TOXAPHENE $C_{10}H_{10}Cl_8$

1. General. Toxaphene is a chlorinated camphene containing about 68 percent chlorine. Insoluble in water but highly soluble in organic solvents and oils, it is widely used in the control of insects. When used against agricultural crops, the normal dosage is in the range 1-3 lbs/acre. Dusts, emulsifiable concentrates, and wettable powders are available commercially. Because of its persistence and particularly high toxicity to fish, drifting sprays and dusts are hazardous near water courses (364, 3005).

2. Cross References. Chlorinated Hydrocarbons, Rotenone, DDT, Benzene Hexachloride.

3. Effects Upon Beneficial Uses.

a. Domestic Water Supplies. The estimated fatal dose of toxaphene for a 70 kg man has been cited as 5 grams (2995). Another report cites a slightly lower figure, 60 mg/kg (3233). Owing to the turpene odor, it is unlikely that toxic concentrations will be consumed by man or animals (677). The threshold odor of toxaphene has been reported as 0.0052 mg/l (2984). The threshold odor of a formulation containing rotenone, rotenoids, toxaphene, solvent, emulsifier, and clay has been reported as 0.020 mg/l (2984). In most cases, toxaphene treatments will result in concentrations of toxaphene of less than 25 μg/l (3208). However, water that is to be used for domestic purposes should not be treated with toxaphene (3024, 3233).

b. Irrigation Waters. Although it has been reported that toxaphene at low concentration may depress the growth of seedlings (677), no specific reference to the effect of toxaphene in irrigation water was noted in this survey.

c. Stock and Wildlife Watering. The acute oral LD_{50} value of toxaphene to rats has been reported at 69 to 90 mg/kg of body weight (2992, 2999, 3002, 3073). The chronic oral toxicity to rats fed daily for two years was 5.0 mg/kg of body weight (3003). The highest level fed to rats without gross effects was 100 mg/kg in the diet (3005). Dogs are considerably more sensitive to toxaphene than rats. Death in dogs occurred at chronic levels as low as 10 mg/kg in the diet per day. Dogs fed toxaphene at levels of 4 mg/kg/day lost weight and exhibited abnormal behavior in the first few days (3005). Toxaphene at 313 mg/kg in the diet killed dogs in 33 days. For cattle, sublethal doses are between 35 and 110 mg/kg. The lowest lethal dose was 144 mg/kg (3070). It is estimated that a cow would have to drink 18,000 gallons of water a day which had been treated with toxaphene at 0.1 mg/l to produce toxic symptoms (3233).

Toxaphene and oil caused a heavy mortality to young birds (3069). It has been implicated in the poisoning of fish-eating birds (3100). Ducks and other waterfowl have suffered significant losses from single sprayings of toxaphene at 1.5 lb/acre (3006). The amount of toxaphene that will produce at least 50 percent mortality in bobwhite quail and ringneck pheasant considering all stages of the life cycle are 500 and 450 mg/kg respectively (2994, 2996). For a rather complete discussion of the effects of toxaphene on wildlife see Rudd and Genelly (3005), and also Von Oettingen (3292).

d. Fish and Other Aquatic Life. During the past few years, toxaphene has been gaining widespread use in fish eradication. Although it is advantageous from the aspects of cost and ease of application, its principal disadvantage is its stability which often results in long periods of toxicity (3213). Some lakes treated with toxaphene have retained toxicity for 3-4 years (3208). It appears that the breakdown in toxicity is much more rapid in highly turbid lakes (3234). Laboratory studies have indicated that hard water detoxifies toxaphene more rapidly than soft water, that autoclaving hinders detoxifying, and that stirring increases detoxifying (3239). All these variables result in a broad range of toxicity figures cited in the literature. The reader is referred to (3235) for a more complete discussion of the duration of toxicity of toxaphene treated lakes and to (3033) for a discussion of variation in results.

The numerous finite values of toxaphene concentrations and their effects on various fish as obtained from the literature are summarized in the following tabulation:

Concentration of Toxaphene mg/l	Type of Fish	Results	Reference
0.005	Trout	Killed	679
0.005	Goldfish	TL_m in 10 days	680
0.025	Goldfish	Loss of equilibrium in 24 hours	680
0.05	Bass fingerlings	Killed	681
0.05	Bluegill fingerlings	Killed	681
0.05	Goldfish	Toxic	681
0.013	Fatheads	96-hour TL_m	3215
.0135-.0165	Trout fingerlings	96-hour TL_m	2199
0.1	Carp	Total kill in 72 hours (18°C)	3233
0.01	Bluegill	Toxicity threshold	3007, 3048
0.005	Trout, rainbow and brown	Toxicity threshold (1" long)	3048
0.025	Brown trout (4-7" long)	Killed 70% in 24 hours	3049
0.1	Carp	Killed	3005
0.1	Bonefish	Killed	3005
0.1	Largemouth bass	Killed	3005
0.1	Bullhead catfish	Killed	3005
0.1	Yellow perch	Killed	3005
0.1	Brown trout	Killed	3005
0.125	Carp	Toxic	3236
0.125	Rainbow trout	Toxic	3236
0.02	Bluegills	Killed	3007
0.02	Creek chubs	Killed	3007
0.02	Blacknose dace	Killed	3007
0.02	Shiners	Killed	3007
0.02	Fallfish	Killed	3007
0.02	Minnows	Killed	3007
0.02	Sculpins	Killed	3007
0.02	Darters	Killed	3007
0.04	Goldfish	Killed	3007

Other toxic values of toxaphene toward fish are as follows:

Concentration of Toxaphene mg/l	*Result*	*Reference*
0.006	Lethal in 10 days	3004
0.036	Complete kills	918, 920
0.04	Lethal	679
1.0–10.0	Toxic	919

Later studies are summarized in the following tabulation:

Concentration of Toxaphene mg/l	Temperature °C	Type of Water	Type of Fish	Result	Reference
0.0035	25	(3)	Bluegills	96-hour TL_m	3000
0.005	19–22	--	Guppies	Distress in 12 hours, death in 30 hours	3073
0.005	19–22	--	Goldfish	Distress in 12 hours, death in 30 hours	3073
0.0051	25	(2)	Fathead minnows	96-hour TL_m	3000
0.0056	25	(3)	Goldfish	96-hour TL_m	3000
0.0075	25	(3)	Fathead minnows	96-hour TL_m	3000
0.01	13	--	Bluegill fingerlings	50-75% died	3007
0.0135	13	(4)	Rainbow trout	96-hour TL_m	3124
0.0145	13	(5)	Rainbow trout	96-hour TL_m	3124
0.0165	13	(6)	Rainbow trout	96-hour TL_m	3124
0.02	17	—	Bluegill fingerlings	Killed all	3007
0.020	25	(3)	Guppies	96-hour TL_m	3000
0.05	21	--	Guppies	Distress in 5 hours, death in 24 hours	2992
0.05	19–22	--	Guppies	Distress in 30 minutes, death in 20 hours	3073
0.05	19–22	--	Goldfish	Distress in 30 minutes, death in 20 hours	3073
1.9 *	20	(1)	Catfish	96-hour TL_m	2981

* ppm by volume

(1) Tap water of the University of Oklahoma, 4-gallon aquaria, 10 fingerling channel catfish per aquarium, 100 or 200 fish per experiment.
(2) Hard water, pH 8.2, DO 8.0 mg/l, alkalinity 360 mg/l, hardness 400 mg/l.
(3) Soft water, pH 7.4, DO 8.0 mg/l, alkalinity 18 mg/l, hardness 20 mg/l.
(4) Dissolved solids 190 mg/l, pH 7.9, DO 9 mg/l, total alkalinity 225 mg/l, total hardness 154 mg/l, 5 fingerlings per 10 liters.
(5) Dissolved solids 115 mg/l, pH 7.5, DO 8 mg/l, total alkalinity 96 mg/l, total hardness 85.5 mg/l.
(6) Dissolved solids 25 mg/l, pH 7.2, DO 10 mg/l, total alkalinity 51 mg/l, total hardness 34 mg/l.

Fish that have been killed by toxaphene have not been found to be off-flavor (3208). Frogs and water snakes have exhibited toxic effects (3233). The 24-hour TL_m values of toxaphene for aquatic sow bugs (*Asellus*), scuds (*Gammarus*), *Daphnia*, and mayflies (*Ephemera*) are greater than for fish (3208). Applications of toxaphene of about 0.1 mg/l killed chironomids within three weeks but the lake was completely repopulated again after nine months. *Chaoborus* larvae showed no immediate effects from toxaphene although larvae were absent from a sample taken six months later and had not reappeared by the end of the study. Oligochaetes increased in numbers during these studies (3219). Eight alkaline lakes in British Columbia were treated with toxaphene at concentrations of 0.01-0.10 mg/l. Most of the fish which were killed died within 120 hours. All fish were killed and the lakes were still toxic to fish eight or nine months later. Amphipods were eliminated at all concentrations and were still absent nine months later. Dragon flies, damselflies, and midge larvae were killed at a concentration of 0.03 mg/l (3237). Toxaphene at a concentration of 5 μg/l can reduce the population of small fish in hard-water lakes without greatly affecting the population of larger fish (3238). Toxaphene, used to control field mice, has been washed by rain into a stream thereby causing the death of fish (3236). Frogs and water snakes are the only vertebrates, other than fish, that have exhibited toxic symptoms in toxaphene-treated waters. Insect life is severely affected but not eliminated (3233). In earthen ponds, toxaphene at 0.2 mg/l remained toxic to bluegills for more than ten weeks, but a concentration of 0.05 mg/l did not kill fingerlings in a 5-week period. Trout fingerlings were disabled by concentrations of toxaphene ranging from 2.0 mg/l in Velsicol solution to 8.0 mg/l in acetone solution (755).

Doudoroff et al. (1449) tested the toxicity at 20°C to goldfish of a dust containing 20 percent toxaphene and 80 percent inert compounds. Goldfish survived 10 days at a concentration of 0.0032 mg/l as toxaphene, but most were killed within 10 days by 0.0056 mg/l, and all were killed by 0.01 mg/l. About 0.025 mg/l caused half of the fish to turn over in less than 24 hours. In earthen ponds, 0.2 mg/l killed bluegills and bass (681). In aquaria, goldfish were killed by 0.05 mg/l; in earthen ponds by 0.2 mg/l. Toxaphene at 0.2 mg/l remained toxic to bluegills for over 10 weeks in earthen ponds (681). A concentration of 10.0 mg/l has been reported lethal to bivalve larvae (2989). A solution of toxaphene (60 percent) at 2.0 mg/l inhibited the growth of 50 percent of the test algae during a 21-day, 22°C incubation period in laboratory tests (2041).

It does not appear that important hazards will arise from the addition of toxaphene in the quantity required for fish eradication to waters not used as drinking water supplies. Even overdosages would not appear to be dangerous. The amount of chemical absorbed by fish at the time of death appears to be very small and there is little chance of acquiring a dangerous dosage from fish flesh. Nevertheless, toxaphene has the greatest toxicity to fish of any of the chlorinated hydrocarbons except endrin.

See Applegate et al. (2976) for the results of screening tests of a number of toxaphene formulations on trout, bluegill, and the sea lamprey.

TOXICHLOR

(see Chlordane)

TRICHLOROACETIC ACID

(see TCA)

TRICHLOROBENZENE

(see Chlorinated Benzenes)

TRICHLOROBENZOIC ACID

(see TBA)

2, 4, 5 - TRICHLOROPHENOXYACETIC ACID

(see 2,4,5-T)

TROLENE

(see Organic Phosphates—General)

VANCIDE

(see Captan)

VAPAM $C_2H_4NNaS_2 \cdot 2H_2O$

This crystalline compound, sodium N-methylodithiocarbamate, has an unpleasant odor, is soluble in water to 72.2 g/100 ml at 20°C, moderately soluble in alcohol, and sparingly soluble in other solvents. It disappears from soil in about two weeks (364). The acute oral LD_{50} to rats is 800 mg/kg of body weight (2992), and mice 285 mg/kg (364).

Vapam at a concentration of 1 mg/l at 21°C caused distress in guppies in six hours, death in 30 hours (2992).

VELSICOL 1068

(see Chlordane)

WS - 1492

(see Aromatic Solvents)

ZDD

ZDD, zinc dimethyl dithiocarbamate, was tested as an algicide at a concentration of 2 mg/l, temperature 22°C, for a laboratory incubation period of 21 days. A 65 percent solution controlled 17 percent, and a 100 percent solution controlled 100 percent, of the algae tested (2041). Concentrations of 0.004 mg/l inhibited *Microcystis*, 0.25 mg/l controlled all diatoms tested, 43 percent of blue-green algae, and 18 percent of green algae (3050). In soft water the 96-hour TL_m value to the fathead minnow was 0.008 mg/l (2987).

CHAPTER X

SURFACE ACTIVE AGENTS

by Jack Patton *

This chapter deals exclusively with surface-active agents including their presence in commercial synthetic detergents. Phosphorus compounds and other "builders," which may constitute from 60 to 80 percent of a commercial synthetic detergent, are covered in Chapter VI, Potential Pollutants.

Certain solutes, even when present in low concentrations, have the remarkable property of lowering the surface tension or other interfacial properties of their solvents. Such solutes are known as surface-active agents and their unique effect is called surface activity (24). The surface-active agents include soaps, detergents, emulsifiers, wetting agents, and penetrants. Of these substances, the synthetic detergents are economically most important and are used in the greatest amounts (2115).

GENERAL

Synthetic surface-active agents were developed primarily to overcome the disadvantages of soaps in hard water. Unfortunately, soap is a softening agent and in hard water some of it must combine with the calcium, magnesium, and certain other metallic ions of the solution before the remainder can become an effective detergent. This combination requires that a greater amount of soap be used than actually goes into cleaning. Indeed, Aultman (34) has shown that the annual cost for a family of 5 using water with a total $CaCO_3$ hardness of about 300 mg/l (as contained by the water in Indianapolis, Indiana) (43) will be about $18 more if the family uses ordinary soap instead of a synthetic detergent. The majority of soaps are sodium or potassium salts of fatty acids. The calcium, magnesium, and ferric cations in hard water form insoluble compounds with the fatty acid anions. The resulting water "scum" often causes clothes washed in such a solution to appear dingy.

The history of synthetic surface-active agents dates back considerably further than is commonly known. Frémy sulfonated olive and almond oils as early as 1831. In 1897, mention was made of the sulfonation of cetyl alcohol from sperm oil as a scouring agent. Twitchell's American patent 601,603 of 1898 made use of sodium sulfonate from petroleum oils, and the German patent 336,558 taken out in 1916 described the production of diisopropyl naphthalene solium sulfonate (44).

The critical soap shortage in Germany during World War I led to the development of modern surface-active agents (24). It was not until 1933, however, that serious production of these compounds was undertaken in the United States. In that year "Dreft" appeared as this nation's first packaged household synthetic-detergent washing product, intended primarily for silks and woolens; and the first synthetic surface-active shampoo, "Dreen," was also marketed (2113).

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For over ten years after their introduction, synthetic detergents made no phenomenal gains. In 1940, only three percent of the total soap and detergent sales were synthetic detergents (48). In 1946, however, with the introduction of "Tide," a product designed to do the "whole family wash," synthetic detergent sales began to skyrocket. In 1948, the synthetic detergents constituted 16 percent of the annual soap and detergent sales (49) and in 1957 the figure had grown to over 75 percent (48). Ward (48) reported an estimate that synthetic detergent sales will level off at about 85 percent of the total market for soaps and detergents. Earlier, however, McKinney and Symons indicated that within twenty years synthetic detergents would replace 98-99 percent of the soap sales (50).

CHEMICAL CONSIDERATIONS

The molecules of surface-active agents are essentially dipolar, and the characteristics of the end groups provide a natural system of division. Chemically, there are two classes of surface-active agents: the nonionic and the ionic or ionogenic (24). The nonionic class has high affinity, nonionizable end groups. Hence it does not ionize in aqueous solution. Nonionic surface-active agents are a ripidly growing class. In the last two years, sales of these products have risen from ten percent of the domestic market to 26 percent (1902).

The ionogenic class of surface-active agents has two main divisions based again upon the end groups of the dipolar molecule, which in this class has an elongated portion of low residual affinity and one end of high residual affinity. If the elongated, low-affinity group is included in the cation in aqueous solution, the agent is termed cationic or cation active (24). These substances are more bactericidal than soaps or other synthetic detergents and are used as sanitizing agents in connection with dishwashing and laundering. The production of cationic surface-active agents is relatively small and they probably present few problems from a water treatment or pollution standpoint (43). Flynn, et al. (49) attribute this low acceptance primarily to the effect of cationics on the protein of the skin. If the surface-active agent ionizes in aqueous solution to give a negatively charged group or anion that contains the low-affinity portion of the molecule, the substance is called anion active or anionic. Presently, about 80 percent of all synthetic detergents in this country (53) are of the anionic type. Most of these anionics are cyclic, with dodecylbenzene sulfonates representing about three-quarters of the total anionic production (49).

Based on the preceding three divisions, the following classification of surface-active agents is quoted from Woodard and Calvery (1895):

"I. *Anionic:* Ionizing so that the major part of the molecule is the anion.

A. *Sulfates:* Compounds with carbon-oxygen sulfur linkages
1. Alkyl sulfates—alcohol sulfates
2. Sulfated esters—sulfated monoglycerides
3. Sulfate-substituted amides

B. *Sulfonates:* Compounds with carbon-sulfur linkages
1. Alkyl sulfonates—aliphatic sulfonates
2. Alkyl aryl sulfonates—aliphatic aromatic sulfonates
3. Sulfonated esters
4. Sulfonated amides

"II. *Cationic:* Ionizing so that the major portion of the molecule is the cation.

A. *Substituted ammonium compounds:* Alkyl aryl ammonium compounds

B. *Cyclic quaternary ammonium compounds*

"III. *Nonionic:* Compounds not ionizing when dissolved in water.

A. *Polyethylene glycol fatty acid esters*

B. *Ethers*
1. Alkyl aryl polyether alcohols
2. Polyoxalkylene derivatives of hexitol anhydride fatty acid esters"

Although the fundamental ingredient of all commercial synthetic detergents is a surface-active agent, they also contain a variety of other components. In fact, the over-all composition of synthetic detergents is quite variable. The amount of surface-active agent used as the active part may range from 20 to 40 percent (49). In addition to this active ingredient, 30 to 50 percent of the product is made up of a molecularly dehydrated phosphate (49) which assists in suspension, dispersion, and emulsification of soil (48). Synthetic detergents also contain about 20 percent (2056) of sodium sulfate which acts as an additional electrolyte to aid in wetting and dispersion, and a maximum of 10 percent of sodium silicate which controls the corrosion of metals, particularly aluminum (49). The remainder of the composition of the detergent comprises fatty acids to stabilize foam (48), a bleaching agent (such as sodium perborate) (48), and a fraction of one percent of sodium carboxymethyl cellulose (2056) to prevent redeposition of soil on the fabrics (49). After a packaged product—whatever type of surface-active agent and other component it may represent—is used for laundering or cleaning and released down the drain, however, it loses its individual identity and must be considered on the basis of its separate components.

The specific surface-active material most widely used today in the bulk of synthetic detergents is the group of anionic alkyl benzene sulfonates, generally termed ABS (48). Derived from polypropylene (55), the ABS molecules are saturated, highly branched hydrocarbons that terminate with a quaternary carbon to which the benzene ring is attached (57). McKinney and Symons (58) maintain that the quaternary carbon of the ABS molecule prevents its bacterial degradation. Hence, some ABS compounds persist in sewage and streams in biologically active solutions without appreciable decomposition from either treatment processes or natural purification. The facts that almost the entire annual production of over 3.8 billion pounds (57) of synthetic detergent is discharged to a ground or surface water and that ABS, the most common ingredient, is resistant to biological metabolism, account for the rising incidence of surface-active materials as water-quality problems.

SURFACE WATER POLLUTION

The concentration of surface-active agents (in mg/l of Manoxol OT) at Raleham on the River Thames grew from 0.24 in 1954 to 0.38 in 1956 (2056). The Orsanco monitoring program has disclosed that the average ABS content of the Ohio River has mounted to about 0.2 mg/l (59). The surface-active agents that reach rivers in the effluents of complete sewage treatment plants have resisted the rigors of intense biological activity in secondary sewage treatment processes, and consequently they are not likely to be metabolized quickly in river water (57).

Hammerton (60) maintains that the rates at which different surface-active agents decompose in river water is dependent on the particular configuration of the alkyl group in the molecule. If the alkyl group has a straight chain, the material will be susceptible to biochemical oxidation; but if the alkyl group is branched, bacterial degradation will either be delayed or greatly resisted, according to the degree of branching (63). As a result, sodium *n*-decyl sulfate is biologically decomposed after two days in river water, sodium *p-n*-decyl benzene sulfonate after four days, but sodium kerylbenzene sulfonate and sodium tetrapropylene benzene sulfonate persist longer than twenty days. Sawyer (64), however, reports ABS to have a half-life of about fifteen days, and hence it has been observed (66) that only 37 percent removal of ABS occurred in the Illinois River over a distance of 114 miles during about eight days of travel time.

Sodium lauryl sulfate, an anionic compound, in a concentration of 150 mg/l, was found to increase the BOD of sewage considerably, and to cause an increase in the reduction of nitrate to nitrite (68). The presence of 50-75 mg/l of secondary sodium alkyl sulfate (under the trade name Teepol) had no perceptible effect on biological sewage treatment but it has been estimated that 100-200 mg/l would cause difficulties. Leclerc and Beaujean (68) found that the presence of 300 mg/l of sodium dodecyl benzene sulfonate caused the development of reducing flora, resulting in the formation of sulfides, 60 mg/l retarded the development of proteolytic bacteria, 15-60 mg/l retarded the growth of aerobic bacteria, and 150 mg/l retarded the growth of denitrifying bacteria. The inhibitory effect of this compound on aerobic organisms is attributed to its interference with the uptake of oxygen.

Fischer (70) studied the effects of ABS on the oxygen uptake of several bacteria in two different media at 38°C. The results are presented in a series of sigmoidally shaped curves. Since it is difficult to pin-point a concentration of initial or final effect, the approximate concentrations for 50 percent inhibition of oxygen uptake were as follows (Puffer's Medium is a buffer):

Test Organism	Medium	ABS Concentration at 50% Inhibition of O_2 Uptake (Approximate mg/l)
Proteus vulgaris	Dextrose-Bouillion	2,500
	M/20 Puffer's Medium + Glucose	220
Staphylococcus aureus	Dextrose-Bouillion	40
	M/20 Puffer's Medium + Glucose	200
Mycobacterium phlei	Dextrose-Bouillion	40
	M/20 Puffer's Medium + Glucose	250

Another report states that anionic surface-active agents slightly increased the oxygen uptake of *Pseudomonas fluorescens* and accelerated the respiration of fungal spores (71), but the concentrations that effected these results were not available in the abstract. In another experiment, however, Van Beneden (69) observed that proteolytic bacteria were not affected by the presence of anionic detergents in concentrations of 500-2000 mg/l (85% active product).

Cationic detergents inhibited the development of proteolytic bacteria considerably even in extremely low concentrations, but in the presence of nonionic detergents there was a large increase in the numbers of bacteria. Cationic detergents in concentrations of 1-100 mg/l completely inhibited the development of nitrifying bacteria and algae, and in a concentration of 0.01 mg/l retarded growth for 6-10 days. Anionic detergents in concentrations of 50-600 mg/l completely inhibited the growth of nitrifying bacteria. Detergents of the alkyl sulfate type in a concentration of 5 mg/l did not interfere with the growth of algae but in higher concentrations growth was affected; detergents of the alkyl aryl sulfonate type in concentrations up to 60 mg/l did not inhibit growth of algae but in concentrations of 120-260 mg/l growth was completely stopped. Nonionic detergents had little effect on nitrifying bacteria in low concentrations and growth was only inhibited with a concentration of 50,000 mg/l. Growth of algae was inhibited by nonionic detergents in a concentration of 500 mg/l (69).

Another report (72) investigated effects of 10 to 100 mg/l concentrations of an anionic, a nonionic, and a cationic detergent upon slime growth. At concentrations that can be expected in sewers, the anionic and nonionic types were reported to stimulate slime growth significantly. The same report states that the anionic and nonionic detergents in the 10 to 100 mg/l range had no marked effect on the number of coliforms and did not increase the bactericidal efficiency of chlorine (in sewage). The cationic compounds, however, caused a considerable decrease in the number of coliforms.

There have been numerous reports of foaming in rivers, especially in turbulent reaches and at points where sewage-treatment-plant effluents flow into receiving waters (48). Indeed, it is reported that foaming caused by surface-active agents in the River Lee, in England, is now a normal occurrence (74). On one occasion froth covered an area of 1500 square yards and was built up to a height of 12 feet (48). ABS compounds are the principal surface-active agents causing foam. Under favorable conditions a concentration of 1 mg/l of ABS will cause a light froth (76) and there have been reports of foaming at concentrations as low as 0.5 mg/l (1745).

Even when foaming does not occur, a detergent-containing film may form over the surface of the water, delaying the gas exchange with the atmosphere (78). Gameson et al. (80) have shown that the presence of detergents will reduce many times the rate of re-aeration in a stream. When the "exit coefficient" in the absence of detergents was 30 cm per hour, the addition of sodium dodecyl benzene sulfonate reduced the rate of entry of oxygen by about 50 percent. When the exit coefficient in the absence of detergent was 100 cm per hour, the addition of detergents had no apparent effect. Between these two extremes, the effect of surface-active agents on re-aeration changed approximately linearly. In general, it has been stated (82) that the presence of 1 mg/l of detergent in river water reduces its capacity for reoxygenation by about 60 percent. Observations on the River Thames (1950) report that the introduction of detergents was followed by a marked lengthening of the zone devoid of oxygen.

Baars (85) stated that although the addition of synthetic detergents decreased the rate of oxygenation in diffused-air activated-sludge systems, there is evidence that oxygenation was increased with the brush-aeration method. For further information, including the aeration of mixed detergents in clean water, see Downing et al. (86).

British investigators (88) studied the effects that residual surface-active matter in an estuary may have on the rate of settling of suspended matter. Their preliminary results show that the effect of 1 mg/l of ABS is to decrease the rate of settling of the mud for any given mud concentration. In one experiment in which the detergent concentration was increased ten-fold, the effect on solids in suspension was roughly doubled.

GROUND WATER POLLUTION

Various cities have recently reported the average value of total synthetic detergents in their raw sewage to be over 40 mg/l (92, 93, 94). Many of the constituents of these detergents are not removed appreciably by treatment processes (2112). If the sewage disposal system involves a method whereby the treated or untreated effluent is released into intimate contact with the soil, ground-water pollution may result. ABS compounds are not precipitated by the calcium, magnesium, or ferric ions of the soil. Moreover, they are resistant to biological degradation in saturated ground-water flow (96). Preliminary evidence indicates, however, that some ABS can be decomposed biochemically in percolation through the zone of aeration under special conditions of intermittent application. Nevertheless, large quantities of synthetic detergents ultimately reach ground water and various cases of pollution have been reported. A survey of individual wells in a Minnesota community of 10,000 (97) indicated that 23.6 percent of the supplies contained surfactants.

After about a year of operation of an experimental sewage lagoon at Kearney, Nebraska, nearby wells were observed to have been polluted by detergents. In a study of the area, Neal et al. (99) found that in 14 months synthetic detergents had contaminated ground water within a radius of ¾ of a mile around the lagoon. An

irrigation well 800 feet away was found to contain an ABS concentration of 2.0 mg/l, and a domestic well 1,000 feet away, 1.5 mg/l.

In a rural German town, Netsch (100) reported that the waste water from a laundry was let into a village pond that had no outflow. After about 10 months of operation, changes began to occur in the water of nearby wells. Water foamed in buckets on pumping, had an unpleasant taste and odor, was refused by livestock, and finally became almost unusable. In a study of the area, it was found that synthetic detergents had polluted ground water for over 600 feet around the pond and the effect was still spreading.

In 1960, a water-quality study of the Mojave River Valley in California (102) revealed that ground water to a depth of 100 feet and extending over a distance of about 2 miles was affected by tastes, odors, and ABS. Water from some of the wells of the area frothed. The observed pollution was attributed to effluents from a municipal treatment plant and from a railroad shop disposal system.

A most famous and thoroughly studied case of groundwater pollution occurred in Suffolk County, New York (49, 96, 111, 112, 117). Here, during investigations of unusual tastes and odors in well water, synthetic detergents were found in one-third of the wells tested in concentrations up to 1.4 mg/l. The Federal Housing Administration refused to approve mortgages in this and surrounding areas when a well and sewage disposal system were to be located on the same lot, unless certain strict conditions were met (117). In a study of the area (111), it was found that pollution by synthetic detergents followed the general direction of ground-water flow, and that some of the detergents from test wells had probably been in the ground water for 1 to 3 years.

The significance of synthetic detergents as vehicles to transport bacteria, viruses, or other biological pollutants greater distances than they might normally travel in ground water has become an area of great concern (49). In this vein, Nichols and Koepp (121) maintain that the presence of ABS-type detergents is a better indicator of possible intestinal virus contamination of drinking water than is the presence of coliform organisms. In their work, they found that water samples with 3 to 10 mg/l of detergents represented nearly five times as many bacteriologically unsafe waters as the group free of detergents. In recent studies with Ohio sands, however, Robeck et al. (122) have shown that ABS at 1-10 mg/l did not have a significant influence on the movement of coliform organisms through soils that were saturated with water.

Much remains to be learned about the fate of ABS and other constituents of commercial synthetic detergents in saturated soil and the zone of aeration. Current research at the Robert A. Taft Sanitary Engineering Center (123), California Institute of Technology (124), University of Illinois (2029), University of California at Berkeley (2022), Manhattan College (111), and elsewhere should provide additional information on this subject.

DOMESTIC WATER SUPPLIES

Difficulties caused by detergents in domestic water supplies include dramatic foaming, turbidity, interference with coagulation, and production of taste and odor (1755). Among the more spectacular effects of detergents in water is the foaming phenomenon. Such a demonstration of frothing occurred in 1957 at Chanute, Kansas (125, 2119). During a drought the city officials decided to reutilize sewage effluent after circulation through a natural reservoir. Although this emergency operation was successfully conducted for five months without hazard to health, the increase of surface-active material caused the water to foam at the plant in waves accumulating to a height of 15 feet at the recarbonation basin, and in the filters during backwashing.

Foaming of ABS is reported to occur at a concentration as low as 0.5 mg/l (1745). Another paper reports laboratory work that produced a stable foam on aeration of a good quality water containing as little as 0.7 mg/l of surface-active material (2120). Truesdale (129) showed that the amount of foam produced increased linearly with the concentration of surface-active agent and the tendency to foam was greatest in clean water, decreasing progressively as the pollution was increased. Edwards et al. (136) studied the frothing of surface-active agents in the presence of common food types. This work indicated that low concentrations of casein are effective in preventing frothing of alkyl aryl sulfonates.

The foaming power of alkyl aryl sulfonates and the stability of the foam produced are greater at pH values of 3 and 9 than at 7 (138). Although the presence of surface-active agents reduces the oxygenation capacity of water, the only probable effect in a water-treatment plant aeration system would probably be the foaming that can result.

Synthetic detergents in concentrations exceeding 3.0 to 5.0 mg/l may interfere with coagulation and floc formation (818). Also, Smith et al. (2047) found that dodecyl benzene sulfonates interfered with water softening by coagulation at 8 mg/l. For the most part, however, recent research seems to indicate that the major detriment to coagulation is not the surface-active agents but the complex phosphates that are used as builder compounds in synthetic detergents (57, 2048, 2049, 2050, 2051). The Water Research Association of England investigated these findings. The surface-active alkyl aryl sulfonates were removed from a commercial product by extraction with alcohol and evaporating to an organic residue. Coagulation tests were carried out with the original packaged product, the organic fraction, and the inorganic fraction. It was found that small concentrations, 2 mg/l, of the inorganic fraction caused noticeable deterioration in results, whereas the organic fraction and the original product produced similar effects at 7 mg/l.

In the subsequent settling process, some workers have shown that common synthetic detergents in concentrations up to 100 mg/l have little effect (1704, 2052). However, it might be pointed out that with respect to the sedimentation of sewage, an increased amount of sludge is obtained after addition of detergent. It is noted that this phenomenon is a function also of the purity of the detergent, the hardness, and the pH (2053).

One of the common properties of surface-active agents is their ability to disperse colloidal particles, thus achieving the opposite of flocculation. Langelier et al. (2054) found that an alkyl aryl sulfonate at a level of 17 mg/l

had no effect on floc formation. But greater concentrations—up to 40 mg/l—showed increasing interference. One nonionic detergent at 40 mg/l and a cationic quaternary compound at 20 mg/l caused complete dispersion in a system that otherwise would have developed a good floc.

Alkyl benzene sulfonate is reported to have caused a marked head loss in the performance of rapid sand filters at concentrations greater than 5 mg/l (2055).

The bactericidal efficiency of chlorine in water treatment processes is not affected by a concentration of surface-active materials of 4 mg/l or below (2056). There is some evidence, however, that the chlorination of detergent-containing waters may produce unpleasant tastes and odors. Filicky (819) reports that 5.0 mg/l of detergents in odor-free water caused both tastes and odors. Hammerton (2056) found that the lowest concentration of mixed anionic detergents (Omo, Fab, Surf, Tide, and Wisk) that will cause a soapy taste in clean water is 4 mg/l. At a water treatment plant in Wheeling, West Virginia, Todd (2057) reported that raw water having a daily average of sythentic detergent concentration of 4 mg/l (with peaks of 12 mg/l) caused an intense fishy odor.

Threshold levels at which ABS will cause an off-taste in clean water have been reported to range from 1 mg/l (49, 1745) to higher than 40 mg/l (76, 2058). The threshold value seems to be dependent upon the purity of the compound. Tests made at the Robert A. Taft Sanitary Engineering Center at Cincinnati, Ohio, showed that the odor of purified ABS is rarely detectable at concentrations less than 1,000 mg/l and that only very sensitive individuals can taste it in water as high as 16 mg/l. In similar tests with a common household detergent containing ABS, however, an off-taste in water occurred at 2 mg/l or more of synthetic detergents (49, 1901). In another test also using the entire detergent, Campbell et al. (2059) reported the taste threshold in redistilled water for the familiar anionic "Tide" to be 12 mg/l.

Filicky et al. (2040) conducted numerous tests on odors and tastes resulting from surface-active agents. The minimum concentrations of several detergents required to produce a perceptible taste and odor in water and also the taste and odor threshold values of a 5 mg/l concentration of the compounds are presented in Table 10-1. In this work, solutions were prepared with odor-free water having a total hardness of 100 mg/l and pH of 8.3. Odor tests were conducted at 60°C, taste tests at 20° C. The same authors found that when hydrochloric acid was used to adjust the pH of the surface-active solutions from 8.3 to 5.0, the taste and odor of the alkyl sulfate was greatly intensified. The other surface-active agents showed little change in taste and odor values (see also 2984).

The increasing concentrations of surface-active agents in water supplies have led to many questions concerning the possible detrimental effects or toxicity that may be caused by these compounds. From a review of the available literature, it has been found that the degree of toxicity exhibited by surface-active agents is extremely variable. For instance, some surface-active agents have very low oral LD_{50} values, while others have proved to be excellent food supplements (2060).

As a rule, the cationic surface-active agents exhibit the greatest toxicity. However, those cationics that are effective germicides are not compatible with the anionics; they cannot exist in the same solution in any appreciable amounts and keep their identity (2061). Therefore, as long as there are anion active agents in water—and anionics are the main class causing water pollution—there should not be any reason to worry about the effects of cationics, for they will be neutralized.

Numerous authors have advanced the opinion that neither present nor future expected concentrations of surface-active agents in water supplies present any reason for alarm from a toxicological standpoint. Indeed, the British government committee on synthetic detergents has concluded that there is no evidence pointing to any serious toxic effects from anionic or nonionic detergents (1902, 2120). It may be of interest to note that Schwartz et al. (2044) claim that there is no doubt that dermatitis of the hands is on the increase since the advent of surfactants (see also 2045). However, Coughlin (2046) estimates that a housewife uses about 3000 mg/l of detergent (about 600 mg/l of ABS) in a dishpan to obtain "proper suds."

TABLE 10-1

Minimum Concentration of a Detergent Required to Produce a Perceptible Taste and Odor. Also, Taste and Odor Threshold Values of a 5 mg/l Detergent Concentration

(After Filicky, et al.) (2040)

Surface-Active Agent	*Minimum Concentration Perceptible (mg/l)*	*Threshold Value of 5 mg/l Concentration*	*Type of Taste or Odor*
Taste Results			
Alkyl aryl sulfonate	0.6	8	Limey, chemical
Alkyl aryl sulfonate	0.4	13	Chemical, soapy
Alkyl sulfate	3.0	2	Solvent
Alkyl sulfonate	1.4	4	Bitter, soapy
Sulfonated amide	2.5	2	Chemical, soapy
Odor Results			
Alkyl aryl sulfonate	0.7	7	Limey, chemical
Alkyl aryl sulfonate	0.3	17	Soapy
Alkyl sulfate	0.2	25	Aromatic, solvent
Alkyl sulfonate	0.3	21	Kerosene, soapy
Sulfonated amide	2.0	3	Chemical, soapy

Because reports of contamination of drinking water by surface-active agents are becoming more common, recognition of this contaminant seemed necessary for the recent revision of the Public Health Service Drinking Water Standards (see Chapter V, Quality Criteria for the Major Beneficial Uses of Water). Since the bulk of detergent sales are anionic, with ABS being the principal agent, the maximum permissible concentration was established in terms of ABS (2062). However, the adopted limit of 0.5 mg/l appears to have been based on esthetic rather than toxicological consideration (2063). ABS will not be toxic at this concentration, but it may cause foaming at the tap. Moreover, this concentration acts as an indicator that at least 5 percent of the water is of sewage origin (2062). Jente, et al. (2064) in a recent survey found the average concentration of ABS in

TABLE 10-2

SINGLE ORAL DOSE TOXICITIES (TOWARD MAMMALS) OF SOME SURFACE-ACTIVE AGENTS

Trade Name	Surface-Active Agent or Chemical Form	Test Animal	Approximate LD_{50}	Reference
Anionics				
Aerosol O.T.	Sodium di(2-ethylhexyl) sulfosuccinate*	Mice	4.80 gm/kg	1894
--	Sodium di(2-ethylhexyl) sulfosuccinate*	Mice	1.5 gm/kg	1895
--	Alkyl aryl amine sulfonate	Mice	2.8 gm/kg	1895
Areskap 100	Sodium monobutyl phenylphenol monosulfonate*	Mice	3.80 gm/kg	1894
Aresket 300	Sodium monobutyldiphenyl sulfonate*	Mice	3.50 gm/kg	1894
Aresklene 400	Disodium salt of dibutyl phenylphenol disulfonate*	Mice	2.20 gm/kg	1894
Igepon T	A substituted amide $C_{17}H_{35}CON(CH_3)C_2H_4SO_3Na$	Mice	6.63 gm/kg	1894
Ivory Snow	Soap	Albino Rat	> 16 ml/kg	1897
Monosulph	Highly sulfonated castor oil	Mice	>25.00 gm/kg	1894
Naccanol NRSF	Alkyl aryl sulfonate	Rat	1.4 gm/kg	1893
			1.5 gm/kg	1895
		Mice	2.8 gm/kg	1895
Nopco 2272-R	Sulfated fatty ester	Mice	8.75 gm/kg	1894
--	N-(sodium sulfoethyl) oleamide	Rat	> 4.0 gm/kg	1895
		Mice	6.3 gm/kg	1895
Santomerse D	Sodium decylbenzene sulfonate*	Mice	2.0 gm/kg	1895
		Rat	2.0 gm/kg	1893
		Mice	2.10 gm/kg	1894
Santomerse 3	Sodium dodecylbenzene sulfonate*	Rat	2.0 gm/kg	1893 1894
--	Sodium aryl alkyl polyether sulfonate	Mice	12.6 gm/kg (comm. sol. not 100%)	1895
--	Sodium hydrocarbon sulfonate	Rat	> 4.0 gm/kg	1895
--	Sodium lauryl and myristyl collamide sulfonate	Mice	2.0 gm/kg	1895
		Rat	3.5 gm/kg	1895
--	Sodium lauryl sulfate	Rat	2.7 gm/kg	1895
			1.0 gm/kg	1895
--	Sodium lauryl sulfate (30 percent solution)	Albino Rat	9.1 ml/kg	1897
--	Sodium monoethyl phenylphenol monosulfonate	Rat	2.0 gm/kg	1895
Sulfatex	Sulfated amine	Mice	>25.00 gm/kg	1894
Tergitol 4	Sodium *sec*-tetradecyl sulfate* (25 percent aqueous solution)	Albino Rat	5.0 ml/kg	1897
			1.3 gm/kg	1893
			7.9 ml/kg (purified)	1897
		Guinea Pig	2.6 ml/kg	1897
Tergitol 7	Sodium *sec*-heptadecyl sulfate* (25 percent aqueous solution)	Albino Rat	5.7 ml/kg	1897
			1.3 gm/kg	1893
		Guinea Pig	1.7 ml/kg	1897
Tergitol 08	Sodium 2-ethylhexyl sulfate* (40 percent aqueous solution)	Guinea Pig	3.8 ml/kg	1897
		Albino Rat	10.3 ml/kg	1897
Cationics				
Emcol 888	Polyalkylnaphthalene methyl pyridinium chloride	Rat	0.47 gm/kg	1893 1894
Hyamine 1622	(Diisobutylphenoxyethoxyethyl) dimethyl benzylammonium chloride*	Rat	0.42 gm/kg	1896
Hyamine 2389	Methyldodecylbenzyl trimethylammonium chloride and methyldodecylxylene bis (trimethylammonium chloride)*	Rat	0.39 gm/kg	1896
--	Methyldodecylbenzyl trimethylammonium chloride (10 percent aqueous solution)	Human	28.3 grams	1898
Roccal	Alkyl dimethyl benzyl ammonium chloride	Rat	0.34 gm/kg	1893 1894
			0.35 gm/kg	1895
Tetrosan	Alkyl dimethyl 3:4-dichlorobenzyl ammonium chloride also contains alkenyl dimethyl ethyl ammonium bromide	Mice	2.00 gm/kg	1894
Zephiran lead	--	Rat	0.34 gm/kg	1893
Nonionics				
--	Cottonseed oil monoglyceride	Rat	>90.0 ml/kg	1899
--	Glycerol monolaurate	Rat	53.4 ml/kg	1899
Igepal CA	Polyoxyethylated *tert*-octylphenol*	Mice	3.50 gm/kg	1894
Nopalco 4-0	Polyethylene Glycol 400 monooleate*	Mice	>25.00 gm/kg	1894
Nopalco 6-0	Polyethylene Glycol 600 monooleate*	Mice	>25.00 gm/kg	1894
Nopalco 6-L	Polyethylene Glycol 600 monolaurate*	Mice	>25.00 gm/kg	1894
Nopcogen 14-L	Fatty amino compound	Mice	9.65 gm/kg	1894
--	Polyoxyethylene monostearate	Hamster	19.5 ml/kg	1899
		Rat	53.0 ml/kg	1899
		Rabbit	>15.0 ml/kg	1899
--	Polyoxyethylene-8-monostearate	Hamster	27.0 ml/kg	1899
		Rat	64.0 ml/kg	1899
		Rabbit	>12.0 ml/kg	1899
--	Polyoxyethylene-20-sorbitan monolaurate	Hamster	18.0 ml/kg	1899
		Rat	36.7 ml/kg	1899
--	Polyoxyethylene-40-sorbitan monostearate	Rat	>60.0 ml/kg	1899
Span 60	Sorbitan monostearate	Rat	31.0 ml/kg	1899
Tween 80	Polyoxyethylated sorbitan monooleate (20 moles EtO)*	Mice	>25.00 gm/kg	1894
		Rat	54.5 ml/kg	1899

* Chemical form according to Rosen and Goldsmith (1900).

the drinking waters of the United States to be 0.024 mg/l ABS, with a range of 0.00 to 0.14 mg/l.

Another important aspect of surface-active agents in drinking water as a menace to public health has been pointed out by Borneff (2065). He has found that water supplies containing synthetic detergents are capable of solubilizing carcinogenic compounds such as 3,4-benzopyrene. From his study Borneff concludes: "Drinking water obtained from rivers subject to contamination with waste waters containing detergents must be considered injurious to health."

The available existing information on the toxicological properties of surface-active agents by oral administration has been summarized in Table 10-2 showing single dose (acute) toxicities, and Table 10-3, presenting toxicities from repeated doses (chronic).

Today, although a great amount of research work is being conducted, one must still agree with the statement of Woodard and Calvery (1895): "We are appalled by the many unanswered questions concerning the toxicological properties of surface-active agents."

Since water treatment processes generally remove very little of the surface-active material, the corrosive properties of syndets are deserving of mention. The corrosion of domestic equipment and plumbing, attributable to syndets uncovering the protective, greasy film on surfaces, has been mentioned as an undesirable consequence of syndets by the British Ministry of Housing (2045). This action would not be in reference to potable water lines. The major studies on the corrosive effect of these compounds have involved solutions of 0.1 to 5.0 percent (1,000 to 50,000 mg/l), and these concentrations are certainly not likely to be encountered even in polluted waters (2066, 2067, 2068, 2069, 2070, 2071, 2072). Briefly these studies indicate that temperature, pH, dissolved

TABLE 10-3

REPEATED DOSE ORAL TOXICITIES (TOWARD MAMMALS) OF SOME SURFACE-ACTIVE AGENTS

Trade name	Surface-active agent	Test animal	Medium	Level	Duration	Effects	Reference
			ANIONICS				
Aerosol O.T.	Sodium di(2-ethylhexyl) sulfosuccinate*	Rats	Diet	.25 gm/kg per day	160 days	No effect	1895
				.5 gm/kg per day	160 days	No effect	1895
				.75 gm/kg per day	160 days	Occasional diarrhea	1895
				.87 gm/kg per day	160 days	50 rats, occasional diarrhea	1907
				1.0%	2 years	Retardation of growth	1904
				1.0%	160 days	No effect	1895
				1.25%	160 days	No effect	1895
				8.0%	<4 months	All animals dead	1904
		Rabbits	Gavage	.5 gm/kg per day	6 months	3 out of 5 animals dead	1907
		Monkeys	Gavage	.125 gm/kg per day	6 months	3 monkeys no effect, larger doses vomited	1907
		Dogs	Diet	.1 gm/kg per day	6 months	3 dogs no effect	1907
				.25 gm/kg per day	6 months	3 dogs no effect, larger doses vomited	1907
		Guinea pigs	Water	2000 mg/l	180 days	Slight toxic effect	1895
--	Alkyl aryl sulfonate	Human	Diet	100 mg per day	4 months	No toxic effect on six men	1902
--	Alkyl benzene sulfonate	Humans	Oral	100 mg per day	4 months	No effect	1901
		Dogs	Diet	1 gm per day	6 months	No effect	1902
		Guinea pigs	Water	2000 mg/l	6 months	No effect	1902
		Young pigs	Diet	0.1%	79 days	No effect	1903
				0.2%	79 days	Stimulated growth	1903
				0.4%	79 days	No effect	1903
		Rats	Diet	0.5%	65 days	No effect	1902
				up to 0.5%	2 years	No effect	1909
			Water	500 mg/l	2 years	No effect	1909
Areskap 100	Sodium monobutyl phenylphenol monosulfonate*	Guinea pigs	Water	2000 mg/l	180 days	Slight toxic effect	1895
--	Lorol meta sodium sulfobenzoate	Guinea pigs	Water	2000 mg/l	180 days	Slight toxic effect	1895
--	N-(sodium sulfoethyl) oleamide	Guinea pigs	Water	2000 mg/l	180 days	Slight toxic effect	1895
--	Sodium alkyl aryl sulfonate	Guinea pigs	Water	2000 mg/l	180 days	Slight toxic effect	1895
--	Sodium alkyl sulfate	Rats	Water	1000 mg/l	160 days	No effect	1895
				2500 mg/l	160 days	Diminished growth, intestinal irritation	1895
				5000 mg/l	160 days	Diminished growth, intestinal irritation	1895
				10,000 mg/l	160 days	4 of 11 animals survived	1895
				50,000 mg/l	5 days	All animals dead	1895
				100,000 mg/l	5 days	All animals dead	1895
		Guinea pigs	Water	2000 mg/l	180 days	Slight toxic effect	1895
--	Sodium decyl benzene sulfonate	Guinea pigs	Water	2000 mg/l	180 days	Slight toxic effect	1895
--	Sodium isopropyl naphthylene sulfonate	Guinea pigs	Water	2000 mg/l	180 days	Slight toxic effect	1895
--	Sodium lauryl sulfate	Rats	Diet	1.0%	2 years	No effect	1904
				4.0%	4 months	Decreased growth	1904
				8.0%	<4 months	All animals dead	1904
			Oral	0.1 gm/kg per day	continued ?	Toxic effects in liver and intestine	1908
--	Sulfoethyl methyl olylamide	Rats	Diet	2.0%	4 months	Retardation of growth	1904
				8.0%	4 months	3 of 5 animals survived	1904
Tergitol 4	Sodium sec-tetradecyl sulfate*	Rats	Water	up to 40,000 mg/l	30 days	Possible slight toxic effect	1895
Tergitol 7	Sodium sec-heptadecyl sulfate*	Rats	Water	up to 40,000 mg/l	30 days	Possible slight toxic effect	1895
Tergitol 08	Sodium 2-ethylhexyl sulfate*	Rats	Water	up to 40,000 mg/l	30 days	Possible slight toxic effect	1895
			CATIONICS				
--	Alkyl dimethyl benzyl ammonium chloride	Rats	Diet	0.063%	2 years	Some toxic symptoms	1904
				0.5%	10 weeks	All animals dead	1904
Hyamine 1622	(Diisobutylphenoxy-ethoxyethyl)dimethyl benzylammonium chloride*	Dogs	Diet	up to 500 mg/l	1 year	No effect	1896
		Rats	Diet	<2500 ppm	2 years	Mortality not affected	1896
				>2500 ppm	2 years	Increased mortality in 10 to 30 weeks	1896
Hyamine 2389	Methyldodecylbenzyl trimethyl ammonium chloride and methyldodecylxylene bis(trimethyl) ammonium chloride*	Dogs	Diet	500 mg/l	1 year	No effect	1896
		Rats	Diet	<2500 ppm	2 years	Mortality not affected	1896
				>2500 ppm	2 years	Increased in 5 weeks	1896
Roccal	Alkyl dimethyl benzyl ammonium chloride	Guinea pigs	Water	2000 mg/l	40 days	All of 12 animals dead	1895
			NONIONICS				
--	Aryl alkyl polyether alcohol	Guinea pigs	Water	2000 mg/l	180 days	Slight toxic effect	1895
G-2125	Polyoxyethylene (4) laurate	Cockerel chicks	Diet	up to 2.0%	10 weeks	No effect	1906
G-2127	Polyoxyethylene (8) laurate	Cockerel chicks	Diet	up to 2.0%	10 weeks	No effect	1906
G-2129	Polyoxyethylene (20) laurate	Cockerel chicks	Diet	up to 2.0%	10 weeks	No effect	1906
Myrj 45	Polyoxyethylated stearic acid (8 moles EtO)*	Cockerel chicks	Diet	up to 2.0%	10 weeks	No effect	1906
Myrj 52	Polyoxyethylated stearic acid (40 moles EtO)*	Cockerel chicks	Diet	up to 2.0%	10 weeks	No effect	1906
--	Oleylpolyoxyethylene glycol ether	Rats	Water	1000 mg/l	6 months	No effect	1905
				2500 mg/l	6 months	No effect	1905
				5000 mg/l	6 months	Slight growth retardation	1905
PMS No. 1	Polyoxyethylene monostearate	Hamsters	Oral	0.5 ml per day	8 days	All of 10 survived	1899
				1.0 ml per day	8 days	4 out of 18 dead within 4 days	1899
				2.0 ml per day	8 days	8 out of 8 dead in 3 days	1899
				4.0 ml per day	1 day	All dead after 1 dose	1899
PMS No. 2	Polyoxyethylene-8-monostearate	Hamsters	Oral	1.0 ml per day	8 days	All survived	1899
				2.0 ml per day	8 days	All dead after 8 days	1899
--	Polyethylene glycol monoisooctyl phenol ether	Rats	Diet	2%	4 months	Retardation of growth	1904
Span 20	Sorbitan monolaurate*	Cockerel chicks	Diet	up to 2.0%	10 weeks	No effect	1906
Tween 20	Polyoxyethylated sorbitan monolaurate*	Cockerel chicks	Diet	up to 2.0%	10 weeks	No effect	1906
Tween 60	Polyoxyethylated sorbitan monostearate (20 mols EtO)*	Cockerel chicks	Diet	up to 2.0%	10 weeks	No effect	1906

* Chemical form according to Rosen and Goldsmith (1906).

oxygen, surface roughness, and dissolved solids may be among the important variables, depending upon the agent and concentration as well as the metal. The above references include studies on aluminum, copper, mild steel, nickel, and tin.

The use of natural surface-active agents, such as soaps, for disinfectant purposes has long been established. The work of Valko and Domagk, around 1935, however, stimulated widespread interest in the possible bactericidal potentials of the whole expanding range of synthetic surface-active agents (2044). Since that time the disinfecting power of surface-active agents has been investigated by numerous authors.

Pinsky (71) found that cation-active materials in high concentrations caused agglutination of *Pseudomonas fluorescens*. The effect was neutralized if an anionic detergent was added before or together with the cationic, but the cationic affect was irreversible if the anionic was added after 15 seconds. Cationics also appear to be bactericidal to *Escherichia coli* because they affect a specific enzyme system and not because they alter cell structure (820). Cousins and Clegg (2073) found that the mineral content of the water greatly influenced the action of quaternary ammonium compounds.

Indeed, the efficiencies of all the agents tested at 200 mg/l in hard water were less than at 50 mg/l in distilled water against *E. coli*. Chambers et al. (2074) had previously indicated the interfering minerals causing primary interference to be bicarbonates, sulfates, and chlorides of calcium and magnesium. However, in experiments on the effect of minerals and other elements on the disinfection of water by benzalkonium chloride, Klimek and Bailey (2075) found that hardness up to 450 mg/l or the presence of 5 mg/l of iron or aluminum did not reduce the effectiveness of the disinfectant against bacteria exposed to it for 2 minutes, but did tend to reduce its effectiveness over shorter periods of time. Kravitz and Stedman (2076) found no effect attributable to hardness in their testing procedures. Klimek (2077) claims the "hard water effect" is a laboratory phenomenon of questionable significance.

None of the anionic detergents were effective against cysts of *Entamoeba histolytica* in concentrations up to 1000 mg/l (742, 744), but the best cationic detergents were cysticidal at concentrations of 30 mg/l for 10 minutes or 10 mg/l for 2 hours. A neutral detergent (hexylresorcinol) was much less effective. Fair et al. (744) suggest that the use of detergents in drinking water should remain experimental until psychological and physiological factors are better known. Smith et al. (822) state that detergents are not good bactericidal agents against tubercle bacteria. For results of tests of a large number of surface-active compounds against *Mycobacterium tuberculosis* see Smith, et al. (2078).

INDUSTRIAL WATER SUPPLIES

Reports of detergents in a water supply that interfered indirectly with commercial laundry operations have been published (2079). "Blackish" water found in the distribution system was attributed to iron and manganese that may have been loosened from pipes by detergents. Also, the back-washing of water softening systems was considerably hindered.

The addition of surface-active agents to coal-washing waters prior to vacuum filtration was studied. In the abstract available, no concentrations are given. However, the effect of the detergent additions was to decrease the water content of the filter cake and double or triple the time required for filtration of a one-liter sample. In the presence of surface-active agents, filtration was claimed not to obey Poiseuille's law (2080).

IRRIGATION WATERS

With the concentration of surface-active agents continuing to increase in surface waters, a natural concern arises over the effect that these materials may have on irrigated crops. A review of the available literature, however, revealed only sparse information.

Halevy and Cathey (2081) found that the quaternary Amo-1618 (2 - isopropyl - 4 - dimethylamino - 5 - methylphenyl-1-piperidine carboxylate methyl chloride) retarded the growth of the seeds of the cucumber (*Cucumis sativus* L.) in concentrations greater than $3x10^{-6}$ Molar.

Den Dulk (2082) reported that a dressing of alkyl aryl sulfonate had a harmful effect on the development of spinach, carrots, endive, lettuce, and tomatoes. It seems that the harmful effect is based on the disturbance of the balance of humidity in the soil, the moisture draining too rapidly. Yet, the use of syndets as a soil additive has been suggested to promote water penetration and retention (2083).

Day and Tucker (2118) attributed lower yields of hay in a plot irrigated with sewage effluent to the detergents and soluble salts. They also state that oats and wheat are less sensitive to detergents and soluble salts than barley or hay.

See also Chapter IX—Aromatic Solvents.

STOCK AND WILDLIFE WATERING

The toxicological information available on animals for surface-active agents has been included in Tables 10-2 and 10-3.

An unexpected result of the pollution of waters by surface-active agents has been reported by Chase (2084). It seems the normal protective oily coating on duck feathers tends to be dissolved by synthetic detergents, thus causing the feathers to become waterlogged and the ducks to be drowned.

Wells polluted by detergents from a pond into which laundry wastes drained contained water that was "refused by livestock" (100).

It has been suggested (2083) that small amounts of alkyl aryl sulfonates added to poultry foods might result in increased weights. Results indicate that there may be weight gain, no growth response, or even weight loss. Sodium oronite at 5 mg/l in the diet was reported to have given a five percent increase in weight for chicks (2085).

FISH AND OTHER AQUATIC LIFE

The concentrations of surface-active materials normally found in American rivers (0.0-0.14 mg/l) (2064) are not generally toxic to fish or other aquatic life over short periods of time (2086). There is a possibility, however, that toxicity to aquatic organisms might result if

the organisms are exposed to these materials over a long period of time or at greater concentrations. Although there is a considerable amount of information with respect to the toxicities of detergents to various aquatic life, it is still almost impossible to compare the work of different investigators. Many variables have been noted that are of considerable importance, and unless standardized techniques are agreed upon and used, there appears to be little hope that comparisons can often be made. Investigators are generally more interested in toxicities under a specific set of conditions than in theoretical or comparative toxicities.

It has been reported by Schmassman (753) that solutions of 10 mg/l of a Swiss detergent (containing pyridin) affected the respiration of fish and 20 mg/l of a German detergent killed fish in 12 hours, but fish immersed for a short time in 100 mg/l recovered completely when transferred to fresh water. He suggested that the strong wetting action of the detergents adversely affected the exchange of gas at respiratory surfaces. An American report (2087) possibly attributes a fish kill to an 8 mg/l ABS concentration from a sewage treatment plant effluent.

Degens et al. (821) show that concentrations of 5.0 mg/l of various detergents had a lethal effect on tadpoles, sticklebacks, roach, carp, and *Daphnia* after 5 to 100 hours, depending on the detergent and organism. Fish can become acclimatized to concentrations of 9 to 36 mg/l if the build-up is gradual from 3.0 mg/l. The limiting concentrations tolerated under acclimatized conditions were reported by Degens et al. as follows:

Detergent	*Limiting Concentration in mg/l for*			
	Roach	*Rhodeus*	*Idus*	*Carp*
Sodium secondary alkyl sulfate	9	9	9	18
Alkyl aryl sulfonate	18	18	36	36
Sodium primary alkyl sulfate	9	9	9	18
Polyglycol ether of alkyl phenol	21	21	21	21

Experiments have shown that roach will normally die in waters containing 5 mg/l of detergent, but that this fish may gradually be acclimatized to live in waters containing 20 mg/l (2088). Herbert et al. (2086) observe that concentrations of 3 mg/l of alkyl aryl sulfonate in well-oxygenated, clean water produced a 50 percent mortality among rainbow trout in 12 weeks. This concentration would constitute an immediate danger to the fish if the dissolved-oxygen levels were at or near the lethal point. The work showed that the toxicity to fish of synthetic detergents is due to the surface-active material and not to the builder portions. Also, the paper points out that after sewage treatment the residues of the detergents that remain are much less toxic than the original detergents.

In a similar study, using both alkyl sulfate and alkyl aryl sulfonate, in aerated, 15-liter aquaria, and presumably at room temperature, Sierp and Thiele (2089) reported the following influences on goldfish:

Surface-Active Agent	*Concentration mg/l*	*Mortality, in percent*	*Time of Exposure*
None	0	0	14 days
Alkyl sulfate	5	0	10 days
	10	30	4 days
	15	100	20 hours
Alkyl aryl sulfonate	5	0	14 days
	10	30	2 days
	15	100	6 hours

Mann (2116) states that the characteristics of the toxic effects of surface-active agents on fish include the strong sliming of skin and gills, disturbance of equilibrium, turning to a side position, and finally, death. Mann's studies showed that the action of the toxic mechanism was on the epithelium of the gills and skin. Fish soaked in solutions of 100 mg/l or more prior to being cooked caused a burning feeling on the tongue. Lesser concentrations had no effect on the appearance or taste of the flesh. Even injecting detergents into the intestine of the fish had no effect on flesh taste. Experiments on *Tubifex, Chironomus, Carinogammus,* and *Asellus* demonstrated limiting concentrations for fishfood animals in the range of 5 to 10 mg/l. Later work (2117) showed limiting concentrations for fish of between 10 and 50 mg/l, but with *Daphnia* the first damage was observed at 5 mg/l.

The relative hardness of the water solution seems to have a definite effect upon the toxicity of surface-active agents to fish. Indeed, because of their precipitation as insoluble calcium salts, natural surface-active compounds exhibit a very greatly reduced effect in hard water (1756). Leclerc and Devlaminck (2090) reported the following toxicities to fish of synthetic and natural compounds in hard and soft water:

Surface-Active Compound	*Minimum Lethal Concentration (mg/l active product)*			
	Distilled Water		*Hard Water (244 mg/l $CaCO_3$)*	
	Dose	*Temperature °C*	*Dose*	*Temperature °C*
Synthetic:				
Sodium lauryl sulfate (anionic)	6–7	18°	6–7	23°
Sodium dodecyl benzene sulfonate (anionic)	6–7	18°	6–7	23°
Polyglycol ether (nonionic)	2–3	18°	2–3	23°
Natural				
Sodium palmitate	10–11	20°	900–950	21°
Sodium oleate	11–12	20°	1000	21°
Sodium stearate	10–12	20°	250–300	20°

Henderson et al. (1910) determined the median tolerance limits of various surface-active agents, largely with different percentages of ABS as the active ingredient. The results for both hard and soft waters have been summarized in Table 10-4. The same paper also studied the toxicities of soaps, using Lux Flakes, Ivory Snow, and Fels Naptha. The 96-hour TL_m values in soft water for the soaps varied from 29 to 42 mg/l (34 mg/l average), and in hard water from 920 to 1800 mg/l (1470 mg/l average). The same results for the five commercial synthetic detergents, Cheer, Tide, Dreft, Felso, and Vel, in soft water were 10.6 to 19.0 mg/l (15.1 mg/l average) and in hard water 4.5 to 15.6 mg/l (8.5 mg/l average) expressed in terms of 100 percent surfactant basis.

The State of Washington's Department of Fisheries has published an extensive work on toxicity studies of various pollutants to salmon and trout (2091). These studies included detergents known commercially as Blast, Santomerse D, Santomerse 3, Sterox SE, Sterox SK, and Gamlen CW. Results are presented in extensive tables that cite the fish stock, age, weight, numbers, source of water, analyses, volume, etc. Different parameters are used to express the results, but generally it was attempted to express them as a critical level (in mg/l) at which concentration obvious deleterious effects were observed. Hence, the critical level is recorded as "less than" this observed value. A brief summary of these data is presented on the following page.

Detergent	Fish	Water	Critical Level in mg/l of Total Compound
Santomerse D	Silver Salmon	Lake, aerated	<5.6
Santomerse 3	Silver Salmon	Lake, aerated	<5.6
Sterox SE	Silver Salmon	Lake, aerated	<20.0
Sterox SK	Silver Salmon	Ground, aerated	<5.6
Gamlen CW	Chinook Salmon	Sea, aerated	<17.8
Blast	Silver Salmon	Lake, aerated	<53.2
Blast	Rainbow Trout	Lake, aerated	<112.5 (67 mm fish) <150.0 (118 mm fish)

TABLE 10-4

MEDIAN TOLERANCE LIMITS OF SURFACE-ACTIVE AGENTS TO FATHEAD MINNOWS AT 25°C

(After Henderson et al.) (1910)

	Median Tolerance Limit (mg/l)					
	Soft Water *			Hard Water *		
Surface-Active Compound	24 hr	48 hr	96 hr	24 hr	48 hr	96 hr
Alkyl benzene sulfonates:						
1. 100% ABS	4.8	4.5	4.5	4.0	3.5	3.5
2. 100% ABS**	8.2	7.5	5.6	--	--	--
3. 85% ABS; 15% inert (mostly sodium sulfate)	4.5	4.2	4.2	4.4	4.4	4.4
4. 60% ABS; 40% inert (mostly sodium sulfate)	15	15	15	8.5	8.5	8.5
5. 40% ABS; 60% inert (mostly sodium sulfate)	23	23	23	12	12	12
6. 31% ABS; 57% sodium tripolyphosphate	63	61	61	21	21	17
7. 30% ABS; 27% sodium tripolyphosphate; 20% sodium sulfate	46	44	41	20	16	15
8. 22% ABS; 8% Alkyl sulfate; 65% sodium sulfate	64	62	61	28	28	28
9. 18% ABS; 47% sodium tripolyphosphate; 25% sodium sulfate	85	85	85	51	51	48
10. 9% ABS; 9% alkyl sulfate; 50% sodium tripolyphosphate; 20% sodium sulfate	59	59	59	100	90	87
Alkyl sulfate	5.9	5.1	5.1	6.1	5.9	5.9
Polyoxyethylene ester	42	37	37	56	42	38
Sodium stearate	100	100	100	>1,800	>1,800	>1,800

	EDTA Hardness	Alkalinity	Dissolved Oxygen	pH
* Soft-water analysis:	20 mg/l	18 mg/l	8.0 mg/l	7.4
Hard-water analysis:	400 mg/l	360 mg/l	8.0 mg/l	8.2

** Bluegills were the test fish here

Wurtz-Arlet (2092) published a series of experiments on the toxicity of detergents to rainbow trout (*Salmo gairdnerii*). He used trout of 10 and 65 days age, and 4 and 8 cm length in groups of 10, and of 15 cm length in groups of 2 or 5. The test water was the same in which the fish had been raised: pH 6.9 ± 0.2, alkalinity (as CaO, to methyl orange) 22 mg/l, DO 75% ± 5% of saturation, temperature 9° and 14° C. He defined the dilution limit as being the highest concentration that does not cause any apparent trouble in the fish during the experiment nor during a 24-hour follow-up observation period in which the fish had been removed to uncontaminated water. In this study, he used one-and six-hour experimental periods. Results were as follows in mg/l of active product:

Dilution Limits
(After Wurtz-Arlet) (2092)

	Sodium Alkyl Aryl Sulfonate (mg/l)		Octophenol (nonionic) (mg/l)	
Trout	1 Hour Exposure	6 Hours Exposure	1 Hour Exposure	6 Hours Exposure
10 days old	10	5	10	5
65 days old	10	7	15	7
4 cm long	12	7	25	5
8 cm long	12	7	25	5
15 cm long	12	7	25	5

Wurtz-Arlet then defines "minimum lethal dose" as that concentration which causes the death of the fish during the period of the experiment. In this study he used similar one- and six-hour periods. The results:

Minimum Lethal Dose
(After Wurtz-Arlet) (2092)

	Sodium Alkyl Aryl Sulfonate (mg/l)		Octophenol (nonionic) (mg/l)	
Trout	1 Hour Exposure	6 Hours Exposure	1 Hour Exposure	6 Hours Exposure
10 days old	20	12	30	15
65 days old	20	10	30	15
4 cm long	15	12	35	10
8 cm long	20	12	35	12
15 cm long	15	7	35	12

In a study of the time required to kill all of the fish, a concentration of 5 mg/l of the anionic killed all fish in 26 to 30 hours, whereas a similar concentration of the nonionic took from one to three days. At 30 mg/l, the anionic killed in 35 to 45 minutes whereas the nonionic took 60 to 135 minutes.

The fourth method used by Wurtz-Arlet was that of average tolerance limit, or that concentration effective in killing 50 percent of the fish in 24 hours. These tests showed values of 3 to 7 mg/l for the anionic and 3 to 10 mg/l for the nonionic.

Turnbull et al. (2093) tested the toxicity of several commercial detergents against *Lepomis machrochirus* in carbon-filtered, Philadelphia tap water of the following characteristics. Total alkalinity to methyl orange, as $CaCO_3$, was 33.0-81.0 mg/l, pH 6.9-7.5, total hardness as $CaCO_3$ 84.0-163.0 mg/l, specific conductance 228-301 micromhos, dissolved solids 154-210 mg/l, sulfates as SO_4 56.0-98.0 mg/l, and chlorides as Cl 3.0-26.0 mg/l. Using the oxygenation interface method, and reporting values in terms of the commercial product, the 24- and 48-hour TL_m values and the safe concentration in mg/l for Nalco 21 were 1.5, 1.4, and 0.37, and for alkyl aryl sulfonate were 4.2, 3.7, and 0.86, respectively. Other variables in these tests included: fish size 7 cm, fish weight 5 gms, temperature 20°C, 10 day acclimatization, DO 5 mg/l, and 10 fish per test.

A number of slime-control agents were studied using two bacteria, two molds, and a fish, the emerald shiner. The reader is referred to the original paper (2986) for the variables; the inhibiting concentration in mg/l for each agent was reported as follows:

	Nalco 21	Nalco 23	Pyridyl-mercuric acetate	Pyridyl-mercuric chloride	Roccal
Aerobacter aerogenes	750.0	450.0	1.0	1.0	200.0
Bacillus mycoides	8.0	10.0	0.15	.4.0	7.0
Aspergillis niger	2000.0	35.0	95.0	40.0	3000.
Penicillium expansum	95.0	40.0	25.0	--	3000.
Emerald shiner	0.4	0.4	0.15	0.04	10.0

Roccal, or alkyl dimethyl benzyl ammonium chloride, was tested against silver salmon (2091) and common shiner (2986). The 72-hour TL_m with salmon was 17.8 mg/l, and the TL_m in an unspecified time with shiner was between 15 and 17.5 mg/l. Although the salmon were unaffected by 10 mg/l for 72 hours, Woelke states that a concentration of 1.0 mg/l is lethal to bivalve larvae (2989).

Cairns and Scheier (2094), in an interesting, remarkably illustrated paper, show the extensive damage that is inflicted upon fish gills in relatively dilute solutions of

sodium alkyl benzene sulfonate. Acute tests with the bluegill sunfish at 20° ± 1°C in both hard and soft synthetic dilution waters, using both batch and continuous flow, indicated 24-, 48-, and 96-hour TL_m values of 17.15 to 17.44 mg/l for all test conditions. This test indicated a biologically safe concentration of 5.15 to 5.23 mg/l. Subsequent chronic studies (3 months exposure to 5.6 mg/l ABS) was reflected in severe gill damage, even though the fish's cruising-speed ability was not affected. The exposed fish also had higher oxygen consumption rates, increased erythrocyte counts, and increased parasitism in the mucous laden gill area by lower invertebrates (protozoans, rotifers, etc.) as compared to controls.

As the result of a proposal to use Tricon Oil Spill Eradicator in the San Francisco Bay area, Chadwick (2095) reported on toxicity studies of this proprietary product against striped bass which inhabit these waters. The product is an emulsifier consisting of 20 percent petroleum sulfonate, 5 percent fatty acid esters, 10 percent polyethylene glycol ether, 63 percent petroleum solvents and 2 percent alcohol, according to the manufacturer. Five striped bass ranging from 7 to 11 cm in length were placed in each of the 7500 ml aquaria through which air continuously bubbled. Temperature was maintained at 65°F. Concentrations of the product of 0.001 percent (10 mg/l) killed all fish in 10 hours or less, with the fish showing their first signs of distress in 1.5 hours. Concentrations of 0.0005 percent (5 mg/l) did not affect any death nor distress in the fish in a 48-hour exposure period.

Atlas 2082A is an emulsifier that has been used in aerial spray formulations. It is a preparation of alkyl aryl sulfonate and polyoxyethylene sorbitan esters of mixed fatty and resin acids, and has an estimated 48-hour TL_m to coho salmon underyearlings of 20.7 mg/l. A limiting concentration is estimated to be approximately 2.1 mg/l (2990).

A possible synergistic effect between synthetic detergents and ammonia has been reported by Herbert (2096), who states that a solution containing both ammonia (ammonium chloride, 10 mg/l as N) and synthetic detergents (mixture of seven proprietary powders at 10 mg/l as Manoxol O.T.) was more toxic than either solution alone. In 7.5 hours, no trout died in tests on either of the individual substances. But when combined, 50 percent of the fish died in 36 and 199 minutes (2097). This work also demonstrated that at low oxygen levels (2.16 mg/l), 1.8 mg/l of mixed detergents (as Manoxol O.T.) allowed only 44-percent survival of rainbow trout at 20° ±0.3°C in 12 hours, whereas the control without detergent (at a dissolved-oxygen level of 2.11 mg/l) had no fatalities.

Although Burgess (2962) reports that the toxicity of syndets to fish varies from 7 mg/l for sodium lauryl sulfate to 2 mg/l for the polyglycol ethers, a preliminary study on a quaternary ammonium compound indicates even lower possible toxicity values. Using dodecylacetamido dimethyl benzyl ammonium chloride and the fathead minnow, 24-, 48-, and 96-hour TL_m values were found to be, respectively, 0.72, 0.65, and 0.65 mg/l (2987). These values were obtained at a pH of 7.6, dissolved oxygen 7.8 mg/l, total alkalinity 34 mg/l, and hardness 30 mg/l. Diquat, also a quaternary ammonium compound and which is used as a herbicide, had a 48-hour TL_m for rainbow trout at 18°C of 70 mg/l, and for chinook salmon at 20°C of 28.5 mg/l. The proprietary quaternary Hyamine 1622 had a 48-hour TL_m for coho salmon at 20°C of 53 mg/l (2988).

The gradual decrease in bottom fauna of a carp pond is described to the inflow of synthetic detergents (2098). This paper also reports that decomposition of cellulose by "actively fermenting river mud" is hindered by detergents, but no figures are available in the abstract.

Jacob and Nisbet (2099) claim that quaternary ammonium compounds in concentrations of 1.2 to 2.0 mg/l can be used to control algae without "permanent" injury to fish. The compounds tested were described as "rather toxic" to minnows, varying from a minimum lethal concentration of 1 mg/l to 3-4 mg/l at 20°C depending on the length of the alkyl chain.

Several authors have investigated the effect of surface-active agents on fish-food organisms. Freeman (1712) tested the toxicity of certain sodium sulfonates toward *Daphnia magna* (temperature 23° ±1°C, pH of compound solutions in the range tested not related to effect), determining the average 100-hour toxicity threshold and the toxicity ranges. His results are summarized in the following tabulation:

Toxicity of Sodium Sulfonates to *Daphnia magna* (After Freeman) (1712)

Compound tested	Concentration in mg/l for:		
	0% kill	50% kill	100% kill
Sodium *n*-butylsulfonate	1637	3380	7827
Sodium benzenesulfonate	1895	2840	8000
Sodium *p*-chlorobenzenesulfonate	1687	2394	3007
Sodium *m*-nitrobenzenesulfonate	1000	2335	5618
Sodium *p*-phenolsulfonate	556	1876	5623
Sodium 4-nitrochlorobenzene-2-sulfonate	318	1474	3187
Sodium 2, 5-dichlorobenzene-sulfonate	389	1468	3890
Sodium *p*-bromobenzenesulfonate	200	523	843
Sodium naphthalene-β-sulfate	55	135	308
Sodium anthraquinone-x-sulfonate	10	12	30

The results demonstrated that the more complex the ring structure of the aromatic compound, the more toxic the sulfonate; thus, in order of increasing toxicity: benzene, naphthalene, anthraquinone. The substitution of various groups on the benzene ring increased the toxicity (1712). *Daphnia* was also used as a test organism by Sierp and Thiele (2089). The results of their study with alkyl sulfate and alkyl aryl sulfate are tabulated as follows:

Influence of Surface-Active Agents on *Daphnia* (After Sierp and Thiele) (2089)

	Percent Surviving					
	Alkyl Sulfate (time in hrs.)			*Alkyl Aryl Sulfate (time in hrs.)*		
Concentration m/l	*24*	*48*	*100*	*24*	*48*	*100*
0	>80	>80	>80	>80	>80	>80
5	80	>80	>80	>80	>80	20–80
10	80	>80	>80	>80	20–80	>20
15	20–80	20–80	20–80	20–80	0	0
25	20–80	20–80	20–80	0	0	0
35	20–80	<20	<20	0	0	0
50	>20	0	0	0	0	0

To determine the molluscacidal effect of surface-active agents, Jakowski (2043) tested several anionics and

nonionics against the snail *Stagnicola reflexa*. Some of his results are given in Table 10-5.

Vallejo-Friere et al. (2042) compared the molluscacidal activity of various cationic quaternary ammonium compounds on *Australorbis* snails. Some of their findings are summarized in Table 10-6.

Other diversified effects of surface-active agents on aquatic animals have been reported. Nardone et al. (2100) found that surface-active materials disrupt permeability and pseudopod formation in the rhizopod *Pelomyxa carolinensis*. In tests with radioactive Na-22 and *P. carolinensis*, it was observed that the anionic alkyl aryl sulfonate (G-3300) did not promote or inhibit sodium uptake; the cationic N-soya-N-ethyl morpholiniumethosulfate (G-271) inhibited sodium uptake; and the nonionics polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene sorbitan monopalmitate (Tween 40), polyoxyethylene sorbitan monostearate (Tween 60), polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monolaurate (Span 20), and sorbitan monooleate (Span 80), all caused an increased sodium uptake. According to Butzel et al. (2101) treatment of *Paramecium aurelia* with anionics caused the organism to lose its ability to respond to a difference in electrical potential. Cationics and nonionics were without effect on the normal movement of the animals toward the cathode.

Soaps in concentrations of 1000 to 2500 mg/l were lethal to mosquito pupae by reducing the surface tension of the water so that the pupae were unable to maintain their position and suffocated. Solutions containing 5000 to 10,000 mg/l were directly toxic to the pupae (805). In another paper, Ginsbury (756) claims that soap in a concentration of 2000 mg/l completely destroys mosquito larvae and pupae.

TABLE 10-5

TIME IN MINUTES FOR MOLLUSCACIDAL EFFECT OF SURFACE-ACTIVE AGENTS ON *STAGNICOLA REFLEXA*

(After Jakowski) (2043)

		Detergent Concentration, 1,000 mg/l				
Trade Name	*Surface-Active Agent*	*0.5*	*5.0*	*10*	*20*	*50*
ANIONICS		**MINUTES**				
Areskap 100	Sodium monobutyl phenylphenol monosulfonate*	21	10.5	8	7	5
Aresket 300	Sodium monobutylbiphenyl sulfonate*	19.5	15	12	10.5	9
Aresklene 400	Disodium salt of dibutyl phenyphenol disulfonate*	34.5	18.5	9	6.5	5.5
Duponol 80	*n*-octyl-sodium sulfate	60	30	25.5	4	3
Santomerse D	Sodium decylbenzene sulfonate*	23.25	10.5	8	6	6
Santomerse 3	Sodium dodecylbenzene sulfonate*	9	7	7	6	1
Tergitol 08	Sodium 2-ethylhexyl sulfate*	>300	>300	>300	6	4
NONIONICS						
Sterox SE	Polyoxyethylene thioether	45	22	7	5	3.5
Tergitol NPG	Alkyl-aryl-polyglycolether	13	8.5	7	6	3

* Chemical form according to Rosen and Goldsmith (1900).

14–

Water plants, too, may be affected by the presence of surface-active agents. Experiments have shown that the rooted plants *Ranunculus aquatilis, Potamogeton pectinatus,* and *Potamogeton densus* are unable to grow in 2.5 mg/l of the alkyl aryl sulfonate detergent, Santomerse. The alga *Cladophora* and the moss *Eurhynchium,* however, grow normally in 5 mg/l of the detergent (1756). Degens et al. (821) found that concentrations of 40 mg/l of synthetic detergents were not harmful to several species of water plants.

Roberts (2102) immersed strands of the plants *Callitriche, Ranunculus aquatilus,* and *Apium* in dishes filled with a treated sewage effluent, 2.5 mg/l alkyl benzene sulfonate diluted in spring water, and a spring water control. Within two weeks, the effluent (with 2-6 mg/l ABS) and the 2.5 mg/l ABS samples showed browning, loss of leaves, and turgidity, whereas the control was unaffected. Teepol, an alkyl sulfate, gave similar results. Lissapol, a nonionic, showed no adverse effect even at doses of 20 mg/l against the same three plants.

According to Wurtz-Arlet (2103) concentrations of 1.0, 5.0 and 10.0 mg/l of a nonionic surface-active agent stimulated green algal growth during the first few days, but later caused a retardation. Growth was retarded from the start by 50 mg/l. Five mg/l of a cationic surface-active agent hindered growth of green algae and 10.0 mg/l of an anionic proved slightly toxic for 25 days, after which growth commenced. Concentrations of the cationic quaternary Diquat of as low as one mg/l dem-

TABLE 10-6

48-HOUR PERCENTAGE KILL OF *AUSTRALORBIS* SNAILS IN AQUEOUS SOLUTIONS OF QUATERNARY AMMONIUM COMPOUNDS

(After Vallejo-Friere) (2042)

Trade Name	*Compound*	*Concentration mg/l*				
		1	*2.5*	*5*	*10*	*20*
Ammonix Dme	Cetyldimethylethylammonium Br	80	100	100	100	100
Ammonix G	Cetyldimethylbenzyl-Ammonium Cl	0	54	96	100	100
Ammonix Trm	Cetyltrimethylethylammonium Br	80	100	100	100	100
Bromat	Cetyltrimethylammonium Br	26	92	100	100	100
BTC	Alkyldimethylbenzylammonium Cl	0	0	0	32	100
BTC 471	Alkylethylbenzyldimethylammonium Cl	0	18	100	100	100
BTC 927	Alkyldimethylbenzyldimethylammonium Cl	0	62	84	100	100
----	Cetyldimethylammonium Br	4	64	100	100	100
----	Cetylpyridinium Cl	1	92	100	100	100
Hyamine 10X	Diisobutylcresoxyethoxyethyldimethylbenzylammonium Cl	0	24	100	100	100
Hyamine 1622	Diisobutylphenoxyethoxyethyldimethylbenzylammonium Cl	2	20	64	100	100
Onyxide	Octadecenyldimethylethylammonium Br	4	90	100	100	100
Roccal	Dimethylbenzylammonium Cl	0	10	80	100	100
Tetrosan 3, 4D	Alkyldimethyl-3, 4-dichlorobenzylammonium Cl	0	16	70	94	100
Triton K60	Lauryldimethylbenzylammonium Cl	0	8	46	64	100

onstrated some toxicity to *Ceratophyllum demersum* and *Elodea densa* in laboratory screening tests. Hyamine 1622, another proprietary quaternary, killed *E. densa* at a concentration of 2 mg/l (2988).

Williams et al. (2104) observed that species of the blue-green alga *Oscillatoria* were inhibited in growth by 5 mg/l of either one of the ammonium chloride cationic complexes known as Arquad C and Arquad D. Pearson et al. (2105) reported that 10 mg/l of sodium dodecyl sulfate or 1.0 mg/l of zephiran chloride showed marked inhibitory effects on kelp. In the same study using giant kelp *Macrocystis pyrifera*, Clendenning and North (2106) reported that the concentration of zephiran chloride causing a 50 percent inactivation of photosynthesis in bottom kelp fronds during a 4-day exposure was less than 1 mg/l, while 5-10 mg/l of sodium dodecyl sulfate were required for a similar effect (also see 2107). ABS at 5 mg/l and 2 mg/l gave complete inactivation in 48 and 96 hours, but at 0.5 mg/l it was only slightly detrimental in 96 hours, not at all in 48 hours. It is expected that the 96-hour TL_m to photosynthesis is in the range of 0.5-1.5 mg/l of ABS (2108).

TABLE 10-7

TOXICITY OF QUATERNARY AMMONIUM COMPOUNDS TO ALGAE (22°C)
(After Palmer and Maloney) (2041)

2 mg/l Concentration of the Compound or Mixture Indicated	Period of Incubation in Days											
	3	7	14	21	3	7	14	21	3	7	14	21
	Cylindrospermum				*Microcystis*				*Scenedesmus*			
Methyldodecylbenzyl trimethyl ammonium chloride (50%), 50% water	T	P	N	N	P[1]	N	N	N	T	T	T	N
Cetyldimethyl ammonium bromide (2%), alkylate ether alcohol (10%), 85% inert	N	N	N	N	N[1]	N	N	N	N	N	N	N
Dodecylacetamido dimethyl benzyl ammonium chloride (100%)	P	P	P	N	T[1]	P	P	N	T	T	T	T
High molecular alkyl-dimethyl-ammonium chloride (10%)	P	P	N	N	N[1]	N	N	N	N	P	N	N
Mixed trimethyl and trimethyl-octadecadrenyl ammonium chlorides (25%), 75% water	N	N	N	N	N[1]	N	N	N	N	P	N	N
Methyl dodecyl benzyl trimethyl ammonium chloride plus tridecyl methyl hydroxy ethyl imidazolinium chloride (10%), 90% water	N	N	N	N	N	N	N	N	P	P	P	N
	Chlorella				*Gomphonema*				*Nitzschia*			
Methyldodecylbenzyl trimethyl ammonium chloride (50%), 50% water	P	P	N	N	T	T	T	N	T	T	T	N
Cetyldimethyl ammonium bromide (2%), alkylate ether alcohol (10%), 85% inert	N	N	N	N	N	N	N	N	N	N	N	N
Dodecylacetamido dimethyl benzyl ammonium chloride (100%)	T	T	T	T	T	T	T	T	T	T	T	T
High molecular alkyl-dimethyl-ammonium chloride (10%)	P	N	N	N	N	N	N	N	P	N	N	N
Mixed trimethyl and trimethyl-octadecadrenyl ammonium chlorides (25%), 75% water	N	S	N	N	N	N	N	N	N	N	N	N
Methyl dodecyl benzyl trimethyl ammonium chloride plus tridecyl methyl hydroxy ethyl imidazolinium chloride (10%), 90% water	P	P	N	N	N	N	N	N	N	N	N	N

T = Toxic. No growth occurred in presence of test chemicals, but did in controls.
P = Partially toxic. Growth occurred in presence of test chemical, but not as profuse as in control.
N = Non-toxic. Growth in test chemical and control the same.
N[1], P[1], T[1] = *Gloeocapsa* substituted for *Microcystis*.

In a series of preliminary screenings, Palmer and Maloney (2041) tested the toxicity of several quaternary ammonium compounds to various algae. Their results are summarized in Table 10-7. The culture medium approximated Gerloff's modification of Chu No. 10 with the amount of nitrate doubled. Tests were carried out in 25 ml Erlenmeyer flasks in an illuminated room at 22°C with algae inoculations of approximately 125,000 per ml.

The Orsanco Aquatic Life Advisory Committee, in a recommendation to the Commission (see Ohio River Valley Water Sanitation Commission, Chapter III), summed up the available data on surface-active agents as follows. The average TL_m values for the alkyl benzene sulfonates range from 3.5 to 6 mg/l. Laboratory tests for the sodium dodecyl benzene sulfonates indicate a 96-hour TL_m of 3.5 mg/l. However, amounts of surfactants that are safe for aquatic life under continuous exposure remain unknown (2109).

CONCLUSION

In conclusion, it is interesting to cite some European developments. One of the recommendations of the British Committee on Synthetic Detergents of 1956 was that "manufacturers of household synthetic detergents should investigate the feasibility of producing efficient washing products based on materials which can be readily oxidized or eliminated, and will not cause persistent foaming, during sewage treatment by present available methods" (2120). Consequently, whereas the ordinary synthetic detergent of today is only capable of about 67 percent decomposition, attempts have been made to manufacture new types that will be capable of 94 percent decomposition. Some of these compounds have already given encouraging experimental results in effluents at Luton and the River Lee in England (82). Finding effective substitutes for ABS has been the main area of research in the development of these new detergents (2110). Surface-active agents based on sucrose esters, for instance, have proven very satisfactory in preliminary tests (2111).

McGauhey and Klein (2114) state that presently in both England and Germany serious consideration is being given to requiring the householder to use a detergent readily decomposed by the normal biochemical processes currently used in sewage treatment. Where these possible restrictions or new products will lead is a matter of conjecture. Chances are, however, that surface-active agents will continue to be a major water-quality problem for several more years.

APPENDIX A-1

WATER QUALITY OBJECTIVES FOR THE EXPORTED AND SUBSURFACE OUTFLOW OF GROUND WATER FROM BUNKER HILL BASIN

Santa Ana Regional Water Pollution Control Board

RESOLUTION 54-4 (Amended)

WHEREAS on October 17, 1952 this Board adopted a policy statement which reads in part as follows: "Water quality objectives for all surface streams, underground water basins, the ocean, its bays and tidewater area, will be formulated from time to time," and

WHEREAS the State Engineer on December 29, 1953 submitted a report to this Board concerning a discharge of industrial waste within Bunker Hill Basin in which were set forth certain tentative "standards to be considered the reasonable upper limit for the principal constituents in the ground waters of Bunker Hill Basin," and

WHEREAS the aforesaid standards were considered by this Board at its June 11, 1954 meeting in connection with the establishment of water quality objectives for Bunker Hill Basin and this Board at the said meeting ordered a further hearing on this matter, and

WHEREAS on August 12, 1954 a hearing was held at which all agencies and persons who had expressed an interest in the subject of water quality objectives for Bunker Hill Basin were invited to attend and at which certain valuable suggestions were presented to this Board by those attending, and

WHEREAS this Board finds and declares as a result of the aforesaid report, meeting and hearing and from its own knowledge and belief, the exported and subsurface outflow of ground water from Bunker Hill Basin is beneficially used and reused in the lower basins for domestic, agricultural and industrial purposes, and

WHEREAS this Board finds and declares likewise that ground water extracted and used within the Bunker Hill Basin is beneficially used and reused for domestic, agricultural and industrial purposes, and

WHEREAS this Board finds that in order to protect the aforesaid beneficial uses of water, it is necessary to establish water quality objectives for the exported and subsurface outflow of ground water from Bunker Hill Basin and likewise it is necessary to establish water quality objectives for ground water extracted and used within Bunker Hill Basin;

NOW THEREFORE BE IT RESOLVED that this Board hereby declares that it intends to protect the aforesaid beneficial uses of water from unreasonable impairment in quality.

BE IT FURTHER RESOLVED that, until further notice and hearing, the following water quality objectives are hereby adopted for the exported and subsurface outflow of ground water from Bunker Hill Basin, such objectives representing the maximum tolerable concentration of each of the constituents named:

Total dissolved solids	500 parts per million
Total hardness (as $CaCO_3$)	300 parts per million
Chloride	60 parts per million
Bicarbonate	300 parts per million
Boron	0.20 parts per million
Fluoride	0.8 parts per million
Sodium percent	40

BE IT FURTHER RESOLVED that, in order to maintain the foregoing water quality objectives for the exported and subsurface outflow of ground water from Bunker Hill Basin, and until further notice and hearing, the following water quality objectives are hereby adopted for the ground water extracted and used within said basin, said objectives to be used as the basis for prescribing requirements for the discharge of sewage and/or industrial waste within said Basin and to represent the maximum tolerable increment in concentration of each of the substances named over that normally found in adjacent ground water unaffected by sewage and/or industrial waste disposal:

Total dissolved solids	250 parts per million
Total hardness (as $CaCO_3$)	50 parts per million
Chloride	40 parts per million
Bicarbonate	100 parts per million
Boron	0.1 parts per million
Fluoride	0.4 parts per million
Dissolved sulfide	None
Sodium percent	20

BE IT FURTHER RESOLVED that, as future changes in conditions indicate or as the quality of ground water within Bunker Hill Basin is or may be affected by factors other than sewage and/or industrial waste disposal, the foregoing water quality objectives for the ground water extracted and used within said Basin are subject to modification in any or all respects necessary to carry out the purpose of this resolution as hereinbefore stated.

BE IT FURTHER RESOLVED that, when requirements are prescribed for a particular discharge of sewage and/or industrial waste, nothing in the foregoing shall preclude: (1) The prescribing, when necessary, of requirements in addition to compliance with the foregoing water quality objectives for ground water extracted and used within Bunker Hill Basin (2) The modification of said objectives, when such modification is found to be reasonable and in conformity with the purpose of this resolution as hereinbefore stated.

BE IT FURTHER RESOLVED that, when requirements are prescribed for each major discharge of sewage and/or industrial waste within Bunker Hill Basin, where such discharge may affect ground water, and after study of available pertinent hydrological data, at least

three indicator water wells which are in active production or capable of active production of water shall be selected to measure compliance with requirements prescribed, said wells and the method of determining such compliance to be described in the resolution prescribing such requirements.

BE IT FURTHER RESOLVED that, whenever it is found that the increase in concentration in one or more of the constituents of the water sampled from any such aforesaid indicator well has reached 50 percent of any prescribed increment in concentration, the discharger or discharger deemed responsible shall be notified in writing that a pollution is considered to be threatened.

BE IT FURTHER RESOLVED that this resolution is hereby made a supplement to the preliminary statement of long-range plans and policies with respect to water pollution control as adopted by this Board on October 17, 1952.

(Adopted January 28, 1955)

APPENDIX A-2

STATE OF CALIFORNIA

REGIONAL WATER POLLUTION CONTROL BOARD NO. 4

LOS ANGELES REGION

RESOLUTION NO. 60-76

Prescribing Requirements for the Disposal of Sewage Treatment Plant Effluent—Whittier Narrows Water Reclamation Plant Sanitation Districts of the County of Los Angeles
(File 60-129)

WHEREAS, in compliance with Section 13054 of the Water Code of the State of California, the Sanitation Districts of the County of Los Angeles have filed with this Regional Water Pollution Control Board a Report on Waste Discharge in their letter of August 24, 1960, proposing:

a) Through the joint efforts of the County of Los Angeles, the Los Angeles County Flood Control District, the Central and West Basin Replenishment District, and the Los Angeles County Sanitation Districts, a program has developed which will result in the construction of a 10 m.g.d. demonstration water reclamation plant located just above the Whittier Narrows Dam;

b) The sewage that will be treated from the water reclamation plant will be diverted from the Districts' trunk sewer which passes through the plant site, a line that serves the western portion of San Gabriel Valley.

c) This plant will be designed, constructed, and operated by the Los Angeles County Sanitation Districts, and the effluent from the plant will be spread by the Los Angeles County Flood Control District in percolating areas to the south along the Rio Hondo River;

d) Los Angeles County will furnish the money to build the plant, and the Water Replenishment District will buy the water developed; and

WHEREAS, this Board has caused the following investigation to be made with respect to this proposed waste discharge:

1. Field investigations were conducted by the staff of this Board.
2. Copies of the proposal were sent to interested governmental agencies. Replies were received from:

a) Los Angeles County Health Department—letters dated September 22 and October 24, 1960.

b) Los Angeles County Flood Control District—letter dated October 26, 1960.

c) State Department of Water Resources—communication dated October 27, 1960;

d) State Department of Public Health—letter dated November 10, 1960; and

WHEREAS, based upon the foregoing investigations this Board finds that:

1. This proposal is a direct result of an engineering report submitted to the Los Angeles County Board of Supervisors in November, 1958, on the potential reclamation of sewage in Los Angeles County, which in turn was based on a study of potential reclamation of water from sewage in Los Angeles County, reported by a Board of Engineers in 1949. One of the chief recommendations of the 1958 report was "That said waste water reclamation envision the construction and operation of a demonstration plant at the Whittier Narrows Reservoir area to provide 10 million gallons daily of reclaimed water for recharge of ground water."
2. The proposed sewage reclamation plant will be located north of San Gabriel Boulevard and west of Rosemead Boulevard between the cities of South El Monte and Montebello, Los Angeles County.
3. The proposed plant will employ the standard-rate activated sludge process followed by chlorination with provision for step aeration. All solids removed in the course of treatment, including sludge, grease, and screenings, will be pumped back into the Los Angeles County Sanitation Districts' Joint Outfall "B" for ultimate disposal through the Districts' sewerage system. The design will permit partial or complete bypassing of the plant.
4. The sewage which it is proposed to reclaim will be essentially domestic in character. Sewage samples taken in 1955 and 1960 from Joint Outfall "B" in the vicinity of the proposed plant, and analyzed by the Department of Water Resources, averaged 1435 mmho electrical conductivity, 164 ppm hardness, 121 ppm chloride, 129 ppm sulfate, 0.6 ppm boron, and 52% equivalent sodium ratio. The mineral quality of such waters is acceptable for domestic uses and Class 2 for irrigation.
5. Effluent from the proposed reclamation plant will be spread in the following areas at various times by the Los Angeles County Flood Control District:

a) *San Gabriel River,* near Siphon Boulevard, by conveyance by means of an unlined canal and pipeline. Waste water flows will then be spread within the unlined river bed downstream as far as Florence Avenue, and/or within the San Gabriel Spreading Grounds.

b) *Rio Hondo,* by direct discharge upstream of San Gabriel Boulevard. Waste water flows will percolate in the stream bed above the Rio Hondo outlet works of Whittier Narrows Dam and thence either be introduced into the unlined rising water channel for spreading in the Rio Hondo Spreading Grounds West Side or be con-

veyed downstream in the lined Rio Hondo and diverted into the spreading grounds on the east side of the channel.

c) *A spreading grounds area within Whittier Narrows Reservoir,* generally enclosed by Rosemead Boulevard, the Southern California Edison Company right-of-way for transmission lines near Legg Lake, and the Flood Control District's Zone I Bypass Ditch.

6. There is one surface diversion in the Whittier Narrows area, a diversion from the San Gabriel River about 0.5 miles downstream of the dam for citrus irrigation.
7. The principal aquifers underlying the reclamation plant site and downstream area are in hydraulic continuity with the ground surface and with one another. Liquids discharged on the surface may percolate to all of these aquifers.
8. Ground waters of the Whittier Narrows and Montebello Forebay are beneficially used for domestic, agricultural, and industrial purposes, and constitute the principal source of recharge to the producing aquifers of the Central Basin and West Coast Basin. These waters are of excellent quality, generally containing less than 60 ppm chloride, less than 150 ppm sulfate, and 200-500 ppm total dissolved solids. However, the effects of spreading imported Colorado River water have already been indicated by increasing concentrations of mineral constituents; and

WHEREAS, on November 18, 1960, a letter transmitting a proposed copy of this resolution was submitted to the Los Angeles County Sanitation Districts and copies thereof sent to interested governmental agencies and individuals with advice that objections thereto would be considered by this Board, if submitted in writing on or before December 7, 1960; and

WHEREAS, objections and recommendations received by the Board have been reviewed and considered;

NOW, THEREFORE, BE IT RESOLVED, that in order to prevent a condition of pollution of the receiving waters and to prevent creation of a nuisance, this Regional Water Pollution Control Board in accordance with authority granted by Division 7 of the Water Code of the State of California, prescribes the following requirements for the disposal of reclaimed sewage wastes into the proposed spreading areas of the Los Angeles County Flood Control District, as described hereinbefore, subject to the provisions of Section 13054 of said Water Code, which reads in part that requirements may be revised from time to time:

1. The discharge of raw sewage or any wastes containing visible solids of sewage origin shall be prohibited.
2. The effluent shall be well oxidized and stable.
3. The discharge of wastes shall not cause odors of sewage origin.
4. The wastes discharged shall at no time contain substances in concentrations toxic to human, animal, plant or fish life.
5. The wastes discharged shall not contain phenols, cresols, or any other substances in concentrations sufficient to impart objectional tastes, odors or foaming to useable waters.
6. The quality of the effluent discharged from the sewage treatment plant shall at no time exceed the following maximum limits of the constituents listed:

CONSTITUENTS	*MAXIMUM LIMIT*
Total dissolved solids, ppm	1000
Sulfate plus chloride, ppm	500
Chloride, ppm	250
Boron, ppm	2.0
Sodium equivalent ratio	60%
Lead, ppm	0.1
Fluoride, ppm	1.5
Alkyl benzene sulfonate detergents (ABS), ppm	2.0
Arsenic, ppm	0.05
Selenium, ppm	0.05
Hexavalent chromium, ppm	0.05
Copper, ppm	3.0
Iron and manganese together	0.3
Zinc	15

BE IT FURTHER RESOLVED, that this Board does hereby inform the County Sanitation Districts of Los Angeles County that the State Department of Public Health has advised the Board that they believe, in order to protect the public health the following conditions should be observed.

"1. The public should be prevented from having contact with the sewage and sewage effluent.
2. The chemical and bacterial content of the sewage percolated into the ground water should be regulated so that waters taken therefrom for domestic use are not contaminated.
3. Use of the sewage effluent for irrigation should comply with the provisions of the regulations of the State Department of Public Health.
4. The breeding of nuisance and/or disease vectors in the sewage effluent should be prevented.
5. A monitoring program of the effluent quality and the quality of the receiving waters should be maintained."

BE IT FURTHER RESOLVED, that no condition or conditions shall exist or be permitted to exist which may constitute a public nuisance under the laws of the State of California as the result of the operation of the sewage reclamation plant and disposal of effluent as proposed; and

BE IT FURTHER RESOLVED, that for the purpose of checking compliance with the conditions hereinbefore specified, and pursuant to the authority granted by Section 13055 of the Water Code of the State of California, the Los Angeles County Sanitation Districts shall commence on the first of the month following the start of operation of the sewage reclamation plant, and shall continue thereafter a monitoring program for observing, sampling, analyzing, and reporting as follows:

A. *Location of Sampling Stations.*

1. A sampling station shall be located just before the reclaimed sewage effluent leaves the water reclamation plant.

B. *Type, Frequency and Techniques of Sampling and Analyzing.*

1. All samples shall be taken and all analyses shall be performed in accordance with the latest edition of "Standard Methods for Examination of Water, Sewage, and Industrial Wastes" published by the American Public Health Association except that for specific analyses newer techniques and newer analytical instruments, such as the spectrophotometer, may be used if, in the judgment of the Executive Officer, results are obtained consistent with the objectives of these waste discharge requirements.
2. Once per month the sewage reclamation plant effluent shall be sampled hourly during the period operators are on duty and composited in proportion to flow. On this composite sample the following analyses shall be made:

   BOD (5 day at 20°C)
   Suspended solids
   Total dissolved solids
   Sulfate
   Chloride
   Boron
   Sodium equivalent ratio
   ABS detergents
   Phenols

   This series of analyses shall be known as the "partial analysis".
3. Once each quarter the analyses on the composite sample for that day from the sewage reclamation plant shall be expanded to include the following determinations:

   Lead
   Fluoride
   Arsenic
   Selenium
   Hexavalent chromium
   Copper
   Iron and manganese together
   Zinc

   This series of analyses, plus the "partial analyses" shall be known as the "extended analysis".

C. *Records and Reports.*

1. Complete records shall be maintained of all aspects of the monitoring program and results therefrom.
2. Two copies of all such reports shall be furnished upon completion to the Los Angeles Regional Water Pollution Control Board for each calendar month within fifteen (15) days after the end of such calendar month.
3. Said records and reports are public documents and shall be available for inspection during business hours at the office of the Los Angeles Regional Board; and

BE IT FURTHER RESOLVED, that the staff of this Board be, and hereby is, directed to coordinate with the Los Angeles County Sanitation Districts, the Los Angeles County Flood Control District, and the Department of Water Resources in a program to establish several appropriate wells as sampling stations for obtaining data to determine the effect, if any, which the proposed waste water disposal will have on underground receiving waters. Such data would consist of, but would not be limited to, analyses of samples pumped once every quarter at each station for the following constitutents:

Total dissolved solids
Sulfate
Chloride
ABS detergents;

and

BE IT FURTHER RESOLVED, that in prescribing these requirements it is the intent of this Board to:

1. Preserve the quality of the receiving waters, insofar as they may be affected by this waste discharge, suitable for domestic, agricultural, and industrial uses.
2. Prevent creation of a nuisance as a result of this discharge; and

BE IT FURTHER RESOLVED, that the requirements listed above do not authorize commission of any act resulting in injury to the person or property of another; and

BE IT FURTHER RESOLVED, that the County Sanitation Districts of Los Angeles County are hereby advised that these requirements do not exempt the operator of this waste disposal facility from any other law which may be applicable. The requirements listed above are not a permit; they do not legalize this proposed waste disposal facility, and they leave unaffected any further restraints on the disposal of wastes at this site which may be contained in other statutes; and

BE IT FURTHER RESOLVED, that the Executive Officer be authorized and he is hereby directed to certify and submit copies of this resolution to the County Sanitation Districts of Los Angeles County and to such other agencies and individuals as may have need therefor or as may request same.

APPENDIX B

KANSAS STATE BOARD OF HEALTH, RADIATION PROTECTION REGULATIONS

28-35-11. Maximum Permissible Dose and Concentrations. The maximum permissible doses specified in this regulation shall not be considered to apply to intentional radiation exposures applied for diagnostic or therapeutic purposes by persons licensed to practice one or more of the healing arts within the authority granted to them by the Kansas healing arts statute or by persons licensed to practice dentistry or podiatry within the authority granted to them by Kansas licensing laws applying to dentists and podiatrists.

a. The radiation doses to which individuals are exposed shall be kept to the lowest practicable level at all times.

b. The maximum permissible dose for an individual shall be considered to include all doses, from internal and external sources, from all types and energies of radiation, whether delivered simultaneously or successively, to the region of interest, during the period of measurement.

c. When the source of radiation is outside the body, the maximum permissible dose rate for individuals occupationally engaged in radiation work shall not exceed those values specified in Appendix B of these regulations.

d. When the source of radiation is within the body, radiation doses shall be controlled by limiting the average rates at which radioactive materials are taken into the body, either by inhalation or by ingestion. Where such intake results from the occurrence of radionuclides in air or water, the average concentration of the radionuclides in air or water used by individuals occupationally engaged in radiation work shall not exceed the maximum permissible concentration specified in Appendix F, Table I, of these regulations.

e. No owner or user shall utilize any radiation source so as to cause any individual in his employ under 18 years of age to receive radiation exposure exceeding one-tenth the maximum permissible dose for occupationally exposed individuals, as established in section c, of this regulation, or to be exposed to airborne or water-borne radioactive materials in concentrations in excess of the limits specified in Appendix F, Table II, of these regulations.

f. The radiation dose to any population group shall be limited to one-tenth the maximum permissible amounts stated in Appendix B and the exposure of population groups to radioactive materials in air or water shall be limited to the concentrations specified in Appendix F, Table II, of these regulations.

g. No owner or user shall possess or utilize any radiation source so as to cause any individual outside the confines of the controlled area or any population group to receive radiation exposure exceeding one-tenth the maximum permissible dose for occupationally exposed individuals, as established in section c of this regulation, or to be exposed to airborne or water-borne radioactive materials in concentrations in excess of the limits specified in Appendix F, Table II, of these regulations.

h. The determination of the dose received by individuals and degree of radiation hazard present in all places to which these regulations apply shall be guided by nationally recognized standards such as, (1) the recommendations of the National Committee on Radiation Protection and Measurements as published in Handbooks of the National Bureau of Standards, (2) recommendations and reports of the Federal Radiation Council, and (3) standards established in regulations of the United States Atomic Energy Commission.

(Authorized by G.S. 1959 Supp., 48-1103) (Effective September 1, 1961)

28-35-26

Maximum Permissible Concentrations of Radionuclides in Water *

Element Atomic Number	*Isotope*[1,4]		*Table I (uc/ml)*	*Table II (uc/ml)*
Actinium 89	Ac^{227}	S	6×10^{-5}	2×10^{-6}
		I	9×10^{-3}	3×10^{-4}
	Ac^{228}	S	3×10^{-3}	9×10^{-5}
		I	3×10^{-2}	9×10^{-5}
Americium 95	Am^{241}	S	1×10^{-4}	4×10^{-6}
		I	8×10^{-4}	2×10^{-5}
	Am^{243}	S	1×10^{-4}	4×10^{-6}
		I	8×10^{-4}	3×10^{-5}
Antimony 51	Sb^{122}	S	8×10^{-4}	3×10^{-5}
		I	8×10^{-4}	3×10^{-5}
	Sb^{124}	S	7×10^{-4}	2×10^{-5}
		I	7×10^{-4}	2×10^{-5}
	Sb^{125}	S	3×10^{-3}	1×10^{-4}
		I	3×10^{-3}	1×10^{-4}
Argon 18	A^{37}	Sub[2]	-----	-----
	A^{41}	Sub	-----	-----
Arsenic 33	As^{73}	S	1×10^{-2}	5×10^{-4}
		I	1×10^{-2}	5×10^{-4}
	As^{74}	S	2×10^{-3}	5×10^{-5}
		I	2×10^{-3}	5×10^{-5}
	As^{76}	S	6×10^{-4}	2×10^{-5}
		I	6×10^{-4}	2×10^{-5}
	As^{77}	S	2×10^{-3}	8×10^{-5}
		I	2×10^{-3}	8×10^{-5}
Astatine 85	At^{211}	S	5×10^{-5}	2×10^{-6}
		I	2×10^{-3}	7×10^{-5}
Barium 56	Ba^{131}	S	5×10^{-3}	2×10^{-4}
		I	5×10^{-3}	2×10^{-4}
	Ba^{140}	S	8×10^{-4}	3×10^{-5}
		I	8×10^{-4}	2×10^{-5}
Berkelium 97	Bk^{249}	S	2×10^{-2}	6×10^{-4}
		I	2×10^{-2}	6×10^{-4}
Beryllium 4	Be^{7}	S	5×10^{-2}	2×10^{-3}
		I	5×10^{-2}	2×10^{-3}
Bismuth 83	Bi^{206}	S	1×10^{-3}	4×10^{-5}
		I	1×10^{-3}	4×10^{-5}
	Bi^{207}	S	2×10^{-3}	6×10^{-5}
		I	2×10^{-3}	6×10^{-5}
	Bi^{210}	S	1×10^{-3}	4×10^{-5}
		I	1×10^{-3}	4×10^{-5}
	Bi^{212}	S	1×10^{-2}	4×10^{-4}
		I	1×10^{-2}	4×10^{-4}

* The values are taken from Appendix B of 10 CFR 20.
(Authorized by G. S. 1959 Supp., 48-1103)
(Effective September 1, 1961)

28-35-26—Continued

Maximum Permissible Concentrations of Radionuclides in Water *

Element Atomic Number	*Isotope*[1,2]		*Table I (uc/ml)*	*Table II (uc/ml)*
Bromine 35	Br^{82}	S	8×10^{-3}	3×10^{-4}
		I	1×10^{-3}	4×10^{-5}
Cadmium 48	Cd^{109}	S	5×10^{-3}	2×10^{-4}
		I	5×10^{-3}	2×10^{-4}
	Cd^{115m}	S	7×10^{-4}	3×10^{-5}
		I	7×10^{-4}	3×10^{-5}
	Cd^{115}	S	1×10^{-3}	3×10^{-5}
		I	1×10^{-3}	4×10^{-5}
Calcium 20	Ca^{45}	S	3×10^{-4}	9×10^{-6}
		I	5×10^{-3}	2×10^{-4}
	Ca^{47}	S	1×10^{-3}	5×10^{-5}
		I	1×10^{-3}	3×10^{-5}
Californium 98	Cf^{249}	S	1×10^{-4}	4×10^{-6}
		I	7×10^{-4}	2×10^{-5}
	Cf^{250}	S	4×10^{-4}	1×10^{-5}
		I	7×10^{-4}	3×10^{-5}
	Cf^{252}	S	7×10^{-4}	2×10^{-5}
		I	7×10^{-4}	2×10^{-5}
Carbon 6	C^{14}	S	2×10^{-2}	8×10^{-4}
	(CO_2)	Sub	———	———
Cerium 58	Ce^{141}	S	3×10^{-3}	9×10^{-5}
		I	3×10^{-3}	9×10^{-5}
	Ce^{143}	S	1×10^{-3}	4×10^{-5}
		I	1×10^{-3}	4×10^{-5}
	Ce^{144}	S	3×10^{-4}	1×10^{-5}
		I	3×10^{-4}	1×10^{-5}
Cesium 55	Cs^{131}	S	7×10^{-2}	2×10^{-3}
		I	3×10^{-2}	9×10^{-4}
	Cs^{134m}	S	2×10^{-1}	6×10^{-3}
		I	3×10^{-2}	1×10^{-3}
	Cs^{134}	S	3×10^{-4}	9×10^{-6}
		I	1×10^{-3}	4×10^{-5}
	Cs^{135}	S	3×10^{-3}	1×10^{-4}
		I	7×10^{-3}	2×10^{-4}
	Cs^{136}	S	2×10^{-3}	9×10^{-5}
		I	2×10^{-3}	6×10^{-5}
	Cs^{137}	S	4×10^{-4}	2×10^{-5}
		I	1×10^{-3}	4×10^{-5}
Chlorine 17	Cl^{36}	S	2×10^{-3}	8×10^{-5}
		I	2×10^{-3}	6×10^{-5}
	Cl^{38}	S	1×10^{-2}	4×10^{-4}
		I	1×10^{-2}	4×10^{-4}
Chromium 24	Cr^{51}	S	5×10^{-2}	2×10^{-3}
		I	5×10^{-2}	2×10^{-3}
Cobalt 27	Co^{57}	S	2×10^{-2}	5×10^{-4}
		I	1×10^{-2}	4×10^{-4}
	Co^{58m}	S	8×10^{-2}	3×10^{-3}
		I	6×10^{-2}	2×10^{-3}
	Co^{58}	S	4×10^{-3}	1×10^{-4}
		I	3×10^{-3}	9×10^{-5}
	Co^{60}	S	1×10^{-3}	5×10^{-5}
		I	1×10^{-3}	3×10^{-5}
Copper 29	Cu^{64}	S	1×10^{-2}	3×10^{-4}
		I	6×10^{-3}	2×10^{-4}
Curium 96	Cm^{242}	S	7×10^{-4}	2×10^{-5}
		I	7×10^{-4}	3×10^{-5}
	Cm^{243}	S	1×10^{-4}	5×10^{-6}
		I	7×10^{-4}	2×10^{-5}
	Cm^{244a}	S	2×10^{-4}	7×10^{-6}
		I	8×10^{-4}	3×10^{-5}
	Cm^{245}	S	1×10^{-4}	4×10^{-6}
		I	8×10^{-4}	3×10^{-5}
	Cm^{246}	S	1×10^{-4}	4×10^{-6}
		I	8×10^{-4}	3×10^{-5}
Dysprosium 66	Dy^{165}	S	1×10^{-2}	4×10^{-4}
		I	1×10^{-2}	4×10^{-4}
	Dy^{166}	S	1×10^{-3}	4×10^{-5}
		I	1×10^{-3}	4×10^{-5}
Erbium 68	Er^{169}	S	3×10^{-3}	9×10^{-5}
		I	3×10^{-3}	9×10^{-5}
	Er^{171}	S	3×10^{-3}	1×10^{-4}
		I	3×10^{-3}	1×10^{-4}
Europium 63	Eu^{152} (T/2=9.2 hrs)	S	2×10^{-3}	6×10^{-5}
		I	2×10^{-3}	6×10^{-5}
	Eu^{152} (T/2=13 yrs)	S	2×10^{-3}	8×10^{-5}
		I	2×10^{-3}	8×10^{-5}
	Eu^{154}	S	6×10^{-4}	2×10^{-5}
		I	6×10^{-4}	2×10^{-5}
	Eu^{155}	S	6×10^{-3}	2×10^{-4}
		I	6×10^{-3}	2×10^{-4}
Fluorine 9	F^{18}	S	2×10^{-2}	8×10^{-4}
		I	1×10^{-2}	5×10^{-4}
Gadolinium 64	Gd^{153}	S	6×10^{-3}	2×10^{-4}
		I	6×10^{-3}	2×10^{-4}
	Gd^{159}	S	2×10^{-3}	8×10^{-5}
		I	2×10^{-3}	8×10^{-5}
Gallium 31	Ga^{72}	S	1×10^{-3}	4×10^{-5}
		I	1×10^{-3}	4×10^{-5}
Germanium 32	Ge^{71}	S	5×10^{-3}	2×10^{-3}
		I	5×10^{-3}	2×10^{-3}
Gold 79	Au^{196}	S	5×10^{-3}	2×10^{-4}
		I	4×10^{-3}	1×10^{-4}
	Au^{198}	S	2×10^{-3}	5×10^{-5}
		I	1×10^{-3}	5×10^{-5}
	Au^{199}	S	5×10^{-3}	2×10^{-4}
		I	4×10^{-3}	2×10^{-4}
Hafnium 72	Hf^{181}	S	2×10^{-3}	7×10^{-5}
		I	2×10^{-3}	7×10^{-5}
Holmium 67	Ho^{166}	S	9×10^{-4}	3×10^{-5}
		I	9×10^{-4}	3×10^{-5}
Hydrogen 1	H^{3}	S	1×10^{-1}	3×10^{-3}
		Sub	———	———
Indium 49	In^{113m}	S	4×10^{-2}	1×10^{-3}
		I	4×10^{-2}	1×10^{-3}
	In^{114m}	S	5×10^{-4}	2×10^{-5}
		I	5×10^{-4}	2×10^{-5}
	In^{115m}	S	1×10^{-2}	4×10^{-4}
		I	1×10^{-2}	4×10^{-4}
	In^{115}	S	3×10^{-3}	9×10^{-5}
		I	3×10^{-3}	9×10^{-5}
Iodine 53	I^{126}	S	5×10^{-5}	2×10^{-6}
		I	3×10^{-3}	9×10^{-5}
	I^{129}	S	1×10^{-5}	4×10^{-7}
		I	6×10^{-3}	2×10^{-4}
	I^{131}	S	6×10^{-5}	2×10^{-6}
		I	2×10^{-3}	6×10^{-5}
	I^{132}	S	2×10^{-3}	6×10^{-5}
		I	5×10^{-3}	2×10^{-4}
	I^{133}	S	2×10^{-4}	7×10^{-6}
		I	1×10^{-3}	4×10^{-5}
	I^{134}	S	4×10^{-3}	1×10^{-4}
		I	2×10^{-2}	6×10^{-4}
	I^{135}	S	7×10^{-4}	2×10^{-5}
		I	2×10^{-3}	7×10^{-5}
Iridium 77	Ir^{190}	S	6×10^{-3}	2×10^{-4}
		I	5×10^{-3}	2×10^{-4}
	Ir^{192}	S	1×10^{-3}	4×10^{-5}
		I	1×10^{-3}	4×10^{-5}
	Ir^{194}	S	1×10^{-3}	3×10^{-5}
		I	9×10^{-4}	3×10^{-5}
Iron 26	Fe^{55}	S	2×10^{-2}	8×10^{-4}
		I	7×10^{-3}	2×10^{-3}
	Fe^{59}	S	2×10^{-3}	6×10^{-5}
		I	2×10^{-3}	5×10^{-5}
Krypton[3] 36	Kr^{85m}	Sub	———	———
	Kr^{85}	Sub	———	———
	Kr^{87}	Sub	———	———
Lanthanum 57	La^{140}	S	7×10^{-4}	2×10^{-5}
		I	7×10^{-4}	2×10^{-5}
Lead 82	Pb^{203}	S	1×10^{-2}	4×10^{-4}
		I	1×10^{-2}	4×10^{-4}
	Pb^{210}	S	4×10^{-6}	1×10^{-7}
		I	5×10^{-3}	2×10^{-4}
	Pb^{212}	S	6×10^{-4}	2×10^{-5}
		I	5×10^{-4}	2×10^{-5}
Lutetium 71	Lu^{177}	S	3×10^{-3}	1×10^{-4}
		I	3×10^{-3}	1×10^{-4}
Manganese 25	Mn^{52}	S	1×10^{-3}	3×10^{-5}
		I	9×10^{-4}	3×10^{-5}
	Mn^{54}	S	4×10^{-3}	1×10^{-4}
		I	3×10^{-3}	1×10^{-4}
	Mn^{56}	S	4×10^{-3}	1×10^{-4}
		I	3×10^{-3}	1×10^{-4}
Mercury 80	Hg^{197m}	S	6×10^{-3}	2×10^{-4}
		I	5×10^{-3}	2×10^{-4}
	Hg^{197}	S	9×10^{-3}	3×10^{-4}
		I	1×10^{-2}	5×10^{-4}
	Hg^{203}	S	5×10^{-4}	2×10^{-5}
		I	3×10^{-3}	1×10^{-4}
Molybdenum 42	Mo^{99}	S	5×10^{-3}	2×10^{-4}
		I	1×10^{-2}	4×10^{-5}
Neodymium 60	Nd^{144}	S	2×10^{-3}	7×10^{-5}
		I	2×10^{-3}	8×10^{-5}
	Nd^{147}	S	2×10^{-3}	6×10^{-5}
		I	2×10^{-3}	6×10^{-5}
	Nd^{149}	S	8×10^{-3}	3×10^{-4}
		I	8×10^{-3}	3×10^{-4}
Neptunium 93	Np^{237}	S	9×10^{-5}	3×10^{-6}
		I	9×10^{-4}	3×10^{-5}
	Np^{239}	S	4×10^{-3}	1×10^{-4}
		I	4×10^{-3}	1×10^{-4}
Nickel 28	Ni^{59}	S	6×10^{-3}	2×10^{-4}
		I	6×10^{-2}	2×10^{-3}
	Ni^{63}	S	8×10^{-4}	3×10^{-5}
		I	2×10^{-2}	7×10^{-4}
	Ni^{65}	S	4×10^{-3}	1×10^{-4}
		I	3×10^{-3}	1×10^{-4}
Niobium (Columbium) 41	Nb^{93m}	S	1×10^{-2}	4×10^{-4}
		I	1×10^{-2}	4×10^{-4}
	Nb^{95}	S	3×10^{-3}	1×10^{-4}
		I	3×10^{-3}	1×10^{-4}
	Nb^{97}	S	3×10^{-2}	9×10^{-4}
		I	3×10^{-2}	9×10^{-4}

* The values are taken from Appendix B of 10 CFR 20.
(Authorized by G. S. 1959 Supp., 48-1103)
(Effective September 1, 1961)

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Maximum Permissible Concentrations of Radionuclides in Water *

Element Atomic Number	*Isotope* [1, 2]		*Table I (uc/ml)*	*Table II (uc/ml)*
Osmium 76	Os^{185}	S	2×10^{-3}	7×10^{-5}
		I	2×10^{-3}	7×10^{-5}
	Os^{191m}	S	7×10^{-2}	3×10^{-3}
		I	7×10^{-2}	3×10^{-3}
	Os^{191}	S	5×10^{-3}	2×10^{-4}
		I	5×10^{-3}	2×10^{-4}
	Os^{193}	S	2×10^{-3}	6×10^{-5}
		I	2×10^{-3}	5×10^{-5}
Palladium 46	Pd^{103}	S	1×10^{-2}	3×10^{-4}
		I	8×10^{-3}	3×10^{-4}
	Pd^{109}	S	3×10^{-3}	9×10^{-5}
		I	2×10^{-3}	7×10^{-5}
Phosphorus 15	P^{32}	S	5×10^{-4}	2×10^{-5}
		I	7×10^{-4}	2×10^{-5}
Platinum 78	Pt^{191}	S	4×10^{-3}	1×10^{-4}
		I	3×10^{-3}	1×10^{-4}
	Pt^{193m}	S	3×10^{-2}	1×10^{-3}
		I	3×10^{-2}	1×10^{-3}
	Pt^{197m}	S	3×10^{-2}	1×10^{-3}
		I	3×10^{-2}	9×10^{-4}
	Pt^{197}	S	4×10^{-3}	1×10^{-4}
		I	3×10^{-3}	1×10^{-4}
Plutonium 94	Pu^{238}	S	1×10^{-4}	5×10^{-6}
		I	8×10^{-4}	3×10^{-5}
	Pu^{239}	S	1×10^{-4}	5×10^{-6}
		I	8×10^{-4}	3×10^{-5}
	Pu^{240}	S	1×10^{-4}	5×10^{-6}
		I	8×10^{-4}	3×10^{-5}
	Pu^{241}	S	7×10^{-3}	2×10^{-4}
		I	4×10^{-2}	1×10^{-3}
	Pu^{242}	S	1×10^{-4}	5×10^{-6}
		I	9×10^{-4}	3×10^{-5}
Polonium 84	Po^{210}	S	2×10^{-5}	7×10^{-7}
		I	8×10^{-4}	3×10^{-5}
Potassium 19	K^{42}	S	9×10^{-2}	3×10^{-4}
		I	6×10^{-4}	2×10^{-5}
Praseodymium 59	Pr^{142}	S	9×10^{-4}	3×10^{-5}
		I	9×10^{-4}	3×10^{-5}
	Pr^{143}	S	1×10^{-3}	5×10^{-5}
		I	1×10^{-3}	5×10^{-5}
Promethium 61	Pm^{147}	S	6×10^{-3}	2×10^{-4}
		I	6×10^{-3}	2×10^{-4}
	Pm^{149}	S	1×10^{-3}	4×10^{-5}
		I	1×10^{-3}	4×10^{-5}
Protoactinium 91	Pa^{230}	S	7×10^{-3}	2×10^{-4}
		I	7×10^{-3}	2×10^{-4}
	Pa^{231}	S	3×10^{-5}	9×10^{-7}
		I	8×10^{-4}	2×10^{-5}
	Pa^{233}	S	4×10^{-3}	1×10^{-4}
		I	3×10^{-3}	1×10^{-4}
Radium 88	Ra^{223}	S	2×10^{-5}	7×10^{-7}
		I	1×10^{-4}	4×10^{-6}
	Ra^{224}	S	7×10^{-5}	2×10^{-6}
		I	2×10^{-4}	5×10^{-6}
	Ra^{226}	S	4×10^{-7}	1×10^{-8}
		I	9×10^{-4}	3×10^{-5}
	Ra^{228}	S	8×10^{-7}	3×10^{-8}
		I	7×10^{-4}	3×10^{-5}
Radon 86	Rn^{220}	S	-----	-----
		I	-----	-----
	Rn^{222}	S	-----	-----
Rhenium 75	Re^{183}	S	2×10^{-2}	6×10^{-4}
		I	8×10^{-3}	3×10^{-4}
	Re^{186}	S	3×10^{-3}	9×10^{-5}
		I	1×10^{-3}	5×10^{-5}
	Re^{187}	S	7×10^{-2}	3×10^{-3}
		I	4×10^{-2}	2×10^{-3}
	Re^{188}	S	2×10^{-3}	6×10^{-5}
		I	9×10^{-4}	3×10^{-5}
Rhodium 45	Rh^{103m}	S	4×10^{-1}	1×10^{-2}
		I	3×10^{-1}	1×10^{-2}
	Rh^{105}	S	4×10^{-3}	1×10^{-4}
		I	3×10^{-3}	1×10^{-4}
Rubidium 37	Rb^{86}	S	2×10^{-3}	7×10^{-5}
		I	7×10^{-4}	2×10^{-5}
	Rb^{87}	S	3×10^{-3}	1×10^{-4}
		I	5×10^{-3}	2×10^{-4}
Ruthenium 44	Ru^{97}	S	1×10^{-2}	4×10^{-4}
		I	1×10^{-2}	3×10^{-4}
	Ru^{103}	S	2×10^{-3}	8×10^{-5}
		I	2×10^{-3}	8×10^{-5}
	Ru^{105}	S	3×10^{-3}	1×10^{-4}
		I	3×10^{-3}	1×10^{-4}
	Ru^{106}	S	4×10^{-4}	1×10^{-5}
		I	3×10^{-4}	1×10^{-5}
Samarium 62	Sm^{147}	S	2×10^{-3}*	6×10^{-5}
		I	2×10^{-3}	7×10^{-5}
	Sm^{151}	S	1×10^{-2}	4×10^{-4}
		I	1×10^{-2}	4×10^{-4}
	Sm^{153}	S	2×10^{-3}	8×10^{-5}
		I	2×10^{-3}	8×10^{-5}
Scandium 21	Sc^{46}	S	1×10^{-3}	4×10^{-5}
		I	1×10^{-3}	4×10^{-5}
	Sc^{47}	S	3×10^{-3}	9×10^{-5}
		I	3×10^{-3}	9×10^{-5}
	Sc^{48}	S	8×10^{-4}	3×10^{-5}
		I	8×10^{-4}	3×10^{-5}
Selenium 34	Se^{75}	S	9×10^{-3}	3×10^{-4}
		I	8×10^{-3}	3×10^{-4}
Silicon 14	Si^{31}	S	3×10^{-2}	9×10^{-4}
		I	6×10^{-3}	2×10^{-4}
Silver 47	Ag^{105}	S	3×10^{-3}	1×10^{-4}
		I	3×10^{-3}	1×10^{-4}
	Ag^{110m}	S	9×10^{-4}	3×10^{-5}
		I	9×10^{-4}	3×10^{-5}
	Ag^{111}	S	1×10^{-3}	4×10^{-5}
		I	1×10^{-3}	4×10^{-5}
Sodium 11	Na^{22}	S	1×10^{-3}	4×10^{-5}
		I	9×10^{-4}	3×10^{-5}
	Na^{24}	S	6×10^{-3}	2×10^{-4}
		I	8×10^{-4}	3×10^{-5}
Strontium 38	Sr^{85m}	S	2×10^{-1}	7×10^{-6}
		I	2×10^{-1}	7×10^{-6}
	Sr^{85}	S	3×10^{-3}	1×10^{-4}
		I	5×10^{-3}	2×10^{-4}
	Sr^{89}	S	3×10^{-4}	1×10^{-5}
		I	8×10^{-4}	3×10^{-5}
	Sr^{90}	S	4×10^{-6}	1×10^{-7}
		I	1×10^{-3}	4×10^{-5}
	Sr^{91}	S	2×10^{-3}	7×10^{-5}
		I	1×10^{-3}	5×10^{-5}
	Sr^{92}	S	2×10^{-3}	7×10^{-5}
		I	2×10^{-3}	6×10^{-5}
Sulfur 16	S^{35}	S	2×10^{-3}	6×10^{-5}
		I	8×10^{-3}	3×10^{-4}
Tantalum 73	Ta^{182}	S	1×10^{-3}	4×10^{-5}
		I	1×10^{-3}	4×10^{-5}
Technetium 43	Tc^{96m}	S	4×10^{-1}	1×10^{-2}
		I	3×10^{-1}	1×10^{-2}
	Tc^{96}	S	3×10^{-3}	1×10^{-4}
		I	1×10^{-3}	5×10^{-5}
	Tc^{97m}	S	1×10^{-2}	4×10^{-4}
		I	5×10^{-3}	2×10^{-4}
	Tc^{97}	S	5×10^{-2}	2×10^{-3}
		I	2×10^{-2}	8×10^{-4}
	Tc^{99m}	S	2×10^{-1}	6×10^{-3}
		I	8×10^{-2}	3×10^{-3}
	Tc^{99}	S	1×10^{-2}	3×10^{-4}
		I	5×10^{-3}	2×10^{-4}
Tellurium 52	Te^{125m}	S	5×10^{-3}	2×10^{-4}
		I	3×10^{-3}	1×10^{-4}
	Te^{127m}	S	2×10^{-3}	6×10^{-5}
		I	2×10^{-3}	5×10^{-5}
	Te^{127}	S	8×10^{-3}	3×10^{-4}
		I	5×10^{-3}	2×10^{-4}
	Te^{129m}	S	1×10^{-3}	3×10^{-5}
		I	6×10^{-4}	2×10^{-5}
	Te^{129}	S	2×10^{-2}	8×10^{-4}
		I	2×10^{-2}	8×10^{-4}
	Te^{131m}	S	2×10^{-3}	6×10^{-5}
		I	1×10^{-3}	4×10^{-5}
	Te^{132}	S	9×10^{-4}	3×10^{-5}
		I	6×10^{-4}	2×10^{-5}
Terbium 65	Tb^{160}	S	1×10^{-3}	4×10^{-5}
		I	1×10^{-3}	4×10^{-5}
Thallium 81	Tl^{200}	S	1×10^{-2}	4×10^{-4}
		I	7×10^{-3}	2×10^{-4}
	Tl^{201}	S	9×10^{-3}	3×10^{-4}
		I	5×10^{-3}	2×10^{-4}
	Tl^{202}	S	4×10^{-3}	1×10^{-4}
		I	2×10^{-3}	7×10^{-5}
	Tl^{204}	S	3×10^{-3}	1×10^{-4}
		I	2×10^{-3}	6×10^{-5}
Thorium 90	Th^{228}	S	2×10^{-4}	7×10^{-6}
		I	4×10^{-4}	1×10^{-5}
	Th^{230}	S	5×10^{-5}	2×10^{-6}
		I	9×10^{-4}	3×10^{-5}
	Th^{232}	S	5×10^{-5}	2×10^{-6}
		I	1×10^{-3}	4×10^{-5}
	Th natural	S	3×10^{-5}	1×10^{-6}
		I	3×10^{-4}	1×10^{-5}
	Th^{234}	S	5×10^{-4}	2×10^{-5}
		I	5×10^{-4}	2×10^{-5}
Thulium 69	Tm^{170}	S	1×10^{-3}	5×10^{-5}
		I	1×10^{-3}	5×10^{-5}
	Tm^{171}	S	1×10^{-2}	5×10^{-4}
		I	1×10^{-2}	5×10^{-4}

* The values are taken from Appendix B of 10 CFR 20. (Authorized by G. S. 1959 Supp., 48-1103) (Effective September 1, 1961)

28-35-26—Continued

Maximum Permissible Concentrations of Radionuclides in Water *

Element Atomic Number	*Isotope* [1, 2]		*Table I (uc/ml)*	*Table II (uc/ml)*
Tin 50	Sn^{113}	S	2×10^{-3}	9×10^{-5}
		I	2×10^{-3}	8×10^{-5}
	Sn^{125}	S	5×10^{-4}	2×10^{-5}
		I	5×10^{-4}	2×10^{-5}
Tungsten (Wolfram) 74	W^{181}	S	1×10^{-2}	4×10^{-4}
		I	1×10^{-2}	3×10^{-4}
	W^{185}	S	4×10^{-3}	1×10^{-4}
		I	3×10^{-3}	1×10^{-4}
	W^{187}	S	2×10^{-3}	7×10^{-5}
		I	2×10^{-3}	6×10^{-5}
Uranium 92	U^{230}	S	1×10^{-4}	5×10^{-6}
		I	1×10^{-4}	5×10^{-6}
	U^{232}	S	8×10^{-4}	3×10^{-5}
		I	8×10^{-4}	3×10^{-5}
	U^{233}	S	9×10^{-4}	3×10^{-5}
		I	9×10^{-4}	3×10^{-5}
	U^{234}	S	9×10^{-4}	3×10^{-5}
		I	9×10^{-4}	3×10^{-5}
	U^{235}	S	8×10^{-4}	3×10^{-5}
		I	8×10^{-4}	3×10^{-5}
	U^{236}	S	1×10^{-3}	3×10^{-5}
		I	1×10^{-3}	3×10^{-5}
	U^{238}	S	1×10^{-3}	4×10^{-5}
		I	1×10^{-3}	4×10^{-5}
	U natural	S	5×10^{-4}	2×10^{-5}
		I	5×10^{-4}	2×10^{-5}
Vanadium 23	V^{48}	S	9×10^{-4}	3×10^{-5}
		I	8×10^{-4}	3×10^{-5}
Xenon 54	Xe^{131m}	Sub	------	------
	Xe^{133}	Sub	------	------
	Xe^{135}	Sub	------	------
Ytterbium 70	Yb^{175}	S	3×10^{-3}	1×10^{-4}
		I	3×10^{-3}	1×10^{-4}
Yttrium 39	Y^{90}	S	6×10^{-4}	2×10^{-5}
		I	6×10^{-4}	2×10^{-5}
	Y^{91m}	S	1×10^{-1}	3×10^{-3}
		I	1×10^{-1}	3×10^{-3}
	Y^{91}	S	8×10^{-4}	3×10^{-5}
		I	8×10^{-4}	3×10^{-5}
	Y^{92}	S	2×10^{-3}	6×10^{-5}
		I	2×10^{-3}	6×10^{-5}
	Y^{93}	S	8×10^{-4}	3×10^{-5}
		I	8×10^{-4}	3×10^{-5}
Zinc 30	Zn^{65}	S	3×10^{-3}	1×10^{-4}
		I	5×10^{-3}	2×10^{-4}
	Zn^{69m}	S	2×10^{-3}	7×10^{-5}
		I	2×10^{-3}	6×10^{-5}
	Zn^{69}	S	5×10^{-2}	2×10^{-3}
		I	5×10^{-2}	2×10^{-3}
Zirconium 40	Zr^{93}	S	2×10^{-2}	8×10^{-4}
		I	2×10^{-2}	8×10^{-4}
	Zr^{95}	S	2×10^{-3}	6×10^{-5}
		I	2×10^{-3}	6×10^{-5}
	Zr^{97}	S	5×10^{-4}	2×10^{-5}
		I	5×10^{-4}	2×10^{-5}

Footnotes.

[1]Soluble (S); Insoluble (I).

[2]"Sub" means that values given are for submersion in an infinite cloud of gaseous material.

Note 1. In any case where there is a mixture of more than one radionuclide, the limiting values for (these) purposes should be determined as follows:

a. If the identity and concentration of each radionuclide in the *mixture* are *known*, the limiting values should be derived as follows: Determine, for each radionuclide in the mixture, the ratio between the quantity present in the mixture and the limit otherwise established in this Appendix for the specific radionuclide when not in a mixture. The sum of such ratios for all the radionuclides in the mixture may not exceed "1" (i.e., "unity").

Example: If radionuclides A, B, and C are present in concentrations C_A, C_B, and C_C, and if the applicable MPC's are MPC_A, MPC_B, and MPC_C respectively, then the concentrations shall be limited so that the following relationship exists:

$$\frac{C_A}{MPC_A} + \frac{C_B}{MPC_B} + \frac{C_C}{MPC_C} \leqq 1$$

b. If either the identity or the concentration of any radionuclide in the mixture is not known, the limiting values for purposes of this Appendix shall be:

(1) For purposes of Table I, Col. 2—3×10^{-7}
(2) For purposes of Table II, Col. 2—1×10^{-8}

c. If the conditions specified below are met, the corresponding values specified below may be used in lieu of those specified in paragraph 2 above.

Element (atomic number) and Isotope	*Table I (uc/ml)*	*Table II (uc/ml)*
If it is known that Sr^{90}, I^{129}, Pb^{210}, Po^{210}, At^{211}, Ra^{223}, Ra^{224}, Ra^{226}, Ac^{227}, Ra^{228}, Th^{230}, Pa^{231}, Th^{232}, and Th-nat are not present	9×10^{-5}	3×10^{-6}
If it is known that Sr^{90}, I^{129}, Pb^{210}, Po^{210}, Ra^{223}, Ra^{226}, Ra^{228}, Pa^{231}, and Th-nat are not present	6×10^{-5}	2×10^{-6}
If it is known that Sr^{90}, Pb^{210}, Ra^{226}, and Ra^{228} are not present	2×10^{-5}	6×10^{-7}
If it is known that Ra^{226} and Ra^{228} are not present	3×10^{-6}	1×10^{-7}
If it is known that alpha-emitters and Sr^{90}, I^{129}, Pb^{210}, Ac^{227}, Ra^{228}, Pa^{230}, Pu^{241}, and Bk^{249} are not present	----	----
If it is known that alpha-emitters and Pb^{210}, Ac^{227}, Ra^{228}, and Pu^{241} are not present	----	----
If it is known that alpha-emitters and Ac^{227} are not present	----	----
If it is known that Ac^{227}, Th^{230}, Pa^{231}, Pu^{238}, Pu^{239}, Pu^{240}, Pu^{242}, and Cf^{249} are not present	----	----
If Pa^{231}, Pu^{238}, Pu^{240}, Pu^{242}, and Cf^{249} are not present	----	----

Note 2. For the purposes of 28-35-19, section *a*, paragraph (1), subparagraph (b), the values in Table II of this Appendix shall be multiplied by 5,000.

Note 3. For the purposes of 28-35-19, section *a*, paragraph (2), subparagraph (b), the values in Table II of this Appendix shall be multiplied by 500.

* The values are taken from Appendix B of 10 CFR 20.
(Authorized by G. S. 1959 Supp., 48-1103)
(Effective September 1, 1961)

APPENDIX C

STATE OF MISSOURI

WATER QUALITY OBJECTIVES—MISSISSIPPI RIVER

GENERAL OBJECTIVES

All wastes, including sanitary sewage, storm water, and industrial effluents, shall be in such condition when discharged to the Mississippi River that they will not create conditions in that stream which will adversely affect the use of those waters for the following purposes: source of domestic water supply or industrial water supply, navigation, fish and wildlife, recreation, agriculture and other riparian activities.

In general, adverse conditions are caused by:

A. Excessive bacterial, physical or chemical contamination.

B. Unnatural deposits in the stream, interfering with fish and wildlife, recreation, or destruction of aesthetic values.

C. Materials imparting objectionable colors, tastes or odors to waters used for domestic or industrial purposes.

D. Floating materials, including oils, grease, garbage, sewage solids, or other refuse.

SPECIFIC OBJECTIVES

In more specific terms, adequate controls of pollution will necessitate the following objectives for:

A. Sanitary Sewage and Storm Water

Sufficient treatment for adequate removal or reduction of solids, bacteria and chemical constituents which may interfere unreasonably with the use of these waters for the purposes afore-mentioned. Adequate protection for these waters would be provided by:

(a) Substantially complete romoval of floating and settleable solids; and

(b) Removal of not less than forty-five percent of the total suspended solids.

B. Industrial Wastes

(1) Chemical Wastes—Phenolic Type

Industrial waste effluents from phenolic hydrocarbon and other chemical plants will cause objectionable tastes or odors in drinking or industrial water supplies and may taint the flesh of fish. Adequate protection should be provided for these waters if the concentration of phenol or phenol equivalents does not exceed a monthly average of 2 parts per billion and a maximum of 5 parts per billion at any point in these waters following initial dilution.

(2) Chemical Wastes—Other Than Phenolic

Adequate protection should be provided if:

(a) The pH of these waters following initial dilution is not less than 6.7 or more than 8.5.

(b) The odor-producing substances in the effluent are reduced to a point that following initial dilution with these waters the mixture does not have a monthly average threshold odor number in excess of 30.

(c) Oils and floating solids are reduced to a point such that they will not create fire hazards, coat hulls of water craft, injure fish or wildlife or their habitat, or will adversely affect public or private recreational development or other legitimate shoreline developments or uses. In order to accomplish this it will be necessary to reduce concentrations of such materials discharged to the point where after initial dilution the stream shall be free of noticeable floating solids, oil, grease and sleek.

(3) Highly Toxic Wastes

Adequate protection should be provided for these waters if substances highly toxic to human, fish, aquatic or wildlife are eliminated or reduced to safe limits.

(4) Deoxygenating Wastes

Adequate protection of these waters should result if sufficient waste reduction process changes or if sufficient treatment is provided to accomplish substantially the same objectives as are herein set up for sanitary sewage.

WATER QUALITY OBJECTIVES—BLUE RIVER JACKSON COUNTY, MISSOURI

The Missouri Water Pollution Board, in cooperation with the Missouri Conservation Commission, has established Water Quality Objectives for the Blue River in Jackson County, Missouri. The Blue River has been divided into three zones which we believe will allow the maximum use of the stream for the various users. *Zone 1*—Above the entrance of Brush Creek. This zone is established for recreational usage. The allowable concentrations of pollutants are to be based upon a stream flow of 1.88 cfs., which is exceeded 90% of the time according to the U.S.G.S. gauge at Bannister Road. *Zone 2*—From Brush Creek to Guinotte Dam. This zone allows for industrial use of the stream, and will also allow fish to survive for short periods of time. This zone is not utilized for fishing. The allowable concentrations of pollutants are to be based upon a stream flow of 1.88 cfs., plus additional flow not pumped from Blue River as provided by industry on a sustained basis. *Zone 3*—Below Guinotte Dam—Essentially the same Water Quality Objectives as for the Missouri River in the Metropolitan Kansas City area.

Each industry will be required to maintain effective waste reduction and prevention programs irrespective of any objectives established for pollutants in the Blue River.

WATER QUALITY OBJECTIVES—BLUE RIVER ZONE 1 (UPSTREAM FROM BRUSH CREEK)

No taste and odor producing substances to be discharged.
Dissolved Oxygen—greater than 5.0 mg/l.
Biochemical oxygen demand—3-4 mg/l.
pH—6.5-8.5
Odor—none
Color—not objectionable
Scum and floating debris—none
Sludge deposits—none
Iron—1.0 mg/l.
Copper—0.2 mg/l.
Total Chromium—1.0 mg/l.
Cyanide—0.02 mg/l.
Zinc—0.01 mg/l.
Cadmium—0.2 mg/l.
Emulsified Crude Oil—0.3 mg/l.
Chloride—less than 250 mg/l.
Sulfate—less than 250 mg/l.
Nickel—0.8 mg/l.
Aluminum—0.07 mg/l.
Ammonia—0.3 mg/l.
Lead—0.1 mg/l.
Fluoride—1.5 mg/l.
Floating Oil—none

Complete treatment of sanitary sewage. Unpolluted cooling water may be discharged to stream provided the temperature elevation does not kill fish.

WATER QUALITY OBJECTIVES—BLUE RIVER ZONE 2 (BRUSH CREEK TO GUINOTTE DAM)

Floating Oil—none
Sludge Deposits—none
No taste and odor producing substances to be discharged.
Treatment of organic waste to maintain Dissolved Oxygen of 3 mg/l. in stream. Complete treatment of sanitary sewage.
pH—6.0-9.5
Iron—5 mg/l.
Copper—1.0 mg/l.
Total Chromium—5.0 mg/l.
Cyanide—0.02 mg/l.
Zinc—0.3 mg/l.
Cadmium—0.4 mg/l.
Emulsified Crude Oil—0.3 mg/l.
Chloride—less than 250 mg/l.
Sulfate—less than 250 mg/l.
Nickel—0.8 mg/l.
Aluminum—0.07 mg/l.
Ammonia—0.3 mg/l.
Lead—0.1 mg/l.
Fluoride—1.5 mg/l.
Unpolluted cooling water may be discharged to stream provided temperature elevation does not kill fish.
Odor—none
Color—not objectionable
No scum or floating debris.

WATER QUALITY OBJECTIVES—BLUE RIVER ZONE 3 (BELOW GUINOTTE DAM)

1. Primary treatment of sanitary waste until such time as Kansas City pipes waste to Missouri River.
2. No taste and odor producing substances to be discharged.
3. No floating oil.
4. Primary treatment of wastes containing floating and settleable organic solids to prevent formation of sludge banks and floating materials.
5. Eliminate batch discharges of toxic wastes.
6. Additional treatment of organic wastes as necessary to prevent a nuisance in the stream.

WATER QUALITY OBJECTIVES—MISSOURI RIVER

"Subject to (a) such higher objectives as may be desired by any State and (b) such other modification as may be deemed necessary, after appropriate investigation, by the State Water Pollution Control Agency concerned, the Engineering Section, Missouri Basin Health Council recommends the following for consideration by the member States as a tentative guide for water pollution control activities in the Missouri Drainage Basin:

1. Toxic substances including metallic ions, phenolic compounds, oils, alkalies, and acids should be virtually eliminated from sewage effluents.
2. Removal of settleable and floating solids to prevent sludge banks, floating materials, and nuisance conditions should be required as the minimum acceptable treatment.
3. For industrial wastes similar in nature to municipal wastes, treatment should be based on the same premise as recommended for municipalities. For industrial wastes not comparable to municipal wastes, treatment should be provided as necessary to prevent deterioration of water quality for beneficial uses in the receiving stream.
4. Additional treatment over that specified in item 2 above should be provided as necessary to protect downstream water uses. Quality objectives for certain water uses are:
   (a) For water serving as a source of domestic supply, raw water bacteriological quality should conform to that recommended in "Manual of Recommended Water Sanitation Practices," 1946, Public Health Bulletin 296, pages 11-13.
   (b) 'For bathing or swimming waters, monthly arithmetical average 'most probable number' of coliform organisms should not exceed 1,000 per 100 ml. during any month of the recreation season; nor exceed this number in more than 20 percent of the samples examined during any such month; nor exceed 2,400 per 100 ml. on any day. For nonbathing or nonswimming waters, the monthly arithmetical average 'most probable number' of coliform organisms should not exceed 5,000 per 100 ml. in any month of the recreational season, nor should exceed this number in more than 20 percent of the samples examined during any such month.'

(c) For fish and aquatic life a minimum oxygen concentration of 5.0 ppm. is desirable in waters otherwise offering suitable fishery habitat, with 6.0 ppm. for trout streams.

(d) For bacteriological quality of irrigation water:

(1) Sewage used for irrigation of forage crops should be given treatment insuring a consistent reduction in coliform bacteria of not less than 90 percent.

(2) Sewage used for irrigation of human food crops should be given treatment insuring a consistent reduction (a) in suspended solids of not less than 75%, (b) in 5 day 20°C. B.O.D. of not less than 75% and (c) in coliform bacteria of not less than 98 percent, unless discharged into a stream where the volume of dilution water, available during the low flow periods during which irrigation diversions may occur, is at least 10 times greater than the anticipated maximum daily volume of sewage, in which case a bacterial reduction of 90 percent may be acceptable.

Provided, however, that where the time of passage from plant discharge to irrigation diversion is such as to permit the normal death rate of bacteria as evidenced by the die-away curve to provide equivalent bacterial reduction, the requirements of (1) and (2) above may be appropriately modified."

APPENDIX D

CLASSIFICATIONS AND STANDARDS OF QUALITY AND PURITY

Adopted by New York Water Pollution Control Board
Pursuant to Article 12, Public Health Law

ADOPTING ORDER

Pursuant to the authority contained in Article 12 of the Public Health Law, the Board having held public hearings as therein provided, hereby adopts the following classifications and standards of quality and purity for the waters of New York State:

I. GENERAL CLASSIFICATION-STANDARDS SYSTEM

1. DEFINITIONS

The several terms, words or phrases hereinafter mentioned shall be construed as follows:

a. Best usage of waters as specified for each class shall be those uses as determined by the Board in accordance with the considerations prescribed by Section 1209 of the Public Health Law.
b. Approved treatment as applying to water supplies means treatment accepted as satisfactory by the authorities responsible for exercising supervision over the sanitary quality of water supplies.
c. Source of water supply for drinking, culinary or food processing purposes shall mean any source, either public or private, the waters from which are used for domestic consumption or used in connection with the processing of milk, beverages, foods or for other purposes which require finished water meeting U. S. Public Health Service Drinking Water Standards.
d. Fishing shall include the propagation of fish and other aquatic life.
e. Agricultural shall include use of waters for stock watering, irrigation and other farm purposes but not as source of water supply for drinking, culinary or food processing purposes.
f. Tidal salt waters shall mean all tidal waters which are so designated by the Board and which generally shall have a chloride ion content in excess of 250 parts per million.

2. CONDITIONS APPLYING TO ALL CLASSIFICATIONS AND STANDARDS

a. In any case where the waters into which sewage, industrial wastes or other wastes effluents discharge are assigned a different classification than the waters into which such receiving waters flow, the standards applicable to the waters which receive such sewage or wastes effluents shall be supplemented by the following:

"The quality of any waters receiving sewage, industrial wastes or other wastes discharges shall be such that no impairment of the best usage of waters in any other class shall occur by reason of such sewage, industrial wastes or other wastes discharges."

b. Natural waters may on occasion have characteristics outside of the limits established by the standards. The standards adopted herein relate to the condition of waters as affected by the discharge of sewage, industrial wastes or other wastes.

3. CLASSES AND STANDARDS FOR FRESH SURFACE WATERS

CLASS AA

Best usage of waters: Source of water supply for drinking, culinary or food processing purposes and any other usages.

Conditions related to best usage: The waters, if subjected to approved disinfection treatment, with additional treatment if necessary to remove naturally present impurities, meet or will meet U. S. Public Health Service Drinking Water Standards and are or will be considered safe and satisfactory for drinking water purposes.

Quality Standards for Class AA Waters

Items	Specifications
1. Floating solids; settleable solids; oil; sludge deposits; tastes or odor producing substances.	None attributable to sewage, industrial wastes or other wastes.
2. Sewage or wastes effluents.	None which are not effectively disinfected.
3. pH.	Range between 6.5 and 8.5
4. Dissolved oxygen.	For trout waters, not less than 5.0 parts per million; for non-trout waters, not less than 4.0 parts per million.
5. Toxic wastes, deleterious substances, colored or other wastes or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to be injurious to fish life, make the waters unsafe or unsuitable as a source of water supply for drinking, culinary or food processing purposes or impair the waters for any other best usage as determined for the specific waters which are assigned to this class.

NOTE No. 1: In determining the safety or suitability of waters in this class for use as a source of water supply for drinking, culinary or food processing purposes after approved treatment, the Water Pollution Control Board will be guided by the standards specified in the latest edition of "Public Health Service Drinking Water Standards" published by the United States Public Health Service.

NOTE No. 2: With reference to certain toxic substances as affecting fish life, the establishment of any single numerical standard for waters of New York State would be too restrictive. There are many waters, which because of poor buffering capacity and composition, will require special study to determine safe concentrations of toxic substances. However, based on non-trout waters of approximately median alkalinity (80 p.p.m.) or above for the state, in which groups most of the waters near industrial areas in this state will fall, and without considering increased or decreased toxicity from possible combinations, the following may be considered as safe stream concentrations for certain substances to comply with the above standard for this type of water. Waters of lower alkalinity must be specially considered since the toxic effect of most pollutants will be greatly increased.

Ammonia or Ammonium compounds	Not greater than 2.0 parts per million (NH_3) at pH of 8.0 or above
Cyanide	Not greater than 0.1 part per million (CN)
Ferro- or Ferricyanide	Not greater than 0.4 parts per million ($Fe(CN)_6$)
Copper	Not greater than 0.2 parts per million (Cu)
Zinc	Not greater than 0.3 parts per million (Zn)
Cadmium	Not greater than 0.3 parts per million (Cd)

CLASS A

Best usage of waters: Source of water supply for drinking, culinary or food processing purposes and any other usages.

Conditions related to best usage: The waters, if subjected to approved treatment equal to coagulation, sedimentation, filtration and disinfection, with additional treatment if necessary to reduce naturally present impurities, meet or will meet U.S. Public Health Service Drinking Water Standards and are or will be considered safe and satisfactory for drinking water purposes.

Quality Standards for Class A Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	None which are readily visible and attributable to sewage, industrial wastes or other wastes or which deleteriously increase the amounts of these constituents in receiving waters after opportunity for reasonable dilution and mixture with the wastes discharged thereto.
2. Sewage or waste effluents.	None which are not effectively disinfected.
3. Odor producing substances contained in sewage, industrial wastes or other wastes.	The waters after opportunity for reasonable dilution and mixture with the wastes discharged thereto shall not have an increased threshold odor number greater than 8, due to such added wastes.
4. Phenolic compounds.	Not greater than 5 parts per billion (Phenol).
5. pH.	Range between 6.5 and 8.5.
6. Dissolved oxygen.	For trout waters, not less than 5.0 parts per million; for non-trout waters, not less than 4.0 parts per million.
7. Toxic wastes, oil, deleterious substances, colored or other wastes or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to be injurious to fish life, make the waters unsafe or unsuitable as a source of water supply for drinking, culinary or food processing purposes or impair the waters for any other best usage as determined for the specific waters which are assigned to this class.

NOTE: Refer to Notes 1 and 2 under Class AA, which are also applicable to Class A standards.

CLASS B

Best usage of waters: Bathing and any other usages except as source of water supply for drinking, culinary or food processing purposes.

Quality Standards for Class B Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	None which are readily visible and attributable to sewage, industrial wastes or other wastes or which deleteriously increase the amounts of these constituents in receiving waters after opportunity for reasonable dilution and mixture with the wastes discharged thereto.
2. Sewage or waste effluents.	None which are not effectively disinfected.
3. pH.	Range between 6.5 and 8.5.
4. Dissolved oxygen.	For trout waters, not less than 5.0 parts per million; for non-trout waters, not less than 4.0 parts per million.
5. Toxic wastes, oil, deleterious substances, colored or other wastes or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to be injurious to fish life, make the waters unsafe or unsuitable for bathing or impair the waters for any other best usage as determined for the specific waters which are assigned to this class.

NOTE: Refer to Note No. 2 under Class AA, which is also applicable to Class B standards.

CLASS C

Best usage of waters: Fishing and any other usages except for bathing or as source of water supply for drinking, culinary or food processing purposes.

Quality Standards for Class C Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	None which are readily visible and attributable to sewage, industrial wastes or other amounts of these constituents in receiving waters after opportunity for reasonable dilution and mixture with the wastes discharged thereto.
2. pH.	Range between 6.5 and 8.5.
3. Dissolved oxygen.	For trout waters, not less than 5.0 parts per million; for non-trout waters, not less than 4.0 parts per million.
4. Toxic wastes, oil, deleterious substances, colored or other wastes or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to be injurious to fish life or impair the waters for any other best usage as determined for the specific waters which are assigned to this class.

NOTE: Refer to Note No. 2 under Class AA, which is also applicable to Class C standards.

CLASS D

Best usage of waters: Agricultural or source of industrial cooling or process water supply and any other usage except for fishing, bathing or as source of water supply for drinking, culinary or food processing purposes.

Conditions related to best usage: The waters will be suitable for fish survival; the waters without treatment and except for natural impurities which may be present will be satisfactory for agricultural usages or for industrial process cooling water; and with special treatment as may be needed under each particular circumstance, will be satisfactory for other industrial processes.

Quality Standards for Class D Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	None which are readily visible and attributable to sewage, industrial wastes or other wastes or which deleteriously increase the amounts of these constituents in receiving waters after opportunity for reasonable dilution and mixture with the wastes discharged thereto.
2. pH.	Range between 6.0 and 9.5.
3. Dissolved oxygen.	Not less than 3.0 parts per million.
4. Toxic wastes, oil, deleterious substances, colored or other wastes, or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to prevent fish survival or impair the waters for agricultural purposes or any other best usage as determined for the specific waters which are assigned to this class.

NOTE: Refer to Note No. 2 under Class AA, which is also applicable to Class D standards.

CLASS E

Best usage of waters: Sewage or industrial wastes or other wastes disposal and transportation or any other usages except agricultural, source of industrial cooling or process water supply, fishing, bathing, or source of water supply for drinking, culinary or food processing purposes.

Quality Standards for Class E Waters

Items	Specifications
1. Floating solids; settleable solids; oil; sludge deposits.	None attributable to sewage, industrial wastes or other wastes in sufficient amounts to interfere with navigation or cause a public nuisance as defined by the Penal Law.
2. pH.	Not lower than 5.0.
3. Dissolved oxygen.	Sufficient dissolved oxygen to prevent odor nuisances due to anaerobic decomposition unless other effective means are used to control odors.
4. Odor producing substances.	None in sufficient amounts to cause a public nuisance as defined by the Penal Law.

CLASS F

Best usage of waters: Sewage or industrial wastes or other wastes disposal.

Quality Standards for Class F Waters

Items	Specifications
1. Floating solids; settleable solids; oil; sludge deposits.	None attributable to sewage, industrial wastes or other wastes in sufficient amounts to cause a public nuisance as defined by the Penal Law.
2. Dissolved oxygen.	Sufficient dissolved oxygen to prevent odor nuisances due to anaerobic decomposition unless other effective means are used to control odors.
3. Odor producing substances.	None in sufficient amounts to cause a public nuisance as defined by the Penal Law.

4. CLASSES AND STANDARDS FOR TIDAL SALT WATERS

CLASS SA

Best usage of waters: Shellfishing for market purposes and any other usages.

Quality Standards for Class SA Waters

Items	Specifications
1. Floating solids; settleable solids; oil; sludge deposits.	None attributable to sewage, industrial wastes or other wastes.
2. Garbage, cinders, ashes, oils, sludge or other refuse.	None in any waters of the Marine District as defined by State Conservation Law.
3. Sewage or waste effluents.	None which are not effectively disinfected.
4. Dissolved oxygen.	Not less than 5.0 parts per million.
5. Toxic wastes, deleterious substances, colored or other wastes or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to be injurious to edible fish or shellfish or the culture or propagation thereof, or which in any manner shall adversely affect the flavor, color, odor or sanitary condition thereof or impair the waters for any other best usage as determined for the specific waters which are assigned to this class.
6. Organisms of Coliform group.	The median MPN value in any series of samples representative of waters in the shellfish growing area shall not be in excess of 70 per 100 milliliters.

CLASS SB

Best usage of waters: Bathing and any other usages except shellfishing for market purposes.

Quality Standards for Class SB Waters

Items	Specifications
1. Floating solids; settleable solids; oil; sludge deposits.	None attributable to sewage, industrial wastes or other wastes.
2. Garbage, cinders, ashes, oils, sludge or other refuse.	None in any waters of the Marine District as defined by State Conservation Law.
3. Sewage or waste effluents.	None which are not effectively disinfected.
4. Dissolved oxygen.	Not less than 5.0 parts per million.
5. Toxic wastes, deleterious substances, colored or other wastes or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to be injurious to edible fish or shellfish or the culture or propagation thereof, or which in any manner shall adversely affect the flavor, color, odor or sanitary condition thereof; and otherwise none in sufficient amounts to make the waters unsafe or unsuitable for bathing or impair the waters for any other best usage as determined for the specific waters which are assigned to this class.

CLASS SC

Best usage of waters: Fishing and any other usages except bathing or shellfishing for market purposes.

Quality Standards for Class SC Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	None which are readily visible and attributable to sewage, industrial wastes or other wastes or which deleteriously increase the amounts of these constituents in receiving waters after opportunity for reasonable dilution and mixture with the wastes discharged thereto.

Items	Specifications
2. Garbage, cinders, ashes, oils, sludge or other refuse.	None in any waters of the Marine District as defined by State Conservation Law.
3. Dissolved oxygen.	Not less than 5.0 parts per million.
4. Toxic wastes, oil, deleterious substances, colored or other wastes or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to be injurious to edible fish or shellfish or the culture or propagation thereof, or which in any manner shall adversely affect the flavor, color, odor or sanitary condition thereof or impair the waters for any other best usage as determined for the specific waters which are assigned to this class.

CLASS SD

Best usage of waters: Any usages except fishing, bathing, or shellfishing for market purposes.

Quality Standards for Class SD Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	None which are readily visible and attributable to sewage, industrial wastes or other wastes or which deleteriously increase the amounts of these constituents in receiving waters after opportunity for reasonable dilution and mixture with the wastes discharged thereto.
2. Garbage, cinders, ashes, oils, sludge or other refuse.	None in any waters of the Marine District as defined by State Conservation Law.
3. Dissolved oxygen.	Not less than 3.0 parts per million.
4. Toxic wastes, oil, deleterious substances, colored or other wastes.	None alone or in combination with other substances or wastes in sufficient amounts to prevent survival of fish life or impair the waters for any other best usage as determined for the specific waters which are assigned to this class.

5. CLASSES AND STANDARDS FOR UNDERGROUND WATERS

CLASS GA

Best usage of waters: Source of water supply for drinking, culinary or food processing purposes and any other usages.

Conditions related to best usage: The waters, if subjected to approved disinfection treatment if necessary, with additional treatment if necessary to reduce naturally present impurities, meet or will meet U. S. Public Health Service drinking water standards and are or will be considered safe and satisfactory for drinking water purposes.

Quality Standards for Class GA Waters

Items	Specifications
1. Sewage or industrial wastes or ineffectively treated effluents; fecal matter, garbage, manure, or other refuse.	None within limiting distances of well, spring or infiltration gallery as specified in rules and regulations enacted under provisions of the Public Health Law for protection of a public water supply; none within limiting distances of well, spring, or infiltration gallery as specified in decisions of Water Power and Control Commission; otherwise, if no limiting distances have been specified as indicated above none within 200 feet of a well, spring, or infiltration gallery used as source of a public water supply and none within 100 feet of well, spring, or infiltration gallery used as a source for any other kind of drinking water supply unless a lesser or greater distance is approved, permitted or required by the local health authorities or the State Department of Health; otherwise none in such a way or in sufficient amounts to impair the waters for use as a source of water supply for drinking, culinary or food processing purposes. Nothing in these specifications shall be construed as preventing the location of new wells under the jurisdiction of the Water Power and Control Commission.
2. Tastes or odor producing substances.	None attributable to sewage, industrial wastes or other wastes in sufficient amounts to make the waters unsafe or unsuitable as a source of water supply for drinking, culinary or food processing purposes.
3. Toxic wastes, deleterious substances, other wastes or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to make the waters unsafe or unsuitable as a source of water supply for drinking, culinary or food processing purposes, or impair the waters for any other best usage as determined for the specific waters which are assigned to this class.

NOTE: In determining the safety or suitability of waters in this class for use as a source of water supply for drinking, culinary or food processing purposes after approved treatment if necessary, the Water Pollution Control Board will be guided by the standards specified in the latest edition of "Public Health Service Drinking Water Standards" published by the United States Public Health Service.

CLASS GB

Best usage of waters: Source of industrial or other water supply and any other usages except as source of water supply for drinking, culinary or food processing purposes.

Conditions related to best usage: The waters without treatment and except for natural impurities which may be present will be satisfactory for agricultural water supply or industrial process cooling water; and with special treatment as may be needed under each particular circumstance, will be satisfactory for other industrial processes.

Quality Standards for Class GB Waters

Items	Specifications
1. Toxic wastes, deleterious substances, other wastes or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to make the waters unsafe or unsuitable for any best usage as determined for the specific waters which are assigned to this class.

The foregoing classifications and standards of quality and purity have state-wide application.

6. SPECIAL CLASSES—SURFACE WATERS

CLASS A—SPECIAL (INTERNATIONAL BOUNDARY WATERS)

This special class with its quality standards, adopted by the Water Pollution Control Board on December 6, 1954, has been assigned to the waters of the Niagara

River, which constitute a portion of the boundary waters covered by a treaty between the United States and Canada and under the supervision of an International Joint Commission. The definition, specifications and standards applying to this class have been filed with the Secretary of State for publication in the "Official Compilation of Codes, Rules and Regulations of the State of New York."

CLASS AA—SPECIAL (LAKE CHAMPLAIN DRAINAGE BASIN)

This special class with its quality standards, adopted by the Water Pollution Control Board on December 11, 1956, has been assigned to the waters of Lake Placid and its tributaries, and Lake George and its tributaries. The definition, specifications and standards applying to this class have been filed with the Secretary of State for publication in the "Official Compilation of Codes, Rules and Regulations of the State of New York."

SPECIAL CLASS (LOWER GENESEE RIVER)

This special class with its quality standards, adopted by the Water Pollution Control Board on April 20, 1959, has been assigned to the waters of the Genesee River between its outlet into Lake Ontario and the Barge Canal Dam at Court Street, Rochester. The definition, specifications and standards applying to this class have been filed with the Secretary of State for publication in the "Official Compilation of Codes, Rules and Regulations of the State of New York."

APPENDIX E

CLASSIFICATIONS AND WATER QUALITY STANDARDS FOR NORTH CAROLINA

(Adopted by the State Stream Sanitation Committee on 19 November 1953)

SECTION I. DEFINITIONS

The definition of any word and phrase used in Section II, III, or IV shall be the same as given in Section 143-212, Article 21 of the General Statutes of North Carolina. The following words and phrases, which are not defined in said section, shall be construed to have the following meanings:

1. Source of water supply for drinking, culinary or food-processing purposes shall mean any source, either public or private, the waters from which are used for human consumption, or used in connection with the processing of milk, beverages, food or for other purposes which require water meeting the "Public Health Service Drinking Water Standards."

2. Approved treatment, as applied to water supplies, means treatment accepted as satisfactory by the health authorities responsible for exercising supervision over the sanitary quality of water supplies.

3. Bathing shall include swimming, diving, wading, and similar uses but shall be regarded as a best usage only for waters in which such uses are or may be expected to be subjected to effective sanitary supervision and control.

4. Fishing shall include the propagation of fish and such other aquatic life as is necessary to provide a suitable environment for fish.

5. Shellfish culture shall include the use of waters for the propagation, storage and gathering of oysters, clams and other shellfish for market purposes.

6. Agricultural shall include the use of waters for stock watering, irrigation, and other farm purposes but not as sources of water supply for drinking, culinary or food-processing purposes.

7. Waste disposal shall include the use of waters for the disposal of sewage, industrial waste or other waste either before or after treatment.

8. Tidal salt waters shall mean all tidal waters which are so designated by the State Stream Sanitation Committee and which generally have a natural chloride ion content in excess of 500 parts per million.

9. Swamp waters shall mean those waters which are so designated by the State Stream Sanitation Committee and which are topographically located so as to generally have very low velocities and certain other characteristics which are different from adjacent streams draining steeper topography.

10. Offensive condition shall be construed to mean and include any condition or conditions resulting from the presence of sewage, industrial wastes or other wastes within the waters of the State or along the shorelines thereof which shall either directly or indirectly cause foul or noxious odors, unsightly conditions, the breeding of abnormally large quantities of mosquitoes or other insect pests, or shall damage private or public water supplies or other structures, result in the development of gases which destroy or damage surrounding property, herbage or grasses, or which shall affect the health of any person residing or working in the area.

11. Best usage of waters as specified for each class shall be those uses as determined by the State Stream Sanitation Committee in accordance with the stipulations in Article 21, General Statutes of North Carolina.

SECTION II. RULES APPLICABLE TO CLASSES AND STANDARDS

1. In cases where sewage, industrial wastes or other wastes are discharged directly into waters which are assigned a different classification than the waters into which such receiving waters flow, the standards applicable to the waters which receive such sewage, industrial wastes or other wastes shall be supplemented by the following:

> "The quality of any waters receiving sewage, industrial wastes or other waste discharges shall be such that no impairment of the best usage of waters in any other class shall occur by reason of such sewage, industrial wastes or other waste discharges."

2. Tests or analytical procedures to determine conformity or nonconformity with standards will, insofar as practicable and applicable, be made in accordance with the methods given in the latest edition of "Standard Methods for the Examination of Water and Sewage," published by the American Public Health Association, or, where other tests or analytical procedures have been found to be more applicable and satisfactory, same may be used upon acceptance and approval by the Committee.

3. In making tests or analytical determinations of classified waters to determine conformity or non-conformity with the established standards, samples shall be collected in such manner and at such locations as approved by the Executive Secretary of the Committee as being representative of the receiving waters after reasonable opportunity for dilution and mixture with the waste discharged thereto. When, in the opinion of the Executive Secretary it becomes necessary or desirable, samples will be collected from sewage and industrial waste outfalls and discharges from such outfalls will be

metered or otherwise measured to determine the volume of flow.

4. Natural waters may on occasion have characteristics outside of the limits established by the standards. The standards adopted herein relate to the condition of waters as affected by the discharge of sewage, industrial wastes or other wastes.

SECTION III. ESTABLISHED CLASSES FOR FRESH SURFACE WATERS AND THE STANDARDS OF WATER QUALITY AND PURITY WHICH SHALL BE APPLIED THERETO

Class A-I

Best Usage of Waters: Source of water supply for drinking, culinary, or food-processing purposes or any other usage requiring waters of lower quality.

Conditions Related to Best Usage: This class is intended primarily for waters having watersheds which are uninhabited and otherwise protected as required by the State Board of Health and which require only approved disinfection, with additional treatment when necessary to remove naturally present impurities, in order to meet the "Public Health Service Drinking Water Standards" and will be considered safe for drinking, culinary, and food-processing purposes.

Quality Standards for Class A-I Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits; tastes or odor-producing substances.	None attributable to sewage, industrial wastes or other wastes.
2. Sewage, industrial wastes or other wastes.	None
3. Toxic wastes; oils; deleterious substances; colored or other wastes.	None

Note No. 1: In determining the safety or suitability of waters in this class for use as a source of water supply for drinking, culinary or food-processing purposes after approved disinfection, the Committee will be guided by the physical, chemical and bacteriological standards specified in the latest edition of the "Public Health Service Drinking Water Standards" and the requirements of the State Board of Health as set forth in the "Rules Relating to the Protection of Unfiltered Public Water Supplies."

Class A-II

Best Usage of Waters: Source of water supply for drinking, culinary or food-processing purposes and any other best usage requiring waters of lower quality.

Conditions Related to Best Usage: The waters, if subjected to approved treatment equal to coagulation, sedimentation, filtration and disinfection, with additional treatment if necessary to remove naturally present impurities, will meet the "Public Health Service Drinking Water Standards" and will be considered safe for drinking, culinary or food-processing purposes.

Quality Standards for Class A-II Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	Only such amounts attributable to sewage, industrial wastes or other wastes as will not, after reasonable opportunity for dilution and mixture of same with the receiving waters, make the waters unsafe or unsuitable as a source of water supply for drinking, culinary, or food-processing purposes, injurious to fish and wildlife, or impair the waters for any other best usage established for this class.
2. Sewage, industrial wastes or other wastes.	None which are not effectively treated to the satisfaction of the Committee and in accordance with the requirements of the State Board of Health.
3. Odor-producing substances contained in sewage, industrial wastes or other wastes.	Only such amounts, whether alone or in combination with other substances or wastes, as will not, after reasonable opportunity for dilution and mixture of same with the receiving waters, cause taste and odor difficulties in water supplies which cannot be corrected by treatment as specified under "Conditions Related to Best Usage," impair the palatability of fish, or have a deleterious effect upon any best usage established for waters of this class.
4. Phenolic compounds.	Not greater than 3.0 parts per billion (phenols).
5. pH.	Shall be normal for the waters in the area, which generally shall range between 6.0 and 8.5, except that swamp waters may have a low of 4.3.
6. Total hardness.	Not greater than 100 parts per million as $CaCO_3$.
7. Dissolved oxygen.	Not less than 5.0 parts per million for trout producing waters; not less than 4.0 parts per million for non-trout waters, except that swamp waters may have a minimum of 3.0 parts per million.
8. Toxic wastes; oils; deleterious substances; colored or other wastes; or heated liquids.	Only such amounts, whether alone or in combination with other substances or wastes, and only such temperatures as will not render the waters unsafe or unsuitable as a source of water supply for drinking, culinary, or food-processing purposes, injurious to fish and wildlife or adversely affect the palatability of same, or impair the waters for any other best usage established for this class.

Note No. 1: In determining the safety or suitability of waters in this class for use as a source of water supply for drinking, culinary or food-processing purposes after approved treatment, the Committee will be guided by the physical, chemical and bacteriological standards specified in the latest edition of the "Public Health Service Drinking Water Standards."

Note No. 2: It is recognized that certain toxic substances will seriously affect fish life; however, the establishment of a single numerical standard for North Carolina waters would be too restrictive. In view of this and since there are many waters which, because of poor buffering capacity and composition will require special consideration, limiting values for such toxic materials will be established on the basis of the characteristics of the particular waters under consideration.

Class B

Best Usage of Waters: Bathing and any other usage except as source of water supply for drinking, culinary or food-processing purposes.

Conditions Related to Best Usage: The waters, under proper sanitary supervision by the controlling health authorities, will meet accepted standards of water quality for outdoor bathing places and will be considered safe and satisfactory for bathing purposes. Also, suitable for other uses requiring waters of lower quality.

Quality Standards for Class B Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	Only such amounts attributable to sewage, industrial wastes or other wastes as will not, after reasonable opportunity for dilution and mixture of same with the receiving waters, make the waters unsafe or unsuitable for bathing, injurious to fish and wildlife, or impair the waters for any other best usage established for this class.
2. Sewage, industrial wastes or other wastes.	None which are not effectively treated to the satisfaction of the Committee. In determining the degree of treatment required for such waste when discharged into waters to be used for bathing, the Committee will take into consideration the quantity and quality of the sewage and wastes involved and the proximity of such discharges to the waters in this class.
3. Phenolic compounds.	Not to exceed 3.0 parts per billion (phenols).
4. pH.	Shall be normal for the waters in the area, which generally shall range between 6.0 and 8.5, except that swamp waters may have a low of 4.3.
5. Dissolved oxygen.	Not less than 5.0 parts per million for trout producing waters; not less than 4.0 parts per million for non-trout waters, except that swamp waters may have a minimum of 3.0 parts per million.
6. Toxic wastes; oils; deleterious substances; colored or other wastes; or heated liquids.	Only such amounts, whether alone or in combination with other substances or wastes, and only such temperatures as will not render the waters unsafe or unsuitable for bathing, injurious to fish and wildlife or adversely affect the palatability of same, or impair the waters for any other best usage established for this class.

Note No. 1: Refer to Note 2 under Class A-II which also applies to Class B Standards.

Note No. 2: In assigning this classification to waters intended for bathing, the Committee will take into consideration the relative proximity of sources of pollution and will recognize the potential hazards involved in locating swimming areas close to sources of pollution and will not assign this classification to waters, the bacterial quality of which is dependent solely upon adequate disinfection, and where the interruption of such treatment would render the water unsafe for bathing.

Class C

Best Usage of Waters: Fishing and any other usage except for bathing or as a source of water supply for drinking, culinary or food-processing purposes.

Conditions Related to Best Usage: The waters will be suitable for fish and wildlife propagation. Also suitable for other uses requiring waters of lower quality.

Quality Standards for Class C Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	Only such amounts attributable to sewage, industrial wastes or other wastes as will not, after reasonable opportunity for dilution and mixture of same with the receiving waters, make the waters unsafe or unsuitable for fish and wildlife, or impair the waters for any other best usage established for this class.
2. pH.	Shall be normal for the waters in the area, which generally shall range between 6.0 and 8.5, except that swamp waters may have a low of 4.3.
3. Dissolved oxygen.	Not less than 5.0 parts per million for trout producing waters; not less than 4.0 parts per million for non-trout waters, except that swamp waters may have a minimum of 3.0 parts per million.
4. Toxic wastes; oils; deleterious substances; colored or other wastes; or heated liquids.	Only such amounts, whether alone or in combination with other substances or wastes, and only such temperatures as will not render the waters injurious to fish and wildlife or adversely affect the palatability of same, or impair the waters for any other best usage established for this class.

Note No. 1: Refer to Note No. 2 under Class A-II which is also applicable to Class C Standards.

Class D

Best Usage of Waters: Agriculture, industrial cooling and process water supply, fish survival and any other usage, except fishing, bathing or as a source of water supply for drinking, culinary or food-processing purposes.

Conditions Related to Best Usage: The waters without treatment and except for natural impurities which may be present therein will be suitable for agricultural uses and will permit fish survival. The waters will also be usable after special treatment by the user as may be needed under each particular circumstance for industrial purposes, including cooling and process waters.

Quality Standards for Class D Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	Only such amounts attributable to sewage, industrial wastes or other wastes as will not, after reasonable opportunity for dilution and mixture of same with the receiving waters, render the waters unsuitable for agriculture, industrial cooling purposes and fish survival.
2. pH.	Shall be normal for the waters in the area, which generally shall range between 6.0 and 8.5, except that swamp waters may have a low of 4.3.
3. Dissolved oxygen.	Not less than 3.0 parts per million.
4. Toxic wastes; oils; deleterious substances; colored or other wastes or heated liquids.	Only such amounts attributable to sewage, industrial wastes or other wastes and only such temperatures as will not render the waters unsuitable for agriculture, industrial cooling purposes and fish survival.

Note No. 1: Refer to Note No. 2 under Class A-II which is also applicable to Class D Standards.

Class E

Best Usage of Waters: Navigation and disposal of sewage, industrial wastes, or other wastes, and any other uses except agriculture, source of industrial cooling waters, fishing, bathing, or source of water supply for drinking, culinary or food-processing purposes.

Conditions Related to Best Usage: Waters will be suitable for navigation where navigable waters are involved, and may be used for waste disposal to the extent that the stream will accommodate same within the limits of the prescribed specifications for this class. This class will not be assigned to waters which can, in the light of consideration prescribed by the Statutes, be properly assigned to a higher class.

Quality Standards for Class E Waters

Items	Specifications
1. Floating solids; settleable solids; oils; sludge deposits.	Only such amounts attributable to sewage, industrial wastes or other wastes as will not interfere with navigation in navigable waters, and will not create an offensive condition in waters, whether or not navigable.
2. pH.	Not lower than 4.3 in waters used for navigation. Otherwise, it shall be maintained at such levels as not to adversely affect the quality of waters in any other class to which these waters may be tributary.
3. Dissolved oxygen.	Sufficient to prevent the development of an offensive condition.
4. Odor-producing substances.	Only such amounts as will not create an offensive condition.

SECTION IV. ESTABLISHED CLASSES FOR TIDAL SALT WATERS AND THE STANDARDS OF WATER QUALITY AND PURITY WHICH SHALL BE APPLIED THERETO

Class SA

Best Usage of Waters: Shellfishing for market purposes and any other usage requiring waters of lower quality.

Conditions Related to Best Usage: Waters will meet the sanitary and bacteriological standards given in the latest edition of "Manual of Recommended Practices for Sanitary Control of the Shellfish Industry," recommended by the Public Health Service and will be considered safe and suitable for shellfish culture.

Quality Standards for Class SA Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	None attributable to sewage, industrial wastes or other wastes.
2. Sewage, industrial wastes, or other wastes.	None which are not effectively treated to the satisfaction of the Committee and in accordance with the requirements of the State Board of Health.
3. pH.	Range between 6.8 and 8.5.
4. Dissolved oxygen.	Not less than 4.0 parts per million, except that swamp waters may have a minimum of 3.0 parts per million.
5. Toxic wastes; oils; deleterious substances; colored or other wastes; or heated liquids.	Only such amounts, whether alone or in combination with other substances or wastes, and only such temperatures as will not make the waters unsafe or unsuitable for fish and shellfish or their propagation, impair the palatability of same, or impair the waters for any other best usage established for this class.
6. Organisms of coliform group.	The coliform bacterial content of the water shall not exceed the limits specified in the latest edition of "Manual of Recommended Practices for Sanitary Control of the Shellfish Industry," recommended by the Public Health Service.

Note No. 1: Refer to Note No. 2 under Class A-II which will also apply to Class SA Standards.

Class SB

Best Usage of Waters: Bathing and any other usage except shellfishing for market purposes.

Conditions Related to Best Usage: The waters, under proper sanitary supervision by the controlling health authorities, will meet accepted sanitary standards of water quality for outdoor bathing places and will be considered safe and satisfactory for bathing purposes.

Quality Standards for Class SB Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	None attributable to sewage, industrial wastes or other wastes.
2. Sewage, industrial wastes, or other wastes.	None which are not effectively treated to the satisfaction of the Committee. In determining the degree of treatment required for such wastes when discharged into waters to be used for bathing, the Committee will take into consideration the quantity and quality of the sewage and wastes involved and the proximity of such discharges to the waters in this class.
3. pH.	Shall be normal for the waters in the area, which generally shall range between 6.0 and 8.5, except that swamp waters may have a low of 4.3.
4. Dissolved oxygen.	Not less than 4.0 parts per million, except that swamp waters may have a minimum of 3.0 parts per million.
5. Toxic wastes; oils; deleterious substances; colored or other wastes; or heated liquids.	Only such amounts, whether alone or in combination with other substances or wastes, and only such temperatures as will not make the waters unsafe or unsuitable for bathing, injurious to fish or shellfish, or adversely affect the palatability of same, or impair the waters for any other best usage established for this class.

Note No. 1: In assigning waters to this class, the Committee will take into consideration the proximity of points of sewage, industrial waste and other waste discharges and will recognize the potential hazards involved in locating bathing areas close to sources of pollution. Waters will not be assigned to this class if the bacterial quality of these waters is solely dependent upon adequate disinfection and where the interruption of such treatment would render the waters unsafe for bathing purposes.

Note No. 2: Refer to Note No. 2 under Class A-II which will also apply to Class SB Standards.

Class SC

Best Usage of Waters: Fishing, and any other usage except bathing or shellfishing for market purposes.

Conditions Related to Best Usage: The waters will be suitable for fishing and fish propagation. Also suitable for other uses requiring waters of lower quality.

Quality Standards for Class SC Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	Only such amounts attributable to sewage, industrial wastes or other wastes as will not, after reasonable opportunity for dilution and mixture of same with the receiving waters, make the waters unsafe or unsuitable for fish, shellfish and wildlife, or impair the waters for any other best usage established for this class.
2. pH.	Shall be normal for the waters in the area, which generally shall range between 6.0 and 8.5, except that swamp waters may have a minimum of 4.3.
3. Dissolved oxygen.	Not less than 4.0 parts per million, except that swamp waters may have a minimum of 3.0 parts per million.

Items	Specifications
4. Toxic wastes; oils; deleterious substances; colored or other wastes; or heated liquids.	Only such amounts, whether alone or in combination with other substances or wastes, and only such temperatures as will not render the waters injurious to fish and shellfish, adversely affect the palatability of same, or impair the waters for any other best usage established for this class.

Note No. 1: Refer to Note No. 2 under Class A-II which will also apply to Class SC Standards.

Class SD

Best Usage of Waters: Navigation and any other best usage, except fishing, bathing, or shellfishing for market purposes.

Conditions Related to Best Usage: Waters will be suitable for navigation, and in certain instances where there is either no practical means of treatment known or where the degree of treatment required to satisfy the standards for a higher class is economically impractical, these waters may be used for the disposal of sewage, industrial wastes or other wastes until feasible treatment processes become available, but in no instance shall any such usage result in the development of an offensive condition.

Quality Standards for Class SD Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	Only such amounts attributable to sewage, industrial wastes or other wastes as will not interfere with navigation, or cause an offensive condition.
2. pH.	Not lower than 4.3 in waters used for navigation. Otherwise, it shall be maintained at such levels as not to adversely affect the quality of waters in any other class to which these waters may be tributary.
3. Dissolved oxygen.	Sufficient to prevent the development of an offensive condition.
4. Toxic wastes; oils; deleterious substances; colored or other wastes.	Only such amounts, whether alone or in combination with other substances or wastes and only such temperatures as will not render the waters unsuitable for navigation, or create an offensive condition.

APPENDIX F

MIAMI RIVER DRAINAGE BASIN

Objectives

The water pollution survey of the streams in the Miami River drainage basin was made to obtain information upon which regulatory pollution measures could be based. The general objective or desired result of the regulatory measures is to restore and preserve the main stream and its tributaries for uses consistent with public health and welfare requirements, giving due consideration to the social and economic progress within the communities.

Recommended reductions of sewage and industrial wastes pollution are presented elsewhere in this report. Compliance with these will result in degrees of modification permitting uses of the streams for (1), domestic water supply; (2) industrial water supply; (3), fish and wildlife and; (4), at least limited recreation.

The policy which has been adopted is to fix water quality objectives consistent with water uses. For instance, where municipal water supplies are involved, this department has exercised its authority to revise upward these objectives. Streams and sections thereof consequently fall into zones of water quality.

Polluting materials are usually divided into three broad classifications: deoxygenating, highly toxic, and chemical. More specific objectives for these materials follow:

Deoxygenating Wastes: These wastes are usually organic compounds, either in suspension or solution, which use the oxygen in the water in the process of decomposition. Where the organic pollution is proportionately great, the oxygen content in the water is depleted, and fish and other wildlife are killed, oxidation ceases, and anaerobic decomposition or septic action increases with the attendant disagreeable odors and toxic effects. Adequate protection of the waters against unreasonable oxygen depletion should result if sufficient treatment is provided sewage and industrial wastes for substantially complete removal of settleable solids together with sufficient removal of other organic matter so as to hold the dissolved oxygen content above a critical point. The minimum dissolved oxygen content for the Miami River and its tributaries has been established at 4 ppm. A few exceptions are made where water use requires higher quality.

Highly Toxic Wastes: The highly toxic wastes are those which are poisonous in nature due to specific compounds or mixtures used in some types of industry. Cyanides, chrome and copper from metal plating operations, cyanides from gas plants, and lead from special manufacturing operations are typical.

Adequate protection will probably be provided the waters of the Miami River if substances highly toxic to human, fish, aquatic or wildlife are eliminated or reduced to the following concentrations after initial dilution at times of critical flow:

Cyanide (as CN)	not over 0.15 ppm
Lead (as Pb)	not over 0.35 ppm
Copper (as Cu)	not over 0.40 ppm
Chromium (hexavalent) (as Cr)	not over 2.00 ppm.

Chemical Wastes: Industrial wastes other than those in the deoxygenating and highly toxic classification are presented herein under the general classification of chemical wastes. Deleterious effects of these wastes include objectionable tastes and odors, tainting of fish flesh, excessive sludge deposits and oil concentrations, and excessive acidity or alkalinity. Probably the most offensive wastes from the standpoint of tastes and odors are the phenolic wastes from coke, synthetic resin, oil refining, petroleum cracking, tar, road oil, creosoting, wood distillation, and dye manufacturing industries. Industries producing chemical wastes other than phenolic are oil well and oil refining, gasoline filling and bulk stations, synthetic pharmaceutics, synthetic fibers, iron and steel, alkali chemicals, rubber fabrication, dye manufacturing, and acid manufacturing.

Adequate protection will probably be provided the waters of the Miami River and its tributaries if, after initial dilution at times of critical flow, the following values are maintained.

pH	Between 6.3 and 9.0
Chlorides	Not over 250 ppm
Phenols	Substantially complete removal (Tentatively not over 25 ppb. to 30 ppb. especially during critical flow periods in cold weather.)
Iron	Not over 5 ppm
Manganese	Not over 5 ppm
Oils	Substantially complete removal
Zinc (as Zn)	Not over 15 ppm
Nitrates	Not over 10 ppm
Free Acid	None
Color and Odor	To be non-offensive
Other toxic wastes, deleterious substances and high temperature liquids	None alone or in combination with other substances in sufficient amounts to impair water usage

LAKE ERIE POLLUTION SURVEY INTERIM REPORT OF 1951

Objectives for Maximum Allowable Concentrations of Coliform Bacteria:

Water Supply (Raw waters subject to filtration treatment): The monthly average "most probable numbers" of coliform organisms in water from lake intakes should not exceed 5,000 per 100 ml in any month, nor exceed

this number in more than 25 per cent of the samples of such waters examined during any month.

Recreational: For waters used for recreational purposes involving any bathing or swimming activities, the monthly arithmetical average "most probable numbers" of coliform organisms should not exceed 1,000 per 100 ml during any month of the recreational season, nor should exceed this number in more than 25 per cent of the samples examined during any such month. For waters used for recreational purposes not involving bathing or swimming, the monthly average "most probable numbers" of coliform organisms should not exceed 5,000 per 100 ml in any month of the recreation season, nor should exceed 5,000 per 100 ml in more than 25 per cent of the samples examined during any such month.

MAUMEE RIVER BASIN

Objectives

Bacteria: At water works intakes on streams of the Maumee River Basin the monthly arithmetical average of "most probable numbers" or, as an alternate, of "indicated numbers" of coliform organisms should not exceed 5,000 per 100 milliliters (5,000 per 100 ml) in any month; nor exceed this density in more than 20% of the samples of such waters examined during any month; nor exceed 20,000 per 100 ml in more than 5% of such samples.

For non-bathing or non-swimming recreation the monthly arithmetical average of "most probable numbers" or of "indicated numbers" should not exceed 5,000 per 100 ml in any month of the recreational season, nor should exceed this number in more than 20% of the samples examined during such a month. For bathing and swimming waters the monthly arithmetical average of "most probable numbers" or of "indicated numbers" of coliform organisms should not exceed 1,000 per 100 ml; nor should exceed this number in more than 20% of the samples examined in such month; nor should exceed 2,400 per 100 ml on any one day.

Dissolved Oxygen: The minimum dissolved oxygen objective for the Maumee River and its tributaries has been established at 4 ppm which, during critical summer conditions, is comparable to 50% dissolved oxygen saturation.

Toxic Wastes: Pending the results of studies and research now in progress by qualified technical groups, the following should serve as a guide to the reasonable protection of the waters of the basin:

	Parts per million (ppm)
Cyanide (as CN)	not over 0.15
Lead (as Pb)	not over 0.35
Copper (as Cu)	not over 0.4
Chromium (hexavalent as Cr)	not over 2.0
Cadmium (as Cd)	not over 0.4
Zinc (as Zn)	not over 1
Nickel (as Ni)	not over 5
Nitrate (as N)	not over 10

It is emphasized that exceptions to the above must be made where water uses require higher quality; and, further, that some of the above substances may cause damages in lower concentrations than noted under certain conditions and in certain combinations.

Taste and Odor: In general, wastes producing tastes and odors shall be limited to the extent required to prevent undue damage to reasonable and necessary water uses. Treatment shall be given to wastes containing phenols, oils, and allied derivatives to obtain substantially complete removal of these substances at their source.

Color: Discharge of color producing substances which impair reasonable and necessary water uses will not be permitted. At least partially pertinent to this category are iron and manganese. The concentrations of each of these substances shall not exceed 5 ppm (as Fe and Mn, respectively). This objective will provide reasonable protection for all water uses.

pH: No substance will be permitted which will cause the pH of the receiving water to fall outside a pH range between 6.3 and 9.0.

Suspended Solids and Floating Materials: Substantially complete removal of settleable solids and floating materials will be required to prevent nuisance conditions and sludge deposits. Additional removal of suspended solids may be required to prevent turbidity causing excessive damages to water uses.

Dissolved Solids: Objectives concerning damages from dissolved solids related to toxicity, oxygen depletion, taste and odors, pH, and color are discussed under these effects. Additional limitations on dissolved solids will be applied to prevent excessive hardness or other damaging effects. Chlorides (as Cl) should not exceed 100 ppm at any location within the basin.

Temperature: Operations resulting in excessive stream temperatures which impair other water uses, reasonable and necessary in the public interest, will not be permitted.

Fluorides: In case control of these wastes becomes necessary the maximum tolerable at water works intakes is fixed at 1.5 ppm.

Special Conditions: Insofar as practicable the effect of sewage or waste discharges on the receiving waters shall be considered after they are mixed with the waters and beyond a zone of immediate effect. The extent to which this is practicable depends on local conditions and the proximity and nature of other water uses.

MUSKINGUM RIVER BASIN

Objectives

The major objective of a proposed water pollution control program in Muskingum River Basin is to secure and maintain the quality of the water in the various streams and sections thereof suitable for those uses which in the public interest are reasonable and necessary.

The establishment of waste treatment requirements or other corrective measures to obtain effluents consistent with the basic objective involves the proper correlation of several basic factors. These include the quantity of the waste discharged, the concentration of the pollutants, dilution and natural purification capacity of the receiving stream, population and industrial growth trends, and the water quality required for downstream uses. Data pertinent to evaluating the magnitude of these factors are presented in this report.

It is recognized that water uses may vary for different streams and even for different sections of the same

stream. Also, the maximum allowable concentration of polluting substances will vary for each particular water use. Due allowance must also be made for the variation of the effects of a particular substance alone and in the presence of other substances.

In the application of water quality objectives, consideration must be given for proper sharing of water resources, future developments, and other special needs in the public interest.

The use of surface water as a source of public water supply is in general, always a major consideration. Although at present only a small part of the waters of this basin are so used, the future growth in the population of the basin may require greater use of these waters for such purposes. At present, recreational, wildlife preservation, and industrial uses are given the major consideration, except where public water supplies are involved. Eleven communities in the basin, having a total population of 159,273 persons (1950), obtain their water supplies from surface sources. In addition, two other communities having a combined population of 30,249 persons, obtain part of their water from surface sources. Waterways furnishing these supplies must be maintained at the highest possible quality.

For recreational and wildlife preservation purposes, a stream should be free of settleable solids, toxic concentration of heavy metals, and acids or alkalies; and should have a dissolved oxygen concentration equal to that required for maintaining aquatic life. For the latter, a concentration of at least 4.0 ppm is considered essential.

In general, the water quality required for industrial use is similar to that given above for sustaining wildlife and for permitting recreation. Heavy metals, and acids or alkalies such as mentioned above, may be injurious to industrial processes and to equipment. In addition, water supplies for industrial use should be free from high concentrations of such salts as the chlorides and sulfates of sodium, calcium, and magnesium. These are also injurious to industrial piping and process equipment.

Due to the multipurpose use of most waters in this basin, water quality objectives to maintain these waters in a condition suitable for reasonable and necessary uses must be sufficiently flexible to meet the varying conditions which exist. Where limits for polluting characteristics are given in the following paragraphs, they are to be considered as the maximum permissible. They will serve as guides in determining pollution abatement and control measures. If, in the public interest, a higher quality of water is needed, more restrictive limits may be required. Conversely, future conditions may warrant lesser requirements.

In considering water quality objectives, pollution may be classified on the basis of effects on water quality as follows: Bacterial contamination, deoxygenation, toxicity, taste and odor, color, hydrogen-ion concentration, suspended and floating matter, excessive dissolved solids, and high temperatures.

Bacterial: Bacterial quality is particularly pertinent with respect to uses of water for public water supply and for recreational purposes. Bacterial pollution usually is associated with domestic sewage. This is understandable by reason of the disease organisms that may be contained in human excretions. However, bacterial increases are coincident with organic decomposition, and bacterial concentrations will vary with organic content and with stages of deoxygenation and decomposition. The densities of coliform organisms (intestinal bacteria) furnish accepted criteria for the evaluation of public water supplies and recreational waters.

The accepted standard method for estimating coliform organisms is the fermentation dilution tube method. From this method a most probable number (MPN) index can be obtained. It should be realized, however, that this is merely an index of the number of coliform bacteria which, more probably than any other number, would give the results shown by the laboratory examination.

Recently, a membrane filter technique has been developed for determining directly the actual number of coliform organisms in a sample of water. Studies have indicated that this procedure and the standard dilution tube method do not measure precisely the same group of micro-organisms. When the limitations of the test are fully recognized and the difficulties of interpretation are known, this technique may be used. It is likely, that, when the sanitary significance of results, as well as the interpretation that may be given to coliform densities determined by this method, are established, it will be adopted as a standard method.

A rough estimate of the density of coliform organisms may be obtained by the use of the indicated number. This number is obtained by taking the reciprocal of the smallest positive dilution in a decimal series of plantings in fermentation tubes. This procedure was recommended in the 1917 Standard Methods and remained the only official method for the expression of coliform results until the publication of the 1936 edition of Standard Methods. Many water works operators still use this method, due to their familiarity with the procedures of calculation. For all practical purposes, the estimation of the coliform concentration by any one of the three methods described, allowing for the limitations of each, will furnish a satisfactory basis for establishing waste treatment requirements.

At water works intakes in the area covered by this report, the monthly arithmetical average estimate of coliform organisms should not exceed 5,000 per 100 milliliters (5,000 per 100 ml) in any month; nor exceed this density in more than 20 per cent of the samples of such waters examined during any month; nor exceed 20,000 per 100 ml in more than five per cent of such samples. It is felt that, if coliform reductions are accomplished to attain such quality, accepted practices of water treatment will assure safe public supplies.

Meeting the objective for public water supplies at existing intakes may assure satisfactory bacterial water quality for certain recreational uses in protected stream zones and may assure water quality suitable for bathing or swimming in a few stream areas. For non-bathing or non-swimming recreation, the monthly arithmetical average estimate of coliform organisms should not exceed 5,000 per 100 ml in any month of the recreational season, nor should exceed this number in more than twenty per cent of the samples examined during such month. For bathing and swimming waters, the monthly arithmetical

average estimate of coliform organisms should not exceed 1,000 per 100 ml; nor should exceed this number in more than twenty per cent of the samples examined in such month; nor should exceed 2,400 per 100 ml on any one day. It must be emphasized, however, that even though all bacteria from sewage pollution were to be removed, other factors beyond the control of water pollution control agencies may still make waters of the inland streams of the basin unsuitable for bathing. Careful consideration must be given to natural, public health, and economic factors in justifying the application of bathing water quality objectives to waters of the basin.

Deoxygenation: Deoxygenating wastes may utilize the dissolved-oxygen content of a stream by biochemical oxidation processes or purely chemical processes. Organic wastes such as sanitary sewage and those from food processing are examples of the first; spent pickle liquor is an example of the latter. In either case, the material causing the oxygen demand may be in suspension or solution. Where such wastes are discharged to a stream in excessive amounts, they may deplete the dissolved-oxygen content of a stream; cause the destruction of fish and aquatic life, or inhibit their propagation; and contribute to anaerobic decomposition with attendant nuisances, disagreeable odors, toxic effects, and public health menace.

Biochemical demands for oxygen take place over a period of several days to a month whereas chemical demands for oxygen may take place over a period of several hours. The rate of oxidation of a specific substance is an important factor in determining the allowable concentration of that substance in the stream.

Adequate protection of the main stream in this basin from unreasonable oxygen depletion, in general, should result if sewage and industrial wastes are treated to obtain substantially complete removal of settleable solids. On tributaries, especially the smaller ones, treatment facilities removing from 65 per cent to 90 per cent of the oxygen demanding properties of wastes discharged to them should provide adequate water quality. These statements are predicated on the complete elimination of spent pickle liquor in all instances.

Toxic Wastes: There are many substances, alone or in combination, which at critical concentration are toxic to humans, animals, fish and other wildlife. The effects of these concentrations may range from serious conditions affecting the public health or from conditions causing direct fish kills to minor disturbances which may be serious only if continued over a prolonged period of time.

The chemical characteristics of the receiving water have a significant effect on the toxicity of individual pollutants to fish and other aquatic life. Also some substances alone may have only a slightly toxic effect but in combination with certain other substances the effect may be much greater. As an example, zinc is slightly toxic alone, however, in the presence of copper the effect is very toxic.

Although, as mentioned above, there are many substances and combinations of substances which are toxic, it is likely that a great many of these will not be discharged to the streams in sufficient quantity to be a problem, possibly because such wastes are not normally found in the basin or because their value tends to preclude their discharge as waste products. However, some substances were encountered during this investigation which are troublesome to certain uses in concentrations above certain limits. Occurrence of these substances above such limits will be prima-facie evidence that excessive pollution exists. Pending the results of studies and research now in progress by qualified technical groups, the following should serve as a guide to the reasonable protection of the waters of this basin:

	Parts per million (ppm)
Cyanide (as CN)	not over 0.15
Lead (as Pb)	not over 0.1
Copper (as Cu)	not over 0.3
Chromium (as Cr)	not over 1.0 (trivalent + hexavalent)
Cadmium (as Cd)	not over 0.4
Zinc (as Zn)	not over 1.0
Nickel (as Ni)	not over 2
Nitrate (as N)	not over 10

It is emphasized that exceptions to the above must be made where water uses require higher quality; and, further, that some of the above substances may cause damages in lower concentrations than noted under certain conditions and in certain combinations.

pH and Alkalinity: The greatest source of acid waste in this basin is spent acid pickle liquors which also contain appreciable amounts of ferrous iron. These wastes originate primarily in the Canton, Massillon, Dover, Zanesville, Shelby and Mansfield areas. The effects of these wastes on a stream include: The conversion of carbonate hardness to sulfate hardness; the reduction of alkalinity; the formation of sludge banks as a result of the coagulation of the iron; and, the lowering of the pH of stream water.

The drainage from coal mines which are principally located in the southeastern section of the basin comprises another source of acid iron wastes. The effects of these wastes on a stream are not unlike acid iron wastes incident to processing steel in steel mills and allied industries. The problem not only involves drainage from active mines but, more difficult of correction, drainage from abandoned mines. Drainage from worked out or abandoned facilities becomes a public problem primarily because of inability to fix responsibility. Studies and research in progress by the Coal Industry Action Committee sponsored by interstate and Federal agencies should give possible guidance toward satisfactory correction of waste disposal from active and abandoned mines.

The program for acid control involves: (1) Effective treatment, salvage or control of concentrated spent pickle liquors to prevent discharges of acids or iron; (2) good operating practices to reduce these constituents of rinse waters; and, (3) the treatment and control of acid rinse waters or other acid wastes for reducing critical constituents to meet requirements of the receiving stream. The main streams generally will be protected if the total acid content of all acid wastes is reduced to insure a residual from each source of 15% of the total acid consumed in process; tributaries generally will be protected if the total acid content of all sources is reduced to insure a residual of 4% of the total acid consumed.

Under present conditions, it is estimated that such a policy will result in water having a satisfactory pH

(meeting an objective of not less than 6.3) and with a desirable residual alkalinity. A residual alkalinity of not less than 30 parts per million is established as an objective.

Encouragement will be given to control of acid wastes by methods other than neutralization and precipitation of the iron salts with lime, because of addition of hardness by the latter method. Where neutralization is practiced, use of high calcium lime, as opposed to material having high magnesium content, is recommended in order to reduce hardness due to highly soluble magnesium sulfate. Sedimentation and proper disposal of iron and undissolved sulfates will be required.

Industrial wastes with caustic alkalinity will require treatment or control to insure that the pH of the receiving stream will not greatly exceed 9.5.

Suspended Solids and Floating Material: Substantially complete removal of settleable solids and floating material will be required to prevent nuisance conditions and sludge deposits. In the Tuscarawas River the problem of suspended solids is aggravated by high concentrations of iron principally as oxides and sulfates. Measurements indicate that the total load of iron as Fe on the streams of this basin averaged approximately 50 tons per day. Concentrations of greater than 100 ppm were observed in the Tuscarawas River during the survey. The formation and settling of iron "floc" in an area where major pollution originates, results in the entrapment and deposition in sludge beds of oils, greases, and other foul smelling materials. These are picked up in concentrated forms during freshets and carried to other points of use, or redeposited on the stream bed as flow decreases, to be picked up again and carried further downstream.

In view of these conditions, the minimum requirements shall be substantially complete removal of settleable iron, particularly in blast furnace gas washer wastes and in rolling mill wastes. Also, in the control of waste pickle liquor . . . provision shall be made to prevent the discharge of iron with the waste. If the acid in the waste pickle liquor is controlled by neutralization, the measures must include precipitation of the iron.

Dissolved Solids: Objectives concerning damages from dissolved solids related to toxicity, oxygen depletion, taste and odors, pH, and color, are discussed under these effects. Dissolved solids are of concern also with respect to hardness. Large quantities of pickling acid, principally sulfuric acid used in the steel industry, result in wastes which react with alkalinity in the river or with alkalinity added to neutralize the acid and form sulfate hardness. Unless proper care is exercised in the control and treatment of acid and iron salts, (see preceding section on pH and alkalinity) excessive sulfate hardness will be added to the river. Some carbonate hardness will be changed to sulfate hardness by discharge of dilute pickle rinse waters. Also, some hardness will be added if neutralization of concentrated pickle wastes is practiced. However, in consideration of the economic aspects of a higher degree of control, the general objectives will be met if discharges of residual acid wastes are limited as previously stated and if, in the treatment or control of concentrated pickle liquors, practical methods are followed to reduce the discharge of hardness to the river.

The elimination of excessive chloride (Cl^-) concentrations as contributed by brine wastes from existing sources represents a major objective in the pollution abatement program of Tuscarawas and Muskingum Rivers. Pending the time when process changes, connected with which are problems of most significant economic magnitude, can be instituted, it will be necessary to give diligent attention to control of brine wastes discharges. The effort must be to keep concentrations in stream waters to a minimum based on the relation of existing chloride loads to average daily stream flows (yearly average).

CUYAHOGA RIVER BASIN

Objectives

The basic objective of the pollution survey of streams of the Cuyahoga River Basin was the establishment of criteria upon which regulatory pollution abatement and control measures could be based for the restoration and maintenance of water quality consistent with reasonable and necessary water uses.

The formulation of treatment requirements or other corrective measures for sewage and industrial wastes discharges to obtain effluents which will be consistent with stream water quality objectives involves the proper correlation of several basic factors. These include the quantity and characteristics of wastes discharged; dilution and natural purification capacity of the receiving stream, population and industrial growth trends; and, the water quality required for downstream uses. Information pertaining to these factors is included in this report.

It is recognized that water uses may vary for different streams and for different sections of a particular stream. Also, the maximum allowable concentrations of polluting substances will vary with the different water uses. Furthermore, the effects of a particular substance may also vary, depending on the presence or absence of other substances in the receiving waters.

The cost of pollution prevention or abatement is not necessarily the same for reclamation of polluted waters as for the preservation of relatively unpolluted water; consequently, water quality objectives formulated for the purpose of restoring a polluted stream or section of a stream to a minimum quality consistent with downstream water uses cannot be considered as inviting the lowering of the water quality of cleaner streams throughout the basin.

In the application of water quality objectives, consideration must be given to provision for proper sharing of water resources, to provision for further development, and to other special needs in the public interest.

Use of the surface waters of the upper portion of this basin for public water supplies is a high priority need. Water quality objectives, designed to meet the requirements of this use, will be applied to protect existing and future supplies.

For the reasons discussed above, water quality objectives established to maintain the basin's waters in a condition suitable for reasonable and necessary uses

must be sufficiently flexible to meet the varying conditions which exist. Where limits for polluting wastes are given in the following paragraphs, they are to be considered as the maximum permissible. They will serve as guides in determining pollution abatement and control measures. More restrictive limits may be required if consideration of all factors at specific locations shows that a higher quality of water is needed in the public interest. Conversely, if changes or future conditions warrant, requirements less restrictive will be given consideration.

In applying the principles stated in the preceding paragraphs to the Cuyahoga River Basin it is particularly pertinent to note that surface water use, in general, may be classified according to area.

In the upper part of the basin, including the area tributary to the Cuyahoga River above Kent, and in the Little Cuyahoga River, a major tributary, there are surface water uses which require water of good quality. Existing uses include public water supplies, industrial water supplies, recreational uses (including fishing), and livestock watering. Also, it is highly important that water from drainage areas not now specifically developed for such uses, may need to be preserved for future developments. Availability of adequate quantities of water within the basin for all of the uses in the area is somewhat limited and may become critical in the foreseeable future. For these reasons emphasis is placed on the desirability of preserving water quality in the upper portion of Cuyahoga River Basin.

Under present conditions, heavy population concentrations and natural drainage conditions, result in a separate subdivision of the basin from Kent downstream to the confluence of Mill Creek with Cuyahoga River insofar as water uses are concerned. The objectives for this section of the stream are to abate nuisance conditions, to improve water quality for industrial uses, and to eliminate or reduce substances which interfere with the natural purification processes in the stream.

The objectives for Cuyahoga River from the confluence of Mill Creek to its mouth and for tributaries to this section are to abate nuisance conditions and to improve water quality for legitimate water uses.

In considering water quality objectives, pollution may be classified on the basis of effects on water quality as follows: bacterial contamination; deoxygenation; toxicity; taste and odor; undesirable pH conditions; suspended solids and floating materials.

Bacterial: Bacterial quality is particularly pertinent with respect to uses of water for public water supply and for recreational purposes. Bacterial pollution usually is associated with domestic sewage. This is understandable by reason of the disease organisms that may be contained in human excretions. However, bacterial increases are coincident with organic decomposition, and bacterial concentrations will also vary with organic content and with stages of deoxygenation and decomposition. The densities of coliform organisms (intestinal bacteria) furnish accepted criteria for the evaluation of public water supplies and recreational waters.

Two acceptable methods are used for determining the density of coliform organisms in water, namely: (1) The fermentation dilution tube method; and (2) the membrane filter technique. The fermentation dilution tube method was accepted as a standard method in 1936 whereas the membrane filter technique was given limited acceptance as an alternate method in 1956. From the first method a most probable number (MPN) index is obtained. This number is merely an index of the number of coliform bacteria which more probably than any other number, would give the results shown by laboratory examination.

The membrane filter technique determines directly the actual number of coliform organisms in a sample of the water tested. Studies have indicated that this procedure does not measure precisely the same group of microorganisms as the fermentation dilution tube method. In applying the results of tests by this method to standards developed for use with the dilution tube method, consideration should be given to the differences between the two tests.

A rough estimate of the density of coliform organisms may be obtained by the use of the indicated number (Phelps index). This number is obtained by taking the reciprocal of the smallest positive dilution in a decimal series of plantings in fermentation tubes. This procedure was recommended in the third edition of "Standard Methods for Water Analyses" (1917) and remained the only official method for the expression of coliform results until the publication of the eighth edition of "Standard Methods for the Examination of Water and Sewage" (1936). Many water works operators still use this method due to their familiarity with the procedures of calculation. For all practical purposes the estimation of the coliform concentration by any one of the three methods described, allowing for the limitations of each, will furnish a satisfactory basis for establishing waste treatment requirements.

At water works intakes in the area covered by this report, the monthly arithmetical average estimate of coliform organisms should not exceed 5,000 per 100 milliliters (5,000 per 100 ml) in any month; nor exceed this density in more than 20 percent of the samples of such waters examined during any month; nor exceed 20,000 per 100 ml in more than five percent of such samples. It is felt that, if coliform reductions are accomplished to attain such quality, accepted practices of water treatment will assure safe public supplies.

Meeting the objective for public water supplies at existing intakes may assure satisfactory bacterial water quality for certain recreational uses in protected stream zones and may assure water quality suitable for bathing or swimming in a few stream areas. For non-bathing or non-swimming recreation, the monthly arithmetical average estimate of coliform organisms should not exceed 5,000 per 100 ml in any month of the recreational season, nor should exceed this number in more than twenty percent of the samples examined during such month. For bathing and swimming waters, the monthly arithmetical average estimate of coliform organisms should not exceed 1,000 per 100 ml; nor should exceed this number in more than twenty percent of the samples examined in such month; nor should exceed 2,400 per 100 ml on any one day. It must be emphasized, however, that even though all bacteria from sewage pollution were to be removed, other factors beyond the control of water pollution con-

trol agencies may still make waters of the inland streams of the basin unsuitable for bathing. Careful consideration must be given to natural, public health, and economic factors in justifying the application of bathing water quality objectives to waters of the basin.

Deoxygenation: Deoxygenating wastes may utilize the dissolved oxygen content of a stream by biochemical oxidation processes or purely chemical processes. Organic wastes such as sanitary sewage or industrial wastes from food processing industries are examples of the first; spent pickle liquor is an example of the latter. In either case, the material causing the oxygen demand may be in suspension or solution. Where such wastes are discharged to a stream in excessive amounts, they may deplete the dissolved oxygen content of a stream; cause the destruction of fish and aquatic life, or the inhibition of their propagation; and, give rise to anaerobic decomposition which is attendant with nuisances, disagreeable odors, toxic effects, and public health menaces.

Biochemical demands for oxygen usually take place over a period of several days to a month whereas chemical demands for oxygen may take place over a period of several hours. The rate of oxidation of a specific substance is an important factor in determining the allowable concentration of that substance in the stream.

Adequate protection of the main streams in this basin from unreasonable oxygen depletion, in general, should result if sewage and industrial wastes are treated to obtain substantially complete removal of settleable solids, and the removal of at least 85 per cent of the oxygen-demanding constituents of these wastes (complete treatment). The required degree of treatment will prevent the depletion of dissolved oxygen in the streams below established objectives. For the Cuyahoga River upstream from its confluence of Little Cuyahoga River, the minimum dissolved-oxygen objective is 4.0 milligrams per liter. This criterion meets requirements for normal water uses, including the protection of fish common to the waters of the basin and other aquatic life. For that portion of the Cuyahoga River downstream from confluence with Little Cuyahoga River, the dissolved oxygen objective is 3.0 mg/l. This criterion is based on the avoidance of nuisance conditions and the maintenance of satisfactory self-purification processes which require aerobic conditions.

Toxic Wastes: There are many substances, alone or in combination, which, at critical concentrations are toxic to humans, animals, fish and other wildlife. The effects of these concentrations may range from serious conditions affecting the public health or from conditions causing direct fish kills to minor disturbances which may be serious only if continued over a prolonged period of time.

The chemical characteristics of the receiving water has a significant effect on the toxicity of individual pollutants to fish and other aquatic life. Also, some substances alone may have only a slightly toxic effect, but in combination with certain other substances the effect may be much greater. As an example, zinc is slightly toxic alone, however, in the presence of copper the effect is very toxic.

Although, as mentioned above, there are many substances and combinations of substances which are toxic, it is likely that a great many of these will not be discharged to the streams in sufficient quantity to be a problem possibly because such wastes are not normally found in the basin or because their value tends to preclude their discharge as waste products. However, some substances were encountered during this investigation, which are troublesome to a few uses in concentrations above certain limits. Occurrence of these substances above such limits will be prima-facie evidence that excessive pollution exists. Pending the results of studies and research now in progress by qualified technical groups, the following should serve as a guide to the reasonable protection of the waters of this basin:

	Milligrams per liter (mg/l)
Cyanide (as CN)	Not over 0.15
Lead (as Pb)	Not over 0.1
Copper (as Cu)	Not over 0.3
Chromium (as Cr)	Not over 1.0 (Total)
Cadmium (as Cd)	Not over 0.4
Zinc (as Zn)	Not over 1.0
Nickel (as Ni)	Not over 2
Nitrate (as N)	Not over 10

It is emphasized that exceptions to the above must be made where water uses require higher quality; and further, that some of the above substances may cause damages in lower concentrations than noted under certain conditions and in certain combinations.

Tastes and Odors: Prevalent offensive wastes produced in the basin, from the standpoint of tastes and odors, are the phenolic and other types of wastes from the production and use of coke, the processing and use of by-products from the coke plant, and the refining of petroleum products. It is recognized that there are taste and odor substances from other industries, such as chemical, tanning, etc., as well as from natural resources. The objectives, with respect to wastes containing phenols, oils, greases and allied derivatives, and certain organic and inorganic chemicals are based on their reduction by means of known practical methods of operation or treatment. By the use of these methods it is expected that some residuals will remain in the industrial wastes discharged to the lower section of the Cuyahoga River.

pH and Alkalinity: Waste pickle liquor acids and iron salts, originating for the most part in the Cleveland area, are the major cause of serious damaging effects on water quality in the Cuyahoga River resulting from acid discharges. The effects of pickle liquor discharges are: (1) Carbonate hardness is converted to sulfate hardness, (2) alkalinity is reduced, (3) sludge banks are formed by the coagulation and precipitation of suspended matter by iron salts; and the pH is lowered.

The program for acid control in the Cuyahoga River Basin includes: (1) The effective treatment, salvage, or control of concentrated waste pickle liquors to prevent the discharge of acid and iron; and (2) the practice of good operations to reduce acid and iron in rinse waters. The treatment of dilute acid rinses in the lower section of the Cuyahoga River will not be required, at least for the present, provided acceptable practices are followed with respect to control of the amount of iron and acid in the rinses. Normally, it is considered that this condition will be met if the total acid in all wastes does not exceed 10% of the acid used. On tributary streams of the basin a further reduction of acid and iron will be required.

The above criterion not only takes into consideration the effects of the acid constituent of the pickle liquor, but also takes into consideration the deoxygenating effect of the iron constituent of spent pickle liquor. For the streams of the Cuyahoga River Basin the deoxygenating effect of the iron constituent of spent pickle liquor is much more critical than the effect of the discharged acid on the alkalinity of the stream. The above given limits of acids that may be discharged is based on the effects of the iron constituent of pickle liquor on the oxygen resources of the stream.

Under present conditions, it is estimated that such a policy will result in stream water having a satisfactory hydrogen-ion concentration meeting an objective of 6.3 (pH index) and with desirable residual alkalinity. A residual alkalinity of not less than 75 mg/l is established as an objective. These objectives are established to protect industrial process equipment and ships from corrosive waters.

Suspended Solids: Substantially complete removal of settleable solids and floating material will be required to prevent nuisance conditions and sludge deposits. The problem of suspended solids in the lower reaches of the Cuyahoga River is aggravated by high concentrations of iron. Data collected during our survey indicate that nearly 90 tons of iron as Fe were discharged daily to the Cuyahoga River. Concentrations of nearly 100 mg/l of iron were found in samples of the lower Cuyahoga River. The presence of iron in the river concentrations of 50-100 mg/l as Fe causes the coagulation of suspended solids, oils and greases which in turn settle to the bottom of the river to form sludge deposits. Upon undergoing anaerobic decomposition, these deposits give rise to the production of foul smelling gases which in turn cause the flotation of some of the sludge.

In view of the above, the minimum requirement shall be substantially complete removal of settleable iron (ferric iron) particularly in blast furnace gas washer wastes and in mill scale wastes from rolling mills. Also, in the control of spent pickle liquor . . . provision shall be made to prevent the discharge of iron with the waste. If acid in the pickle liquors is controlled by neutralization, the measures must include the precipitation of the iron.

Dissolved Solids: Objectives concerning damages from dissolved solids related to toxicity, oxygen depletion, taste and odors, pH and color, are discussed under these effects. Dissolved solids are also of concern with respect to hardness. Large quantities of pickling acid, principally sulfuric, used in the steel industry, result in wastes which react with alkalinity in the river or with alkalinity added to neutralize the acid and form sulfate hardness. Unless proper care is exercised in the control and treatment of acid and iron salts (see preceding section on pH and alkalinity), excessive sulfate hardness will be added to the river. In addition, some carbonate hardness will be changed to sulfate hardness by discharge of dilute pickle rinse waters. However, in consideration of the economical aspects of a higher degree of control, the general objectives will be met if discharges of residual acid wastes are limited as previously stated and if, in the treatment or control of concentrated spent pickle liquors, practical methods are followed to reduce the discharge of hardness to the river.

The discharge of wastes containing high concentrations of chlorides such as brine wastes shall be controlled so that the concentration of chlorides in the receiving stream shall not exceed 250 mg/l as Cl^-.

Color: The objective is control of color-producing substances as required to prevent impairment of reasonable and necessary uses. Iron and manganese are significant with respect to color. Iron is particularly significant because of the large quantities in wastes in the low level section of the Cuyahoga River.

The objectives for color due to iron will be met if the concentration of iron is kept within the objectives listed under suspended solids and deoxygenating wastes.

Temperature: High temperatures occur in the Cuyahoga River in the Akron and Cleveland area. This is due to the use of large volumes of river water for cooling purposes. High stream water temperatures are objectionable because it increases the rate of deoxygenation of pollution loads, decreases the solubility of oxygen in the stream, and thus lowers the critical point on the oxygen "sag" curve. An increase in water temperature also intensifies the effects of toxic substances on aquatic life.

At present the effects of temperature on necessary and desirable water uses is not now significant and, therefore, state regulatory action with respect to temperature is not considered necessary at this time.

Special Conditions: Insofar as practicable, the effect of sewage or waste discharges on the receiving waters shall be considered after they are mixed with the waters and beyond a zone of immediate effect. The extent to which this is practicable depends on local conditions and the proximity and nature of other water uses.

Where limitations on waste discharges are determined by public water supplies and where these limits are more restrictive than for other water uses, the objectives shall be applied to conditions at the water supply intakes.

Every effort will be made by the regulatory agency (1) to preserve the present cleanliness of basin waters and (2) to improve the water quality insofar as such action is consistent with the public interest. Objectives as offered, therefore, do not invite the loading of waters to the maximum allowable, but do serve as a guide to a sound pollution abatement and control program.

MAHONING RIVER BASIN

Objectives

The basic objective of the proposed program for abatement and prevention of pollution in the Mahoning River Basin is to secure and maintain a water quality in the various streams and sections of streams suitable for those uses which, in the public interest, are reasonable and necessary.

Establishment of treatment requirements, or other corrective measures, for wastes discharged to the streams involves the proper correlation of several factors so that the final waste effluents will be consistent with the basic objective. The factors involved include quality and quantity of wastes discharged, dilution and natural purification capacities of the receiving streams, water uses, population and industrial growth trends, technical knowledge with respect to corrective measures, costs of such measures, and quality of water required for the various uses.

The water pollution survey by the Ohio Health Department and information from other sources provide data on wastes discharged, stream flows, population trends, and water uses which are presented elsewhere in this report. This section is concerned primarily with water quality objectives to be applied in correlation with other factors.

It is recognized that the water uses may vary for different streams and for different sections of a particular stream. Also, the maximum allowable concentrations of polluting substances will vary with the different water uses. Furthermore, the effects of a particular substance may also vary, depending on the presence or absence of other substances in the receiving waters.

The cost of pollution prevention or abatement is not necessarily the same for reclamation of polluted waters as for the preservation of relatively unpolluted water; consequently, water quality objectives formulated for the purpose of restoring a polluted stream or section of a stream to a minimum quality consistent with downstream water uses cannot be considered as inviting the lowering of the water quality of cleaner streams throughout the basin.

In the application of water quality objectives, consideration must be given to provision for proper sharing of water resources, provision for further development, and other special needs in the public interest.

Use of the surface waters of the basin for public water supplies is a high priority need. Water quality objectives, designed to meet the requirements of this use, will be applied to protect existing and future supplies.

For the reasons discussed above, water quality objectives established to maintain the basin's waters in a condition suitable for reasonable and necessary uses must be sufficiently flexible to meet the varying conditions which exist. Where limits for polluting wastes are given in the following paragraphs, they are to be considered as the maximum permissible. They will serve as guides in determining pollution abatement and control measures. More restrictive limits may be required if consideration of all factors at specific locations shows that a higher quality of water is needed in the public interest. Conversely, if changes or future conditions warrant, requirements less restrictive will be given consideration.

In applying the principles stated in the preceding paragraphs to the Mahoning River Basin, it is particularly pertinent to note that a rather definite classification of stream areas based on water uses results from existing conditions.

In the upper part of the basin, including the area tributary to the Mahoning River above Newton Falls, Mosquito Creek a short distance above Niles, the Meander Creek drainage area, and areas drained by several smaller tributaries, there are water uses which require water of good quality. Existing uses include public water supplies, industrial water supplies, recreational uses (including fishing), and live stock water supplies. Also, it is highly important that water from drainage areas not now specifically developed for such uses be preserved for future developments. Availability of an adequate quantity of water for all uses in the area has been a serious problem for many years and is becoming more critical. For these reasons, discussed in more detail in the section on water uses, emphasis is placed on the desirability of preserving water quality in the upper part of the Mahoning Basin.

Under present conditions, heavy industrial concentration along the Mahoning River in Ohio, defined as the Newton Falls to Ohio-Pennsylvania State line section of the river, results in separate subdivision of the basin insofar as uses are concerned. The objectives for this section of the stream are to abate nuisance conditions, to improve quality for existing industrial uses, to eliminate or reduce substances which interfere with the natural purification processes in the stream, and to apply all practical control measures necessary to prevent undue damaging effects on necessary and desirable high priority water uses downstream in Pennsylvania.

The third stream area, considered from the standpoint of present water use, concerns water quality in the lower Mahoning River as it affects public water supplies taken from the Beaver River in Pennsylvania. Public water supplies affected by Mahoning River water quality include the West Pittsburgh water supply and the supplies in the Beaver Falls and New Brighton areas. Other public water supplies farther downstream in the Ohio River may be adversely affected. However, the supplies mentioned are the closest to the sources of pollution and govern the present objectives for improvement in quality.

In considering water quality objectives, pollution may be classified on the basis of effects on water quality as follows: bacterial contamination; deoxygenation; toxicity; taste and odor; color; undesirable pH conditions; suspended solids and floating material; excessive dissolved solids; and high temperature.

Bacterial: Bacterial quality is particularly pertinent with respect to uses of water for public water supply and for recreational purposes. Bacterial pollution usually is associated with domestic sewage. This is understandable by reason of the disease organisms that may be contained in human excretions. However, bacterial increases are coincident with organic decomposition, and bacterial concentrations will vary with organic content and with stages of deoxygenation and decomposition. The densities of coliform organisms (intestinal bacteria) furnish accepted criteria for the evaluation of public water supplies and recreational waters. Two methods are in general use for enumeration of coliform organisms; namely: the most probable number method (MPN); and the indicated number (IN) based on Phelps Index values. The Ohio Department of Health is prone to use the indicated number method as a time saving procedure. In its relations with other States in the Ohio River Basin, objectives are enumerated on the basis of most probable numbers. For all practical purposes, the use of either method as an index of pollution furnishes a satisfactory basis for establishing waste treatment requirements to protect public water supplies.

At waterworks intakes in the area covered by this report, the monthly arithmetical average of "most probable numbers" or, as an alternate, of "indicated numbers" of coliform organisms should not exceed 5000 per 100 milliliters (5000 per 100 Ml.) in any month; nor exceed this density in more than 20 percent of the samples of such waters examined during any month; nor exceed 20,000 per 100 Ml. in more than five percent of

such samples. It is felt that, if coliform reductions are accomplished to attain such quality, accepted practices of water treatment will assure safe public supplies.

Meeting the objective for public water supplies at existing intakes may assure satisfactory bacterial water quality for certain recreational uses in protected stream zones and may assure water quality suitable for bathing or swimming in a few stream areas. For non-bathing or non-swimming recreation, the monthly arithmetical average of "most probable numbers" or of "indicated numbers" should not exceed 5000 per 100 Ml. in any month of the recreational season, nor should exceed this number in more than twenty percent of the samples examined during such month. For bathing and swimming waters, the monthly arithmetical average of "most probable numbers" or of "indicated numbers" of coliform organisms should not exceed 1000 per 100 Ml.; nor should exceed this number in more than twenty percent of the samples examined in such month; nor should exceed 2400 per 100 Ml. on any one day. It must be emphasized, however, that even though all bacteria from sewage pollution were to be removed, other factors beyond the control of water pollution control agencies may still make waters of the inland streams of the basin unsuitable for bathing. Careful consideration must be given to natural, public health, and economic factors in justifying the application of bathing water quality objectives to waters of the basin.

Deoxygenation: Deoxygenating wastes may utilize the dissolved oxygen content of the stream by biochemical oxidation processes or by purely chemical processes. The former are generally organic in nature and may consist of sewage or be contained in effluents from industrial plants. The latter are of industrial origin. In either case, the material causing the oxygen demand may be either in suspension or solution. Where such wastes are discharged to streams in excessive amounts, the oxygen content of the water is depleted; fish and other aquatic life are killed, or their propagation inhibited; oxidation ceases, and anaerobic decomposition or septic action takes place with attendant nuisances, disagreeable odors, toxic effects, and public health menaces. Corrective action should consist of the removal of settleable solids from sewage and industrial wastes and the removal of a sufficient amount of the remaining deoxygenating waste, so that the dissolved oxygen in the stream will be maintained above a critical point required by those water uses considered desirable. For the upper Mahoning River Basin and tributaries, as defined previously, the minimum dissolved oxygen objective has been established at four parts per million. This criterion meets requirements for normal water uses, including the protection of fish common to the waters of the basin and other aquatic life. For the industrial section of the Mahoning River, the dissolved oxygen objective is a minimum of three parts per million. This criterion is based on avoidance of nuisance conditions and maintenance of satisfactory self-purification processes. If this condition is met at the critical points in the industrial section of the river, adequate levels for normal uses will be maintained below the Ohio-Pennsylvania line.

Toxic Wastes: There are literally hundreds of substances, alone or in combination, which, at critical concentrations, are toxic to humans, animals, fish, and other wildlife. The toxic effects may range from serious conditions affecting public health or causing direct fish kills to minor disturbances which may be serious only over a long period.

The chemical characteristics, or mineral content, of the receiving water has a great influence on the toxicity of individual pollutants to fish and other aquatic life. Also concentrations of certain substances, considered individually, may not be serious, whereas they may be quite injurious in combination with other substances.

In some instances concentrations of toxic materials, not sufficient to cause direct kills of fish but inimical to fish propagation, possibly may be tolerated in short, most critical, stream sections. Objectives with respect to fish and aquatic life do not apply to the industrial section of the Mahoning River except as they affect the condition of the River below the State line.

The above discussion leads to the conclusion that, in light of existing knowledge, it is impractical to fix final limits on concentrations of toxic substances. The general objective will be to eliminate or prevent concentrations which will be unduly injurious to the reasonable and necessary uses. Insofar as fish and other aquatic life are concerned, determination of whether or not the objective is being met will be based on biological indices of pollution. Application of toxicity limits to protect fish and other aquatic life will generally provide satisfactory protection for other water uses. Should there be exceptions, limits will be based on the specific uses affected.

Although, as mentioned above, there are many substances and combinations of substances which are toxic, it is likely that a great many of these will not be discharged to the streams in sufficient quantity to be a problem, possibly because such wastes are not normally found in the basin or because their value tends to preclude their discharge as waste products. However, some substances were encountered during this investigation which are troublesome to certain uses in concentrations above certain limits. Occurrence of these substances above such limits will be prima facie evidence that excessive pollution exists. Pending the results of studies and research now in progress by qualified technical groups, the following should serve as a guide to the reasonable protection of the waters of this basin:

	Parts per million (ppm)
Cyanide (as CN)	substantially none
Lead (as Pb)	not over 0.1
Copper (as Cu)	not over 0.3
Chromium (as Cr)	not over 1.0—trivalent not over 0.05—hexavalent
Cadmium (as Cd)	not over 0.4
Zinc (as Zn)	not over 0.3
Nickel (as Ni)	not over 2
Nitrate (as N)	not over 10

It is emphasized that exceptions to the above must be made where water uses require higher quality; and, further, that some of the above substances may cause damages in lower concentrations than noted under certain conditions and in certain combinations.

Fluorides: During the course of the survey it was found that there were industrial establishments in the basin which had significant fluoride contents. It is the intent that proper control measures will be provided to

assure that excessive concentrations are not discharged. In conformance with this intent an objective of not more than 1.2 ppm of fluoride (as F) in the river at waterworks intakes is established.

Taste and Odor: In general, wastes producing tastes and odors shall be limited to the extent required to prevent undue damage to reasonable and necessary water uses.

The most prevalent offensive wastes produced in the basin, from the standpoint of tastes and odors, are the phenolic and other types of wastes from production and use of coke and from processing of by-products from the coke plants. It is recognized that there are taste and odor substances from other industries and natural sources. The objective with respect to wastes containing phenols, oils, greases and allied derivatives, is based on their reduction by means of known practical methods of operation or treatment. Using presently known methods of controlling phenolic wastes, it is to be expected that some residuals will remain in the water in the industrial section of the Mahoning River. Every effort will be made initially to reduce these to such a minimum that undue damage will not persist at downstream water plants. It is believed that known practical methods will reduce phenolic loads to the extent that not over 75 parts per billion (p.p.b.) will be present in the water at the lower end of the industrial section of the river, (i.e., at the State line). As used herein, phenolic loads are intended to include cresols and other phenolic type substances normally measured by current analytical methods. It is contemplated that dissipation in the Mahoning and Beaver Rivers, together with dilution from the Shenango River, will be such that acceptable taste and odor conditions in the finished water at downstream water plants may be obtained with present water treatment practice.

Color: The objective is control of color producing substances as required to prevent impairment of reasonable and necessary water uses.

Iron and manganese are significant with respect to color. Iron is particularly significant because of the large quantities in wastes in the industrial section of the river.

In establishing a program to meet the above objectives with respect to iron, it is pertinent to consider the conditions prevailing at the time of the survey. The majority of iron wastes were discharged in the Warren to Lowellville section of the Mahoning River. The iron wastes, as discharged, were in both the dissolved and suspended state, principally as sulphates and oxides. The quantities were great, measurements indicating a total load averaging 170 tons per day, and largely uncontrolled. Upon mixing with the oxygen and alkalinity in the water, the dissolved iron was converted to the undissolved state, and during normal stream flow conditions practically all of the iron settled to the bottom, forming sludge banks. During such flow periods, records at downstream water plants indicate a relatively low iron content. However, during fluctuating flow conditions due to rains or other conditions, iron content at downstream plants increased greatly, reaching values in excess of 100 parts per million. Most of the iron is in suspension and settles readily with normal treatment processes. Another undesirable condition, considered more important than the iron concentration itself, accompanies the situation just described. The formation and settling of iron floc in the area where major pollution originates, results in the entrapment and deposition in sludge beds of oils, greases, and other foul smelling materials. These are picked up in concentrated form during freshets and carried to other points of use, redeposited on the stream bed as flow decreases, to be picked up again and carried further downstream.

In view of these conditions, the minimum requirement shall be substantially complete removal of settleable iron, particularly in blast furnace gas washer wastes and in rolling mill wastes. Also, in the control of waste pickle liquor (discussed later) provision shall be made to prevent the discharge of iron with the waste. If the acid in the waste pickle liquor is controlled by neutralization, the measures must include precipitation of the iron.

pH and Alkalinity: Pickle liquor acid and iron salts, originating for the most part between Warren and Lowellville, are the cause of serious damaging effects on water quality. Carbonate hardness is changed to sulphate hardness, alkalinity is reduced, sludge banks of iron compounds and other materials carried down with the iron floc are formed, and the pH is lowered.

The program for acid control includes effective treatment or control of concentrated waste pickle liquor to prevent discharge of acid or iron, and good operation practices to reduce the substances in rinse water. Treatment of dilute acid rinses probably can be postponed under present conditions of industrial development and stream flow, provided acceptable practices are followed with respect to control of the amount of acid and iron in the rinses. Normally, it is considered that this condition will be met if the total acid in all wastes does not exceed 15 percent of the acid used. In the upper section of the river and on tributaries, further reduction of acid and iron will be required if needed to protect water uses.

Under present conditions, it is estimated that such a policy will result in water having a satisfactory pH (meeting an objective of not less than 6.3) and with a desirable residual alkalinity. A residual alkalinity of not less than 30 parts per million is established as an objective. The objectives for pH and alkalinity are applicable to the water at the State line, below sources of major industrial wastes. A program directed toward equitable industrial use of water and treatment of wastes will improve water quality for industrial uses throughout the industrial section of the river.

Encouragement will be given to control of acid wastes by methods other than neutralization and precipitation of the iron salts with lime, because of addition of hardness by the latter method. Where neutralization is practiced, use of high calcium lime, as opposed to material having high magnesium content, is recommended in order to reduce hardness due to highly soluble magnesium sulphate. Settling out and proper disposal of iron and undissolved sulphates will be required.

Suspended Solids and Floating Materials: Substantially complete removal of settleable solids and floating material will be required to prevent nuisance conditions and sludge deposits.

Dissolved Solids: Objectives concerning damages from dissolved solids related to toxicity, oxygen depletion, taste and odors, pH, and color, are discussed under these effects. Dissolved solids are of concern also with respect to hardness. Large quantities of pickling acid, principally sulphuric, used in the steel industry, result in wastes which react with alkalinity in the river or with alkalinity added to neutralize the acid and form sulphate hardness. Unless proper care is exercised in the control and treatment of acid and iron salts, (see preceding section on pH and alkalinity) excessive sulphate hardness will be added to the river. Some carbonate hardness will be changed to sulphate hardness by discharge of dilute pickle rinse waters. Also, some hardness will be added if neutralization of concentrated pickle wastes is practiced. However, in consideration of the economic aspects of a higher degree of control, the general objectives will be met if discharge of residual acid waste is limited as previously stated and if, in the treatment or control of concentrated pickle liquors, practical methods are followed to reduce the discharge of hardness to the river.

Temperature: High temperatures occur in the heavily industrialized section of the river. Outside of the section of the river principally used for industrial water supply, for which the objective is to prevent nuisance conditions, temperature as related to necessary and desirable water uses is not now a significant factor. State regulatory action with respect to temperature is not considered necessary at this time.

This statement of policy is not intended to minimize the importance of the problem of excessive temperature in the industrial section of the river. Alleviation of this problem, a limiting factor on industrial production, is probably of more direct economic significance than other pollution problems. However, the problem is of most concern to a group of industries in a contiguous area. In the past, these industries have cooperated to improve the situation by provision of storage and flow regulation. Studies are under way for cooperating in additional similar improvements.

Special Conditions: Insofar as practicable, the effect of sewage or waste discharges on the receiving waters shall be considered after they are mixed with the waters and beyond a zone of immediate effect. The extent to which this is practicable depends on local conditions and the proximity and nature of other water uses.

Where limitations on waste discharges are determined by public water supplies and where these limits are more restrictive than for other water uses, the objectives shall be applied to conditions at the water supply intakes.

Every effort will be made by the regulatory agency (1) to preserve the present cleanliness of basin waters and (2) to improve the water quality insofar as such action is consistent with the public interest. Objectives as offered, therefore, do not invite the loading of waters to the maximum allowable, but do serve as a guide to a sound pollution abatement and control program.

SCIOTO RIVER BASIN

Objectives

The basic objective of the proposed program for the abatement and prevention of water pollution in the streams of the Scioto River Basin is to secure and maintain stream water quality suitable for those uses which, in the public interest, are necessary and reasonable.

The establishment of treatment requirements or other corrective measures for the discharge of municipal or industrial wastes to the streams involve the proper correlation of several factors so that the final effluents will be consistent with the basin objective. The factors involved include the quantity and quality of the discharged wastes, the dilution available in the receiving stream, the capacity of the stream for the assimilation of waste discharges and downstream water uses. Other pertinent data to take into consideration are growth trends of urban population centers as well as the growth trend of industrial development. Most of the information for evaluating many of these items was obtained during the field investigation for this report.

In establishing the treatment requirements presented in this report, it was recognized that water uses may vary for different streams or even for different sections of the same stream. Also, the maximum allowable concentration of a pollutant may vary with different water uses. Furthermore, the effects of a particular substance may also vary, depending on the presence or absence of other substances in the receiving waters.

It is also recognized that consideration must be given to the economic balance between the cost of pollution prevention or abatement and the benefits received. The cost of pollution prevention or abatement is not necessarily the same for reclamation of polluted waters as for the preservation of relatively unpolluted water; consequently, water quality objectives formulated for the purpose of restoring a polluted stream or section thereof to a minimum quality consistent with downstream water uses cannot be considered as inviting the lowering of the water quality of cleaner streams throughout the basin.

In the application of water quality objectives, consideration must be given to provisions for proper sharing of water resources, future development, and other special needs in the public interest.

Use of the surface waters in the upper portion of this basin for public water supply is a high priority need. Water quality objectives, designed to meet the requirements of this use, will be applied to protect existing and future supplies.

For reasons discussed above, water quality objectives established to maintain the surface water of this basin in a condition suitable for reasonable and necessary uses must be sufficiently flexible to meet the varying conditions which exist. Where limits for pollutants are given in the following paragraphs, they are to be considered as the maximum permissible at critical low flows. They will also serve as a guide for determining pollution control measures. More restrictive limits may be required at specific locations if a higher quality of water is needed in the public interest.

In considering water quality objectives, pollution may be classified on the basis of effects on water quality as follows. Bacterial contamination, deoxygenation, toxicity, taste and odor, color, undesirable pH conditions, suspended and floating material, excessive dissolved solids, and high temperature.

Bacterial: Bacterial quality is particularly pertinent with respect to uses of water for public water supply and for recreational purposes. Bacterial pollution of surface waters usually is associated with domestic sewage. This is understandable by reason of the disease organisms that may be contained in human excretions. However, a natural increase in the number of nonpathogenic bacteria in a stream is coincident with organic decomposition, and bacterial concentrations will vary also with organic content and with stages of deoxygenation and decomposition. The densities of coliform organisms (intestinal bacteria) furnish accepted criteria for the evalution of public water supplies and recreational waters.

Two acceptable methods are used for determining the density of coliform organisms in water, namely: (1) The fermentation dilution tube method, and (2) the membrane filter technique. The fermentation dilution tube method was accepted as a standard method in 1936, whereas the membrane filter technique was not established as an alternative standard method until 1956. From the first method a most probable number (MPN) index is obtained. This number is merely an index of the number of coliform bacteria which more probably than any other number, would give the results shown by laboratory examination on a series of decimal plantings.

The membrane filter technique determines directly the actual number of coliform organisms in a sample of the water tested. Studies have indicated that this procedure does not measure precisely the same group of microorganisms as the fermentation dilution tube method. In applying the results of tests by this method to standards developed for use with the dilution tube method, consideration should be given to the differences between the two tests.

A rough estimate of the density of coliform organisms may be obtained by the use of the indicated number. This number is obtained by taking the reciprocal of the smallest positive dilution in a decimal series of plantings in fermentation tubes. This procedure was recommended in 1917 Standard Methods and remained the only official method for the expression of coliform results until the publication of the 1936 edition of Standard Methods. Many water works operators still use this method due to their familiarity with the procedures of calculation. For all practical purposes, the estimation of the coliform concentration by any one of the three methods described, allowing for the limitations of each, will furnish a satisfactory basis for establishing waste treatment requirements.

At water works intakes in the area covered by this report, the monthly arithmetical average estimate of coliform organisms should not exceed 5,000 per 100 milliliters (5,000 per 100 ml.) in any month; nor exceed this density in more than 20 per cent of the samples of such waters examined during any month; nor exceed 20,000 per 100 ml. in more than five per cent of such samples. It is felt that if coliform reductions are accomplished to attain such quality, accepted practices of water treatment will assure safe public supplies.

Meeting the objective for public water supplies at existing intakes may assure satisfactory bacterial water quality for certain recreational uses in protected stream zones and may assure water quality suitable for bathing or swimming in a few stream areas. For non-bathing or non-swimming recreation, the monthly arithmetical average estimate of coliform organisms should not exceed 5,000 per 100 ml. in any month of the recreational season, nor should exceed this number in more than twenty per cent of the samples examined during such month. For bathing and swimming waters, the monthly arithmetical average estimate of coliform organisms should not exceed 1,000 per 100 ml.; nor should exceed this number in more than twenty per cent of the samples examined in such month; nor should exceed 2,400 per 100 ml. on any one day. It must be emphasized, however, that even though all bacteria from sewage pollution were to be removed, other factors beyond the control of water pollution control agencies may still make waters of the inland streams of the basin unsuitable for bathing.

Careful consideration must be given to natural, public health, and economic factors in justifying the application of bathing water quality objectives to waters of the basin.

Deoxygenation: Deoxygenating wastes utilize the dissolved oxygen content of a stream by either biochemical oxidation processes or purely chemical processes. Organic wastes such as sanitary sewage or the industrial wastes from food processing industries are examples of the first, whereas spent acid pickling liquors are an example of the latter. In either case, the material causing the oxygen demand may be in suspension or solution. Where such wastes are discharged to a stream in excessive amounts they may utilize all of the dissolved oxygen content of the stream, thereby inhibiting propagation or destroying fish and other aquatic life, and give rise to anaerobic decomposition which is attendant with nuisances, disagreeable odors, toxic effects, and public health menaces.

Biochemical demands for oxygen on the dissolved oxygen resources of a stream usually take place at a nearly constant rate over a period of at least several days to a much longer period. In contrast chemical demands for oxygen often take place in a relatively short period of time. From the above it can therefore be seen that the rate at which a particular pollutant exerts its demand for oxygen is an important factor in determining the necessary waste reduction requirements for that load.

The effect of a pollution load on the oxygen resources of a stream is also influenced by the hydraulic characteristics of the receiving stream. The rate of absorption of oxygen from the atmosphere into a stream per unit volume of water increases with surface area. Thus a wide shallow stream would receive more oxygen by this process than a narrow deep stream having the same volume of flow.

The minimum dissolved oxygen objective set forth for the Scioto River and all of its tributary streams is 4.0 milligrams per liter (mg/l). An exception is made for that section of the Scioto River immediately downstream from the outfall sewer of the Columbus sewage treatment plant to the confluence of Big Walnut Creek. For this section of Scioto River, at least currently, a minimum dissolved oxygen objective of 3.0 mg/l has been established. Unless low flow augmentation is provided, a higher objective during periods of critical stream flow would be impractical to obtain with known methods of sewage treatment.

For the present, consideration may be given to a minimum dissolved oxygen concentration of somewhat less than 4.0 mg/l in certain other stretches of streams in this basin which now flow through a relatively inhabited area.

The required degree of treatment of deoxygenating wastes set forth in this report are those which have been determined as necessary to maintain the established minimum dissolved oxygen objectives at critical stream flows under existing wastes loads. In making these determinations, due consideration was given to the self-purification capacity of the receiving stream.

Toxic Wastes: There are many substances, alone or in combination, which at critical concentrations are toxic to humans, animals, fish and other wildlife. The effects of these concentrations may range from serious conditions affecting the public health or conditions causing direct fish kills to minor disturbances which may be serious only if continued over a prolonged period of time.

The toxicity of an individual pollutant to fish or other aquatic life is significantly influenced by the chemical characteristics of the stream water. Also a particular ion which may only have a slight toxic effect under normal stream conditions may become much more toxic in combination with a second ion. As an example, zinc is only slightly toxic alone, however, in the presence of copper the effect is very toxic.

Although, as mentioned above, there are many substances and combinations of substances which are toxic, it is likely that a great many of these will not be discharged to the stream in sufficient quantity to be a problem, because such wastes may not normally be found in the basin or because their value tends to preclude their discharge as wastes products. However a few toxic ions were encountered during this survey in sufficient concentrations to be objectionable to existing water uses. Pending the results of studies and research now in progress by qualified technical groups, the following list of stream water quality objectives should serve as a guide to the reasonable protection of the waters of this basin.

Toxic Ions	*Concentrations—mg/l*
Cyanides as (CN)	not over 0.15
Lead as (Pb)	not over 0.1
Copper as (Cu)	not over 0.3
Chromium as (Cr)	not over 1.0
Cadmium as (Cd)	not over 0.4
Zinc as (Zn)	not over 1.0
Nickel as (Ni)	not over 2
Nitrate as (N)	not over 10

Occurrence of the above ions in concentrations greater than those given will be prima facie evidence of pollution. It should be noted that exceptions to the above must be made where water uses require a higher quality; and, further, that some of the above ions may cause damages in lower concentrations than noted under certain conditions and in certain combinations.

Taste and Odor: In general, the discharge of industrial wastes that produce tastes and odors shall be modified to the extent required to prevent undue damage to reasonable and necessary water uses.

Probably the most prevalent industrial wastes as far as tastes and odors are concerned are those from the following manufacturing processes: By-product coke, synthetic resins, synthetic fibre, oil refining and associated processes, creosoting, wood distillation, and dye manufacturing. Treatment shall be given to wastes containing phenols, oils, and allied derivatives to obtain substantially complete removal of these substances at their source.

Color: Discharge of color producing substances shall be limited to those concentrations that will not impair reasonable and necessary water uses.

Iron and manganese are significant with respect to color. Discharges containing significant quantities of either of these ions shall be modified so as to maintain the concentration of each of these ions at less than 5 mg/l in the receiving stream.

pH and Alkalinity: The discharge of strong acid or alkali wastes shall be modified so as to maintain the hydrogen ion concentration within the pH range of 6.3 to 9.0, inclusive. In general, this will provide protection to public and industrial water supplies as well as fish and aquatic life and to other uses.

Suspended Solids and Floating Materials: Substantially complete removal of settleable solids and floating materials will be required to prevent nuisance conditions and sludge deposits.

Dissolved Solids: Objectives concerning damages from dissolved solids related to toxicity, oxygen depletion, taste and odors, pH, and color are discussed under these effects. Dissolved solids are of concern also with respect to hardness. The discharge of sulphuric acid, which is frequently used for the pickling of steel, reacts with the alkalinity of the river water or with alkalinity added to neutralize the acid and form sulphates which increase the hardness of the water.

The quantity of acid wastes discharged to the streams of the Scioto River Basin at the time of this survey was relatively insignificant and thus compliance with pH and alkalinity objectives will be the only requirements imposed on such discharges at this time.

Objectives with respect to the dissolved solids also include the discharge of concentrated brine wastes. The discharge of chloride-bearing wastes shall be controlled in such a manner as to minimize the effect of these wastes on the receiving stream.

Temperature: High temperatures occur in streams of this basin due to discharges of cooling waters from industries and electric generating stations. Water temperature as related to necessary and desirable water uses may be a pollutant but is not now a significant factor in this basin. State regulatory action with respect to temperature, however, may need to be considered at a future time.

Special Conditions: Insofar as practical, the effects of sewage or industrial waste discharges on the receiving stream shall be considered after they are mixed with the stream waters and beyond the zone of immediate effect. The extent to which this is practical depends on local conditions and the proximity and nature of other water uses.

Where limits of concentration of critical constituents of sewage and industrial wastes are determined by the use of a stream for public water supply and where these limits are more restrictive than for other uses, the objectives shall be applied to conditions at the water supply intakes.

Every effort will be made by the regulatory agency to preserve the present cleanliness of basin waters and to improve the water quality insofar as such action is consistent with the public interest.

The water quality objectives as set forth in this report do not invite the loading of stream waters of this basin to the maximum allowable, but do serve as a guide to a sound pollution abatement and control program.

APPENDIX G

OREGON ADMINISTRATIVE RULES—STATE SANITARY AUTHORITY

Standards of Purity for Waters and Disposal Therein of Sewage and Industrial Wastes

11-010 RESTRICTIONS ON THE DISCHARGE OF SEWAGE AND INDUSTRIAL WASTES. No sewage or industrial waste shall be discharged into any waters of the State of Oregon which in itself or in conjunction with other wastes will cause:

(1) The dissolved oxygen content of the waters to be less than five (5) parts per million.
(2) The hydrogen-ion concentration (pH) of the waters to be outside of the range of 6.5 to 8.5.
(3) The liberation of dissolved gases, such as carbon dioxide, hydrogen sulfide or any other gases, in sufficient quantities to be deleterious to fish or related forms of aquatic life, navigation or other reasonable uses made of such waters.
(4) The development of fungi or other growths having a deleterious effect on stream bottoms, fish or related forms of aquatic life, or which are injurious to health, recreation or industry.
(5) The creation of toxic conditions that are deleterious to fish or related forms of aquatic life or affect the potability of drinking water.
(6) The formation of appreciable bottom or sludge deposits or the formation of any organic or inorganic deposits deleterious to fish or related forms of aquatic life or injurious to public health, recreation or industry.
(7) Objectionable discoloration, turbidity, scum, oily sleek or floating solids, or coat the aquatic life with oil films.
(8) Bacterial pollution or other conditions deleterious to waters used for domestic purposes, livestock watering, irrigation, bathing, or shellfish propagation, or be otherwise injurious to public health.

11-015 MAINTENANCE OF STANDARDS OF PURITY. (1) The degree of sewage or waste treatment required to restore and maintain the above standards of purity shall be determined in each instance by the State Sanitary Authority and shall be based upon the following:

(a) The uses which are or may likely be made of the receiving stream.
(b) The size and nature of flow of the receiving stream.
(c) The quantity and quality of the sewage or wastes to be treated, and
(d) The presence or absence of other sources of pollution on the same watershed.

(2) At its discretion, the State Sanitary Authority may require chlorination or equally efficient disinfection of sewage and waste treatment plant effluents wherever and whenever the discharge of unchlorinated effluents shall or may affect the quality of water used for public or domestic water supplies, irrigation, shellfish growing areas, or swimming and recreational areas.

11-020 CLASSIFICATION OF STATE WATERS. (1) For the purpose of facilitating the determination of the degree of treatment required for municipal and sanitary sewage the waters of the state of Oregon shall be classified in three divisions as follows:

Class "A"

Bodies of water which are used or which may be used for public water supplies, swimming and recreation, for irrigation, for the propagation of game and commercial fish, or for the propagation of shellfish and which because of insufficient flow cannot receive, without detriment, wastes other than those that have been given a high degree of treatment.

Class "B"

Bodies of water which are used or which may be used for public water supplies, swimming and recreation, for irrigation, for the propagation of game and commercial fish, or for the propagation of shellfish, and which, in the opinion of the State Sanitary Authority, provide sufficient dilution to obviate the necessity for the degree of treatment required under Class "A" waters.

Class "C"

Bodies of water into which the temporary discharge of untreated sewage or wastes may be permitted, if, in the opinion of the State Sanitary Authority, such discharge may not be detrimental to any reasonable use of said waters.

(2) Unless a higher degree of treatment is found to be necessary by the State Sanitary Authority to meet the requirements of section 11-010 above, municipal or sanitary sewage discharged into the above described classes of waters of the state of Oregon shall, in each instance, receive the following degree of treatment as a minimum:

(a) Municipal or sanitary sewage discharged into Class "A" waters shall be so treated that the effluent from the treatment plant:

(A) Shall be free of noticeable floating solids, oil, grease, sleek, and practically free of suspended solids.

(B) Shall indicate an average five-day bio-chemical oxygen demand reduction of not less than 85 per cent and at no time shall the residual effluent B. O. D. be in excess of 50 parts per million.

(b) Municipal or sanitary sewage discharged into Class "B" waters shall be so treated that the effluent from the treatment plant:

(A) Shall be free of noticeable floating solids, oil, grease, and sleek.

(B) Shall indicate an average suspended solids reduction of at least 55 per cent.

(C) Shall indicate an average five-day bio-chemical oxygen demand reduction of not less than 35 per cent, and at no time shall the residual effluent B. O. D. be in excess of 125 parts per million.

(c) Municipal or sanitary sewage may be discharged without treatment into Class "C" waters; provided, that a temporary permit for such discharge has first been obtained from the State Sanitary Authority, that no such permit shall be valid for a period of time longer than three years, and further that such permits may be revoked for cause at any time after the date of issuance thereof.

APPENDIX H

RULES AND REGULATIONS OF THE PENNSYLVANIA SANITARY WATER BOARD WITH RESPECT TO THE DISPOSAL OF WASTES INTO THE UNDERGROUND

(Adopted January 10-11, 1951)

WHEREAS, the underground waters are by law part of the waters of the Commonwealth and their pollution is subject to the provisions of the antipollution law; and

WHEREAS, the course which will be followed by wastes discharged into the underground and the effect of any polluting substance contained in such wastes, cannot, in general, be determined with the same facility as in the case of wastes discharged to surface waters; but

WHEREAS, under certain conditions wastes have been successfully disposed of by discharge into appropriate underground horizons without any known prejudicial effect; but

WHEREAS, such disposal may be responsible for the pollution of water supplies when the discharge is made to inappropriate horizons; and

WHEREAS, it is the responsibility of each producer of polluting wastes to dispose of them without harm to others and the Sanitary Water Board will not relieve him of such responsibility;

NOW THEREFORE, the said Board makes of record its general policy with respect to such disposal as follows:

1. The Board will, except as otherwise provided, consider the disposal of wastes into the underground as potential pollution unless the disposal is within such proximity of the surface that the wastes will be absorbed in the soil mantle and be acted upon by the bacteria naturally present therein before reaching the underground or surface waters.

2. Discharge of inadequately treated wastes, except coal fines into the underground workings of active or abandoned mines is prohibited.

3. Discharge of wastes into abandoned wells is prohibited.*

4. Disposal of wastes into underground horizons will only be accepted as an abatement of pollution when the applicant can show by the log of the strata penetrated and by the stratigraphic structure of the region that it is improbable that such disposal will be prejudicial to the public interest, but any such acceptance will be conditioned and will not relieve the applicant of responsibility for any pollution of the waters of the Commonwealth which may occur, in which case such disposal shall be stopped forthwith.

RULES AND REGULATIONS OF THE SANITARY WATER BOARD

Article 600—Section 16, Heated Wastes

A. The temperature of the waters of the Commonwealth shall not be increased artificially by amounts that shall be inimical or injurious to the public health or to animal or aquatic life or prevent the use of water for domestic, industrial or recreational purposes.

B. The heat content of discharges shall be limited to an amount that could not raise the temperature of the entire stream at the point of discharge above 93°F assuming complete mixing. The heat content of discharges may be increased or further limited where local conditions would be benefited thereby.

C. Where downstream circumstances warrant, the area in which the temperature may be artificially raised above 93°F will be prescribed.

D. A fishway will be required in streams receiving heated discharges where this is essential for the preservation of migratory pathways of game fish, or for the preservation of important aquatic life.

E. Paragraphs B and C do not apply to streams so impregnated with acid mine drainage that they cannot support a fish population typical of the region except for heated discharges which adversely affect domestic or industrial uses or secondary streams.

F. There shall be no new discharge to waters providing a suitable environment for trout if as a result the temperature of the receiving stream exceeds 58°F or stream temperature, whichever is higher.

G. Reduction of the heat content of discharges to estuarial waters will be required where necessary to protect the public interest. Estuarial waters are those containing ocean salts. Tidal waters not containing ocean salts are considered as fresh water streams.

COMMONWEALTH OF PENNSYLVANIA
DEPARTMENT OF HEALTH
Sanitary Water Board

INDUSTRIAL WASTES PERMIT NO. 1832

This permit is issued subject to the following Special Conditions:

(A—D omitted)

E. The permittee is hereby directed to immediately notify the Pennsylvania Department of Health whenever there is a spill or an accidental discharge of radioactive material and shall advise that Department promptly concerning the pertinent facts and probable danger. The permittee shall maintain rosters of Pennsylvania Department of Health personnel and of downstream users of river water who shall be notified. The necessary information for such rosters shall be furnished to the permittee by the Department of Health. In the event of any such accidental discharge, the Department of Health shall determine whether or not downstream users shall be notified, and by whom.

Moreover, the permittee is required to see to the training and supervision of all operating personnel, in order

* Note that the drilling of new wells for waste disposal is subject to Nos. 1 and 4 of this regulation.

to prevent the discharge of such material, fluid or solid, to the waters of the Commonwealth or to the site, without adequate treatment.

F. All industrial wastes discharged to the portion of the Ohio River on which the permittee's plant is located shall meet at least the requirements of the Sanitary Water Board for primary treatment. "Primary treatment" does not apply to the radioactive characteristics of the wastes.

The term "primary treatment," as here used, is such treatment of sewage as, in the opinion of the Board, will remove practically all of the settleable solids; will remove at least 35 percent of the organic pollution load as measured by the biochemical oxygen demand test; will accomplish the removal of oils, greases, acids, alkalis, toxic, putrescible, taste- and odor-producing substances, and other substances inimical to the public interest in the receiving stream; will provide effective disinfection to control disease-producing germs; will provide for satisfactory disposal of sludge; and will produce a final effluent that is suitable for discharge into the receiving stream. "Primary treatment of industrial wastes," as here used, is treatment to a degree equivalent to that set forth herein for sewage.

Moreover, because of the radioisotopes which these wastes may carry, such wastes shall be substantially free of turbidity and suspended solids over and above the amounts of such characteristics in the intake water.

G. The treated radioactive liquid wastes as discharged to the Ohio River shall comply with the Pennsylvania Department of Health Radiation Protection Regulation 433 and any subsequent regulations of the Department of Health.

H. With respect to the amount of radioactivity released in the liquid wastes to the waters of the Commonwealth, this permit is issued subject to the following conditions: (A) that the average radioactivity, exclusive of tritium activity, of these wastes over any consecutive 365-day period, shall not exceed 15,900 microcuries per day, with the maximum discharge not exceeding 62,000 microcuries per day, (B) these wastes shall at no time carry more radioactivity, exclusive of tritium activity, than 10^{-7} microcuries per milliliter (U^{226} and U^{228} not present) in excess of that of the plant intake water from the Ohio River, and (C) that the discharge of tritium in these wastes shall be not more than 10 curies per day averaged over any consecutive 365-day period, nor more than 50 curies per day maximum. Moreover, the company shall conduct all its pressurized water reactor operations so as to produce and discharge the least practicable amounts of radioactivity.

I. Although tritium has a short biological half-life (19 days) and a maximum permissible concentration (MPC) of 0.2 microcuries per ml (according to Handbook 52 of the National Bureau of Standards) as compared with an MPC of 10^{-8} microcuries per milliliter for the general population for unidentified isotopes, its radiological half-life is long enough (4,600 days) to make its continued presence in the stream undesirable and possibly inimical to aquatic and other life. At the present time, it is reported, there are no practical means for its separation from a liquid. The permittee, however, shall continue to investigate and apply more effective means to remove this potential hazard as rapidly as such means are developed, or attempt to devise some other means or to attempt to find some other substance which will have advantages similar to that of lithium hydroxide without producing the present discharge of tritium. The attention of the permittee is directed to the possibility that tritium may prove objectionable as a constituent of water used for certain industrial processes.

J. The radioactive and other liquid wastes discharged to the effluent channel shall be well admixed with the cooling water so that the waste content of this channel shall be substantially uniform below the effluent weir or at the sampling rake.

K. The treated effluent shall be discharged to the river in a manner acceptable to the Sanitary Water Board. If any structure is necessary to accomplish this, it shall be well protected against the effects of flood waters, ice, and other hazards and the design of such structure shall be acceptable to the Sanitary Water Board.

L. Provision shall be made for regulating discharge of the treated effluent so that it shall be equally distributed over the longest practicable period, in order that advantage may be taken of maximum possible dilution by the receiving stream, and means to accomplish uniform 24-hour distribution shall be provided if in the opinion of the Department of Health this shall be found necessary.

M. The wastes after treatment and prior to discharge to the effluent channel shall contain no more than 0.05 ppm of hexavalent chromium and 1.0 ppm of trivalent chromium.

(N—P omitted)

Q. Acknowledgment is made of the company's study now in progress to determine temperatures and percentage of river volume that flows through a narrow river channel between Phillis Island and the mainland to determine the expected rise in river water temperature in this channel when the condenser cooling water is discharged to it. The permittee is hereby advised that the river water temperature rise is tentatively limited to 2° F at points to be determined by the Sanitary Water Board after the results of the thermal studies of the channel and of other data have been reviewed, and that if this limit cannot be maintained with the proposed method of discharge, then complete dispersal of the condenser effluent in the river, or other means for temperature control, may be necessary. The report on the river channel study shall be submitted in duplicate to the Sanitary Water Board within 60 days of completion of the study which is expected to terminate in the last months of 1958, with progress reports on a quarterly basis.

(R—T also omitted)

APPENDIX I

STATE OF SOUTH CAROLINA WATER POLLUTION CONTROL AUTHORITY

Section III of Classification-Standards System

Established Classes for Fresh Surface Waters and the Standards of Quality and Purity Which Shall be Applied Thereto:

CLASS AA

Waters meeting State Board of Health regulations as suitable for use for domestic and food-processing purposes with sterilization as only treatment required. Suitable also for uses requiring waters of lesser purity.

Quality Standards for Class AA Waters

Items	Specifications
1. Floating solids; settleable solids; oil; sludge deposits; tastes or odor-producing substances.	None attributable to sewage, industrial wastes or other wastes.
2. Sewage or waste effluents.	None.
3. Dissolved oxygen.	Not less than 5 ppm.
4. Toxic wastes, deleterious substances, colored or other wastes or heated liquids.	None in amounts to exceed limitations set by State Board of Health for waters for this use.

CLASS A

Waters meeting the State Board of Health regulations as suitable for use as swimming water. Suitable also for other uses requiring waters of lesser purity.

Quality Standards for Class A Waters

Items	Specifications
1. Floating solids; settleable solids; oil; sludge deposits.	None which are readily visible and attributable to sewage, industrial wastes or other wastes and which measurably increase the amounts of these constituents in receiving waters after opportunity for reasonable dilution and mixture with the wastes discharged thereto.
2. Sewage or waste effluents.	None in such quantity or quality as to raise the coliform bacteria count of these waters above 1,000 per 100 ml.
3. Odor-producing substances contained in sewage, industrial wastes or other wastes.	The waters after opportunity for reasonable dilution and mixture with the wastes discharged thereto shall not have an increased threshold odor number greater than 8, due to such added wastes.
4. Phenolic compounds.	Not greater than 5 parts per billion (Phenol).
5. pH.	Range between 6.0 and 8.0, except that swamp waters may range from pH 5.0 to pH 8.0.
6. Dissolved oxygen.	Not less than 4.5 ppm, except that swamp waters may have a low of 2.5 ppm.
7. Toxic wastes, deleterious substances, colored or other wastes, or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to be injurious to fish life or make the waters unsafe or unsuitable for bathing.

CLASS B

Suitable for domestic supply after complete treatment in accordance with regulations of the State Board of Health. Suitable also for uses requiring water of lesser purity.

Quality Standards for Class B Waters

Items	Specifications
1. Floating solids; settleable solids; oil; sludge deposits.	None which are readily visible and attributable to sewage, industrial wastes or other wastes and which measurably increase the amounts of these constituents in receiving waters after opportunity for reasonable dilution and mixture with the wastes discharged thereto.
2. Sewage or waste effluents.	None in such quantities or qualities as to make the coliform bacteria count of the waters exceed 5,000 per 100 ml.
3. pH.	Range between 6.0 and 8.0, except that swamp waters may range from pH 5.0 to pH 8.0.
4. Dissolved oxygen.	Not less than 4.0 ppm, except that swamp waters may have a low of 2.5 ppm.
5. Phenolic compounds.	Not greater than 5 parts per billion.
6. Toxic wastes, deleterious substances, colored or other wastes, or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to be injurious to fish or make the waters unamendable to standard treatment processes intended to prepare them for domestic use.

CLASS C

Waters suitable for propagation of fish, industrial and agricultural uses and other uses requiring water of lesser quality.

Quality Standards for Class C Waters

Items	Specifications
1. Floating solids; settle- or other wastes or heated liquids.	None which are readily visible and attributable to sewage, industrial wastes or other wastes and which measurably increase the amounts of these constituents in receiving waters after opportunity for reasonable dilution and mixture with the wastes discharged thereto.
2. pH.	Range between 6.0 and 8.5, except that swamp waters may range between 5.0 and 8.5.
3. Dissolved oxygen.	Not less than 4 ppm, except that swamp waters may have a low of 2.0 ppm.
4. Toxic wastes, deleterious substances, colored or other wastes, or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to be injurious to fish life or impair the waters for any other best usage as determined by the Water Pollution Control Authority for the specific waters which are assigned to this class.

CLASS D

Temporary classification to be assigned waters receiving wastes for which no practicable means of treatment is known. Dischargers of wastes shall make every reasonable effort to avoid creation of nuisance conditions.

As soon as feasible treatment processes are available, Class D waters shall be reclassified to an appropriate class and further waste discharges governed accordingly.

CLASSIFICATION-STANDARDS SYSTEM FOR THE STATE OF SOUTH CAROLINA APPLYING TO FRESH SURFACE WATERS

(Adopted by the South Carolina Water Pollution Control Authority August 20, 1954)

Class C_s

Waters suitable for fish survival,* industrial and agricultural uses and other uses requiring water of lesser quality.

Quality Standards for Class C_s Waters

Items	Specifications
1. Floating solids; settleable solids; oil; sludge deposits.	None which are readily visible and attributable to sewage, industrial wastes or other wastes and which measurably increase the amounts of these constituents in receiving waters after opportunity for reasonable dilution and mixture with the wastes discharged thereto.
2. pH.	Range between 6.0 and 8.5, except that swamp waters may range between 5.0 and 8.5.
3. Dissolved oxygen.	Not less than 2.0 parts per million.
4. Toxic wastes, deleterious substances, colored or other wastes, or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to be injurious to fish survival or impair the waters for any other best usage as determined by the Water Pollution Control Authority for the specific waters which are assigned to this class.

SOUTH CAROLINA WATER POLLUTION CONTROL AUTHORITY CLASSES AND STANDARDS FOR TIDAL SALT WATERS

(Adopted by the South Carolina Water Pollution Control Authority March 13, 1952)

Class SA

Best Usage of Waters: Shellfishing for market purposes and any other usages.

Quality Standards for Class SA Waters

Items	Specifications
1. Floating solids; settleable solids; oil; sludge deposits.	None attributable to sewage or industrial wastes.
2. Garbage, cinders, ashes, oils, sludge or other refuse.	None.
3. Sewage or waste effluents.	None which are not effectively disinfected.
4. Dissolved oxygen.	Not less than 5.0 parts per million.
5. Toxic wastes, deleterious substances, colored or other wastes or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to be injurious to edible fish or shellfish or the culture or propagation thereof, or which in any manner shall adversely affect the flavor, color, odor or sanitary condition thereof or impair the waters for any other best usage as determined for the specific waters which are assigned to this class.
6. Organisms of coliform group.	The median MPN value in any series of samples representative of waters in the shellfish growing area shall not be in excess of 70 per 100 milliliters.

Class SB

Best Usage of Waters: Bathing and any other usages except shellfishing for market purposes.

Quality Standards for Class SB Waters

Items	Specifications
1. Floating solids; settleable solids; oil; sludge deposits.	None attributable to sewage or industrial wastes.
2. Garbage, cinders, ashes, oils, sludge or other refuse.	None.
3. Sewage or waste effluents.	None which are not effectively disinfected.
4. Dissolved oxygen.	Not less than 5.0 parts per million.
5. Toxic wastes, deleterious substances, colored or other wastes or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to be injurious to edible fish or the culture or propagation thereof, or which in any manner shall adversely affect the flavor, color, odor or sanitary condition thereof; and otherwise none in sufficient amounts to make the waters unsafe or unsuitable for bathing or impair the waters for any other best usage as determined for the specific waters which are assigned to this class.
6. Organisms of coliform group.	None in excess of the requirements of the South Carolina State Board of Health for bathing water.

Class SC

Best Usage of Waters: Crabbing, non-commercial fishing and any other usages except bathing or other shellfishing for market purposes.

Quality Standards for Class SC Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	None which are readily visible and attributable to sewage or industrial wastes or which deleteriously increase the amounts of these constituents in receiving waters after opportunity for reasonable dilution and mixture with the wastes discharged thereto.
2. Garbage, cinders, ashes, oils, sludge or other refuse.	None.

* "Fish survival" as used in this standard means the continued existence of individual fish normally indigenous to waters of this type.

Items	Specifications
3. Dissolved oxygen.	Not less than 4.0 parts per million.
4. Toxic wastes, oil, deleterious substances, colored or other wastes or heated liquids.	None alone or in combination with other substances or wastes in sufficient amounts or at such temperatures as to be injurious to edible fish or the culture or propagation thereof, or which in any manner shall adversely affect the flavor, color, odor or sanitary condition of fish or impair the waters for any other best usage as determined for the specific waters which are assigned to this class.

Class SD

Best Usage of Waters: Any usages except fishing, bathing, or shellfishing for market purposes.

Quality Standards for Class SD Waters

Items	Specifications
1. Floating solids; settleable solids; sludge deposits.	None which are readily visible and attributable to sewage or industrial wastes or which deleteriously increase the amounts of these constituents in receiving waters after opportunity for reasonable dilution and mixture with the wastes discharged thereto.
2. Garbage, cinders, ashes, oils, sludge or other refuse.	None.
3. Dissolved oxygen.	Not less than 3.0 parts per million.
4. Toxic wastes, oil, deleterious substances, colored or other wastes.	None alone or in combination with other substances or wastes in sufficient amounts to prevent survival of fish life or impair the waters for any other best usage as determined for the specific waters which are assigned to this class.

APPENDIX J

UTAH WATER POLLUTION CONTROL BOARD THE STANDARDS OF QUALITY AND THE REGULATIONS FOR WATER CLASSIFICATION

Adopted by the Utah Water Pollution Control Board, November 19, 1954 and made official by order of the Board, February 24, 1955, pursuant to authority of Section 73-14-6, Utah Code Annotated, 1953 as amended by Session Laws of 1953. Amended by order of the Board, September 7, 1960.

CLASS "A" WATERS

Class "A" waters shall be so protected against pollution as to be suitable at all times without treatment for domestic water supplies, irrigation, stock watering, fish and wildlife propagation, recreation, as a source for industrial supplies, and for other uses as may be determined by the Board.

Regulations and Standards: No person shall discharge any waste directly into Class "A" waters, or dispose of any wastes in such a way as to result in

(1) characteristics of said waters exceeding the limits prescribed by U.S. Public Health Service Drinking Water Standards as of February 6, 1946, or

(2) chemical characteristics of said waters exceeding the recommendations for irrigation water quality as outlined in Chapter 5 of the U.S. Department of Agriculture "Agriculture Handbook No. 60," issued February, 1954.

CLASS "B" WATERS

Class "B" waters shall be so protected against pollution as to be suitable at all times for domestic supplies which are treated before use by disinfection only. Class "B" waters shall be suitable without treatment for irrigation, stock watering, fish and wildlife propagation, recreation as a source for industrial supplies, and for other uses as may be determined by the Board.

Regulations and Standards: No person shall discharge any wastes directly into Class "B" waters or dispose of any wastes in such a way as to result in

(1) physical and chemical characteristics of said waters exceeding the limits prescribed by U.S. Public Health Service Drinking Water Standards as of February 6, 1946, or

(2) chemical characteristics of said waters exceeding the recommendations for irrigation water quality as outlined in Chapter 5 of the U.S. Department of Agriculture "Agriculture Handbook No. 60," issued February, 1954, or

(3) a monthly arithmetical average "most probable number" (MPN) of coliform organisms in said waters exceeding 50 per 100 milliliters; or in an MPN exceeding this number in more than 20% of the samples collected during any month; or in an MPN exceeding 100 per 100 milliliters in more than 5% of such samples.

CLASS "C" WATERS

Class "C" waters shall be so protected against pollution as to be suitable at all times for domestic water supplies which are treated before use by coagulation, sedimentation, filtration, and disinfection. Class "C" waters shall be suitable without treatment for irrigation, stock watering, fish and wildlife propagation, recreation (except swimming), as a source for industrial supplies, and for other uses, as may be determined by the Board.

Regulations and Standards: No person shall discharge into Class "C" waters any wastes

(1) which result in chemical characteristics of said waters exceeding the limits prescribed by U.S. Public Health Service Drinking Water Standards as of February 6, 1946, or

(2) which result in chemical characteristics of said waters exceeding the recommendations for irrigation water quality as outlined in Chapter 5 of the U.S. Department of Agriculture "Agriculture Handbook No. 60," issued February, 1954, or

(3) which result in a monthly arithmetical average "most probable number" (MPN) of coliform organisms in said waters exceeding 5,000 per 100 milliliters; or in an MPN exceeding this number in more than 20% of the samples collected during any month; or in an MPN exceeding 20,000 per 100 milliliters in more than 5% of such samples, or

(4) which result in a monthly arithmetical average biochemical oxygen demand (BOD) in said waters exceeding 5 parts per million (ppm); or in a BOD exceeding this amount in more than 20% of the samples collected in any month; or in a BOD exceeding 10 ppm in more than 5% of such samples, or

(5) which result in any slicks, floating solids, suspended solids or sludge deposits in said waters which are readily visible, or which result in an appreciable change in color of said waters, or

(6) which result in a pH of said waters lower than 5.0 or greater than 9.0, or

(7) which contain any toxic wastes, phenols, or other deleterious substances in such concentrations or at such temperatures as will render said waters injurious to fish life and waterfowl, or unsafe or unsuitable as sources of water supply for domestic use, food processing or industrial use, or unsuit-

able for agricultural purposes, stock watering, or recreation (except swimming).

CLASS "D" WATERS

Class "D" waters shall be so protected against pollution as to be suitable at all times for general irrigation, but not for irrigation of lawns, recreational areas, pastures used for dairy cattle, root crops or any low growing crops produced for human consumption. Such waters shall be suitable also as a source for industrial supplies and for other uses as may be determined by the Board.

Regulations and Standards: No person shall discharge into Class "D" waters any wastes

(1) which result in chemical characteristics of said waters exceeding the limits prescribed by U. S. Public Health Service Drinking Water Standards as of February 6, 1946, or

(2) which result in chemical characteristics of said waters exceeding the recommendations for irrigation water quality as outlined in Chapter 5 of U. S. Department of Agriculture "Agriculture Handbook No. 60," issued February, 1954, or

(3) which result in a monthly arithmetical average "most probable number" (MPN) of coliform organisms in said waters exceeding 5,000 per 100 milliliters; or in an MPN exceeding this number in more than 20% of samples collected during any month; or in an MPN exceeding 20,000 per 100 milliliters in more than 5% of such samples or

(4) which result in a monthly arithmetical average biochemical oxygen demand (BOD) in said waters exceeding 25 parts per million (ppm); or in a BOD exceeding this amount in more than 20% of the samples collected in any month; or in a BOD exceeding 50 ppm in more than 5% of such samples, or

(5) which result in any slicks, floating solids, suspended solids, or sludge deposits in said waters which are readily visible, or which result in an appreciable change in color of said waters, or

(6) which result in a pH of said waters lower than 5.0 or greater than 9.0, or

(7) which contain any toxic wastes, phenols, or other deleterious substances in such concentrations or at such temperatures as will render said waters unsuitable for the uses designated in the introductory paragraph of this section.

CLASS "E" WATERS

Class "E" waters shall be protected against such pollution as may result in nuisances. They shall have no beneficial uses.

Regulations and Standards: No person shall discharge into Class "E" waters any wastes which will create a condition constituting or resulting in a nuisance. The Board may direct that such waters be suitably isolated by closed conduit, approved fencing, or other means.

The following notes were adopted as modifications to The Standards of Quality and The Regulations for Water Classification on September 7, 1960, and are hereby made a part of said standards and regulations.

NOTE 1: The Board may assign any one of the foregoing classifications to a given water notwithstanding the presence in said water of natural pollutants in excess of limits established by the classification, in which case the subscript "1" shall be added to the usual classification designation (A_1, B_1, C_1, etc.) to denote the modification of water quality. No change in waste discharge restrictions of the basic classification shall be inferred, except that the discharge of any waste in such a way as to increase the concentration of any of the excessive natural pollutants in the classified water is prohibited.

NOTE 2: Wastes discharged to waters of the state under limitations imposed by a given classification shall be further controlled as required to protect water quality designated by all downstream classifications.

APPENDIX K

WATER SUPPLY AND POLLUTION ABATEMENT COMMITTEE

Water Quality Objectives for
The Potomac River in Washington Metropolitan Area

June 1959

A clean Potomac is the only acceptable water quality objective for the Washington Metropolitan Area. Its early attainment within the greater portion of the river between the Monocacy and Hallowing Point is believed to be feasible. Substantial improvement in the remainder of this zone is an immediate necessity.

These objectives, therefore represent a thoughtful indication of the uses of the Potomac to which the people of the area may aspire within the foreseeable future. Their acceptance by the Washington Metropolitan Regional Conference is in no sense to be considered as an encroachment on the enforcement agencies within the States.

The accomplishment of pollution control to the extent required to meet these objectives will call upon all jurisdictions and agencies in the area concerned with the problem to exert extensive and persistent efforts to this end. This the committee is confident they will do—to and even beyond the limits of their immediate resources.

By the acceptance of these objectives the governmental representatives of the principal jurisdictions will have set for themselves on a voluntary basis the first guidelines as to water quality ever applied to the metropolitan area. These will thus provide the first understandings upon which all can proceed in the development of their respective programs for construction and operation of the necessary control works. The concentration of population and the rapid expansion of the densely occupied land areas dictate the necessity for such coordination in the development of sewage collection and disposal facilities throughout the region.

The following text has been based on the recommendations of the Interstate Commission on the Potomac River Basin. Certain changes have been made therein primarily for purposes of clarification and internal consistency of text. Other changes have been made to reflect the previously stated policy of this committee with respect to effluent discharges above the water intakes.

These objectives have been prepared in the light of present knowledge. With a growth of knowledge and experience as to the behavior of the Potomac, changes in the objectives and the related specific water quality criteria are to be expected. Such changes must always, however, be directed toward ever higher uses as may be in the best regional interest.

The water quality objectives for the Sections indicated below are as follows:

SECTION I

POTOMAC RIVER: MONOCACY RIVER TO GREAT FALLS

General

Objectives: The establishment of conditions:

1. suitable for domestic water supplies, fish propagation, and recreational uses;
2. conducive to reduction of existing silt loading and prevention of future increases in this loading.

Criteria: Limiting values of the following water quality characteristics shall be as indicated in 95% of the samples analyzed monthly:

1. Coliform Group: Monthly median MPN not to exceed 2,000 per 100 ml.
2. pH Range between 6.5 and 8.5.
3. D. O.: Monthly median not less than 6.5 ppm; minimum content shall be 4.0 ppm.
4. Turbidity: After opportunity for reasonable dilution in the river, the turbidity of the receiving water shall not be measurably increased by any discharges so received.
5. Other Conditions: There shall be no floating solids, oil, settleable solids, or sludge deposits attributable to sewage, industrial wastes or other wastes. There shall be no toxic wastes, deleterious substances, colored or other wastes, or heated liquids, taste- or odor-producing substances, either alone or in combinations sufficient to be injurious to fish life or to make the waters unsafe or unsuitable as a source of municipal water supply or other desirable uses. Development should be so designed as to prevent discharge of effluent from sewage treatment plants either into the river or its tributaries.

SECTION II

POTOMAC RIVER: GREAT FALLS TO LITTLE FALLS

Objective: The establishment of conditions:

1. suitable for domestic water supplies, fish propagation and recreational uses;
2. conducive to reduction of existing silt loading and prevention of future increases in this loading.

Criteria: Limiting values of the following water quality characteristics shall be as indicated in 95% of the samples analyzed monthly:

1. Coliform Group: Monthly median MPN not to exceed 2,000 per 100 ml.
2. pH Range between 6.5 and 8.5.
3. D. O.: Monthly median not less than 6.5 ppm; minimum content shall be 4.0 ppm.
4. Turbidity: After opportunity for reasonable dilution in the river, the turbidity of the receving water shall not be measurably increased by any discharges so received.
5. Other Conditions: There shall be no floating solids, oil, settleable solids, or sludge deposits attributable to sewage, industrial wastes or other wastes. There shall be no toxic wastes, deleterious substances, colored or other wastes or heated liquids, taste- or odor-producing substances, either alone or in combinations in sufficient amounts to be injurious to fish life or to make the waters unsafe or unsuitable as a source of municipal water supply or other desirable uses. Development should be so designed as to prevent discharge of effluent from sewage treatment plants either into the river or its tributaries.

SECTION III

POTOMAC RIVER: LITTLE FALLS TO KEY BRIDGE

Objective: The establishment of conditions:

1. suitable for swimming, fishing, boating, shore recreation, and safe for all species of fish life with favorable conditions prevailing for their propagation;
2. conducive to reduction of existing silt loading and prevention of future increases in this loading.

Criteria: Limiting values of the following water quality characteristics shall be as indicated in 95% of the samples analyzed monthly:

1. Coliform Group: Monthly median MPN not to exceed 2,000 per 100 ml.
2. pH Range between 6.5 and 8.5.
3. D. O.: Monthly median not less than 6.5 ppm; minimum content shall be 4.0 ppm.
4. Turbidity: After opportunity for reasonable dilution in the river, the turbidity of the receiving water shall not be measurably increased by any discharges so received.
5. Other Conditions: There shall be no floating solids, oil, settleable solids, sewage and waste effluent or sludge deposits attributable to sewage, industrial wastes or other wastes. There shall be no wastes, deleterious substances, colored or other wastes or heated liquids, taste- or odor-producing substances, either alone or in combinations, in sufficient amounts to be injurious to fish life or to make the waters unsafe for swimming or shore recreation.

SECTION IV

POTOMAC RIVER: KEY BRIDGE TO FORT WASHINGTON

Objectives: The establishment of conditions:

1. suitable for boating, shore recreation, industrial water supply and safe for the passage of all species of fish, with favorable conditions prevailing for the propagation of the hardier types;
2. conducive to reduction of existing silt loading and prevention of future increases in this loading.

Criteria: Limiting values of the following water quality characteristics shall be as indicated in 95% of the samples analyzed monthly:

1. Coliform Group: Monthly median MPN not to exceed 10,000 per 100 ml.
2. pH Range between 6.5 and 8.5.
3. D. O.: Monthly median not less than 5.0 ppm; with the minimum value greater than 4.0 ppm 97% of the time and greater than 3.0 ppm 99% of the time.
4. Turbidity: After opportunity for reasonable dilution in the river, the turbidity of the receiving water shall not be measurably increased by any discharges so received.
5. Other Conditions: There shall be no floating solids, oil, settleable solids, or sludge deposits attributable to sewage, industrial wastes or other wastes. There shall be no toxic wastes, deleterious substances, colored or other wastes or heated liquids, taste- or odor-producing substances, either alone or in combinations, in sufficient amounts to make the waters unsafe or unsuitable as a source of industrial process water supply, or for boating, shore recreation, passage of all species of fish or propagation of the hardier species of fish.

SECTION V

POTOMAC RIVER: FORT WASHINGTON TO HALLOWING POINT

Objective: The establishment of conditions:

1. suitable for boating, fishing, swimming, and shore recreation;
2. conductive to reduction of existing silt loading and prevention of future increases in this loading.

Criteria: Limiting values of the following water quality characteristics shall be as indicated in 95% of the samples analyzed monthly:

1. Coliform Group: Monthly median MPN not to exceed 2,000 per 100 ml.
2. pH Range between 6.5 and 8.5.

3. D. O.: Monthly median not less than 6.0 ppm with the minimum value greater than 4.0 ppm 97% of the time and greater than 3.5 ppm 99% of the time.
4. Turbidity: After opportunity for reasonable dilution in the river, the turbidity of the receiving water shall not be measurably increased by any discharges so received.
5. Other Conditions: There shall be no floating solids, oil, settleable solids, or sludge deposits attributable to sewage, industrial wastes or other wastes. There shall be no toxic wastes, deleterious substances, colored or other wastes or heated liquids, taste- or odor-producing substances, either alone or in combinations, in sufficient amounts to be injurious to propagation of fish life or to make the waters unsafe or unsuitable for boating, swimming, fishing, or shore recreation.

As a basic consideration applying to all Sections, any sewage or industrial wastes discharged or permitted to flow into tributaries of the Potomac should be treated to that extent, if any, which may be necessary to maintain such waters in a sanitary and satisfactory condition at least equal to the criteria recommended above for the waters of the Potomac immediately above the confluence of the tributary with the main stream.

The reference "other wastes" under condition No. 5 of the foregoing objectives shall be interpreted to include trash, garbage, dirt, soil or any matter causing or aiding pollution.

APPENDIX L

INTERNATIONAL JOINT COMMISSION OBJECTIVES FOR BOUNDARY WATERS QUALITY CONTROL

In order to permit a more accurate evaluation of the nature and extent of pollution, its effects and the remedial measures necessary, the Commission, in the course of the investigation, adopted the following statement of objectives for boundary waters quality control which was developed by the technical advisers:

"The term 'boundary waters' as herein used shall include the waters defined in the references to the International Joint Commission dated April 1, 1946, October 2 and 3, 1946, and April 2, 1948, and are as follows:

St. Clair River, Lake St. Clair, the Detroit River, St. Mary's River from Lake Superior to Lake Huron, and Niagara River from Lake Erie to Lake Ontario.

These objectives are for the boundary waters in general, and it is anticipated that in certain specific instances, influenced by local conditions, more stringent requirements may be found necessary.

General Objectives

"All wastes, including sanitary sewage, storm water, and industrial effluents, shall be in such condition when discharged into any stream that they will not create conditions in the boundary waters which will adversely affect the use of those waters for the following purposes: source of domestic water supply or industrial water supply, navigation, fish and wildlife, bathing, recreation, agriculture and other riparian activities.

"In general, adverse conditions are caused by:

(A) Excessive bacterial, physical or chemical contamination.

(B) Unnatural deposits in the stream, interfering with navigation, fish and wildlife, bathing, recreation, or destruction of aesthetic values.

(C) Toxic substances and materials imparting objectionable tastes and odors to waters used for domestic or industrial purposes.

(D) Floating materials, including oils, grease, garbage, sewage solids, or other refuse.

Specific Objectives

"In more specific terms, adequate controls of pollution will necessitate the following objectives for:

(A) Sanitary Sewage, Storm Water, and Wastes from Water Craft

Sufficient treatment for adequate removal or reduction of solids, bacteria and chemical constituents which may interfere unreasonably with the use of these waters for purposes aforementioned. Adequate protection for these waters, except in certain specific instances influenced by local conditions, should be provided if the coliform M.P.N. median value does not exceed 2,400 per 100 ml at any point in the waters following initial dilution.

(B) Industrial Wastes

(1) Chemical Wastes—Phenolic Type

Industrial waster effluents from penolic hydro-carbon and other chemical plants will cause objectionable tastes or odors in drinking or industrial water supplies and may taint the flesh of fish.

Adequate protection should be provided for these waters if the concentration of phenol or phenol equivalents does not exceed an average of 2 ppb and a maximum of 5 ppb at any point in these waters following initial dilution. This quality in the receiving waters will probably be attained if plant effluents are limited to 20 ppb of phenol or phenol equivalents.

Some of the industries producing phenolic wastes are: coke, synthetic resin, oil refining, petroleum cracking, tar, road oil, creosoting, wood distillation, and dye manufacturing plants.

(2) Chemical Wastes—Other Than Phenolic

Adequate protection should be provided if:

(a) The pH of these waters following initial dilution is not less than 6.7 nor more than 8.5 This quality in the receiving waters will probably be attained if plant effluents are adjusted to a pH value within the range of 5.5 and 10.6.

(b) The iron content of these waters following initial dilution does not exceed 0.3 ppm. This quality in the receiving waters will probably be attained if plant effluents are limited to 17 ppm of iron in terms of Fe.

(c) The odor-producing substances in the effluent are reduced to a point that following initial dilution with these waters the mixture does not have a threshold odor number in excess of 8 due to such added material.

(d) Unnatural color and turbidity of the wastes are reduced to a point that these waters will not be offensive in appearance or otherwise unattractive for the aforementioned purposes.

(e) Oils and floating solids are reduced to a point such that they will not create fire hazards, coat hulls of water craft, injure fish or wildlife or their habitat, or will

adversely affect public or private recreational development or other legitimate shoreline developments or uses. Protection should be provided for these waters if plant effluents or storm water discharges from premises do not contain oils, as determined by extraction, in excess of 15 ppm, or a sufficient amount to create more than a faint iridescence. Some of the industries producing chemical wastes other than phenolic are: oil wells and petroleum refineries, gasoline filling stations and bulk stations, styrene copolymer, synthetic pharmaceutical, synthetic fiber, iron and steel, alkali chemical, rubber fabricating, dye manufacturing, and acid-manufacturing plants.

(3) Highly Toxic Wastes

Adequate protection should be provided for these waters if substances highly toxic to human, fish, aquatic, or wildlife are eliminated or reduced to safe limits.

Some of the industries producing highly toxic wastes are: metal plating and finishing plants discharging cyanides, chromium or other toxic wastes; chemical or pharmaceutical plants and coke ovens. Wastes containing toxic concentrations of free halogens are included in this category.

(4) Deoxygenating Wastes

Adequate protection of these waters should result if sufficient treatment is provided for the substantial removal of solids, bacteria, chemical constituents and other substances capable of reducing the dissolved oxygen content of these waters unreasonably. Some of the industries producing these wastes are: tanneries, glue and gelatin plants, alcohol, including breweries and distilleries, wool scouring, pulp and paper, food-processing plants such as meat packing and dairy plants, corn products, beet sugar, fish-processing and dehydration plants."

NOTE: The methods of determination of the chemical constituents referred to in the preceding Objectives are as given in "Analytical Methods for Boundary Waters Quality Control," as prepared by the Board of Technical Advisers. Bacterial determinations are to include the presumptive and confirmed tests for the coliform group of bacteria as given in "Standard Methods for the Examination of Water and Sewage," American Public Health Association, New York.

APPENDIX M

OHIO RIVER VALLEY WATER SANITATION COMMISSION REGULATORY ACTIONS

BACTERIAL-QUALITY OBJECTIVES FOR THE OHIO RIVER

Adopted April 4, 1951

As a guide in the establishment of treatment requirements for sewage discharged in the Ohio River, and as a yardstick for evaluating sanitary conditions in waters used for potable supplies and recreational purposes, the Ohio River Valley Water Sanitation Commission on April 4, 1951 adopted these bacterial-quality objectives:

Water Supply Uses—The monthly arithmetical average "most probable number" of coliform organisms in waters of the river at water intakes should not exceed 5,000 per 100 ml in any month; nor exceed this number in more than 20 percent of the samples of such waters examined during any month; nor exceed 20,000 per 100 ml in more than 5 percent of such samples.

Recreational Uses—For bathing or swimming waters, monthly arithmetical average "most probable number" of coliform organisms should not exceed 1,000 per 100 ml during any month of the recreation season; nor exceed this number in more than 20 percent of the samples examined during any such month; nor exceed 2,400 per 100 ml on any day. For non-bathing or non-swimming waters, the monthly arithmetical average "most probable number" of coliform organisms should not exceed 5,000 per 100 ml in any month of the recreational season, nor should exceed this number in more than 20 percent of the samples examined during any such month.

OHIO RIVER SEWAGE TREATMENT STANDARDS

Treatment Standard No. 1—CINCINNATI POOL
(Adopted April 6, 1949)

All sewage from municipalities or other political subdivisions, public or private institutions or corporations discharged or permitted to flow into that portion of the Ohio River, commonly known as the "Cincinnati Pool", extending from U. S. Corps of Engineers Dam No. 36, located about three miles upstream from Cincinnati, Ohio, and being 461 miles downstream from Pittsburgh, Pennsylvania, to U. S. Corps of Engineers Dam No. 37, located at Fernbank, Cincinnati, Ohio, and being 483 miles downstream from Pittsburgh, shall be so treated as to provide for:

(a) Substantially complete removal of settleable solids; and

(b) Removal of not less than forty-five percent of the total suspended solids; and, in addition

(c) Reduction by not less than 65% of the biochemical oxygen demand of organic wastes, provided, however, that whenever conditions permit, such lesser degree of reduction of biochemical oxygen demand, but not lower than 35%, may be applied to organic wastes if as a result there will be no impairment in the Cincinnati Pool of a water-quality standard of 4.0 parts per million of dissolved oxygen at the bottom of the oxygen sag in the Ohio River below Cincinnati.

Treatment Standard No. 2—HUNTINGTON, W. VA., TO CINCINNATI, OHIO
(Adopted April 2, 1952)

All sewage from municipalities or other political subdivisions, public or private institutions or corporations discharged or permitted to flow into that portion of the Ohio River extending from U. S. Corps of Engineers Dam No. 27, located about five miles upstream from Huntington, West Virginia, and being 301 miles downstream Pittsburgh, Pennsylvania, to U. S. Corps of Engineers Dam No. 36, located about three miles upstream from Cincinnati, Ohio, and being 461 miles downstream from Pittsburgh, Pennsylvania, shall be so treated as to provide for:

(a) Substantially complete removal of settleable solids; and

(b) Removal of not less than forty-five percent of the total suspended solids; and, in addition

(c) Reduction in coliform organisms in accordance with the following schedule:

Not less than 90% reduction during the months May through November.

Not less than 80% reduction during the months December through April.

Treatment Standard No. 3—BELOW PITTSBURGH (Mile 15) TO HUNTINGTON
(Adopted April 29, 1953)

All sewage from municipalities or other political subdivisions, public or private institutions or corporations discharged or permitted to flow into that portion of the Ohio River extending from the Allegheny County-Beaver County line in Pennsylvania, located approximately 15 miles downstream from the confluence of the Allegheny and Monongahela Rivers at Pittsburgh, to Corps of Engineers Dam No. 27, located about five miles upstream from Huntington, W. Va., and being 301.0 miles downstream from Pittsburgh, Pennsylvania, shall be so treated as to provide for:

(a) Substantially complete removal of settleable solids; and

(b) Removal of not less than forty-five percent of the total suspended solids; and, in addition

(c) Reduction in coliform organisms in accordance with the following schedule:

Not less than 80% reduction during the months May through October.

Not less than 85% reduction during the months November through April.

Treatment Standard No. 4—PITTSBURGH AND VICINITY (Mile 0.0 to Mile 15) (Adopted April 29, 1953)

All sewage from municipalities or other political subdivisions, public or private institutions or corporations discharged or permitted to flow into that portion of the Ohio River extending from the point of confluence of the Allegheny and Monongahela Rivers at Pittsburgh, designated as Ohio River mile point 0.0, to the Allegheny County-Beaver County line in Pennsylvania, located approximately 15 miles downstream from the confluence of the Allegheny and Monongahela Rivers, shall be so treated as to provide for:

(a) Substantially complete removal of settleable solids; and
(b) Removal of not less than forty-five percent of the total suspended solids; and
(c) Reduction in biochemical oxygen demand of approximately fifty percent; and, in addition
(d) Reduction in coliform organisms in accordance with the following schedule:
Not less than 80% reduction during the months May through October.
Not less than 85% reduction during the months November through April.

Treatment Standard No. 5—CINCINNATI TO OWENSBORO (Adopted January 13, 1954)

All sewage from municipalities or other political subdivisions, public or private institutions or corporations discharged or permitted to flow into that portion of the Ohio River extending from mile point 483.2 (miles below Pittsburgh, Pensylvania), located about ten miles downstream from Cincinnati, Ohio, and at which point is located Dam No. 37, to mile point 750.0 (miles below Pittsburgh, Pennsylvania), located about six miles upstream from Owensboro, Kentucky, shall be so treated as to provide for:

(a) Substantially complete removal of settleable solids; and
(b) Removal of not less than forty-five percent of the total suspended solids.

Treatment Standard No. 6—OWENSBORO TO HENDERSON (Adopted January 13, 1954)

All sewage from municipalities or other political subdivisions, public or private institutions or corporations discharged or permitted to flow into that portion of the Ohio River extending from mile point 750.0 (miles below Pittsburgh, Pennsylvania), located about six miles upstream from Owensboro, Kentucky, to mile point 803.0 (miles below Pittsburgh, Pennsylvania) located near Henderson, Kentucky, shall be so treated as to provide for:

(a) Substantially complete removal of settleable solids; and
(b) Removal of not less than forty-five percent of the total suspended solids; and, in addition
(c) Reduction in coliform organisms in accordance with the following schedule:
Not less than 85% reduction during the months May through October.
Not less than 65% reduction during the months November through April.

Treatment Standard No. 7—HENDERSON TO CAIRO (Adopted January 13, 1954)

All sewage from municipalities or other political subdivisions, public or private institutions or corporations discharged or permitted to flow into that portion of the Ohio River extending from mile point 803.0 (miles below Pittsburgh, Pennsylvania), located near Henderson, Kentucky, to Cairo Point, Illinois, located at the confluence of the Ohio and Mississippi Rivers and being 981.0 miles downstream from Pittsburgh, Pennsylvania, shall be so treated as to provide for:

(a) Substantially complete removal of settleable solids; and
(b) Removal of not less than forty-five percent of the total suspended solids.

CONCLUSIONS REGARDING TREATMENT NEEDS FOR THE WABASH RIVER BETWEEN TERRE HAUTE, IND. AND VINCENNES, IND.

Adopted October 4, 1950

On the basis of observed conditions coupled with stream analysis and in accordance with established quality objectives, the following conclusions are reached:

TERRE HAUTE

Oxygen Conditions—Maintenance of the desired dissolved-oxygen water-quality objectives requires treatment facilities for the Terre Haute pollution load capable of providing substantially complete removal of settleable solids and total reduction of 52 percent in biochemical oxygen demand. Whenever stream flow and temperature conditions permit, an overall reduction in biochemical oxygen demand of less than 52 percent may be applied, if, as a result, there is no impairment to the water-quality objective of 50 percent dissolved-oxygen saturation in the river below Terre Haute. However, under no conditions shall treatment be less than that which will provide for substantially complete removal of settleable solids and the removal of not less than forty-five percent of the total suspended solids, which is the minimum specified under the terms of the Ohio River Compact.

Bacteriological Conditions—Chlorination of Terre Haute sewage is advisable because of the high concentration of coliform organisms discharged, the influence of which is felt as far downstream as Vincennes. Chlorination could be expected to improve the quality of water reaching the Vincennes intake, as well as enhance the recreational potentialities of the stream.

Allocation of Treatment—Allocation of treatment requirements among the various polluters at Terre Haute is a matter that commends itself for attention by the Indiana Stream Pollution Control Board. State, municipal and industrial relationships, as well as

technical considerations, are involved in this decision. Among the factors to be weighed are the relative size of domestic and industrial pollution loads and the responsibility to be assigned for their reduction. The equities involved are a matter for decision by the state.

VINCENNES

At Vincennes treatment of all municipal and industrial wastes providing for substantially complete removal of settleable solids and the removal of not less than forty-five percent of the total suspended solids will be adequate to insure maintenance of desired dissolved-oxygen water-quality objectives in the Wabash River below this city.

CONCLUSIONS AND RECOMMENDATIONS REGARDING TREATMENT NEEDS FOR THE WEST FORK AND MONONGAHELA RIVERS BETWEEN WESTON, W. VA. AND PITTSBURGH, PA.

Adopted February 6, 1959

This investigation was made for purposes of evaluating pollution conditions resulting from sewage discharged to the Monongahela and West Fork rivers and for determination of remedial measures in terms of sewage-treatment requirements.

Recommendations resulting from the investigation reflect the following considerations: First, sewage discharges originating within one signatory state shall not injuriously affect water uses in another state; second, waters of the Ohio Valley Compact district shall be placed and maintained in a satisfactory sanitary condition, in accordance with the provisions of Article I of the Compact.

Quality conditions were evaluated in terms of estimated dissolved-oxygen levels and coliform-bacterial concentrations at river flows of varying magnitude. From this analysis the following conclusions are reached:

1. Treatment of existing sewage discharges to the Monongahela River (which is formed by the confluence of the West Fork and Tygart rivers) in accordance with the minimum degree of treatment specified in the Compact should insure the maintenance of adequate sanitary quality at points of interstate concern. One point of interstate concern is the West Virginia-Pennsylvania state line at Mile 91.2. Also of interstate concern is the quality of water at the mouth of the Monongahela and its consequent effect on conditions in the Ohio River.
2. Sewage discharged in West Virginia to the West Fork River—notably from Weston and Clarksburg—requires something more than primary treatment to achieve satisfactory conditions in the West Fork River as measured by dissolved-oxygen content and coliform bacteria concentrations.
3. To meet local needs with regard to water supplies taken from the Monongahela River at points below certain sewage discharges in Pennsylvania, the sewage-treatment requirements at these places should be somewhat more stringent than those which are necessary to satisfy interstate necessities alone.

Recommendations

It is recommended that:

1. The Commission take such action as will express approval from an interstate point of view, of a program in which all sewage discharged into the Monongahela River is treated so as to provide substantially complete removal of settleable solids and the removal of not less than 45 percent of the total suspended solids.
2. The State of West Virginia and the Commonwealth of Pennsylvania be invited to utilize such findings in this report as they may find useful in establishing sewage-treatment requirements at certain communities where local conditions claim separate consideration.
3. Quality conditions in the Monongahela River be reevaluated at periodic intervals, notably with regard to water-quality changes resulting from the operation of sewage-treatment plants and the anticipated mitigation of mine-drainage pollution. Data being collected at Commission-sponsored monitor stations will be most useful toward this end. Equally important, however, will be such additional data as can be developed by West Virginia and Pennsylvania with regard to: (a) sewage and industrial-waste loads; and, (b) river-water analyses made by them and by water-treatment plants under their respective jurisdiction.

OHIO RIVER VALLEY WATER SANITATION COMMISSION

IW-1

BASIC INDUSTRIAL WASTE REQUIREMENTS

Industrial wastes shall be treated or otherwise modified prior to discharge so as to maintain the following conditions in the receiving waters:

1. Freedom from anything that will settle to form putrescent or otherwise objectionable sludge deposits which interfere with reasonable water uses.
2. Freedom from floating debris, scum and other floating materials in amounts sufficient to be unsightly or deleterious.
3. Freedom from materials producing color or odor in such degree as to create a nuisance.
4. Freedom from substances in concentrations or combinations which are toxic or harmful to human, animal or aquatic-life.

These conditions to be maintained in the receiving waters following the discharge of industrial-waste effluents, are basic or minimum requirements. Investigations will be conducted by the Commission as time and circumstances permit to establish the need and validity of altering or adding to the above basic requirements.

Questions concerning compliance with requirements are to be addressed to the signatory state agency in the state in which the industrial plant is operating. Arrangements will be made by the state agency for such contact

and consultation with the Commission as may be necessary or requested.

OHIO RIVER VALLEY WATER SANITATION COMMISSION

RESOLUTION NO. 17-58

Chloride-control measure

Adopted September 12, 1958

(Several introductory paragraphs omitted)

The following measures are hereby adopted by the Commission to be followed by the signatory states for the joint cooperative control of chloride discharges:

1. Existing and future discharges of wastes containing significant chloride loads into the waters of the Ohio River Valley Water Sanitation District shall be subject to control by impoundment or by such other methods as may be acceptable to the water pollution-control agency of the state in which the discharge originates, so as to insure that the amount thereof shall conform to a discharge schedule to be formulated, as hereinafter provided, for the purpose of maintaining an acceptable proportion between the amount of such chloride-containing wastes and the volume of flow in the stream into which it is discharged;
2. In order to insure appropriate ratios between chloride-waste discharges and volume of stream flows, all impoundment basins and other control methods shall be operated in accordance with schedules to be formulated by the water pollution-control agency of the state in which the discharge originates in consultation with the Ohio River Valley Water Sanitation Commission.

RESOLUTION NO. 18-58

Implementation of chloride-control program

Adopted September 12, 1958

(Introductory paragraph omitted)

(a) Where impoundment basins are employed for control purposes, the discharge from which enters the Ohio River directly or through its tributaries, the preferred minimum storage capacity shall be sufficient to contain three-months production of chloride waste. Deviations from this preferred minimum capacity requirement may be permitted only with the approval of the water pollution-control agency of the state in which the discharge originates after consultation with the Ohio River Valley Water Sanitation Commission.

(b) Each of the signatory states shall compile an inventory of existing industries discharging a significant chloride load, together with the locations and amounts of such discharges. These inventories shall be maintained up-to-date as new industries are granted discharge privileges and shall be made part of the Commission records.

(c) Within one year following the adoption of these chloride-control resolutions each state shall prepare a progress report for presentation at a regular meeting of the Commission on the status of control for each discharger of chloride waste subject to compliance.

(d) Each state shall conduct, and may request collaboration of the Commission in conducting, such investigations of effluents from political subdivisions that discharge sewage into the Ohio River directly or through its tributaries as may be necessary to determine whether these effluents contain abnormal concentrations of chloride. Where abnormal conditions are revealed, the state shall take appropriate steps to insure correction of such conditions.

(e) The Executive Director is hereby instructed to make periodic reports on chloride concentrations in the waters of the Ohio River Valley Water Sanitation District as revealed from monitor-station records.

RESOLUTION NO. 14-60

Significant chloride load

Adopted April 8, 1960

RESOLVED: That for present determination of chloride discharges subject to compliance with the ORSANCO chloride-control measure, a "significant load" is defined as:

1. Any existing discharge to the Ohio River or its tributaries which is equal to or greater than 25 tons per day; or
2. Any discharge from new or expanded operations to the Ohio River or its tributaries which is equal to or greater than 5 tons per day; or
3. Any discharge less than any of the above values which in the opinion of the state agency, causes a local degradation of water quality, although it otherwise satisfies interstate control requirements.

Deviations from the above definitions may be permitted only with the aproval of the water pollution control agency of the state in which the discharge originates after consultation with the Commission.

APPENDIX N

INTERSTATE COMMISSION ON THE DELAWARE RIVER BASIN EXCERPTS FROM THE RECIPROCAL AGREEMENT

Article II—Classification of Zones

It is recognized by the signatory states that due to such variable factors as location, size, character, and flow, and of the many varied uses of the waters of the interstate Delaware River and its aforesaid West Branch, such as water supply, recreation, navigation, industrial developments, maintenance of fish life, shellfish culture, agriculture, and other purposes, that no single standard of sewage and waste treatment and of quality of receiving waters is practical for all parts of the river. Therefore, in order to apply minimum requirements for the attainment of correction and control of pollution which will be appropriate to the varied factors including the existing and potential quality and uses of the waters, the interstate Delaware River is hereby divided into four zones, to wit:

Zone 1: Zone 1 is that part of the Delaware River and its West Branch extending from the New York-Pennsylvania boundary line to the head of tidewater at Trenton, New Jersey and Morrisville, Pennsylvania.

The drainage basin contributory to this zone, excepting part of the Lehigh River Basin, is relatively sparsely inhabited and contains few sewered communities and relatively few industrial establishments producing waste water. The streams draining this area being, in general, relatively clean and of high elevation, are well adapted as sources of public water supplies, after treatment or purification.

The principal uses of the waters of the Delaware River in Zone 1 are expected to be for water supply after such treatment or purification as may be necessary, and for recreation, bathing, maintenance of fish and aquatic life, agriculture, and for other related purposes.

Zone 2: Zone 2 is that part of the Delaware River extending from the head of tidewater at Trenton, New Jersey and Morrisville, Pennsylvania, to a line drawn perpendicular to the channel of the Delaware River from the mouth of Pennypack Creek in Philadelphia, Pennsylvania, to the corresponding point on the New Jersey shore.

The drainage basin contributory to this zone is somewhat more densely populated than that of Zone 1, and it contains more sewered communities and industrial establishments.

The principal uses of the waters of the Delaware River in Zone 2 are expected to be for water supply, after treatment or purification, and for recreation, navigation, maintenance of fish and aquatic life, agricultural, industrial and other purposes.

Zone 3: Zone 3 is that part of the Delaware River extending from the aforesaid line connecting the mouth of Pennypack Creek in Philadelphia and the corresponding point in New Jersey to the Pennsylvania-Delaware boundary line.

The drainage basin contributory to this zone contains populous metropolitan areas including Philadelphia, Pennsylvania and Camden, New Jersey.

The principal uses of the waters of the Delaware River in Zone 3 are expected to be for navigation, industrial water supply, and other purposes.

The water in this zone, however, should be of such sanitary quality that it will not be unfit for use as sources of water supply, will not be harmful to fish life, and will not adversely affect the quality of the waters of the tidal tributaries.

Zone 4: Zone 4 is that part of the Delaware River extending from the Pennsylvania-Delaware boundary line to the Atlantic Ocean.

The principal uses of the waters of the Delaware River in Zone 4 are expected to be for navigation, industrial water supplies, commercial fishing, shellfish culture, recreation and other purposes.

In order to attain conditions of cleanliness and sanitation of the waters of the Delaware River which will be consistent with the appropriate existing and future quality and uses of such waters, the following minimum requirements shall apply to the several zones herein provided. It is the purpose and intent of such requirements to apply to artificial (not natural) causes of pollution.

Article III—Minimum Requirements

In order to put and maintain the waters of the interstate Delaware River and its West Branch as aforesaid, in a clean and sanitary condition, no sewage, industrial wastes or other polluting matter shall be discharged into, or be permitted to flow or fall into, or be placed in any respective zone of the interstate Delaware River as herein established, unless such sewage, industrial waste or other artificial polluting matter shall first have been so treated as to produce an effluent which will meet the following minimum requirements:

Zone 1: 1. Such effluent shall be free of noticeable floating solids, color, oil, grease, or sleek, and practically free of suspended solids.

2. Such effluent shall be sufficiently free of turbidity that it will not cause noticeable turbidity in the water of the Delaware River.

3. Such effluent shall show a reduction of organic substances of at least eighty-five (85) percent as measured by the biochemical oxygen demand, and furthermore, such effluent in no case shall exceed a biochemical oxygen demand of fifty (50) parts per million, and furthermore, the discharge of such effluent, after dispersion in the water of the river, shall not cause a reduction of the dissolved oxygen content of such water of more than five

(5) per cent. The aforesaid reduction in dissolved oxygen content shall be determined by the average results obtained from dissolved oxygen tests made upon samples collected on not less than six (6) consecutive days from points in the river above and below the point or points of effluent discharge.

4. Such effluent shall be of such quality that the most probable number of organisms of the Coli Aerogenes group shall not exceed one (1) per milliliter in more than ten (10) per cent of the samples of sewage effluent tested by the confirmed test, and provided further that no single sample shall contain more than one hundred (100) organisms of the Coli Aerogenes group in one (1) milliliter.

5. Such effluent shall be sufficiently free of acids, alkalis, and other toxic or deleterious substances, that it will not create a menace to the public health through the use of the waters of the Delaware River for public water supplies, for recreation, bathing, agriculture and other purposes; nor be inimical to fish, animal or aquatic life.

6. Such effluent shall be free of offensive odors and also be free of substances capable of producing offensive tastes or odors in public water supplies derived from the Delaware River at any place below the discharge of such effluent.

Zone 2: 1. Such effluent shall be free of noticeable floating solids, color, oil, grease, and practically free of both suspended solids and sleek.

2. Such effluent shall be sufficiently free of turbidity that it will not cause noticeable turbidity in the water of the Delaware River.

3. Such effluent shall show a reduction of organic substances of at least eighty-five (85) per cent as measured by the biochemical oxygen demand, and furthermore, such effluent in no case shall exceed a biochemical oxygen demand of one hundred (100) parts per million, and furthermore, the discharge of such effluent, after dispersion in the water of the river, shall not cause a reduction of the dissolved oxygen content of such water of more than ten (10) per cent. The aforesaid reduction in dissolved oxygen content shall be determined by the average results obtained by dissolved oxygen tests made upon samples collected on not less than six (6) consecutive days from points in the river above and below the point or points of effluent discharge.

4. Such effluent shall be of such quality that the most probable number of organisms of the Coli Aerogenes group shall not exceed one (1) per milliliter in more than twenty-five (25) per cent of the samples of sewage effluent tested by the confirmed test, and provided further that no single sample shall contain more than one hundred (100) organisms of the Coli Aerogenes group in one (1) milliliter.

5. Such effluent shall be sufficiently free of acids, alkalis, and other toxic or deleterious substances, that it will not create a menace to the public health through the use of the water of the Delaware River for public water supplies, for recreation, industrial and other purposes; nor be inimical to fish, animal or aquatic life.

6. Such effluent shall be free of offensive odors and also be free of substances capable of producing offensive tastes and odors in public water supplies derived from the Delaware River at any place above or below the discharge of such effluent.

Zone 3: 1. Such effluent shall be free of noticeable floating solids, oil or grease, and substantially free of both suspended solids and sleek.

2. Such effluent shall be sufficiently free of turbidity that it will not cause substantial turbidity in the water of the Delaware River after dispersion in the water of the river.

3. Such effluent shall show a reduction of at least fifty-five (55) per cent of the total suspended solids and a reduction of not less than thirty-five (35) per cent of the biochemical oxygen demand. (It is the intent of this requirement to restore the dissolved oxygen content of the river water in this zone to at least fifty (50) per cent saturation. To accomplish this, it may be necessary in the case of certain wastes, to obtain reductions greater than those required under this item.)

4. Such effluent, if it be discharged within two miles of a public water works intake or within prejudicial influence thereof, shall at all times be effectively treated with a germicide.

5. Such effluent shall be sufficiently free of acids, alkalis, and other toxic or deleterious substances, that it will not create a menace to the public health through the use of the waters of the Delaware River for public water supples, or render such waters unfit for industrial and other purposes; or cause the water of the Delaware River to be harmful to fish life.

6. Such effluent shall be practically free of substances capable of producing offensive tastes or odors in public water supplies derived from the Delaware River.

Zone 4: 1. Such effluent shall be free of noticeable floating solids, oil, or grease, and substantially free of both suspended solids and sleek.

2. Such effluent shall be sufficiently free of turbidity that it will not cause substantial turbidity in the waters of the Delaware River after dispersion in the water of the river.

3. Such effluent shall show a reduction of at least fifty-five (55) per cent of the total suspended solids and shall be subject to such further treatment as may be needed to prevent a nuisance.

4. Such effluent, if it be discharged within prejudicial influence of a public water works intake, or of recreational areas, or of shell fish grounds, shall at all times be effectively treated with a germicide, except that in the case of recreational area influence, such treatment need not be provided during the period from October 15th to May 15th of each year.

5. Such effluent shall be sufficiently free of acids, alkalis, and other toxic or deleterious substances that it will not create a menace to the public health through the use of the waters of the Delaware River for public water supplies, or render such waters unfit for commercial fishing, shell fish culture, recreational, industrial or other purposes.

6. Such effluent shall be practically free of substances capable of producing offensive tastes or odors in public water supplies derived from the Delaware River.

Intrastate Tributaries

It is further recognized by the signatory states that the quality of the waters of the intrastate tributaries of the Delaware River and its aforesaid West Branch are of interstate concern at their points of confluence with the Delaware River and its West Branch. Therefore, it is also agreed that sewage, industrial waste or other artificial polluting matter discharged into, or permitted to flow or to fall into, or be placed in any intrastate tributary of the aforesaid Delaware River, shall be treated to that degree, if any, necessary to maintain the waters of such intrastate tributary immediately above its confluence with the aforesaid Delaware River in a condition at least equal to the clean and sanitary condition of the waters of the Delaware River immediately above the confluence of such tributary.

Standard Methods

Analyses and tests regarding the minimum requirements herein prescribed, shall be determined in accordance with the provisions contained in the American Public Health Association's latest edition on "Standard Methods for the Examination of Water and Sewage."

Future Requirements

The aforesaid requirements as to treatment of sewage, industrial wastes or other artificial polluting matter and as to the sanitary quality of receiving waters are minima. It is the intent and purpose of these requirements to accomplish reasonable and adequate control of correction of pollution. Due to the many variable factors involved, however, and to the impossibility of forecasting future developments with certainty, it may be necessary in the future to impose additional requirements, particularly in Zones 2 and 3.

The minima herein prescribed, therefore, shall be considered the first steps toward attaining the objectives sought, and if necessary, may be required to be supplemented in the case that the general application of such minimum requirements does not adequately improve and maintain the sanitary quality of the waters of the Delaware River.

APPENDIX O

MINIMUM WATER QUALITY CRITERIA FOR STREAMS IN THE POTOMAC RIVER BASIN

(From Water Quality Criteria, Interstate Commission on the Potomac River Basin)

As Approved 8 August 1946, by the Commission and Recommended to the States of the Basin for Their Use

Principles Involved in the Establishment of Water Quality Criteria

The Water Quality Criteria and Maps showing existing conditions and first step in a progressive program of stream improvement established by the Interstate Commission on the Potomac River Basin shall be used and interpreted upon the basis of the following fundamental principles:

(1) The criteria and maps showing existing conditions and first step in a progressive program of stream improvement shall be considered as a cooperative effort on the part of the signatory bodies to establish a uniform yet flexible yardstick for describing and indicating the water quality in the streams of the basin. This quality can be improved from time to time as the necessary work for its betterment is completed. It is a means by which areas of relatively serious pollution can be shown and progress as it is made can be recorded.

(2) The criteria and maps are not to be considered as placing in any way an ultimate or final objective on any section of stream.

(3) The map indicating existing (present-day) conditions has been prepared upon the basis of information now available to the Commission. Areas will be made more definite and accurate as the state agencies, in pursuing their abatement programs, obtain the necessary information to more completely define the picture.

(4) The map showing recommended conditions represents the first step in the improvement of water quality proposed to the signatory bodies. This is to be followed by succeeding steps as time and conditions warrant.

Class A Waters

Waters in Class A are suitable for a potable water supply with no treatment necessary except chlorination or disinfection. The waters may also be used for bathing, fish life, recreation and industrial process waters. The waters under this classification shall meet the following minimum requirements:

(1) Coliform Bacteria
The most probable number of coliform bacteria present in any 100 ml sample shall not exceed 50.

(2) Color
The color of any sample shall not exceed 10 parts per million.

(3) Turbidity
The turbidity of any sample shall not exceed 10 parts per million.

(4) pH
The pH value of the water shall vary between 6.0 and 8.0.

(5) Dissolved Oxygen
The average of all monthly tests for DO shall not be less than 7.5 parts per million, with no single observation being less than 6.5 parts per million.

(6) Other Conditions
The waters shall contain no toxic substances of any nature such as oils, tars, or free acid at any time. There shall be no floating solids or debris except from natural sources. No taste- or odor-producing substances will be permitted unless they are of natural origin. There shall be no sludge deposits of any nature.

Class B Waters

Waters in Class B are suitable for bathing, fish life, and for other similar recreational purposes. This water is also satisfactory for domestic water supplies with complete treatment, for industrial process water, and other similar uses. The results of sampling shall meet the following minimum requirements:

(1) Coliform Bacteria
The monthly average of all samples shall show the most probable number of coliform bacteria per 100 ml shall vary between the limits of 50 to 500. Any one sample shall not contain more than 1,000 coliform bacteria as the most probable number per 100 ml.

(2) Color
A color of not more than 20 ppm is desirable.

(3) Turbidity
A turbidity of not more than 40 ppm is desirable.

(4) pH
The acidity or alkalinity of the water shall vary between the limits of 6.0 and 8.5.

(5) 5-Day Biochemical Oxygen Demand
The 5-Day 20°C BOD of all samples during any one month shall not exceed 1.5 ppm. The maximum observed BOD in any sample shall not be more than 3.0 ppm.

(6) Dissolved Oxygen
The monthly average of all samples shall show a DO content of not less than 6.5 ppm. No one sample shall show a DO content of not less than 5.0 ppm.

(7) Other Conditions
Other conditions which shall prevail shall be the same as those enumerated for Class A waters.

Class C Waters

Waters in Class C are suitable for domestic water supplies, with complete treatment, also for industrial process water, navigation, and similar uses. The minimum conditions which shall prevail for these waters shall be as follows:

(1) Coliform Bacteria
The monthly average of all samples shall show the most probable number of coliform bacteria varying between the limits of 500 to 5,000 per 100 ml.

(2) Color and Turbidity
Color and Turbidity may be allowed which can be removed by standard equipment and filtration practices.

(3) pH
The acidity or alkalinity of the waters shall vary between the limits of 6.0 and 8.5.

(4) 5-Day Biochemical Oxygen Demand
The 5-Day 20°C BOD shall show a monthly average of all samples of not more than 2.0 ppm. The maximum observation on any one sample shall not be more than 4.0 ppm.

(5) Dissolved Oxygen
The monthly average of all samples for DO shall not be less than 6.5 ppm, and no single sample shall show a DO content of less than 5.0 ppm.

(6) Other Conditions
Other conditions for this class of water shall be the same as those for Class A waters.

Class D Waters

The waters in Class D shall have a satisfactory general sanitary condition and shall not create a nuisance in the surrounding area. The waters can be used for navigation, as cooling water, and for similar purposes. They will in no way affect public health unless used for domestic water supplies or for swimming purposes.

(1) pH
The acidity or alkalinity of the waters shall vary between the limits of 6.0 and 8.5

(2) 5-Day Biochemical Oxygen Demand
The monthly average of all samples for 5-Day 20°C BOD shall not be more than 3.0 ppm. The maximum observation of a single sample shall not be more than 5.0 ppm.

(3) Dissolved Oxygen
The monthly average of all samples for DO shall not be less than 4.0 ppm. The minimum daily average of all samples shall not be less than 3.0 ppm nor shall the observation on any one sample be less than 2.0 ppm.

(4) Other Conditions
The waters shall contain no toxic substances such as oils, tars, or free acid at any time. There shall be no floating solids or debris except for those from natural sources. If unpreventable, slight localized sludge deposits will be permitted. No offensive odors shall be given off by the waters at any time.

NOTE: These criteria are to be used only in conjunction with a sanitary survey as a guide in determining the minimum water quality for the various classes of water use listed. It is intended that these criteria should apply to conditions which are expected to prevail for the major part of the time.

In the use of the criteria given on the previous pages, consideration should be given to the following:

1. They are to be used as a guide to the purity of water in a stream or portion thereof, and only then in connection with a sanitary survey of the area, taking into consideration those factors which may affect materially the interpretation of laboratory results.
2. In the enforcement of pollution control it may prove simpler for each state health department, or other pollution control agency, to set up standards for waste discharges which will provide a water quality in the stream equal to that indicated by the Commission maps.
3. One map shows existing conditions which the Commission believes to exist on the basis of available information. The other map, showing recommended conditions, represents the first step in the improvement of water quality proposed to the signatory bodies which will be followed by succeeding steps. As better information becomes available to the Commission, and as time and conditions warrant, changes in the classification of areas shown on both maps will be recommended.
4. As the criteria and maps showing existing conditions and recommended first step in a progressive stream improvement program are used, and additional information on the condition of the water in the streams becomes available, and as progress in the treatment of wastes advances, the Commission will from time to time review both the criteria and maps and will recommend changes which seem to be desirable. In this respect, it is realized that many points of their practical application, such as sampling in the field, handling, and test procedures, may require standardization. Many of these problems cannot be foreseen now and the solution to many others can be carried out by the state agencies themselves.
5. Examination and tests of water necessary to determine quality shall be made in accordance with the procedures contained in the American Public Health Association's latest edition of "Standard Methods for the Examination of Water and Sewage."

In preparing the criteria, the Commission found that they could not prepare rigid standards since the analysis of stream waters involves highly technical sampling procedures. Wide variations result from natural river conditions, solar radiation, tidal variation, and variations in river cross-section. In time the Commission hopes that information will be available for more definite recommendations on sampling procedures.

APPENDIX P

GUIDE FOR WATER POLLUTION CONTROL ACTIVITIES MISSOURI DRAINAGE BASIN

Reaffirmed January 7, 1954, Engineering Section,
Missouri Basin Health Council

Subject to (a) such higher objectives as may be desired by any State and (b) such other modification as may be deemed necessary, after appropriate investigation by the State Water Pollution Control Agency concerned, the Engineering Section, Missouri Basin Health Council recommends the following for consideration by the member states as a guide for water pollution control activities in the Missouri Drainage Basin:

1. Toxic substances including metallic ions, phenolic compounds, oils, alkalies, and acids should be virtually eliminated from sewage effluents.
2. Removal of settleable and floating solids to prevent sludge banks, floating materials, and nuisance conditions should be required as the minimum acceptable treatment.
3. For industrial wastes similar in nature to municipal wastes, treatment should be based on the same premise as recommended for municipalities. For industrial wastes not comparable to municipal wastes, treatment should be provided as necessary to prevent deterioration of water quality for beneficial uses in the receiving stream.
4. Additional treatment over that specified in item 2 above should be provided as necessary to protect downstream water uses. Quality objectives for certain water uses are:
   (a) For water serving as a source of domestic supply, raw water bacteriological quality should conform to that recommended in "Manual of Recommended Water Sanitation Practices, 1946, Public Health Bulletin No. 296," pages 11-13.
   (b) For bathing or swimming water, monthly arithmetical average "most probable number" of coliform organisms should not exceed 1,000 per 100 ml during any month of the recreational season; nor exceed this number in more than 20 percent of the samples examined during any such month; nor exceed 2,400 per 100 ml on any day. For non-bathing or non-swimming waters, the monthly arithmetical average "most probable number" of coliform organisms should not exceed 5,000 per 100 ml in any month of the recreational season nor should not exceed this number in more than 20 percent of the samples examined during any such month.
   (c) For fish and aquatic life a minimum oxygen concentration of 5.0 ppm is desirable in waters otherwise offering suitable fishery habitat, with 6.0 ppm for trout streams.
   (d) For bacteriological quality of irrigation water:
      (1) Sewage used for irrigation of forage crops should be given treatment insuring a consistent reduction in coliform bacteria of not less than 90 percent.
      (2) Sewage used for irrigation of human food crops should be given treatment insuring a consistent reduction (a) in suspended solids of not less than 75%, (b) in 5-day 20° C BOD of not less than 75% and (c) in coliform bacteria of not less than 98%, unless discharged into a stream where the volume of dilution water, available during the low flow periods during which irrigation diversions may occur, is at least 10 times greater than the anticipated maximum daily volume of sewage, in which case a bacterial reduction of 90 percent may be acceptable.

Provided, however, that where the time of passage from plant discharge to irrigation diversion is such as to permit the normal death rate of bacteria as evidenced by the die-away curve to provide equivalent bacterial reduction, the requirement of (1) and (2) above may be appropriately modified.

APPENDIX Q

U. S. PUBLIC HEALTH SERVICE RECOMMENDED SANITARY REQUIREMENTS FOR WATER TREATMENT SYSTEMS

A. GENERAL REQUIREMENTS

1. Extent of Treatment

For purposes of classification with respect to treatment requirements, waters acceptable for treatment may be divided into four groups. For practical purposes coliform bacterial content of the raw water can be expressed either in terms of "most probable numbers" or "Phelps Index", without changing the limiting coliform averages indicated for the various water groups. (See Public Health Reports Reprint No. 1170 (2), July 15, 1927, pp. 7-11 and 18-19).

Group I. Water Requiring No Treatment. This group is limited to underground waters not subject to any possibility of contamination, and meeting, in all respects, the requirements of the Public Health Service Drinking Water Standards, as shown by satisfactory, regular, and frequent sanitary inspections and laboratory tests.

Group II. Waters Requiring Simple Chlorination, or Its Equivalent. This group includes both underground and surface waters subject to a low degree of contamination, and meeting the requirements of the Public Health Service Drinking Water Standards in all respects except as to coliform bacterial content, which should average not more than 50 per 100 ml in any month.

Group III. Waters Requiring Complete Rapid-Sand Filtration Treatment, or Its Equivalent, Together With Continuous Postchlorination. This group includes all waters requiring filtration treatment for turbidity and color removal; waters of high or variable chlorine demand; and waters polluted by sewage to such an extent as to be inadmissible to groups I and II, but containing numbers of coliform bacteria averaging not more than 5,000 per 100 ml in any one month and exceeding this number in not more than 20 percent of the samples examined in any one month.

Group IV. Waters Requiring Auxiliary Treatment in Addition to Complete Filtration Treatment and Postchlorination. This group includes waters meeting the requirements of group III with respect to the limiting monthly average coliform numbers, but showing numbers of coliform bacteria exceeding 5,000 per 100 ml in more than 20 percent of the samples examined during any one month and not exceeding 20,000 per 100 ml in more than 5 percent of the samples examined during any one month.

Note. "Auxiliary treatment" as used here is presedimentation or prechlorination, or their equivalents, either separately or combined, as may be necessary. Long-time storage, for periods of 30 days or more, represents a permanent and reliable safeguard which in many cases would provide something more than an effective substitute for one or both of the two other methods indicated.

Remarks. (a) Although group I conceivably might include exceptional surface waters free of any possible contamination and further protected by storage, it hardly may be considered as a safe general rule to admit any surface water to a public supply without chlorination as a minimum safeguarding treatment, in view of the present increased hazards of chance contamination resulting from the extension of recreational and migratory travel to many hitherto inaccessible places.

(b) The limiting monthly average coliform numbers stated for waters of groups II and III are intended as guides rather than inflexible rules, though they are based on extensive observational data fairly representing present water treatment practice in this country. Consideration should be given to all aspects of the character and quality of the raw water; its range and suddenness of fluctuation in concentration of constituents which would influence the efficiency of the water-treatment process; the size and complexity of the water-treatment plant, and the type of supervision provided, in addition to the bacteriological characteristics of the raw water. Certain recent improvements in water chlorination and its control offer promise of increasing the margin of safety of water-purification efficiency, with respect to bacterial removal. These improvements have not become fully incorporated into general practice throughout the country, and they should not be regarded as warranting any relaxation in the requirements for raw-water quality which experience and present standards of safety indicate as being necessary for providing adequate protection to sources of water supply in general.

(c) For waters of group IV, which differ from those of group III only in respect to variability, auxiliary treatment is intended mainly as a factor of safety in controlling variations in coliform numbers within the range of 5,000 to 20,000 bacteria per 100 ml. The larger of these two figures represents the maximum safe limit for prechlorination, or its equivalent, in addition to filtration treatment and postchlorination.

(d) Waters failing to meet the requirements of groups I, II, III, or IV are considered as unsuitable for use as a source of water supply, unless they can be brought into conformance with these requirements by means of prolonged preliminary storage, or some other measure of equal permanence and reliability.

2. Other Raw Water Requirements

Waters acceptable for treatment, in addition to meeting the aforesaid bacterial requirements, should not contain any toxic, taste-producing, or otherwise harmful substances, or organisms, not readily and completely removable by ordinary water treatment. Raw waters should be free of excessive amounts of acid, microscopic organisms, and organic matters causing any interference with the normal operation and efficiency of water-treatment processes.

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Large Rivers and Eutrophication

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Abstract

This paper discusses the effects of nutrients on the physical, chemical and biological components of large rivers in the context of both human costs and ecosystem response. In addition to the direct consequences of adding nutrients, such as increased primary productivity and resultant effects on water chemistry, additional interactions also characterize large river nutrient responses: for example, algal community structure is altered leading to proliferation of nuisance taxa, taste and odor problems, increased water treatment costs, increased toxins (cyanobacterial blooms), and loss of habitat. In addition, nuisance macrophytes (rooted aquatic plants) also increase and affect water chemistry and habitat. As a result of these direct and indirect responses, large rivers suffer impacts to aquatic life and recreational uses. Rivers also function to transport nutrients to downstream ecosystems, and some of the impacts of nutrients on large rivers are transported to downstream lake and coastal receiving waters.

1. Introduction

Water quality in rivers is vital to humans and to maintenance of biotic and ecological integrity. Rivers are part of a continuum of flowing water and no clear delineations exist to differentiate between small streams and large rivers. For example, from an agency sampling point of view, large rivers are not wadable, whereas an alternative definition considers large rivers to be navigable. Some quantifiable characteristics often used to delineate large rivers from smaller streams are drainage basin size and discharge. Some of the stream/river size cutoffs that have been used include basins >100 km^2 for small rivers (Meybeck and Helmer 1989), 100-10,000 km^2 for medium, and > 10,000 km^2 for large rivers (Caraco et al. 2003). Discharge in large rivers typically exceeds 100 cfs (2.83 $m^3 s^{-1}$) for at least part of the year.

Eutrophication refers to increases in plant and algal production. In some waterbody types, this can be a natural process, but in the vast majority of instances, eutrophication is brought about by human inputs of nutrients and is termed "cultural eutrophication" (Dodds 2002). Eutrophication occurs widely in large rivers of the United States, perhaps because large rivers integrate such large land areas. This paper reviews what is known about large rivers, their natural ecology, and the effects of nutrient enrichment on these systems. First, we will provide basic descriptions of river morphology, hydrology, baseline nutrients, and food webs. Then we will consider physical and chemical alterations related to food webs. Finally, we will cover effects of eutrophication on river ecosystem structure and function.

River morphology is dependent on basin geology, climate, and vegetation (Leopold 1994). In areas dominated by depositional sand or gravel, rivers tend to be low gradient and become shallow and broad, or braided. These rivers often have broad, poorly constrained floodplains and numerous riparian wetland features (e.g., the lower Mississippi). Steeper gradients are common when rivers flow through rocky, alluvial areas. These rivers become deeper, have incised canyons and constrained flood plains, and often have areas of intense rapids or whitewater (e.g., the Colorado River).

Generally, as discharge increases, rivers get wider more quickly than they get deeper, increasing the width to depth ratio. This pattern is displayed by the Mississippi River, which is approximately 100 times wider than it is deep in the lower segment (Leopold 1994). Width:depth ratio is more typically 1 to 10 for smaller streams. Characteristic widening causes the influence of riparian vegetation to become less important as the river gets larger. Most rivers in a natural state have riparian wetland habitat, such as oxbows and side channels that are sometimes connected to the main river channel through overland flow or groundwater exchange. Therefore, riparian influences are not completely absent, but are more seasonal based on hydrology. These side channels can be important to the nutrient retention of rivers during floods.

River hydrology depends on climate, vegetation, and slope (Dodds 1997). Therefore, water residence time can be quite variable among rivers. Generally, rivers contain a

larger volume of water and have more stable flow than smaller streams. The water column is usually well mixed vertically, but less so laterally, and a distinctly faster moving thalweg (central region with higher water velocity) and slower moving side channels are common.

Ranges of most naturally occurring elements in rivers span from 1 to 2 orders of magnitude (Dodds 2002) and vary with basin geology and climate (Meybeck and Helmer 1989). Rivers are greatly affected by humans (e.g., wastewater effluent, water withdrawal, nonpoint pollution). Rivers tend to receive more inputs from natural and anthropogenic point and nonpoint sources than smaller streams because they drain larger watershed areas. Also, because many major cities are located on large rivers, their watersheds are home to larger human populations. Increased anthropogenic inputs increase dissolved ion concentrations and suspended material in rivers, such as sediments and nutrients. Therefore, rivers are more likely to be turbid than smaller streams in the same basin (e.g., Meybeck et al. 1999, Whiles and Dodds 2002), and nutrient transport is typically greater than retention (net uptake of nutrients, Alexander et al. 2000, Bernot and Dodds 2005).

In rivers, primary production in the main channel can be benthic (i.e., periphyton or macrophytes) or planktonic (suspended algae, also referred to as sestonic). Key factors determining whether the dominant primary producer type is benthic or planktonic are hydraulic residence time, turbidity, and channel morphology. Plankton and macrophytes become more prominent as conditions become more lake-like with slower flushing rates (Van Nieuwenhuyse and Jones1996). Some river food webs can be based mainly on planktonic production (Thorp and Delong 1998). Side channel and oxbow primary production may be driven by macrophytes or emergent vegetation. Some large rivers have lakes periodically connected to them. These mostly act as shallow lakes and the balance of their nutrient input comes from inundation during flooding. If river nutrient load is high when flooding occurs, nutrient loading into lakes can dramatically increase, altering these ecosystems (Hamilton and Lewis 1990).

In silty and sandy rivers with shifting bottoms, most macroinvertebrates are associated with stable patches such as wood, logjams, or rocky areas. In general, large rivers have more diverse fish assemblages than small streams (Matthews 2003). Some large fishes such as sturgeons and paddlefishes are specially adapted to large rivers and not found elsewhere.

Three main theories predicting the major carbon (C) source fueling river food webs are: the river continuum concept (RCC) (Vannote et al. 1980), the flood pulse concept (FPC) (Junk et al. 1989), and the riverine productivity model (RPM) (Thorp and Delong 1994). The major C source according to the RCC is from upstream transport, but planktonic production is acknowledged. The FPC suggests that food webs depend on terrestrial C from the floodplain, and the RPM stresses the importance of local autochthonous C produced in the channel.

Very little is currently known about cycling, processing, and retention of nutrients in rivers other than what can be inferred from other systems. To date, the majority of research has been modeling studies (Alexander et al. 2000, Seitzinger et al. 2002, Darracq and Destouni 2005, Wollheim et al. 2006), and much knowledge of nutrient cycling in lotic systems has been gleaned from smaller streams. Most models predict that nutrient retention per unit length of stream declines with increasing stream size. The model of Alexander et al. (2000), which incorporated data from 374 U.S. stream stations across a large gradient, predicts less in-stream retention and greater transport of nitrogen (N) with increasing stream depth. Additionally, river nutrient concentrations are linked to watershed population, human activity, and energy consumption (Meybeck 1982). The model created by Caraco et al. (2003), using data from 249 North Temperate watersheds, predicted a nitrate export of 360, 630, and 443 kg N km^{-2} watershed area yr^{-1} for small, medium, and large rivers respectively. Population estimates for these watersheds were 38, 117, and 53 individuals per km^2.

Natural N and phosphorus (P) concentrations in rivers vary with basin geology, vegetation, and climate (Meybeck 1996). Natural NO_3 and PO_4 concentrations in rivers range from 0.05 to 0.2 and from 0.002 to 0.025 mg L^{-1}, respectively (Meybeck and Helmer 1989). Rivers tend to have less temporal and spatial variability in nutrient concentrations than smaller streams (Smith et al. 2006). Worldwide it is documented that nutrient concentrations are increasing in rivers by at least 50% (Dodds 2002). Total nitrogen (TN) and total phosphorus (TP) concentrations have been decreasing slightly in the United States over the past 30 years, following a reduction in agricultural intensity (Alexander and Smith 2006). However, changes in river trophic state in response to nutrient reductions are unclear.

Nutrient criteria for streams may be needed to avoid direct toxicity, taste, and odor problems, alterations in biotic integrity, and interference with recreation (Dodds and Welch 2002). Similar issues occur for rivers. For example, many municipalities rely on rivers for drinking water. Nitrate concentrations in drinking water above 10 mg L^{-1} can negatively affect human health by causing methemaglobanemia (blue baby syndrome when it occurs in infants). Excess nutrients can also indirectly harm humans through increased water treatment costs. Algal and cyanobacterial blooms can cause taste and odor problems, and increase toxin levels in river water (e.g., the Murray-Darling River system in Australia, Maier et al. 2001, Davis and Koop 2006). Algal blooms can also interfere with recreational uses such as boating, swimming, fishing, and tourism due to increased phytoplankton, periphyton, and/or macrophyte biomass. Finally, eutrophication of rivers can alter biotic community composition and decrease biotic integrity (Miltner and Rankin 1998).

The objective of this document is to discuss the effects of river eutrophication in terms of a river's physical, chemical, and biological components, and in the context of both human costs and ecological responses. In addition to the direct consequences of adding nutrients, such as increased primary productivity, additional interactions may alter the ecosystem. Due to methodological and technical limitations of large river research, studies on the effects of eutrophication on these systems have been limited. However, the

importance of rivers to watershed, basin, and continental nutrient cycling, storage, and transport is evident.

2. Biological Responses

2.1 Ecosystem Structure

There are few direct effects of eutrophication on the physical structure of rivers. Since eutrophication in rivers causes chemical and biological changes that are tightly linked, we will discuss them collectively. Structural responses in river biota can be altered both directly and indirectly by the addition of nutrients. Primary producers that can use the additional resource directly should be more rapidly affected by the addition than higher trophic levels. There is little empirical evidence of changes in microbial assemblage structure in rivers due to eutrophication. There is no reason to assume, however, that microbes in rivers should behave differently than microbes in smaller streams (Dodds 2006). Dumestre et al. (2001) demonstrated a change in microbial community structure with eutrophication below a reservoir on the Sinnamary River, South America. At this point, not a lot is known about the consequences of a microbial structural change or how such changes would indirectly affect the riverine food web.

Algal responses to nutrients are the most studied feature of river eutrophication. Algal community structure is directly affected by eutrophication, resulting in increased biomass and altered community composition (Butcher 1947). N, P, or both N and P can limit phytoplankton and benthic algal growth in rivers (Watson 1989, Francoeur 2001, Tank and Dodds 2003). Chetelat et al. (2006) found phytoplankton biomass across 46 rivers in Ontario and western Quebec to be more dependent on TP concentration than residence time or light. TP was also found to be strongly correlated to phytoplankton biomass in a study of 115 North American Temperate streams (Van Nieuwenhuyse and Jones 1996).

Indirect effects of eutrophication in rivers are principally reactions to an overgrowth of algal biomass or change in algal community structure, including the proliferation of nuisance and toxic species. For example, there are positive responses of the nuisance alga *Cladophora* to altered nutrient concentrations in the Clark Fork River, Montana (Dodds et al. 1997), which alters the river ecosystem's structure (Dodds 1992). Eutrophication can also alter benthic cyanobacterial species composition and distribution along a nutrient gradient, with many eutrophic cyanobacteria producing taste and odor problems and even toxins (Perona and Mateo 2006). In more lentic riverine habitats, additional nutrients can stimulate *Chara* or *Nitella*, which can also create taste and odor problems. Other problems include hypoxia in parts of, or throughout, the water column, and a reduction in recreation potential of the river. In the Murry-Darling River basin, Australia (Maier et al. 2001, Mitrovic et al. 2003, Davis and Koop 2006) and the Tualatin River, Oregon, reduced flow coupled with high nutrient loading stimulated cyanobacterial blooms and hypoxic conditions in the bottom of the river channel. And in North Carolina, excess phytoplankton biomass was the source of labile carbon for hypoxic conditions in a piedmont river (Mallin et al. 2006).

River macrophytes respond to eutrophication with increases in biomass (Chambers et al. 1994). Empirical evidence for this response was found in the Saskatchewan River, where a reduction in nutrient concentrations led to reduced macrophyte biomass (Chambers et al. 1999). Water hyacinth also does well in high nutrient conditions; it is used in nutrient removal for sewage treatment (Kumar and Garde 1990). Water hyacinth is a nuisance plant in many tropical and sub-tropical regions (Dodds 2002), and increased loading could exacerbate growth problems with this plant and others.

The direct effects of nutrient enrichment on river macroinvertebrates are not known. Indirectly, secondary production should increase with increased primary production and available food sources. For example, deBruyn et al. (2003) found a five-fold increase in macroinvertebrates downstream of a sewage treatment plant effluent in the St. Lawrence River, Montreal. Very little is known to link macroinvertebrates to the quality of food in rivers. Whiles and Dodds (2002) attempted to link suspended sediments to macroinvertebrate biomass in rivers, but a strong pattern could not be established.

Little is known about the influence of increased nutrient loading on vertebrate riverine biota such as fish, amphibians, reptiles, and birds. Miltner and Rankin (1998) analyzed data across 1657 sites in Ohio and found a deleterious effect on fish communities when TN and TP levels exceeded natural background levels in lower order streams, but found no effect in larger rivers. Fish communities depend on a natural nutrient balance within the system, but not much is known about the response of fish communities in large rivers to the cascade of effects caused by an imbalance in nutrients. Any effect of enrichment is most likely indirect and probably unpredictable. The exception is that low dissolved oxygen excursions (hypoxia) can occur in stagnant, nutrient-rich rivers, and these conditions have obvious negative effects on fish.

2.2 Ecosystem Function

Eutrophication of rivers can influence both heterotrophs and autotrophs (Dodds 2006). Major ecosystem functional responses to eutrophication should stem from changes in the structural and functional roles of microbial assemblages and primary producers within the system. However, not much is known about the effects of elevated inorganic nutrient loads on heterotrophic communities except that decomposition of litter in small streams is stimulated by higher nutrients (Dodds 2006). Autotrophic responses are better studied.

General responses of primary production in rivers should be less than in smaller streams because of increased turbidity and light limitation. However, there is no reason to believe that the underlying physiological responses of primary producers to increased nutrients, such as increased biomass and altered community structure, seen in smaller streams should not hold true in rivers. For example, when Dodds et al. (1997) plotted the relationships between nutrients and benthic algal biomass for the Clark Fork River, they did not differ substantially from those derived from many studies of small streams. When environmental conditions are right, primary producer responses can cause whole river effects. For example, more primary production and increased heterotrophic

microbial activity creates greater diurnal oxygen swings that could lead to areas of hypoxia within the river, such as those observed in the Murray-Darling (Maier et al. 2001), Clark Fork River (Dodds et al. 1997), Tualatin River (http://www.waterencyclopedia.com/Mi-Oc/Nutrients-in-Lakes-and-Streams.html), and others. Hypoxia typically occurs during periods of very low discharge or in rivers with limited flushing rates.

Changing biomass and composition of primary producers can alter lotic food webs and the availability of resources to higher trophic levels. Phytoplankton production may supply a significant portion of productivity in medium to large rivers that are not highly turbid and do not completely mix because they have zones of limited water replacement (e.g., Thorp et al. 1998, Wehr and Descy 1998). How increased production cascades up the food web is not well characterized for many rivers.

Structural changes in lotic biota may alter ecosystem function, such as nutrient cycling. However, direct measurements of nutrient and metal cycling are also rare for larger rivers. It is not known if or how increased nutrient loads would change cycling rates, such as nitrification and denitrification, though information published for small streams suggests functional effects, such as decreased proportional retention with increased nutrient loading.

Rivers also function to transmit nutrients to downstream habitats. Turner et al. (2003) estimated river discharge into oceans for major algal nutrients. These values are 16.2, 21, 2.6, 3.7 to 5.6, and 194 Tg yr^{-1} for NO_3-N, total nitrogen (TN), dissolved inorganic phosphorus (DIP), total phosphorus (TP), and dissolved silica (DSi), respectively. If nutrient retention becomes saturated and channelization lowers the ability of rivers to retain nutrients (Bernot and Dodds 2005), then the potential for transmitting eutrophication downstream increases. Increased transport of total nutrients to impoundments, lakes, and coastal waters, would likely lead to hypoxia. The nutrient ratios in the discharge of the world's 10 largest rivers approach the Redfield ratio, which may increase coastal phytoplankton production and occurrence of harmful algal blooms due to a shift to N or Si limitation (Justic et al.1993, Justic et al. 1995).

In conclusion, river food webs and water quality are potentially negatively impacted by eutrophication. While effects of increased nutrients on rivers are not as clearly defined and as well studied as they are in small streams or lakes, several aspects of river ecosystem structure and function may be altered by increases in nutrients. These include changes in community composition and increases in primary production and biomass. Changes in ecosystem structure and function can lead to problems with biological integrity, water quality, and the value of large rivers for recreation.

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BIOLOGICAL PROBLEMS

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WATER POLLUTION

Transactions of a Seminar on Biological Problems
in Water Pollution held at the
Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio
April 23 - 27, 1956

Compiled and Edited

by

CLARENCE M. TARZWELL

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
Public Health Service
Bureau of State Services
Division of Sanitary Engineering Services
Robert A. Taft Sanitary Engineering Center

1957

PREFACE

During the past few years a number of State Health Departments and Water Pollution Control Boards have initiated or expanded investigations in the field of sanitary biology. Universities and other research organizations are showing increased interest in biological problems connected with the detection and abatement of stream pollution. A few universities are now giving courses directed toward the training of sanitary biologists, and several are considering the establishment of curricula for the training of aquatic biologists for work in the water works, sewage treatment, and stream pollution fields. In recent years industries have added sanitary biologists to their staffs, and several aquatic biologists have undertaken consultant activities.

Biologists engaged in pollution investigations and research often work alone and are somewhat isolated. For some time, therefore, there had been recognized a need for a conference of those engaged in the study of biological problems in water pollution control, to acquaint them with current developments and new methods of approach, and to enable them to become acquainted with other workers in the field. The first such gathering was held as a seminar at the Robert A. Taft Sanitary Engineering Center, April 23 - 27, 1956. The meeting was well attended, as ninety persons were registered at the Seminar. Representatives were present from twenty-eight states, the District of Columbia, and the Provinces of Ontario, Quebec, and New Brunswick, Canada. The following states were represented: Alabama, California, Florida, Georgia, Idaho, Illinois, Indiana, Iowa, Louisiana, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Montana, New York, North Carolina, Ohio, Oregon, Pennsylvania, Rhode Island, South Carolina, Tennessee, Texas, Utah, Washington, Wisconsin, and Wyoming. Participants included workers from industry, State Conservation Departments, Universities, State Boards of Health, and Stream Control Commissions. Representatives were also present from the Ohio River Valley Water Sanitation Commission, the Illinois State Natural History Survey, the Atomic Energy Commission, the U. S. Naval Radiological Defense Laboratory, and the Fish and Wildlife Service. A total of twenty-three were in attendance from the Public Health Service, six from outside the Cincinnati Area.

The Dow Chemical Company, Atlantic Refining Company, Sulphite Pulp Mfg. Research League, Institute of Paper Chemistry, General Electric Company, and Pantech, Inc. had representatives at the Seminar. Representatives were present from Conservation Departments of the following states: Alabama, Idaho, Indiana, Iowa, Michigan, Mississippi, Missouri, Montana, New York, Ohio, Pennsylvania, Tennessee, and Wyoming.

Representatives were present from sixteen universities as follows: Pennsylvania State University, University of Florida, Massachussets Institute of Technology, University of Missouri, University of Wisconsin, Michigan State University, University of Utah, Ohio State University, University of

Michigan, University of Miami, University of Southern California, University of Montreal, University of Minnesota, University of Cincinnati. University of Toronto, and University of North Carolina. The State Boards of Health of Florida, Missouri, and Illinois sent representatives as did the Stream Control Commissions of Louisiana and Wisconsin. Michigan led in the number of representatives present, having seven; Ohio was second with six; the Province of Ontario, Pensylvania, and California were third with five each; Wisconsin, Florida, and Alabama each had four; while Iowa and Missouri sent three.

The seminar consisted of panel discussions and was planned so that most of the time was devoted to commentary from the floor with only short presentations by panel members. Subjects discussed were (1) Use and Value of Bioassays; (2) Use and Value of Biological Indicators of Pollution; (3) Current Investigations in Water Pollution Biology; (4) Water Quality Criteria for Aquatic Life; and (5) Training of Sanitary Aquatic Biologists Discussions of each subject were lead by a panel chairman and 4 to 9 panel members, each of whom presented a phase of the problem in 10 to 20 minutes. The remainder of the day was then devoted to discussions from the floor. All presentations and discussions were off the record in order that the participants could feel free to express their opinions. All members of the panel on Biological Indicators of Pollution were asked to prepare papers to be included in the transactions of the meeting. These papers could be discussed in their panel presentations if they wished or their presentations could be entirely different. All panel members and all those in attendance were invited to submit papers for inclusion in the transactions The transactions, therefore, are not a record of the discussions at the meeting, and the papers contained therein may or may not have been presented during the panel discussions, but are the results of the prospect or the events of the sessions.

During the meeting, discussions were free and critical It is believed they were valuable in stimulating those in attendance and will serve to advance research and investigations in the field of water pollution. Numerous letters have been received expressing approval of the meeting and inquiring as to when another similar meeting would be held It is planned to hold a seminar on biological problems in water pollution every three years; hence, the next meeting will occur in 1959. It is requested that all those interested in such a meeting submit their suggestions by June 1958. Suggestions are solicited as to time and length of meeting, program, and manner of carrying on the discussions.

C. M. Tarzwell

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PROGRAM

SEMINAR ON BIOLOGICAL PROBLEMS IN WATER POLLUTION

April 23 - 27, 1956

DATE & TIME	SUBJECT
Monday April 23	
8:00 - 9:00	REGISTRATION
9:00 - 9:15	WELCOME - Harry G. Hanson, Director Robert A. Taft Sanitary Engineering Center
9:15 - 9:30	PLAN OF THE SEMINAR AND ANNOUNCEMENTS - Clarence M. Tarzwell Chief of Aquatic Biology
9:30 - 4:30	Panel Discussion - Use and Value of Bio-Assays Chairman, George Burdick, Dept. of Conservation Albany, New York Extending Acute Toxicity Data to Indicate Toxicity Under Continuous Exposure George Burdick Department of Conservation Albany, New York Bio-Assays for Determining the Tainting of Fish Flesh A. W. Winston Dow Chemical Company Midland, Michigan Use of Bio-Assays in Industry W. B. Hart Director, Industrial Waste Eng. Div. Pantech, Inc. Folcroft, Pennsylvania

DATE & TIME	SUBJECT
Monday April 23 (cont'd)	**Methods of Assay for Paralytic Shellfish Poison** **Earl F. McFarren** **Public Health Service** **Cincinnati, Ohio** **Application Factors to be Applied to the Bio-Assays for the Safe Disposal of Toxic Wastes** **Croswell Henderson** **Public Health Service** **Cincinnati, Ohio**
8:00 P.M.	**Social Evening at Residence of C. M. Tarzwell** **780 Ivy Ave.** **Glendale, Ohio**
Tuesday April 24	
8:30 - 4:30	**Panel Discussion - Use and Value of Biological Indicators of Pollution** **Chairman, Dr. A. R. Gaufin, University of Utah, Salt Lake City, Utah** **Problems in Identification of Microorganisms** **Dr. Wm. Bridge Cooke** **Public Health Service** **Cincinnati, Ohio** **Bacteria** **Dr. Paul Kabler** **Public Health Service** **Cincinnati, Ohio** **Protozoa** **Dr. James Lackey** **University of Florida** **Gainesville, Florida**

DATE & TIME	SUBJECT
Tuesday April 24 (cont'd)	Algae Dr. C. Mervin Palmer Public Health Service Cincinnati, Ohio Fungi Dr. Wm Bridge Cooke Public Health Service Cincinnati, Ohio Mollusca Dr. William Ingram Public Health Service Cincinnati, Ohio Macro-Invertebrates Dr. A. R. Gaufin University of Utah Salt Lake City, Utah Fishes Dr. Peter Doudoroff Public Health Service Oregon State College Corvallis, Oregon Indicators of Lake Pollution Eugene Surber Fish and Wildlife Service Atlanta, Georgia
Wednesday April 25	
8:30 - 4:30	Panel Discussion - Water Quality Criteria for Aquatic Life Chairman, Dr. O. Lloyd Meehean Assistant to the Director, U.S. Fish and Wildlife Service

DATE & TIME	SUBJECT
Wednesday April 25 (cont'd)	Oxygen Requirements Dr. William Spoor University of Cincinnati Cincinnati, Ohio Effects of Turbidity and Silt John Wilson Public Health Service Portland, Oregon Requirements for Aquatic Life Dr.. O. Lloyd Meehean Fish and Wildlife Service Washington, D. C. The Status of Water Quality Criteria Dr. C. M. Tarzwell Public Health Service Cincinnati, Ohio
6:00	Group Supper - Millcroft Inn, Milford, Ohio
Thursday April 26	
8:30 - 4:30	Panel Discussion - Current Investigations in Water Pollution Biology Chairman, Kenneth M. Mackenthun, Committee on Water Pollution, Madison, Wisconsin Investigations and Problems in the Missouri Basin Dr. J. K. Neel Public Health Service Kansas City, Missouri Investigations and Problems in Alabama Dr. I. B. Byrd Alabama Dept. of Conservation Montgomery, Alabama

DATE & TIME	SUBJECT
Thursday April 26 (cont'd)	Biology and Water Pollution in Great Britain Thomas W. Beak Consulting Biologist Hawkesbury, Ontario Investigations at Oregon State College Charles Warren Department of Fish and Game Management Oregon State College Corvallis, Oregon Investigations and Problems in Ontario John Neil Ontario Dept. of Health Toronto, Ontario, Canada
4:30 - 6:00	Program Review and Tour of the Robert A. Taft Sanitary Engineering Center
6:30	Dinner - Center Cafeteria Speaker - Harry A. Faber, PHS, Washington, D. C. The PHS Research Grant Program
8:00 P.M.	Panel Discussion - The Training of Aquatic Sanitary Biologists Chairman, Dr. Herbert Jackson, Public Health Service, Cincinnati, Ohio Panel Members: Dr. Curtis L. Newcombe U.S. Naval Radiological Defense Laboratory San Francisco, California Dr. Lloyd Smith University of Minnesota St. Paul, Minnesota Dr. Charles Renn Department of Sanitary Engineering and Water Resources Johns Hopkins University Baltimore, Maryland Dr. T. H. Langlois Ohio State University Columbus Ohio

DATE & TIME	SUBJECT
Thursday April 26 (cont'd)	
	Dr. William Spoor University of Cincinnati Cincinnati, Ohio
Friday April 27	
9:00 - 4:30	Panel Discussion - Current Investigations in Water Pollution Biology
	Chairman, Dr. A. F. Bartsch, Public Health Service, Cincinnati, Ohio
	Use and Value of Sewage Lagoons
	Dr. A. F. Bartsch Public Health Service Cincinnati, Ohio
	Investigations and Problems in Florida
	Mr. William M. Beck State Board of Health Jacksonville, Florida
	Investigations and Problems in Illinois
	Dr. William C. Starrett Illinois Natural History Survey Urbana, Illinois
	Investigations and Problems in the Disposal of Radioactive Wastes
	Dr. J. J. Davis General Electric Company Richland, Washington
	Investigations and Problems in Ohio
	John Ries Division of Wildlife Dept. of Natural Resources Columbus, Ohio

DATE & TIME	SUBJECT
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List of Registrants

Seminar on Biological Problems in Water Pollution

April 23-27 1956

Public Health Service R A Taft Sanitary Eng. Center

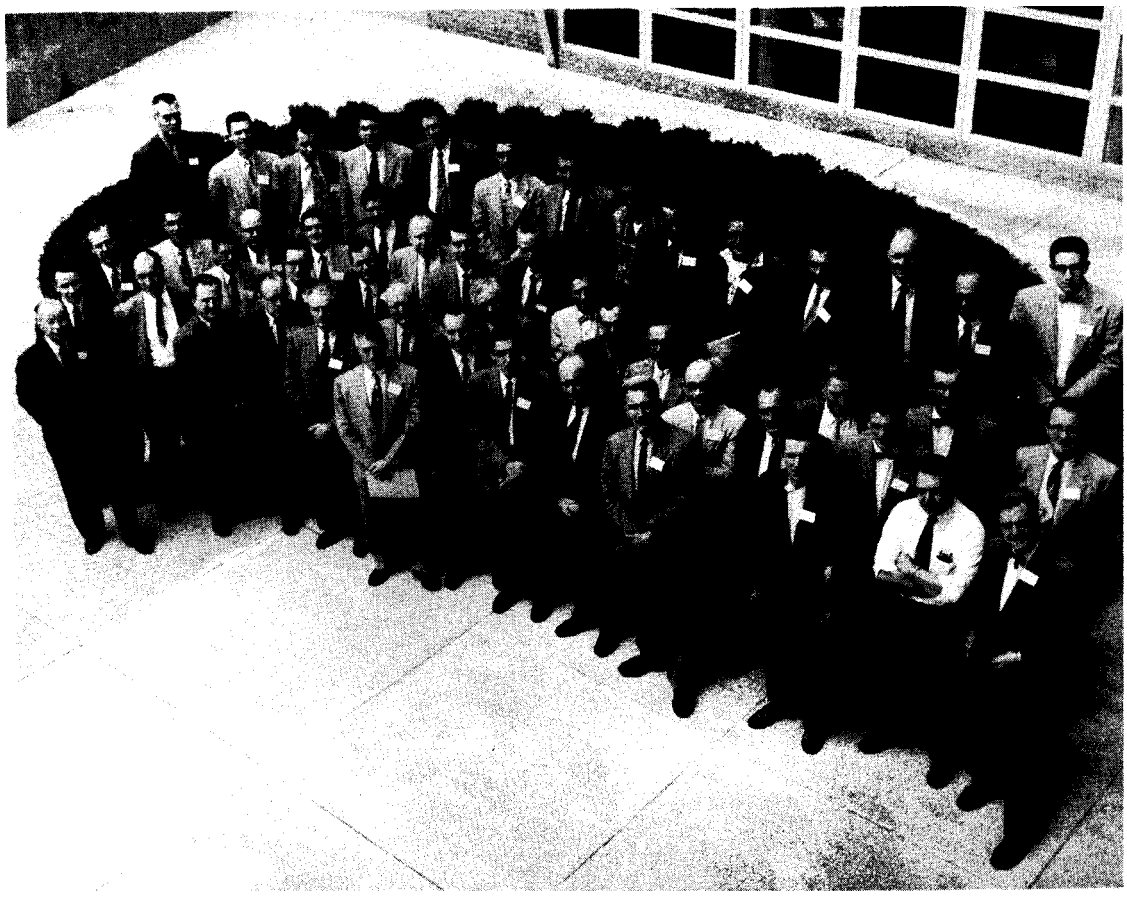
Cincinnati, Ohio

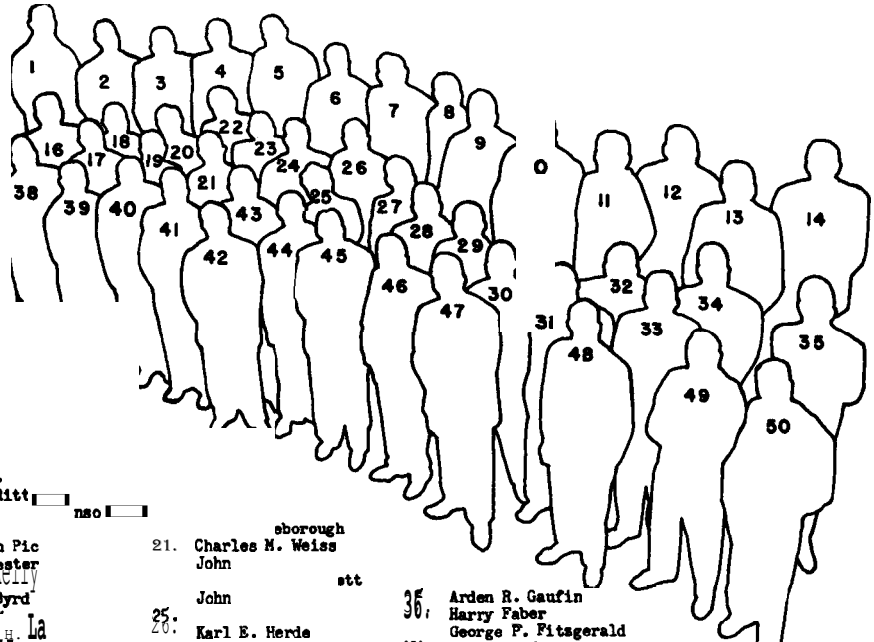
Dr. Bertil G. Anderson	Pennsylvania State University	University Park, Pennsylvania
J. B. Anderson	PHS, Region 7	Dallas, Texas
Robert F. Balch	Inst. of Paper Chemistry	Appleton, Wis.
Dr. Robert C. Ball	Div of Conservation Michigan State College	E. Lansing, Mich.
P. G. Barnickol	Missouri Conservation Comm.	Columbia, Mo.
Dr. A F. Bartsch	PHS	Cincinnati, Ohio
T. W Beak	4 Hamilton Street	Hawkesbury, Ont.
William M Beck Jr	Bureau of San Eng Florida State Bd. of Health	Jacksonville, Fla.
B.B. Berger	PHS	Cincinnati, Ohio
L. Berner	University of Florida	Gainesville, Fla.
K. E. Biglane	Stream Control Comm	Baton Rouge, La.
Mario Boschetti	Lawrence Experiment Station	Lawrence, Mass.
Clifford E, Bosley	Game and Fish Commission	Cheyenne, Wyo.
Arthur Bradford	Pennsylvania Fish Comm.	Harrisburg, Pa.
G. E. Burdick	Conservation Department	Albany 1, N. Y.
LB. Byrd	Dept of Conservation	Montgomery, Ala.
Dr. Robert S. Campbell	University of Missouri	Columbia, Mo.
Robert Cleary	Biology Bldg	Okoboji, Iowa
Dr. Wm Bridge Cooke	PHS	Cincinnati, Ohio
J. Davis	General Electric Co	Richland, Wash.
Dr Peter Doudoroff	PHS	Corvallis, Ore,

Robert J. Ellis	Inst. for Fisheries Research Michigan Dept. of Conservation	Ann Arbor, Mich.
Harry Faber	Eng, Resources Program PHS	Washington, D. C.
Dr Frederick Fish	PHS	Atlanta, Ga.
Dr George P Fitzgerald	University of Wisconsin	Madison, Wis.
Dr. Paul O. Fromm	Michigan State University	E. Lansing, Mich.
Dr. Arden R. Gaufin	University of Utah	Salt Lake City, Utah
Dick Graham	Dept. of Fish and Game	Helena, Montana
G Hamlin	ORSA NCO	Cincinnati, Ohio
Harry Hanson	PHS	Cincinnati, Ohio
Harry Harrison	Biology Building	Okoboji, Iowa
W. B Hart	Industrial Waste Eng. Div Pantech, Inc.	Folcroft, Pa
Forrest Hauck	Dept. of Fish and Game	Boise, Idaho
Croswell Henderson	PHS	Cincinnati, Ohio
Karl E. Herde	AEC	Aiken, S. Carolina
John Hester	Dept of Conservation	Montgomery, Ala.
Dr. Frank F. Hooper	Michigan Dept. of Conservation	Ann Arbor, Mich.
W. Charles Howard	PHS	Cincinnati, Ohio
Dr. Wm, Ingram	PHS	Cincinnati, Ohio.
Dr. Herbert Jackson	PHS	Cincinnati, Ohio
Dr. Paul Kabler	PHS	Cincinnati, Ohio
H D, Kelly	Dept. of Conservation.	Montgomery, Ala.
Dr. M. H A Keenleyside	Fish. Research Bd. of Canada	St. Andrews, N. B.
Harry Kramer	PHS	Cincinnati, Ohio
Dr James B. Lackey	University of Florida	Gainesville, Fla.
Dr. Thomas H. Langlois	Ohio State University	Columbus, Ohio

Dr. George H. Lauff	University of Michigan	Ann Arbor, Mich.
Bernard Lueck	Sulphite Pulp Mfg Res. League	Appleton, Wis,
Kenneth M Mackenthun	Comm on Water Pollution	Madison. Wis.
Earl McFarren	PHS	Cincinnati, Ohio
Kneeland McNulty	Marine Laboratory	Coral Gables, Fla.
H. E. McReynolds	Ind. Dept. of Conservation	Versailles, Ind.
Dr. O. Lloyd Meehean	Fish & Wildlife Service	Washington, D. C.
R. H. Millest	Dept. of Lands and Forests	Toronto, Ontario
John L Mohr	Univ. of Southern California	Los Angeles, California
D D. Moss	Dept. of Conservation	Montgomery, Ala.
J. K Neel	PHS, Region 6	Kansas City, Mo.
John H. Neil	Ontario Dept. of Health	Toronto, Ontario
Dr. Curtis L. Newcombe	U S Naval Radiological Def. Lab.	an Francisco, Calif.
Dr. H. P. Nicholson	PHS, Region 6	Kansas City, Mo.
George Paine	PHS	Cincinnati, Ohio
Ralph Palange	PHS	Cincinnati, Ohio
Dr. C. Mervin Palmer	PHS	Cincinnati, Ohio
Quentin Pickering	PHS	Cincinnati, Ohio
Gustave Prevost	University of Montreal	Montreal, Canada
R. Raneri	PHS	Cincinnati, Ohio
John N. Reis	Aquatic Biology Laboratory	Delaware, Ohio
Dr. S. C. Rittenberg	University of Southern Calif.	Los Angeles, Calif.
Earl T Rose	Biology Building	Okoboji, Iowa
Donald J Reish	University of Southern Calif.	Los Angeles, Calif.
J D. Roseborough	Lands and Forests	Toronto, Ontario

E. R. Roth	Atlantic Refining Co.	Philadelphia, Pa.
C. E. Ruhr	State Game & Fish Comm.	Nashville, Tenn.
Robert Schiffman	Michigan State University	E. Lansing, Mich.
Lloyd L. Smith, Jr.	University of Minnesota	St. Paul, Minn.
Wesley E. Smith	Bar. of Public Health Eng. Division of Health	Jefferson City, Mo.
William G. Spence	Game and Fish Commission	Jackson, Miss.
Dr. Wm. Spoor	University of Cincinnati	Cincinnati, Ohio
John Sprague	University of Toronto	Toronto, Ontario
Dr. William C. Starrett	State Nat. Hist. Survey Div.	Urbana, Illinois
Dr. Robert E. Stevenson	University of Sourthern Calif.	Los Angeles, Calif.
Harold Streeter	ORSA NCO	Cincinnati, Ohio
Eugene W. Surber	Fish and Wildlife Service	Atlanta, Georgia
Dr. CM. Tarzwell	PHS	Cincinnati, Ohio
W. W. Towne	PHS	Cincinnati, Ohio
William J. Tucker	Dept. of Public Health	Springfield, Ill.
John E. Watson	Fish and Wildlife Service	E. Greenwich, R.I.
Charles M. Weiss	University of North Carolina	Chapel Hill, N. C.
John N. Wilson	PHS	Portland, Oregon
A. W. Winston, Jr.	Dow Chemical Company	Midland, Michigan
Charles B. Wurtz	Commercial Trust Building	Philadelphia, Pa.





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USE AND VALUES OF BIOASSAYS

DEVIATION OF THE THRESHOLD VALUE FOR TOXICITY AND THE EQUATION OF THE CURVE BY A GRAPHICAL METHOD

G. E. Burdick
Senior Aquatic Biologist
New York State Conservation Department
Albany, N. Y.

The curvature found in graphs of toxicity when time is plotted against concentration on logarithmic paper is introduced by the failure of the effective range to conform to the axes of the paper.

For many years workers in the field of fish toxicology have been plagued by the curvilinear nature of the graphs of data that cover a wide range of concentrations. The equation must then be formulated by means of multiple degree equations, or, alternatively, the data must be transformed so it may be fitted somewhat approximately by a straight line.

Many methods have been suggested to avoid the necessity of considering the data as curves, such as the use of the reciprocal of time (Powers, 1917, and a number of English authors); the use of only that part of the data conforming most closely to a straight line (Herbert and Merkens 1952, and others) and the conventional plotting as powers, roots, or natural logarithms.

In their TL_m method Hart, Doudoroff and Greenbank (1945) avoid the production of a curve by the use of single values for specified times. However, in their estimation of biologically safe concentrations, which involves the relationship between the 24 and 48-hour TL_m an approximate straight line relationship appears to be assumed.

Shepard (1955) straightened his data on the tolerance of brook trout to low oxygen by applying as a corrective the time for death at zero oxygen.

In most cases concentrations have been plotted as logarithms and consideration of the spread of the data when times for death of individual fish are noted, also indicates time to be logarithmically distributed. When so plotted a curve results which usually can be fitted only approximately by multiple degree equations.

This observation led to an analysis of various methods of plotting and the procedures commonly.used to straighten curvature. As a result of this study the hypothesis which was stated at the start of this paper was formulated.

Time would not permit the complete discussion of the reasoning and calculations which led to this supposition and the suggested method for graphical translation. This has previously been prepared and submitted to the N. Y. Fish and Game Journal for publication.

Briefly, concentration can affebt time only in that range which lies between the threshold value of concentration and a minimum time for death which is independent of actual concentration. Only if both of these approached zero would the data conform to the axes of the paper. If the values were known, translation of the data could readily be accomplished by subtraction. This is not generally feasible.

Usually the minimum time for death at any concentration is small and if there is sufficient spread, this correction becomes negligible at low concentration. Progressive subtraction of different assumed values from the plotted curve in this range will produce a straight line, which is extended to cover the entire spread of the data. Only one such line can be produced on logarithmic paper and the concentration subtracted when it is formed represents the threshold value. Undercorrection produces a curve in the same direction as the original, overcorrection a curve in the reverse direction. If the subtraction is then continued over the remainder of the curve, the points gradually deviate from the straight line and approach the original curve. The space between the straight line and this produced curve represents the minimum time for death. The straight line represents a translation of the data to the axes of the paper. In order to use this method the data must include the part of the curve having maximum inflection.

The procedure also provides a method of deriving an equation representing accurately the original plotted curve. The fit on those curves tested appears better than that given by the use of multiple degree equations, while the labor is obviously much less. The equation of the straight line is first found by any of the usual methods In this equation, the log (x - the threshold value) is substituted for log X and the log (y - the minimum time) is substituted for log Y. This then represents the equation of the original data and closeness of fit can be determined by substituting selected values ot x and solving for y.

While the data to which it has been applied appear to confirm the hypothesis, it cannot yet be considered completely proved, since it should be applied to many toxicity curves. The indications are that it may prove a useful tool for the determination of threshold concentrations, since these are derived not as extrapolated values, but as a function of the plotted curve. Use of the straightline relationship and replotting or re-use of the corrected data would appear also to open the way for the use of a number of statistical analyses which could not be applied to curvilinear data.

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INTERIM PLAN* FOR STANDARDIZING THE BIOASSAY OF PARALYTIC SHELLFISH POISON BY USE OF A REFERENCE STANDARD

DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
Public Health Service
Washington 25, D. C.

INTRODUCTION

Prevention of poisoning due to eating toxic shellfish has been a problem of concern to health and fishery authorities for many years.

On May 26, 1955, the Public Health Service sponsored a conference to discuss recent developments in the assay for shellfish toxicity. The principal objective of the conference was establishment of a uniform procedure for bioassay of shellfish poison.

State and Federal agencies having representatives at the Conference included: Department of the Army, Fish and Wildlife Service, Food and Drug Administration, Massachusetts Department of Public Health, Canadian Department of National Health and Welfare, State of New York Conservation Department, and U. S. Public Health Service.

The most significant development in shellfish poison assay was reported by Dr. E. J. Schantz, Chief, Chemistry Branch, Army Chemical Corps biological research laboratory. Dr. Schantz and his associates were engaged in this field of research for several years and succeeded in isolating the poison in pure form. In working with the purified poison they also found certain color reactions, such as the Jaffe and Benedict-Bahre tests, which could serve as the basis of chemical assay for the poison. The conferees agreed that: (1) purified poison should be used as a tentative reference standard, and (2) results of future bioassays should be reported in terms of weight of poison.

The Army Chemical Corps has provided the Public Health Service with a limited quantity of purified shellfish poison for initiating standardization of the assay procedure. This plan outlines the manner in which the Public Health Service will distribute the reference standard to laboratories interested in standardizing their bioassay procedures.

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* This plan has been developed jointly by representatives of the Public Health Service and the Chemical Corps. Among those primarily responsible for preparing the technical details are Dr. E. J. Schantz, Chief, Chemistry Branch, Fort Detrick, Md.; Mr. E. T. Jensen, Acting Chief, Shellfish Sanitation Section, Milk and Food Program, Washington, D. C., Dr. K. H. Lewis, Chief, and Mr. E. F. McFarren, Chemist, Milk and Food Research, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio. The background data discussed by Mr. McFarren at the seminar will be presented in a series of papers to be published elsewhere.

SECTION A

OBJECTIVES

Primary objectives as stated at the 1955 Conference on Shellfish Toxicology are: (1) National and international standardization of laboratory techniques used in assay of paralytic shellfish poison, and (2) determination of the weight of purified shellfish poison equivalent to one mouse-unit. Schantz and associates have found that approximately 0.2 μg of purified poison is equivalent to one mouse-unit. This relationship needs to be established in each laboratory that employs the reference standard.

Secondary objectives are: (1) Accumulation of data which might be used to evaluate the statistical reliability of the bioassay, and (2) development of a chemical test to supplement or replace the bioassay.

SECTION B

DISTRIBUTION OF REFERENCE STANDARD

Laboratories interested in utilizing the purified shellfish poison for standardizing the bioassay procedure should direct their requests to Public Health Service, Washington 25, D. C., Attention: Shellfish Sanitation Section. Requests from Canadian laboratories can be considered only when endorsed by the Food and Drug Directorate, Department of National Health and Welfare, Ottawa.

In making application for the reference standard the laboratory director agrees:

(1) To use this material only for standardization of assay procedures used in connection with control of research activities on paralytic shellfish poison.

(2) To make no secondary distribution of the reference standard to other laboratories except those which are under his administrative control.

(3) To include in the standardization of the assay procedures the methods recommended herein.

(4) To furnish the Public Health Service with the accumulated standardization data and typical results of assays employing the standardized procedures.

The Public Health Service will send participating laboratories 5 to 10 ml of a reference standard containing 100 μg of poison per ml in an acidified aqueous solution. Each lot of solution will be assayed prior to mailing. Additional supplies of the standard are contingent upon receipt of data noted in item (4) above. Copies of a standardized report form (See page 30) are available from the Public Health Service.

Investigations of paralytic shellfish poison will be continued by the Public Health Service. Emphasis will be given to improvement of chemical assay methods which may supplement or replace the

presently used bioassay techniques.

Requests for information or consultation on technical problems related to utilization of the reference standard or development of assay procedures should be directed to the Robert A. Taft Sanitary Engineering Center, Cincinnati 26, Ohio, Attention: Chief, Milk and Food.

SECTION C

OBLIGATIONS OF PARTICIPATING LABORATORIES

Participating laboratories have three obligations under this program:

(1) Determination of the weight of poison per mouse unit for their own laboratory's conditions. When this relation has been established, assay results can be reported in terms of micrograms of poison per 100 grams of shellfish meats.

(2) Use the purified poison as a periodic check on their operating procedures. (Experience to date indicates that C Falued 1/ should not deviate from the mean by more than + 20%. A recheck is recommended if this value is consistently exceeded).

(3) Fulfill the agreement noted in Section B.

SECTION D

PROCEDURE FOR BIOASSAY OF REFERENCE STANDARD SOLUTION

(1) Select healthy mice weighing 19 to 21 grams from the stock to be used for routine assays. If mice weighing less than 19 grams or more than 21 grams are used a correction factor must be applied to obtain the true death-time. 2/

(2) Place one ml of the Standard Reference Solution in a 100 ml volumetric flask or graduated cylinder and dilute to 100 ml with water. If kept at 3 to 4° C this solution is good for several weeks.

(3) Dilute aliquots of the above solution with distilled water until intraperitoneal injection of 1 ml doses into a few test mice causes the median death time to fall between 5 and 7 minutes. The following dilutions are suggested as a guide:

Parts of poison solution	Parts of water	ug poison per ml
10	10	0.500
10	15	0.400
10	20	0.330
10	25	0.286
10	30	0.250

C F = $\frac{\text{ug poison per ml}}{\text{mouse unit poison per ml}}$

See page 29 for weight correction factors.

Preparation of smaller increments of dilution, as indicated by these preliminary tests, will be necessary to obtain satisfactory results. For example, when 10 parts of poison solution in 25 parts of water kill the initial test mice in 5 to 7 minutes additional dilutions should be tested which contain 10 parts of poison solution in 24 parts of water and 10 parts of poison solution in 26 parts of water. The pH of the dilutions should be between 2 and 4 for assay and must not be higher than 4.5.

(4) Use 10 mice on each of the 2 dilutions--preferably 3 dilutions-- that fall within the 5 to 7 minute median death period. Give a 1 ml dose to each mouse by intraperitoneal injection and determine the death time as the time elapsed from completion of the injection until the last gasping breath of the mouse.

(5) Repeat the assay one or two days later on the dilutions as prepared under (3) above.

(6) Repeat the entire procedure starting with step 1.

(7) Take the median death time of the ten mice for each dilution within each of the 4 trials and determine the number of mouse units contained in one ml of each dilution from the Sommer Tables (page 11). Divide the micrograms of poison in one ml by the mouse units in one ml. The result is a conversion factor (hereafter termed the CF value) expressing the micrograms of poison equivalent to one mouse unit. Compute the average of the individual CF values and use this average value as a reference point to check routine assays. This CF value will vary from one laboratory to another depending on differences in animals and techniques. The individual CF values may be expected to vary significantly within a laboratory if techniques and mice cannot be rigidly controlled. The latter situation would require the continued use of the reference standard or a secondary standard depending upon the volume of assay work undertaken by the laboratory.

SECTION E

PROCEDURE FOR USING THE REFERENCE STANDARD WITH ROUTINE ASSAYS OF SHELLFISH PRODUCTS

The conversion factor (micrograms of poison per mouse unit), determine as indicated in Section D-7 and termed the CF value, is used to calculate the micrograms of poison in a sample of shellfish by multiplying the number of mouse units found in 100 grams of sample by the CF value.

A periodic check on the CF value should be made as follows: If shellfish products are assayed less than once each week, a check run should be made each day assays are performed by injecting 5 mice with the reference standard. If assays are made on several

days during a week only one check need be made each week. The check run should be carried out on a dilution of poison such that the median death time falls between 5 and 7 minutes. The CF value thus determined should check the average CF value within ± 20%. If it does not check within this range complete a group of ten mice by adding 5 more mice to the 5 mice you have already injected, and inject a second group of 10 mice with the same dilution of poison. The CF value determined for the second group should be averaged with that of the first group and the resulting value taken as the new CF value for the conversion of mouse units to micrograms of poison. A variation of more than 20% represents a significant change in the response of the mice to the poison, or in technique of assay. Any changes of this type should be compensated by a change in the CF value.

Repeated checks of the CF value ordinarily produce consistent results within the limits prescribed above; if wider variations are encountered frequently, the possibility of uncontrolled or unrecognized variables in the test procedure should be investigated before proceeding with routine assays,

SECTION F

PROCEDURE FOR BIOASSAY OF CLAMS OR MUSSELS FOR SHELLFISH POISON 3/

(1) Preparation of Sample

a. Shucking

Thoroughly clean the outside of the shellfish with fresh water. Open by cutting the adductor muscles. Rinse the inside with fresh water to remove sand or other foreign material. Remove the meat from the shell by separating the adductor muscles and the tissue connecting at the hinge. Do not use heat or anaesthetics preparatory to opening the shell, and do not cut or damage the body of the mollusk at this state of the procedure. Collect the meats in a glazed dish until about 100-150 grams of material are obtained.

b. Draining

As soon as possible after shucking, transfer the shellfish meats to a ten-mesh sieve without layering and allow to drain for 5 minutes. Discard the drainings.

c. Grinding

Grind the meats in a meat grinder of the household type which has 1/8 to 1/4 inch holes in the grinding face, or macerate in a blender until a homogeneous mixture is obtained.

3/ Modification of a procedure adopted November 19, 1943, by the U. S. Public Health Service, U.S. Fish and Wildlife Service, and the Federal Food and Drug Administration, yvith excerpts from a procedure used by the Laboratory of Hygiene, Department of National Health and Welfare, Canada.

(2) **Extraction**

a. **Weighing**

Weigh out 100 grams of the well mixed material into a tared beaker.

b. **Acidification**

For all species of clams add 100 ml of 0.1 N hydrochloric acid, for sea mussels add 80 ml of 0.1 N hydrochloric acid and 20 ml of distilled water. Stir thoroughly.

c. **Digestion**

Heat the mixture and boil gently for 5 minutes, remove from the heat and allow to cool to room temperature.

d. **pH adjustment**

Adjust the cooled mixture to pH 4.0 to 4.5 as determined by B. D. H. Universal Indicator, brom phenol blue, congo red paper or a pH meter. To lower the pH add 5 N hydrochloric acid drop by drop with stirring. To raise the pH add 0.1 N sodium hydroxide dropwise with constant stirring to prevent local alkalinization and consequent destruction of the poison. After adjustment of pH make the volume up to 200 ml.

e. **Clarification**

Stir the mixture to homogenity and allow to settle until a portion of the supernatant liquid is translucent and can be decanted off free of solid particles large enough to block a 26 gauge hypodermic needle. If necessary it may be centrifuged (5 minutes at 3000 r. p. m.) or filtered through filter paper. It is necessary to obtain only enough liquid to carry out the bioassay.

(3) **Mouse Test**

a. **Inoculation**

Select mice weighing between 18 and 22 grams, if possible, and never over 25 grams. The mice should be of the same strain as used in the standardization procedure (Sections D and E). Inoculate each test mouse intraperitoneally with 1 cc of the acid extract obtained in F-2 above. Note time of inoculation and observe mice carefully for time of death as indicated by the last gasping breath. Record time by means of a stop watch or clock with a sweep second hand. One mouse may be used for the initial determination but 2 or 3 are preferred. If the death time or median death time of several mice is less than 5:00 minutes, make a dilution so as to obtain death

times between 5 and 7 minutes. If the death time of one or two mice injected with an undiluted sample is greater than 7 minutes but one or more mice do die, a total of at least three mice need to be inoculated in order to determine the toxicity of the sample with confidence. If large dilutions are necessary, the pH of the dilution should be adjusted by addition of dilute hydrochloric acid (0.1 or 0.01 N) dropwise so that the pH is between 3.5 and 4.5 (and never higher than 4.5). Inoculate three mice with the dilution that gives death times between 5 and 7 minutes (death determined as the time of the last gasp).

b. Calculation of Toxicity

Determine the median death times4/ of the mice, and from Sommer's table determine the iiumber of mouse units corresponding to the median death time. If test animals weigh less than 18 grams or more than 22 grams, a weight correction must be made for each mouse by determining the mouse units corresponding to the death time for that mouse from Sommer's table, multiplying this value by the correction factor for that mouse from Sommer's table, and then determining the median mouse unit for the mice. To determine the amount of poison per 100 grams of meat multiply the median mouse-unit by the dilution factor used in obtaining this unit and then by 200, since the original clam extract was made up to 200 ml. Convert the mouse units to micrograms of poison per 100 grams of meat by multiplying by the CF value.

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4 Include survivors in determination of median death time.

TIME DOSAGE RELATIONS FOR PARALYTIC SHELLFISH POISON (ACID) 5/

TIME	DOSE	TIME	DOSE	WEIGHT OF MICE	DOSE
		5:00	1.92	10 gm.	0.50
		05	1.89	10-1/2	.53
1:08	100	10	1.86	11	.56
10	66.2	15	1.83	11-1/2	.59
15	38.3	20	1.80	12	.62
20	26.4	30	1.74	12-1/2	.65
25	20.7	40	1.69	13	.675
30	16.5	45	1.67	13-1/2	.70
35	13.9	50	1.64	14	.73
40	11.9	6:00	1.60	14-1/2	.76
45	10.4	15	1.54	15	.785
50	9.33	30	1.48	15-1/2	.81
55	8.42	45	1.43	16	.84
2:00	7.67	7:00	1.39	16-1/2	.86
06	7.04	15	1.35	17	.88
10	6.52	30	1.31	17-1/2	.905
15	6.06	45	1.28	18	.93
20	5.66	8:00	1.25	18-1/2	.95
25	5.32	15	1.22	19	.97
30	5.00	30	1.20	19-1/2	.985
35	4.73	45	1.18		
40	4.48	9:00	1.16	20	1.000
45	4.26	30	1.13		
50	4.06	10:00	1.11	20-1/2	1.015
55	3.88	30	1.09	21	1.03
3:00	3.70	11:00	1.075	21-1/2	1.04
05	3.57	30	1.06	22	1.05
10	3.43	12:00	1.05	22-1/2	1.06
15	3.31	13	1.03	23	1.07
20	3.19	14	1.015	23-1/2	1.075
25	3.08			24	1.08
30	2.98	15	1.000	24-1/2	1.085
35	2.88			25	1.09
40	2.79	16	0.99	26/27	1.10
45	2.71	17	0.98	28/29	1.11
50	2.63	18	0.972	30	1.12
55	2.56	19	0.965		
4:00	2.50	20	0.96		
05	2.44	21	0.954		
10	2.38	22	0.948		
15	2.32	23	0.942		
20	2.26	24	0.937		
25	2.21	25	0.934		
30	2.16	30	0.917		
35	2.12	40	0.898		
40	2.08	60	0.875		
45	2.04				
50	2.00				
55	1.96				

5/ These data are for IP injections as determined by Dr. Sommer, - and are based on the data furnished by him to workers in the field.

FORM FOR REPORTING ASSAY DATA ON REFERENCE STANDARD SHELLFISH POISON SOLUTION

Log Number Reference Standard or RSS* _____

Laboratory _____

Date of Assay _____

Date Reference Standard Solution was prepared or received _____

Date of preparation of 1 to 100 dilution of RSS* _____

Name of Assayer _____

Strain of mice _____

Data on individual mice

			Dilution	
			2	
g poison per ml				
pH of dilution				
Mouse No.	Mouse Weight (gm)	death time (seconds)**	death time (seconds)**	death time seconds)**
2				
3				
4				
5				
6				
7				
8				
9				
10				
Median death time***				
Mouse units per ml				
CF value	$\frac{\text{g poison per ml}}{\text{Mu per ml}}$			

Send 3 copies of data sheets to: Sanitary Engineering Center, Cincinnati 26, Ohio Attn: Chief, Milk and Food.

*RSS - Reference Standard Solution.

** Death time from completion of injection to last gasp.

***Include survivors in determination of median death time.

APPLICATION FACTORS TO BE APPLIED TO BIOASSAYS FOR THE SAFE DISPOSAL OF TOXIC WASTES

Croswell Henderson

ROBERT A. TAFT SANITARY ENGINEERING CENTER
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The tremendous expansion of the chemical industry, especially that dealing with petrochemicals, the newer metals, synthetic fibers, insecticides, and detergents, presents new and difficult problems in the protection of aquatic life from effects of industrial pollution. Necessary chemical methods for the detection and measurement of many substances in these complex wastes have not been developed. Even when chemical methods are available, toxicity information generally is not, nor can toxicity always be attributed to one or more simple materials. In complex wastes there may be a number of different toxicants or there may be synergy or antagonism between substances so that the toxicity of final effluents may be entirely different from that of its components.

By using bioassays correctly, the effect on aquatic life of complex industrial wastes may be determined directly. Relatively simple bioassay methods have been devised and are being currently used. The procedures recommended by the subcommittee on toxicity of the Sewage and Industrial Wastes Federation (1) which are based in part on research previously reported by Hart, Doudoroff, and Greenbank (2) are quite satisfactory.

The basic bioassay procedure consists essentially of preparing different concentrations of an effluent or other test material with a selected dilution water, adding the test fish and observing their reactions over a definite time period. A logarithmic series of concentrations is generally most convenient.

For effluents of unknown toxicity, it is desirable to make exploratory or small scale tests to determine the approximate toxic range. Two fish are added to 2 liters of each test solution over a wide range of concentrations; e. g. , 100, 10, 1, and 0. 1 percent effluent. Observations over a short time period will indicate the necessary concentrations for the full scale experiments.

For the full scale tests, it is desirable to use a minimum of ten fish for each test concentration. This may be conveniently done in five gallon wide mouth glass bottles, using five fish in ten liters with duplicate samples. A series of intermediate concentrations are set up in the range indicated by the exploratory tests. For example, if the exploratory tests indicate an effect on fish between 10 and 1 percent, concentrations of 10, 5. 6, 3. 2, 1.8, and 1.0 percent, or in some cases intermediates between these are set up and fish added to each.

T[illegible] H, [illegible] hardness.

T[illegible] conditions [illegible] relatively [illegible] available. F[illegible] (Pimephales promelas) [illegible] (L[illegible] macrochirus) [illegible] n 1-1/2 [illegible] 2-1/2 [illegible] 1 [illegible] 2-1/2 [illegible] M[illegible] I[illegible].

T[illegible] A[illegible] bv [illegible] H[illegible] S[illegible] intervals, [illegible] successfully. P[illegible] dissolved [illegible] H, [illegible]

O[illegible] generally [illegible] 96 [illegible] T[illegible] 48, [illegible] 96 [illegible] TL_m (median [illegible] 50% [illegible]) values [illegible] interpolation.

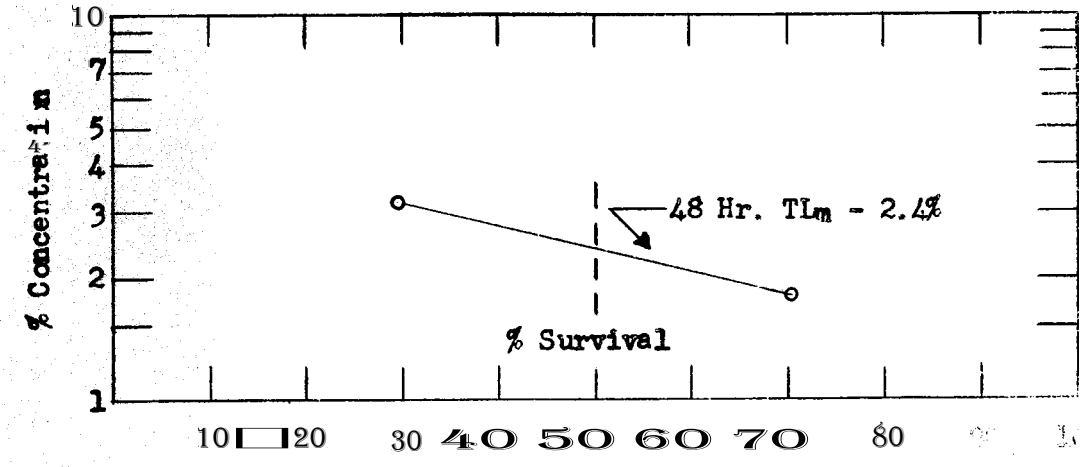
E[illegible] - 5.6% - A[illegible] 48 hrs.

3.2% - 3 [illegible] 10 [illegible] 48 [illegible].

1.8% - 7 [illegible] 10 [illegible] survive 48 [illegible],

1.0% - A[illegible] 48 hrs.

C[illegible] - A[illegible] 48 [illegible],



Once the TL_m value has been obtained, the next step is to determine how to use it. Obviously a concentration that will kill 50% of a test species is not safe for aquatic life. Liberal application factors (sometimes erroneously called safety factors) must be applied. Methods of applying laboratory bioassays have received very little attention. Industry and others are now using bioassays to some extent and more are ready to use them if provided a relatively simple bioassay procedure and reasonable methods of applying the results.

Stepwise, a proposed method of applying bioassays to meet specific industrial waste problems is as follows:

(1) Perform laboratory bioassays to obtain TL_m values.

(2) Calculate the dilution water required.

Example: 48 hour TL_m - 2.4%

Effluent flow - 10 cfs

Dilution Ratio - 2.4:97.6 or 1:40

∴ 10 cfs effluent would require 400 cfs dilution water from the receiving stream to produce a condition which would kill half of the test fish in 48 hours.

(3) Develop and apply a numerical factor that will provide safety to aquatic life in the receiving water. This can be in the form of a whole number when applied to dilution water needed or a fractional value of the TL_m when applied to the effluent.

There are many individual factors to be considered in developing a single application factor for specific wastes. The major ones may be grouped as follows:

A. Relating laboratory bioassays to actual conditions.

(1) Conversion from 50% to 100% survival.

(2) Conversion of acute or short term toxicity to possible long term or chronic effects of a toxicant.

(3) Conversion of toxicity in non-renewed or static test solutions to continuous flow conditions.

B. Relating test fish to other aquatic life.

(1) Effect on test fish versus other more sensitive fish species that may be present in the receiving stream.

(2) Other stages in the life cycle such as fry or eggs may be more sensitive.

(3) Some of the major fish food organisms may be more sensitive.

C. Variability in the toxicity of effluents which may fluctuate considerably over a period of time. Bioassays with 24 hour composite samples of effluents from various industries over a three month period, have produced the following results:

		48 hr. TL % Concentration		
Type of Industry	No. of Samples	Maximum Toxicity	Minimum Toxicity	Average Toxicity
Petrochemical	3	2.6	22	9.6
Oil Refinery	2	37	40	38.5
By products coke	4	3.7	13.5	9.0
Sewage plant (containing industrial wastes)	4	3.3	22	10.2
Chemical & dye 1	6	4.4	16	11.9
" 2	6	13.5	28	20.0
3	7	4.2	24	8.5

It is evident that any one sample taken for bioassay at a particular time may give erroneous results. Either a large number of samples would have to be tested or some factor developed to provide for conditions of maximum toxicity. The above factors are generally of concern in all cases and are not provided for in the experimental procedures.

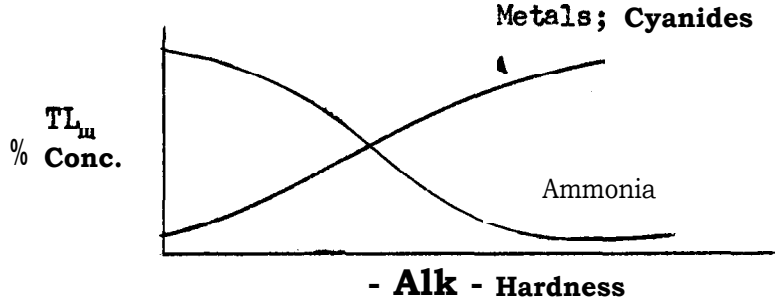
D. Other individual factors may have considerable effect on toxicity but are generally provided for in the tests. Any major differences between test and actual conditions in the following characteristics must be considered.

(1) Change in water quality characteristics

Temperature and Dissolved oxygen - Toxicity is generally .greater at higher temperatures and lower dissolved oxygen.

pH, Alkalinity, Hardness - These characteristics are somewhat interdependent. The buffering capacity would determine the pH which may have an effect on the toxicity.

Example - The toxicity of metals, cyanides, ammonia, and many other toxicants is greatly influenced by pH and other characteristics.



(2) Synergy and antagonism - A combination of materials may produce a more toxic or less toxic effect. It has been reported that copper and zinc in the same solution will produce an effluent eight times more toxic than copper alone. Likewise a material such as calcium may have an antagonistic effect (3).

The addition of several different effluents to a receiving water may give an entirely different toxicity picture from that of the separate effluents. For example, one industry may release an effluent containing metals into a hard water stream. The metals may precipitate or complex into insoluble compounds with materials in the stream waters and become relatively non toxic to aquatic life. Further downstream, another industry may release an acid effluent, lowering the pH and causing the metal to go into solution and become highly toxic. Bioassays with receiving waters would indicate the toxicity but the toxic materials may be attributed to the wrong industry. For this and other reasons, a knowledge of the chemical composition of the effluents and what reactions may be expected are of extreme importance.

If the above factors are carefully examined and related to established experimental evidence, sizeable application factors are apparent. An application factor of 10 times the 48 hour TL_m has been tentatively suggested. Circumstances in a particular study may raise or lower this value. Some individual factors, as already mentioned, may add to the above figure. Others which may detract some from the magnitude of the figure and must be considered are natural purification, loss of volatiles, oxidation or hydrolysis to non toxic products, precipitation, complexing, antagonism, etc.

The following example may illustrate a means of arriving at an application factor, providing the necessary information is available or can be obtained:

Factor	How Derived	Numerical Value
A. Test vs Actual conditions	Experiments Research	2
B. Test fish vs other aquatic life	Experiments Research	3
. Variability of effluents	Experiments Research	2
D. Other variables (see page 34)	Provided for in tests	

Application Factor = 2 x 3 x 2 = 12

Using the previous example of dilution water required for 10 cfs of an effluent having a 48 hr. TL value of 2.4%.

400 x 12 = 4800 cfs dilution water required for safety to aquatic life.

It is evident that considerable research is necessary before definite factors can be assigned which will be accepted. Good experimental evidence and a definite basis for the derivation of these factors will have to be available. Factors should be suggested which will adequately do the job and yet not cause undue expense for treatment.

Several methods of approach to this problem are suggested. The laboratory fundamental research program may give some definite answers or at least methods of mathematical expression. These could consist of long time continuous flow experiments with different wastes or waste components using various species of fish, different life history stages, and other aquatic organisms. The information could be mathematically related to our short term static bioassay. Other fundamental knowledge needed which could be obtained is the effect on toxicity of changes in water quality characteristics such as turbidity, temperature, dissolved oxygen, pH, alkalinity, hardness, etc. Programs to obtain this information could be and to some extent are being carried out here at the Sanitary Engineering Center, as well as in states, universities, industrial laboratories, and other research institutions.

Another approach which may give partial but more immediate answers is the field approach of working with actual effluents under field conditions. Bioassays could be made of the effluents from a particular industry or group of industries. Operating conditions, effluent flows, stream flows, and other conditions could be obtained. A study of the biota of the receiving waters could detect what is happening under present operating conditions. Study of several plants connected with a particular type of industry may give evidence

as to the magnitude of the application factor needed. The information may also be valid for related industries with similar types of effluents.

An ideal situation for a study of this type would be one continuously operating plant on a stream which may lagoon or regulate waste flows. The effect on the biota may be determined at different flow rates and the application factor estimated which affords protection to aquatic life.

These problems can be worked on for an extended period and still no mathematically precise answer obtained as with certain physical and chemical phenomena. However, a reasonably accurate estimate may be obtained. With proper application factors conditions may be estimated which are reasonably safe for aquatic life. How satisfactory they are can only be determined by studying the stream biota after a period of operation when they are in effect.

There are many ways in which industry can use bioassays. Toxicity of final effluents and the probable effects on receiving waters can be determined. Toxic components can be traced in process effluents, which may permit the treatment of much smaller quantities of waste. The effectiveness of treatment processes may be established. In the location of new plants, the amount and quantity of dilution water necessary or the degree of treatment of wastes may be ascertained in advance of construction.

It is believed that the greatest use will be made of bioassays if the methods are relatively simple and easy to use. Reliable and suitable application factors should be developed.

References

1. Doudoroff, P., et al., "Bio-Assay Methods for the Evaluation of Acute Toxicity of Industrial Wastes to Fish." Sew. and Ind. Wastes, 23, 11, 1380-1397, (1951).

2. Hart, W. B., P. Doudoroff, and J. Greenbank, "The Evaluation of Industrial Wastes, Chemicals, and Other Substances to Fresh-Water Fishes." Waste Control Laboratory of the Atlantic Refining Company, (1945).

3. Doudoroff, P., and M. Katz, "Critical Review of Literature on the Toxicity of Industrial Wastes and Their Components to Fish. II. The Metals, as Salts." Sew. and Ind. Wastes, 25, 7, 802-839, (1953).

USE AND VALUE OF BIOLOGICAL INDICATORS OF POLLUTION

SOME PROBLEMS IN THE IDENTIFICATION OF MICROORGANISMS

Wm. Bridge Cooke

ROBERT A. TAFT SANITARY ENGINEERING CENTER
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In studying the populations of algae and fungi on high-rate and standard-rate trickling filters recently, it was found that within groups of well known organisms, such as algae, growing in this habitat there were problems of identification which were difficult if not impossible to solve using preserved materials. For instance, in the case of a coccoid green alga, would you feel confident in determining from a pickled sample whether the cells belonged to Chlamydomonas, Chlorococcum, Protococcus, Palmellococcus, some other coccoid genus, or were only early palmelloid stage cells of Stigeoclonium? Under certain conditions of observation after preservation of a sample in the field any one or several of these names could be applied to a collection. In the case of blue green filamentous algae, could you tell from a preserved specimen which had been broken up in a Waring Blendor so that filament length or branching were not factors in the collection, whether you had Oscillatoria, Phormidium, Lyngbya, or some other form? Yet, we had such problems to answer. Several specialists in the field of green algae were consulted on the identification of the coccoid organisms, and only after prolonged study and as a result of familiarity with the groups did G. W. Prescott come up with an answer which admittedly was only tentative. Dr. Drouet was sent material of the filamentous blue-green, which turned out to be Amphithrix.

This experience led me to the idea that it might be well to develop a discussion of the problem of the identification of organisms that it is hoped may be used a indicative of pollution by their presence or absence in a stream. Some of the pitfalls in the identification of such organisms may be mentioned.

In the taxonomy and systematics of any group of organisms, plant or animal, there are usually two schools of thought. In the colloquial language of the trade of taxonomy these are referred to as the splitters and the lumpers. Modern taxonomy has shown that there is room for both, but in the past the matter has had the finality of either/or. On the basis of minute variations, some species of organisms have been split into many species, while without visible differences but with microscopic variation not visible to a casual observer, species have been lumped together.

In the early years of systematic biology the cataloging of the animals and vegetation of the earth's surface was of prime importance. As time went on, it became important to integrate the studies so begun, and for various regions floras and faunas were produced; lists, annotated or not, of various organisms were developed; and monographic treatments of genera, familes, orders, and even classes were attempted. As mountains of materials were built up in the herbaria, museums, and culture collections

of the world, more and more such synthesizing of our knowledge could be endeavored. Much of this work was done with the assumption, stated or implied, that certain barriers existed between species, barriers which prevented exchange of genetic materials between populations. When such barriers were based on political lines, as in the older works and the earlier catalogues, they were unnatural and the assumption of separate populations invalid within certain groups. Depending on the members of the populations, many geographic barriers may be considered valid, others not.

Where specimens were not available to a student of a group, where reliance had to be placed on records regardless of reliability, the development of generic monographs without regard to unnatural barriers resulted in burdensome lists of species in excess of numbers which could be supposed with little effort to be synonymous. Where specimens were available and critical interpretation of parallel geographic species was carried out, the lists became shorter. Where undue emphasis was placed on minute detail as valid bases for species separation, lists of species became quite lengthy. Where minute detail was considered a non sequitur for species criteria but the result of individual variation, species lists shrank. Based on specimens of leaves from one tree, a species has been based on the shade leaves of the common beech, another on the sun leaves.

In the genetics laboratory, an elementary problem is occassionally presented in which the common fruit-fly, *Drosophila melanogaster*, is used. A group of females of one type is mated with a group of males of another. The types are chosen with the idea of developing the concepts involved in the statistical study of Mendelian ratios. The progeny of such crosses.are obviously different from or similar to their parents. Yet if a taxonomist of the pre-Mendelian school were to observe these he could easily find the bases for several species of *Drosophila*, if not for new genera.

These introductory remarks are used to indicate that as our knowledge of life and our ability to classify its various aspects progress with the increasing knowledge from various fields such as morphology, cytology, anatomy, physiology, genetics, etc., the concepts of species and other categories change. As we find more and more specimens of organisms, some of which appear to vary markedly from known species, our concepts of various groups change. Various myths of taxonomy become exposed as additional materials are obtained. For instance, Dr. W. M. Ingram mentioned recently that many species of Hawaiian snails were shown to have been invalidly proposed when it was discovered that the basic character, that of color of shell, was found to have been altered for gain by those paid to collect additional color variants.

The use of dried specimens of plants, skins of animals, prepared mounts of micro-fauna, dried and otherwise prepared materials of algae, fungi and bacteria, has its place in modern taxonomy. At one time these were the only type of material on which concepts of species were based. More and more, modern taxonomy is using such materials as "type" materials or as records of past accomplishment and historical fact. The type specimen is the basis on which the species is founded, but the living specimen is the basis on which species in many groups of organisms are

best known and studied today. The geneticist needs living material for various types of genetic manipulation, as do the physiologist, the experimental morphologist, and the other scientists who base their work on the types of activity used by the first three mentioned. The systematic botanist today more and more uses genetic techniques for proving or denying the existance of one species or another in developing generic monographs. Experimental taxonomy is being actively used in many fields of botanical and zoological classification as well as in bacteriological and mycological systematics. In many cases, not only taxonomy and genetics are being learned in this way, but, at the same time and with the same organisms, many bits of information useful in "pure" and "applied" science are being discovered.

The present state of systematic identification of micro- and macro-organisms in the average field laboratory is based largely on well established texts in which a limited number of organisms are described and illustrated. Such texts may be considered comparable to a mushroom handbook in which only the most obvious form is illustrated with a photo taken more or less recently or a painting or a pencil sketch made more or less accurately within the last half century. Such description and nomenclature as may be used do not take into consideration the wide variation possible within the species, nor do they consider the fact that two species of mushroom obviously identical to the naked eye may be quite different both in respect to edibility and to micromorphology. The process of revising a genus of bacteria, fungi, algae, protozoans, mollusks, diatoms, or any other group, is long and laborious. Unless it is a genus which includes species of great economic importance such as Penicillium or Aspergillus, the work must be carried out in the "ivory tower" of the University or the Academy, otherwise it will interfere with or conflict with the interests of the employer whose business is the practical application of one process or another, of one species or another, or the development of one product or another.

In the development of a taxonomy which is adequate to the needs of the student of the biological indicators of pollution, not only at the level of specialization represented by the members of this panel but also at the level of usability of the man in the field, many factors will have to be taken into consideration. Not the least of these is the development of monographic studies at the genus or family level, in which by use of the techniques of experimental taxonomy a species may be given a more sound definition comprehensible to the man in the field whether he be biologist, engineer, or chemist, for it is rare that all three professions will be represented adequately on any field staff. Such dynamic rather than static taxonomic studies should be carried out in all fields in which organisms occur that may be found under varying conditions and types of pollution. The studies should overlap into other fields, since organisms occurring in or prevented from occurring in polluted waters may also be of importance in agriculture, forestry, food processing, industrial production, and other fields. Information from the laboratory should be available concerning the pollution tolerances of such species. Following the production of the monographic studies, the information contained therein of interest to the pollution biologist should be gathered together and made available to him in language understandable by a relatively unspecialized college graduate.

Such studies, properly presented, should enable a pollution biologist to interpret readily the role of the more important organisms in the stream, sewage treatment plant, or possibly even water treatment plant under his jurisdiction, in terms of work load or indicator value of which those organisms are capable, much as the forester can determine the possibilities of his forest in terms of board feet of lumber available and potential crop replacement.

USE AND VALUE OF BACTERIAL INDICATORS OF POLLUTION

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Normal Intestinal Bacteria

Following Pasteur's epic work and Kock's discovery of solid media, rapid progress was made in the isolation and description of the bacterial species commonly found in the intestinal tract.

S. typhosa (B. typhosus) described by Eberth in 1880.

Vibrio cholera described by Kock in 1884.

E. coli (Bacillus coli) described by Escherich in 1886.

It was clearly shown that E. typhosa, V. cholera, and the Salmonellae were associated with enteFic disorders and it s hoped that suitable procedures for the identification of these and other etiological agents of human disease might be developed for use as indices of drinking water quality. Several ingenious techniques were developed and remain with certain modifications as valuable research tools. There are many instances on record in which specific pathogenic bacteria have been isolated from suspected water sources, however, such attempts almost always ended in failure when used as a routine test for water quality. It is, therefore, apparent that attempts to isolate pathogenic microorganisms from a water supply are not recommended in the routine examination as an index of pollution for the following reasons:

1. Present available methods are tedious, laborious, and require high technical skill.

2. By the time a pathogen is recovered from a water the harm has long been done.

Subsequent to the demonstration by Escherich that E. coli was a normal intestinal organism, numerous investigators showed that E. coli and related organisms were consistently present in sewage, polluted streams, lakes and wells and in contaminated soil. These data showed that:

1. Coliform organisms are normally present in the feces of all warm blooded animals.

2. Some coliforms are to be found on plants and grains.

3. Feces contain from 5 to 500 million coliforms per gram, about 60 to 95% of which are E. coli.

4. A. aerogenes usually numbers 10,000 to 500,000 per gram of feces.

5. C[illegible] organisms are rarely found in virgin soil, but may be numerous in cultivated soils. From 65 to 80% may be A. aerogenes.

6. C[illegible] may survive weeks or months in fresh water, the predominating type varying with environmental conditions.

7. C[illegible] may live in dry soil 45 to 100 days, but in moist soil may survive a year or more.

Because the [illegible] group is constantly present in alimentary discharges, and because of the comparative ease of enumerating them, the [illegible] organisms have become the accepted indicator of fecal pollution. Also because of the fact that water containing fecal pollution may contain intestinal pathogens, the "[illegible] test" has assumed importance as a criterion in judging the sanitary quality of water.

The C[illegible] Group

"The [illegible] group shall include all of the aerobic and facultative anaerobic Gram-negative [illegible] -forming bacilli which ferment lactose with gas formation in 48 hrs. at 35° C." (Standard Methods for the Examination of Water and Sewage, 10[illegible] edition, [illegible]. 375).

The first [illegible] test was devised at the New York State Department of Health Laboratories by T[illegible] Smith in 1893 using dextrose broth and first edition of "Standard Methods" was published in 1905. The current edition (10[illegible]) of Standard Methods provides for no differentiation of "fecal" and "[illegible]" types. It is stated, "Such differentiation is of little moment in determining the suitability of water for human consumption, as contamination with either type of waste renders the water potentially unsatisfactory and of unsafe sanitary quality". Current methods have certain recognized imperfections as to time required, specificity and reproducibility. Continuous efforts have been directed toward development of better methodology. Some improvements have been:

1. Substitution of lactose for glucose in the presumptive medium, which materially reduced the number of presumptive false positive tests.

2. Churchman's observation that some dyes will selectively inhibit certain species or group of Gram-positive and of spore-forming organisms.

3. Modifications of incubation times, amount of gas, media ingredients and incubation temperatures.

    a. Bile salts by M[illegible] C[illegible] (1908).

    b. Perry and H[illegible] modified E[illegible] test (1933).

    c. McCrady, brilliant green bile (1937).

    [illegible]. M[illegible], [illegible] sulphate [illegible] broth (1941).

The adoption of brilliant green lactose bile broth as a confirmatory medium is perhaps the most important advance in methods in the last fifty years. The use of lauryl sulfate tryptose broth in the presumptive test is also an important improvement.

Recently suggested (tentative method only) membrane filter techniques may prove to have far reaching applications.

Other Organisms as Pollution Indicators

Streptococci

A leading "pretender" as an indicator of pollution is the Streptococcus (Enterococcus).

The Streptococci as a group have wide distribution. Many of them are pathogenic for man and animals. Some strains are strict parasites while others are saprophytic in existence. Many are fastidious in their growth requirements while others will grow with ease.

The sewage Streptococcus or Enterococcus have been proposed as an indicator organism on the basis that:

1. They are present in feces and sewage and are found in known polluted waters.

2. They are not found in pure waters, virgin soil and sites out of contact with human and animal life.

3. They do not multiply outside the animal body (except in such media as milk). (Suckling, Exam. Waters & Water Supplies 1943], Blakiston & Co.)

There is incomplete agreement on these three points. Evidence indicates the Enterococci do not multiply in water. Whether they persist longer in water or disappear at about the same rate as coliforms is a disputed point.

The sewage Streptococci are never present in as large numbers as coliforms. The btreptococcus conform ratio variesfrom 1/100 down to 1/10. As the Streptococcus detection methods are apparently no more sensitive than coliform methods, and as the pollution density is less, this would have the practical effect of reducing the sensitivity of the method.

Streptococcus classification methods have attempted to distinguish between human and animal strains. Mannitol-positive strains "do appear more common in human feces and raffinose-positive strains more common in bovine feces, " but separation on this basis has no place in evaluating the sanitary quality of a water supply.

Streptococcus methods offer a promising line of investigation. At this time the Streptococcus index in not a satisfactory substitute for the coliform group. Its greatest value is as a corroborative test where the coliform datum is suspected.

The Anaerobes

Spore-forming, lactose-fermenting anaerobes have been used as indicator organisms in water. The anaerobic organism is usually referred to as Clostridium welchii in England and Clostridium perfringens in America.

The spore-forming Clostridium is unsuitable as an indicator organism for the following reasons:

1. They are extremely resistant to destruction and survive for long periods.

2. They are abundant in animal manure, cultivated soil, and decomposing organic material.

3. They fail to correlate with results of sanitary surveys.

4. There are small differences in their numbers in heavily polluted and pure waters and they give no evidence of the degree of pollution.

The major interest in anaerobes at present centers about the occasional false positive presumptive test and its carry-over to brilliant green bile broth tube with gas and decolorization. The anaerobe can be eliminated by its failure to grow in formate ricinoleate broth.

The problem of finding a better indicator organism is one of paramount interest. Some show promise, but none at present compete with the fifty odd years of experience with the coliform estimation and its correlated data.

Escherichia coli

Originally Bacterium coli was considered to be a single species. As new methods and tests were devised, this so-called single species was divided into more and more species. Now this group of organisms is considered under the collective term of "coliform organisms".

Hundreds of research workers have added their contributions to the studies to determine the characteristics of the bacteria included in the coliform group, so that the ideal bacterial criterion of pollution might be defined, From this mass of data has evolved a classification of the coliforms into the coli group, the intermediate group, and the Aerobacter group. These differences are not sharp, well-defined distinctions, but are rather differences that shade from one variety to another on the basis of the interpretation of results from multiple testing procedures. It is further complicated by the question of what intermediate varieties to include with the E. coli as pollution indicator organisms.

Lewis and Pittman found no significant difference in the ratio of E. coli to coliforms in polluted water and in water of high sanitary Ruchhoft, et al., found the ratio of E. coli to coliforms in a sewage effluent and at the pumping station to be F6out the same

although the coliform M. P. N. counts were 13,815 and 0.219 per milliliter, respectively. Taylor found the E. coli ratio of no help in estimating the sanitary quality of water in English lakes.

A summation of the evidence of many workers warrants the following conclusions.

1. The ratio of E. coli to that of other coliforms is not an index of water quality.

2. Presence of E. coli usually is no more significant than other members of the coliform group.

3. The additional labor involved in the routine identification of E. coli in the presence of other coliforms is not justified on the basis of the information attained.

4. The sanitary quality of water is dependent on the total number of coliform organisms present. Density is an indication of pollution.

Summary

From a bacteriological point of view the coliform group is currently the best available indicator for use in the estimation of pollution of waters and in the sanitary evaluation of pure waters of potable quality. The coliform test is a quantitative test, Significance of its interpretation is dependent on determining the density (or most probable number). Only when the count exceeds a normally expected minimum number of coliform organisms is pollution indicated. Few samples of water are completely free from coliforms when a sufficiently large sample is examined. The established standard of the Public Health Service for drinking water recognizes the possible occurrence of an occasional coliform.

In the foregoing discussion it has been evident that pathogenic organisms cannot be used as indicator organisms. Their unsuitability is based on infection of the public before their discovery, their abnormal occurrence in the feces of a small percent of any group, and their small number in comparison to other intestinal type organisms.

It is freely admitted that there are normally found in the intestinal tract of man many types of organisms not belonging to the "coliform group". Some of these organisms at various times have been considered as "indicator organisms". The available data eliminated each for one or more reasons.

The substitution of E. coli for the present coliform group is unsuitable because it gives no additional information for the increased labor and because there are not sufficient epidemic studies to interpret the E. coli index.

Butterfield stated that ".... while it is desirable to seek for better bacterial criteria of pollution in water, and these researches should be continued, the results of such studies to date have not produced a criterion which may be considered to have satisfactorily replaced the coliform group." This summation is equally true today.

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PROTOZOA AS INDICATORS OF THE ECOLOGICAL CONDITION OF A BODY OF WATER

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Introduction

The topic as assigned by the program chairman was limited to protozoa. "Protista" would be a better term for several reasons. One is that much of this work has been concerned with various species of colored flagellates, whereas only a few colorless flagellates today are assigned to the protozoa and even these are regarded as colorless algae by some workers. Another is that very few ciliates are regarded as indicator species - some ciliates seem to prefer anaerobic habitats and others aerobic, but there is little to indicate a marked preference for pollution by any of them. The third reason is that the Sarcodina have been far too little investigated in respect to pollution. There is some indication that a small group of the minute amoebas are characteristic of certain stages of domestic sewage treatment but the larger naked amoebas are rarely abundant anywhere. Decloitre (1) is currently tending to show that the thecate amoebas are cosmopolitan, whereas there has been a tendency to regard most of them as sphagnum bog types. Foraminifera and Radiolaria we know are marine - the former in the ooze, the latter pelagic. For these reasons, any discussion should be more widespread than the colorless flagellates, the ciliates and the amoebas.

The Varying Nature of Environments

An organism generally occupies a given niche permanently because that niche offers the most favorable environment for it. Such occupation implies choice on the part of the organism. It does not take into account crowding or other competition, changing the environment as by shutting off light, being forcibly removed or retained as by a current, etc. In other words, we too frequently assume an environment to be relatively constant, whereas it is anything but constant and the organisms in it either must be adaptable to change, or migrate, or die. New kinds, suited to the new environment, may replace them and our concept must be flexible to take care of such changes. There was a lake in Florida which was often referred to as "a good bass lake. " Within one growing season, it became covered with water hyacinths and the bass disappeared, migrating downstream. This did not alter the concept implied by the phrase "good bass lake, " but it certainly showed that such concepts are applicable to transient situations.

Actually, we talk as if we are stating a constant. Over a broad environmental range, such relative constants actually exist. Thus

a coal mine stream, among other things may constantly contain enough sulfuric acid to be highly acid a great part of the time. At such times, it is favorable to a small group of organisms, the most striking being Euglena mutabilis. This Euglena has been noted as abundant also in iron seeps where ferric hydroxide was concentrated and is occasionally found in a variety of other situations, some of them alkaline. What favors Euglena mutabilis in the acid coal mine stream? Does it have a greater physiological tolerance to sulfuric acid than does, say Euglena polymorpha? Or is there less competition for food and sunlight? Or is there a particular food substance in such a stream? Until we have answers to some such questions as these, we are on dangerous ground in talking of indicator organisms. Euglena polymorpha is one of several species of this genus which increase markedly in streams receiving effluents from sewage treatment plants. It has been referred to as an indicator species of recent fecal pollution. But it also occurs as dense blooms in certain types of swamp waters, such as the cedar swamp at Woods Hole, Massachusetts. These two situations must have in common some other factor than fecal pollution.

Ubiquitous Organisms

Some organisms tolerate such a wide range of environmental variation as to almost defy identification of limiting factors. Man is the most conspicuous of these. In our case, we can adapt to extremes of heat, cold, moisture, dryness, pollution of the atmosphere, and so on through a wide range. The blue crab, Callinectes sapidus, occurs along the Atlantic coast from Main to the Florida Keys, as well as the Gulf Coast - a geographic range within which there is a wide fluctuation of temperature, food, enemies, salinities and other factors. In Florida it migrates up the rivers and into some of the fresh springs, or those almost fresh. But what of the small ciliate, Cyclidium glaucoma, which apparently is truly cosmopolitan and which Finley has shown (2) can transfer from fresh to sea water or vice versa? It rarely attains huge numbers anywhere but in a hay infusion it may. The only indicator value it has in such a situation is to indicate that the bacteria on which it feeds are very abundant and that competition for this bacterial food is at a low ebb. This is easily amenable to experimental proof - one has only to grow Cyclidium with and without competing predatory ciliates.

The number of such ubiquitous species may be large. Undoubtedly, we often fail to recognize them. For example, Didinium nasutum, rarely common anywhere, but used by Beers (3) as a fresh water experimental ciliate, is frequent in samples from the high seas. Some Vorticella species belong in this category also and apparently Chlorella among the green algae. Peranema trichophorum and Anisonema ovale of the colorless Euglenophyceae are others.

Organisms of Somewhat Limited Distribution

It seems an easy matter to make a long list of organisms which occur in either salt or fresh water but not both. Or, for marine

TABLE I

Species Of Frequent Occurrence or May Occur in Large Numbers in	Probable Oxygen Relationship Of Species			
	Aerobe	Anaerobe	Faculative	
Sewage treatment plants				
Streams of waters enriched by treated or untreated sewage				
Waters with organic enrichment other than sewage				
Waters fertilized by agricultural drainage - largely inorganic enrichment				
Deliberately fertilized ponds, as for fish production				
Other pollutants such as acid mine drainage, etc. Inorganic				
Unpolluted waters				

Mastigamoeba spp.		X		X	X				
Mastigella spp.		X		X	X				
Helkesimastix faecicola		X		X	X				
Monas spp.			X	X	X	X	X		
Oicomonas spp.			X	X	X	X	X	X	X
Pleuromonas jaculans	X			X	X	X	X		
Tetramitus spp.		X		X	X				
Spiromonas angusta			X	X	X				
Desmarella moniliformis	X				X		X	X	X
Cladospongia elegans			?		X				
Gyromonas ambulans		X		X	X				
Cercomastix parva		X		X	X				
Poteriodendron petiolatum	X			X	X	X	X		
Sterromonas formicina			X	X	X				
Phanerobia pelophila		X		X	X				
Trigonomonas compressa		X		X	X	X			
Trepomonas spp.		X		X	X	X			
Urophagus rostratus		X		X	X	X			
Volvocales									
Carteria spp.			X	X	X	X	X	X	X
Chlamydomonas spp.			X	X	X	X	X	X	X
Chlorogonium spp.			X	X	X	X	X	X	X
Dunaliella salina			X			X			
Eudorina elegans			X		X	X	X	X	
Gonium pectorale			X		X	X	X	X	X
sociale			X		X	X	X	X	X
Pandorina morum			X		X	X	X		
Polytoma uvella			X	X	X	X			
Polytomella citri			X	X	X	X			
Platymonas spp.			X		X	X	X		
Chlorobrachis (Pyrobotrys gracilis)			X		X	X			
Spondylomorum quarternarium			X						
Euglenophyceae - colorless									
Astasia spp			X	X	X	X			
Entosiphon sulcatum			X	X	X	X			X
Notosolenus spp.			X	X	X	X	X		X
Peranema trichophorum			X	X	X	X			
Euglenophyceae - green									
Cryptoglena pigra					X	X	X		X
Euglena acus					X	X	X		X
agilis					X	X	X		X
deses					X	X	X		X
gracilis			X	X	X	X	X		X
mutabilis								X	X
pisciformis			X	X	X	X	X		X
" polymorpha					X	X	X		X
quartana			X	X	X	X			
sanguinea									
tripteris					X	X	X		X
viridis					X	X	X		X

Eutreptia viridis	X				X	X	X	X		X
Lepocinclis butschlii	X				X	X	X	X		X
" ovum	X				X	X	X	X		X
texta	X				X	X	X	X		X
Phacus parvulus			X	X	X	X				
" triqueter			X		X	X	X	X		X
" pyrum			X	X	X	X				X
Trachelomonas urceolata	X				X	X	X			X
volvocina	X					X	X	X		X
Chrysophyceae										
Botryococcus braunii	X				X	X				X
Chromulina ovalis	X							X	X	X
globosa	X				X	X				X
Mallomonas spp.	X					X		X		X
Coccolithophora										
Pontosphaera sp.	X					X		X		X
Dinoflagellata										
Oxyrrhis marina	X					X		X		X
Gymnodinium splendens	X					X				X
Prorocentrum triangulatum	X					X	X	X		X
Cryptophyceae										
Chilomonas paramecium			X	X	X	X	X			
Cryptomonas spp.						X	X			
Rhodomonas spp.	X					X	X	X	X	X
Ciliata										
Carchesium sp.	X			X	X	X				X
Chilodonella spp.	X			X	X	X	X			X
Cinetochilum margaritaceum	X			X	X	X	X			X
Colpoda spp.			X	X	X	X				
Colpidium sp.			X	X	X	X				
Glaucoma scintillans			X	X	X	X	X			
Halteria grandinella	X			X	X	X	X			X
Lionotus faeciola	X			X	X	X				X
Metopus sigmoides			X	X	X	X				
Opercularia spp.	X			X	X	X				
Oxytricha spp.	X			X	X	X	X			X
Paramecium spp.			X	X	X	X				
Saprodinium putrinum		X		X	X	X				
Stylonichia spp.	X			X	X	X				X
Trimyema compressa		X		X	X	X				
Urocentrum turbo			X	X	X	X				
Vorticella spp.	X			X	X	X	X			X
Hastatella radians	X					X				
Enchelyomorpha vermicularis		X		X	X	X				

species, pelagic versus neritic species, as Radiolaria and perhaps certain dinoflagellates. Even boreal, termperate and tropic species may occur: Chrysococcus cingulum is common at Woods Hole and the Chesapeake but has not been found in three years' examination of Florida Gulf Coast samples. Certain species of ciliates, such as Irimyema compressa and Enchelyomorpha vermicularis seem restricted to water - or mud - devoid of oxygen, but are widespread if enough such situations are examined.

In this second category, it is always difficult to determine the limiting conditions. Metopus (possibly more than one species) is often found along with the two ciliates above but will exist also in the presence of some oxygen: In these instances, is it a lack of oxygen or the presence of H_2S which is effective? In the Radiolaria is it the ions of full strength sea water or merely physical osmotic phenomena? The chloromonad Gonyostomum semen is common to the brown waters of cedar and cypress swamps but is almost unknown elsewhere. There are many instances of limited distribution but knowledge of the limitations is virtually lacking.

Rare Organisms

Many species are named in the literature which the average observer has never seen. This may mean poor observation, not having sampled the habitat type for the organism, or it may really be rare. The diatom Attheya zachariasi has frustules of such transparency that they are seen only with considerable difficulty and its chloroplasts and cytoplasm are usually grouped in a small flattened disc. It is seldom reported in the United States, yet seems common. Another is Cephalomonas granulosa, one of the green flagellates. This distinctive organism occurs sparingly in Ohio Valley waters but on one occasion, a slight bloom of them was recorded in Lytle Creek (4); the species was originally described from Maryland (5). Trachelomonas reticulata is colorless and Klebs (6) described it from "faulenden" Itı so it should occur in polysaprobic situations. There are no known records of the species in the United States, except a very dense bloom found by the writer in an Alabama tree hole. A fourth rare organism is the ciliate, Hastatella radians. It is large (100 μ) and unmistakable but has been seldom encountered. On April 8, 1956, it was the dominant ciliate, there being 80 per ml, in a deep sink hole in Orange Lake, Florida. The sink hole acted as a concentrating pit for the slowly drying up lake, there being a slow but steady outflow through the 71 foot deep bottom.

Undoubtedly then, many organisms which we consider rare, will attain high numbers if the environment is favorable. The question generally develops into attempts to recognize the optimal factors.

Discussion

Before stating a relationship between organism species or organism numbers and pollution, we must first define the pollutant. There are many kinds of pollution, varying from an excess of organic

or putrescible matter to heavy concentrations of inorganic salts, Poisons, even physical conditions, enter the picture. It is hardly worthwhile to list types of pollution here but there are chemical and physical tests which are revealing even when a condition is not apparent to the eye. Conditions having been determined, we ascertain the numbers and kinds of organisms present and the permanence of both the physico-chemical condition and the population.

The mere occurrence of an organism in a sewage treatment plant does not mean it is indicative of pollution - Oxytricha may occur either in a sewage treatment plant or in a hay infusion simply because it finds in each place the bacteria it uses as food, as well as ample oxygen. A long list of organisms occurring in sewage treatment plants could be made and the same organisms would be found in other situations in many cases. Table I is such a list in part. It does not cover the green nonflagellated algae and the diatoms. It is based partly on personal experience and partly on the known literature. No references are given, for there would be too many and in addition, the list does not cover all the references which could be made. It is weakest in regard to the occurrence of ciliates.

This table is intended to show that there are few organisms which are common to only one type of habitat. It does not include those which are common to or which sometimes occur in huge numbers in waters not known to contain substances we would regard as pollutants or fertilizers. Nor does it include laboratory cultures, Undoubtedly, there are enriching substances in the gravel pits around Chillicothe, Ohio, which annually bloom with Uroglena, or the Cedar Swamp at Woods Hole which annually blooms with Gonyostomum. But at present we either do not know what they are, or we do not regard them as pollutants. The same is true of the Gulf of Mexico water in which Gymnodinium brevis blooms so heavily, or the once famous Spirostomum pool at Woods Hole. The vast majority of waters will have many species in common and often one or more of these species will bloom. But the organisms do not classify such waters; hence, fall outside the scope of this discussion,

It cannot be too strongly emphasized that a cause and effect relationship does not necessarily exist simply because of abundance of an organism and occurrence of a defined pollutant. Clark (7) has described mechanical trapping of plankton in certain situations and has also called attention to common orientations and attractions of organisms to others of their own kind, "Trapped" populations may remain in a situation, so we cannot be too sure of even this yardstick. Statements as to the indicator value of an organism in a given situation absolutely demand critical experimental analysis and accumulation of much data. Even then the relationship to pollution may be secondary. The outfall of the Tampa sewage treatment plant apparently has little effect on chlorophyll bearers in its section of Tampa Bay but the number of tintinnid ciliates is considerably higher here than in other sampled sections of the Bay. Counts of the

bacterial population have not been made but an increased number there could account for the increased ciliates. The magnitude of the sewage contribution and the size of the Bay are discouraging to attempts at collecting precise and continuous data. It would be necessary not only to secure tidal flow data, but seasonal and vertical biological data here and elsewhere in the Bay, then add chemical analyses. At the end of this costly process, it is questionable where relationships would have been established. In November, there is a bloom of the diatom, Skeletonema, but it varies in density from year to year; it also occurs in other portions of the Bay which receive no sewage. There are repeated dense but local blooms of many dinoflagellates, such as Gymnodinium splendens, Ceratium furca and others in various parts of Tampa Bay, but no explanation of why they occur. Many thousands of dollars have been spent on attempts to determine the cause of the Red Tide organism, Gymnodinium brevis. So far, all it indicates is trouble.

Still there are some hopeful signs. Study of the algae or protozoa in a fairly constant environment reveals some which tend to occur permanently. Yount (8) concluded that no one factor determined the species density in a habitat. He worked in the Silver Springs (Florida) boil area or near it, where many factors are uniform the year round. Even so, light and some other factors fluctuate and since they worked with diatoms this is important. Such seemingly small matters as the surface of a slide soon after immersion, as compared to the same film-covered slide after several days can and do make surprising differences as to what and how many diatoms are found on the slide. There has been too little work of this type but it has been experimental experience that one way to learn what species of colorless Euglenophyceae may be found in a habitat, is to suspend slides for several days.

Studying smaller streams indicates that the more abundant is the oxidizable matter, or the inorganic salts, the more varied and abundant is the suspended population. Lytle Creek (Ohio) studied at five stations and Cowan Creek, nearby, and studied at one station (4) over a period of three months in the summer showed 167 and 89 species respectively. Two of the Lytle Creek stations were in a polluted area, receiving the effluent from a badly overloaded sewage treatment plant. In this area, certain types of Euglenophyceae appeared in large numbers. Others died out, especially most species of Trachelomonas. Chrysophyceae also failed to pass through this polluted zone, although 12 species were present above. Apparently, these are responses to domestic sewage pollution.

Unhappily, most of the Chrysophyceae are also lacking in the small Santa Fe River of Florida. This stream had a large number (332) of species during the time it was studied and 99 of them were the same as those in Lytle Creek. The Santa Fe is unpolluted and receives little drainage from arable land; total numbers of organisms were low. The same story holds for the two forks of the Licking River of Kentucky - that fork draining the steep, wooded areas such as Magoffin County in Eastern Kentucky is fairly rich in species

but low in numbers of organisms. The more westerly fork, draining the rich blue grass country, is high in both species and numbers,

After many years, the status of indicator species among the algae and protozoa seems little near clarification. Immediate sewage pollution of a stream, resulting in oxygen depletion, tends to eliminate all but a few species of anaerobic ciliates and colorless flagellates. Settling the sewage in oxidation ponds results in heavy growths of Chlorococcales and Volvocales. Allum (9) says that raw sewage lagoons in South Dakota showed DO values as high as 370% saturation, attributable to algae. Silva and Papenfuss (10) made a systematic study of the algae of sewage oxidation ponds in California and in an extensive comparative review of the literature recorded some 26 genera from at least 10 of their ponds, which list generally agreed with lists of other investigators. A few other algae were recorded and a few Euglenophyceae.

The trouble with observations such as these lies neither in their accuracy nor in the high counts. Rather, it is the tendency we acquire to regard the organisms found as characteristic of sewage pollution. Such is not the case. In the Orange Lake pool referred to above, many of these same species occurred in great numbers and many of them are common in situations where there is no sewage or sewage effluent. In fact, commercial fertilizers will produce heavy growths of some of them.

Partial sewage treatment, resulting in a sharp decrease in bacteria and BOD increases the number of ciliates and colorless flagellates, and in the effluent channels, attached blue green and green algae, the latter usually two genera. Further reduction of BOD tends to produce some eight or ten species of Euglena and Phacus and to increase the species of Volvocales and Euglenophyceae. There is little doubt that certain species of algae and protozoa are readily associated with sewage pollution. But we should distinguish between "characteristic of" and "indicative of, " the latter being a more restrictive term.

This last distinction might help make the suggestion of Beck (11) work. In it, he adds the numbers of species found which are known to be associated with clean water to the numbers of species found, which are known to be associated with polluted water. He says it should apply only to macroscopic invertebrates found in Florida. However, a detailed analysis of a stream station usually reveals a large number of species of algae and protozoa. If we can satisfactorily allocate them to one or the other of the above classifications, the result should classify the stream at the station studied. Allocation is a difficult task and few workers are in agreement for more than a very few species.

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ALGAE AS BIOLOGICAL INDICATORS OF POLLUTION

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IT APPEARS EVIDENT TO MANY WORKERS THAT PARTICULAR GENERA OR EVEN SPECIES OF ALGAE, WHEN CONSIDERED SEPARATELY, ARE NOT RELIABLE INDICATORS OF THE PRESENCE OR ABSENCE OF ORGANIC WASTES IN WATER. HOWEVER, WHEN A NUMBER OF KINDS OF ALGAE ARE CONSIDERED AS A COMMUNITY, THAT GROUP MAY BE RELIABLE AS SUCH AN INDICATOR. SUCH POPULATIONS MAY BE USEFUL EVEN IN THE DETERMINATION OF THE DEGREE OF PURIFICATION WHICH HAS OCCURRED; BUT THE DATA AVAILABLE ON THIS IS MORE LIMITED. THEREFORE, IT HAS SEEMED ADVISABLE TO OMIT HERE ANY ATTENTION TO INTERMEDIATE STAGES IN THE NATURAL PURIFICATION PROCESS.

EMPHASIS IS PLACED IN THIS ARTICLE ON THE ALGAE WHICH VARIOUS WORKERS HAVE FOUND TO BE TOLERANT TO RELATIVELY UNDECOMPOSED SEWAGE OR CLOSELY RELATED ORGANIC WASTES. THESE ALGAE WOULD, IN GENERAL, BE THOSE FOUND TO BE PRESENT AND RELATIVELY PROMINENT IN THE polysaprobic AND alphameso-saprobic ZONES IN A POLLUTED STREAM. THESE TERMS, HOWEVER, ARE USED BY RELATIVELY FEW WORKERS WHO HAVE REPORTED SEWAGE TOLERANT ALGAE. THE WRITER HAS INTERPRETED THEIR FINDINGS AS THEY APPLY TO SEWAGE pollution. DUE TO THE LARGE NUMBER OF REFERENCES USED, AN OCCASIONAL MISINTERPRETATION SHOULD NOT SERIOUSLY DISTORT THE COMPOSITE RECORD CONCERNING THE ALGAE.

THE REPORTS OF FIFTY-SIX WORKERS HAVE BEEN EXAMINED TO DATE. THE GENERA AND SPECIES OF ALGAE TOLERANT TO SEWAGE OR TO RELATED CONDITIONS HAVE BEEN RECORDED, AND A TOTAL OF MORE THAN 500 KINDS OF ALGAE HAS BEEN COMPILED. THE MAXIMUM NUMBER OF AUTHORS LISTING ANY ONE GENUS WAS 34, AND, ONE SPECIES, 18.

IN ORDER TO TABULATE THE INFORMATION, THE WRITER HAS ALLOTTED ARBITRARY NUMERICAL VALUES TO EACH AUTHOR'S RECORD OF AN ALGA. A VALUE OF TWO WAS GIVEN TO EACH ALGA REPORTED AS VERY HIGHLY TOLERANT AND A VALUE OF ONE, TO EACH ALGA HIGHLY TOLERANT TO SEWAGE. LIGHTLY TOLERANT AND NON-TOLERANT ALGAE WERE NOT RECORDED IN THIS COMPILATION. THE TOTAL POINTS FROM ALL OF THE AUTHORS WAS THEN DETERMINED FOR EACH GENUS AND SPECIES. THE ALGAE WERE ARRANGED IN THE ORDER OF DECREASING EMPHASIS BY THE AUTHORS AS A WHOLE. THE FIFTY GENERA AND THE FIFTY SPECIES OF SEWAGE TOLERANT ALGAE WITH THE HIGHEST TOTAL NUMBER OF POINTS ARE GIVEN IN TABLES 1 AND 2, PLATE 1. "Polluted Water Algae" ILLUSTRATES NINETEEN OF THE FIFTY GENERA LISTED IN TABLE 1.

F[illegible]-[illegible], [illegible]. T[illegible]

T[illegible] T[illegible] 1 [illegible] 2 [illegible]-[illegible]. T[illegible] [illegible], [illegible]. P[illegible].

S[illegible], [illegible], [illegible]. T[illegible] T[illegible] 2 [illegible] T[illegible] 1, [illegible]-[illegible]. T[illegible] N[illegible], S[illegible], [illegible] Chlamydomonas. I[illegible] species. F[illegible], F[illegible] (2) [illegible]-[illegible] U[illegible], [illegible] "[illegible]" [illegible] "[illegible]."

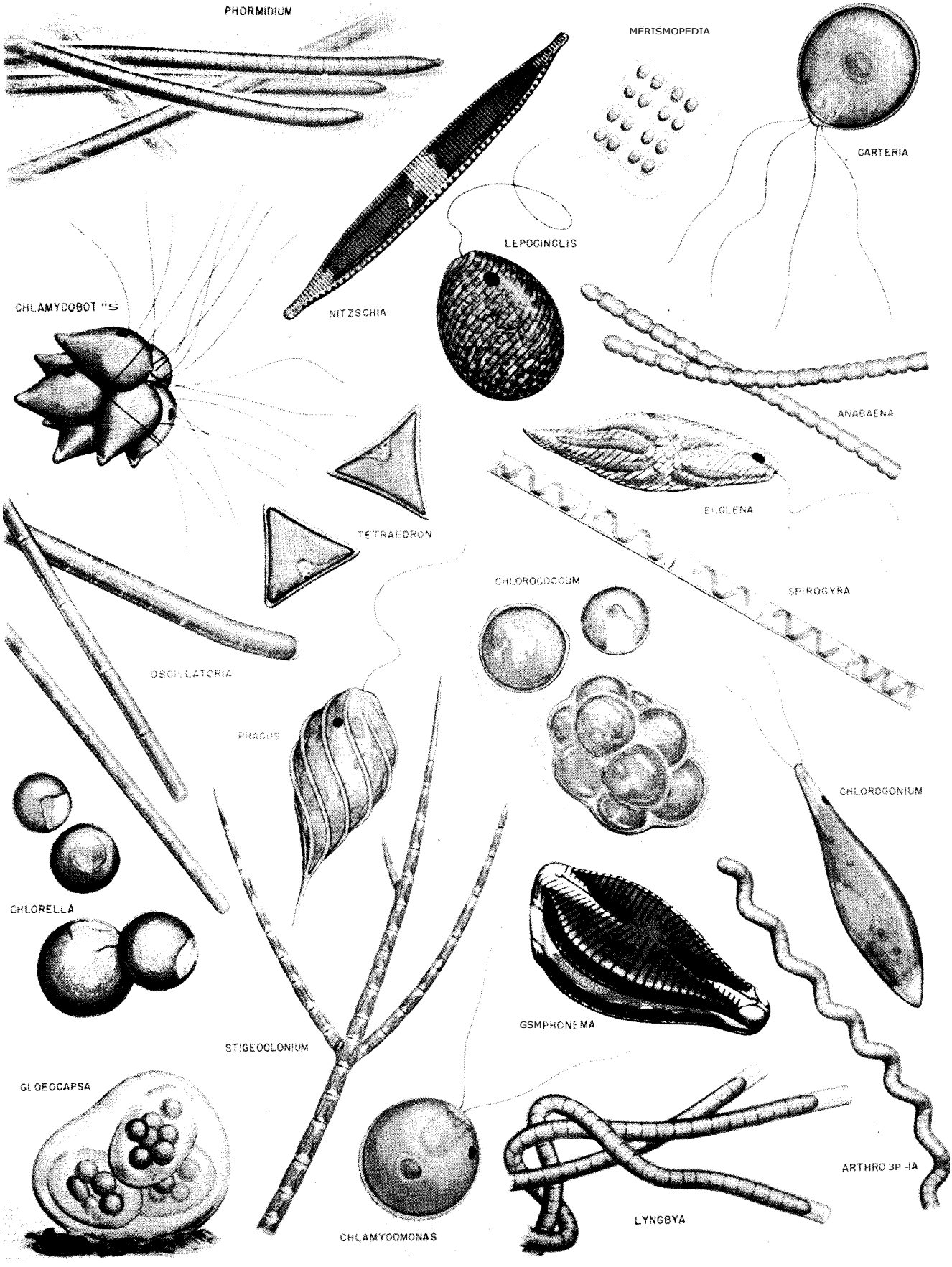
A[illegible]. T[illegible]

T[illegible] T[illegible] 2 [illegible]. T[illegible] C[illegible]., S[illegible]., [illegible] S[illegible]. F[illegible], [illegible]. R[illegible]. M[illegible]. E[illegible] C[illegible], S[illegible], [illegible] S[illegible] communis.

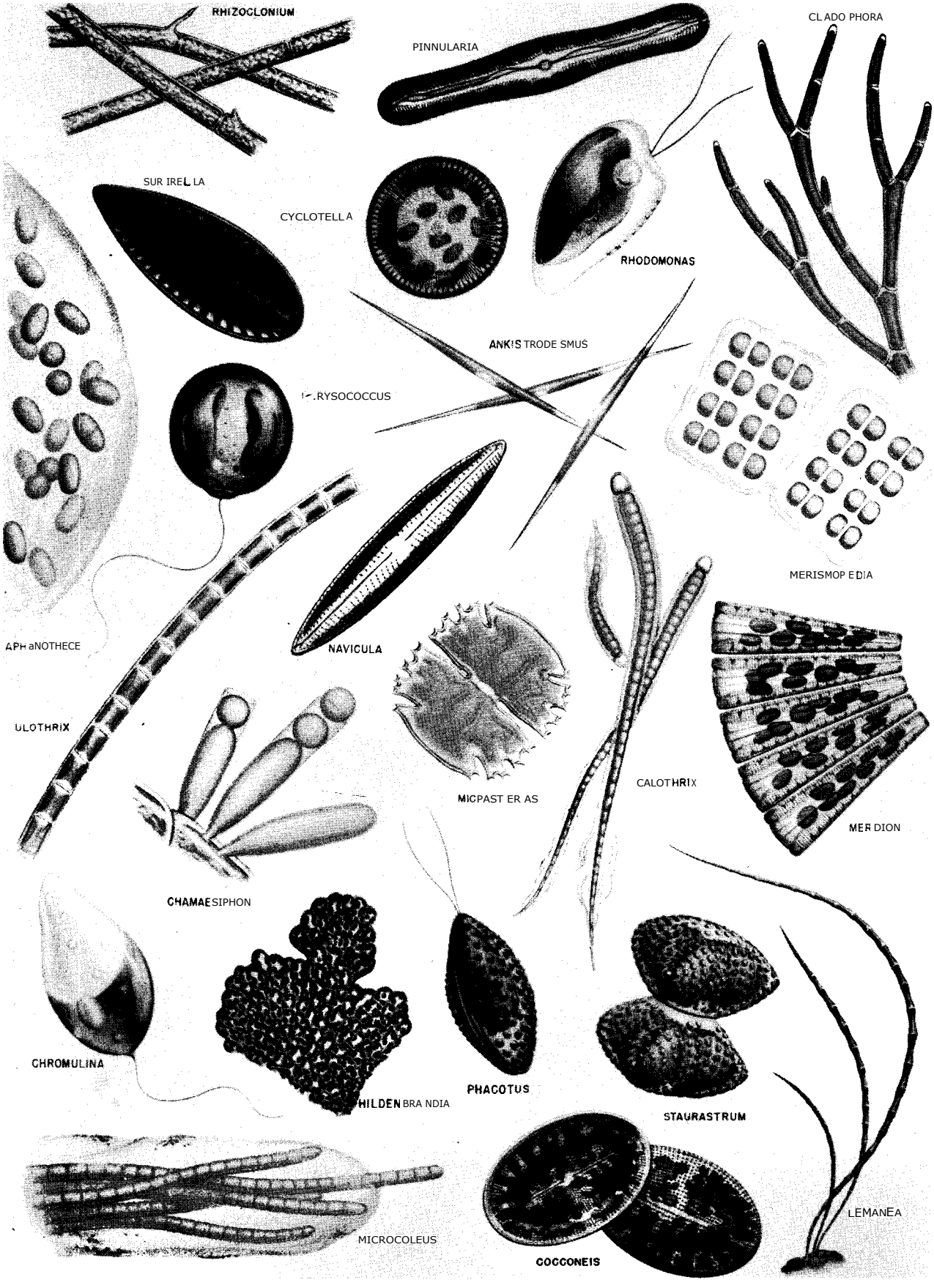
N[illegible] (10). T[illegible]. [illegible], [illegible] P[illegible] II.* T[illegible], listed [illegible]: A[illegible]. [illegible], A[illegible], C[illegible] parietina, Chamaesiphon [illegible], C[illegible], C[illegible] rufescens, C[illegible], C[illegible], C[illegible] [illegible],

*T[illegible], [illegible] M[illegible]. H[illegible] J. W[illegible] R[illegible] A. T[illegible] S[illegible] E[illegible] C[illegible].

POLLUTED WATER ALGAE



CLEAN WATER ALGAE



Hildenbrandia rivularis, Lemanea annulata, Meridion circular e, Merismopedia glauca, Micrasterias truncata, Microcoleus subtorulosus, Navicula Phacotus lenticularis, Pinnularia nobilis, Rhizoclonium hieroglyphicum, Rhodomonas lacustris, Staurastrum punctulatum, Surirella splendida, and Ulothrix aequalis.

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Table 1

Pollution Tolerant Genera of Algae

List of the Fifty Most Tolerant Genera

In order of decreasing emphasis by 56 authorities

	Genera	No. Authors	Total Points*
1.	Oscillatoria	34	57
2.	Euglena	31	53
3.	Navicula	19	32
4.	Chlorella	18	30
5.	Chlamydomonas	17	29
6.	Nitzschia	15	25
7.	Stigeoclonium	18	25
8.	Phormidium	15	22
9.	Scenedesmus	12	18
10.	Synedra	14	17
11.	Arthrospira	9	£6
12.	Spirogyra	10	15
13.	Phacus	9	14
14.	Gomphonema	9	14
15.	Melosira	9	12
16.	Pandorina	9	12
17.	Ulothrix	10	12
18.	Lepocinclis	7	11
19.	Lyngbya	7	11
20.	Chlamydobotrys	6	10
21.	Chlorogonium	6	10
22.	Tribonema	6	10
23	Anabaena	8	10
24.	Spondylomorum	8	10
25	Carteria	5	9

	Genera	No. Authors	Total Points*
26.	Ankistrodesmus	6	9
27.	Hantzschia	7	9
28.	Pediastrum	7	9
29.	Cladophora	8	9
30.	Anacystis	5	8
31.	Eudorina	6	8
32.	Spirulina	6	8
33.	Cyclotella	7	8
34.	Fragilaria	7	8
35.	Cryptomonas	8	8
36.	Cymbella	4	7
37..	Micractinium	4	7
38.	Closterium	5	7
39.	Stauroneis	7	7
40.	Chlorococcum	4	6
41.	Merismopedia	4	6
42.	Stephanodiscus	4	6
43.	Cocconeis	5	6
44.	Cosmarium	5	6
45.	Cryptoglena	5	6
46,	Gonium	5	6
47.	Oocystis	5	6
48.	Stichococcus	5	6
49.	Surirella	5	6
50.	Trachelomonas	5	6

*Tolerance by author "Very High" - 2 points
Tolerance by author "high" - 1 point

Table 2

Pollution Tolerant Species of Algae

List of the Fifty Most Tolerant Species
In order of decreasing emphasis by 56 authorities

	Species	No. Authors	Total Points*
1.	Euglena viridis	18	33
2.	Nitzschia palea	14	23
3.	Oscillatoria limosa	10	15
4.	Oscillatoria tenuis	11	15
5.	Arthrospira jenneri	8	14
6.	Stigeoclonium tenue	9	14
7.	Euglena gracilis	7	12
8.	Chlorella vulgaris	8	12
9.	Oscillatoria formosa	8	11
10.	Phacus pyrum	5	10
11.	Chlamydomonas sp.	(6)	(10)
12.	Euglena polymorpha	7	10
13.	Oscillatoria chlorina	7	10
14.	Oscillatoria putrida	7	10
15.	Spondylomorum quaternarium	7	10
16.	Oscillatoria chalybea	8	10
17.	Phormidium uncinatum	8	10
18.	Chlorella pyrenoidosa	5	9
19.	Gomphonema parvulum	5	9
20.	Oscillatoria lauterbornii	5	9
21.	Euglena oxyuris	6	9
22.	Lepocinclis texta	6	9
23.	Hantzschia amphioxys	7	9
24.	Euglena deses	6	8
25.	Oscillatoria princeps	6	8

	Species	No. Authors	Total Points*
26.	Pandorina morum	6	8
27.	Phorrnidium autumnale	6	8
28.	Anabaena constricta	5	7
29.	Chlorogonium euchlorum	5	7
30.	Melosira varians	5	7
31.	Cryptoglena pigra	6	7
32.	Chlamydobotrys gracilis	4	6
33.	Euglena pisciformis	4	6
34.	Lepocinclis ovum	4	6
35.	Merismopedia tenuissima	4	6
36.	Navicula crytocephala	4	6
37.	Nitzschia acicularis	4	6
38.	Scenedesmus sp.	(4)	(6)
39.	Synedra ulna	4	6
40.	Cyclotella meneghiniana	5	6
41.	Euglena intermedia	5	6
42.	Stichococcus bacillaris	5	6
43.	Oscillatoria splendida	6	6
44.	Phormidium foveolarum	6	6
45.	Pediastrum boryanum	3	5
46.	Spirogyra sp.	(3)	(5)
47.	Eudorina elegans	4	5
48.	Euglena fusca	4	5
49.	Surirella ovata	4	5
50.	Ulothrix zonata	4	5

*Tolerance by author "Very High" - 2 points
Tolerance by author "High" - 1 point

Dr. Ruth Patrick was unable to attend the seminar. However, in order to make our coverage of the subject of indicator organisms more complete she subsequently kindly consented to prepare a paper for inclusion in the Transactions. Her paper on diatoms as indicators of environmental conditions is herewith presented.

DIATOMS AS INDICATORS OF CHANGES IN ENVIRONMENTAL CONDITIONS

By

Ruth Patrick

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Academy of Natural Sciences of Philadelphia

Diatoms, which are one-celled algae belonging to the Chrysophyta, have long been of interest to students of the microscope. The earliest students of these plants were concerned with the structure of the siliceous cell walls and the general morphology. Diatoms were often used as test objects for lenses in order to determine the ability of a lens to define a given structure. One of the first to become interested in the geographical distribution of diatoms was Ehrenberg. In 1829 he published a paper on the geographical distribution of Infusoria (a general grouping which includes diatoms) in North Africa and West Asia. This paper was followed by several others on the distribution of Infusoria in various parts of the world. Other workers such as Greville, Kutzing, W. Smith, Grunow, Van Huerck and Cleve continued to explore this field of interest They recorded not only the geographical distribution of diatoms but also described the conditions in which diatoms were found.

Cleve (1894) pointed out the importance of a knowledge of the habitats and geographical distribution of diatoms to geological research. Considerable information has been obtained by studies of fossil diatoms as to the extent of the invasion of the sea and the effect of glaciation on the temperature of fresh and marine waters. Much of the knowledge of the paleogeography of Scandinavia has been elucidated in studies of fossil diatoms by Cleve (1899), Cleve-Euler (1940, 1944), Hustedt (1939) and Molder (1943)

In North America the extent of the effect of glaciation on fresh waters has been set forth by studies of diatoms (Hanna, 1933; Patrick, 1946). The succession of changes occurring in lake development has also been determined by diatom studies of Patrick (1936, 1943, 1946, 1954), Pennington (1947) and Ross (1950). It is because many species of diatoms have their best development in water with certain specific chemical characteristics that such correlations are possible.

One of the most important works summarizing what was known as to the ecology of diatoms was published by Kolbe (1927). In this work he clearly sets forth a system for classifying diatoms as to their tolerance to various chloride concentrations in water. Krasske (1927), Hustedt (1953), Legler and Krasske (1940) and Petersen (1943) have also contributed to our understanding of the chloride tolerance of diatoms.

The knowledge of the relationship of the occurrence of given species of diatoms to other chemical characteristics of water, such as pH, iron, nitrates, phosphates, and silicon, has developed greatly during the last 25 years. From time to time this kind of information has been brought together and published by such workers as Hustedt (1938 - 39), Schroeder (1939), Patrick (1948) and Fjerdingstad (1950).

Only very recently has the structure of the diatom population been correlated with the presence of sanitary and industrial wastes. Kolkwitz and Marsson (1908) *set* forth a system classifying many species of,diatoms as to their ability to withstand varying degrees of pollution from sanitary sources. Fjerdingstad (1950) published a very good summary of the more important literature on the effect of varying amounts of pollutants on the occurrence of diatoms. Budde (1930b) found out that the association of species and the relative sizes of populations of species produce the best picture of the effects of waste on the diatom flora. Although many of the studies concerned with the effect of wastes on the aquatic life of a stream have been based on plankton, Jurgensen (1935), Nowak (1940), Butcher (1933, 1940), Patrick (1948) and others have pointed out that it is the attached forms, or those organisms which grow and reproduce in a given area, that give the most reliable indication as to whether the environment of an area is suitable for the support of aquatic life.

Diatoms may float or be carried into habitats where they may survive for a period of several days without dying, although the quality of water is unsuitable for growth and reproduction. This is one of the reasons why diatoms, which in natural or unpolluted rivers indicate so well changes in the environment, may under conditions set forth above persist and thus not appear to indicate the character of the water in which they are found.

Another error which has occurred in the study of diatoms has been pointed out by Fjerdingstad (1950). Workers often fail to examine their collections to see if the diatoms found are living and in good condition. Since diatoms have cell walls of silicon, which persist after they are dead they may be included as living in a given area unless the above precaution is taken. In our studies of diatoms taken from various rivers in the United States, large numbers of dead frustules of species not living in an area are often found intermixed in the living collections.

Another cause of error in the handling of collections is contamination. Dirty pipettes, beakers and collecting jars may well result in the transfer of species of diatoms from one collection to another and thus produce a false picture of the structure of the diatom flora being studied.

The methods used in collecting diatoms and of studying the slides must be the same if one wishes to compare the diatom flora of various areas. Because these methods are not standardized among diatomists it is often very difficult to compare results. Such considerations are particularly important if one is comparing the number of species and the sizes of the populations.

Another consideration which is often overlooked in discussing indicators of polluted or of deleterious conditions is the number of variable factors and the combination of such factors which may produce the deleterious effects. As pointed out by Fjerdingstad (1950) the classification assigned by Kolkwitz and Marsson (1908) to certain diatom species often does not hold. This undoubtedly is due to several factors but certainly one of the most important is the fact that Kolkwitz and Mars son's system is based on the reaction of organisms to sanitary wastes or wastes with a heavy organic load.

In this system the greatest degree of pollution is characterized by low oxygen content, high bacterial counts, high biochemical oxygen demand and a heavy organic load. However, when one is considering toxic wastes, a stream may have a high dissolved oxygen content, low biochemical oxygen demand and low bacterial counts and yet be inimical to aquatic life because of the presence of toxic substances. Likewise high temperatures may be lethal and only be associated with a lowering of the dissolved oxygen. Whereas organic wastes, toxic wastes and high temperatures in excessive amounts will kill most organisms in sublethal or threshold concentrations they will affect various species in different ways. In most rivers which we have studied in the United States, pollution is rarely of a single type, but rather is a combination of toxic substances and organic wastes often accompanied by high temperatures.

Fjerdingstad (1950) and others have emphasized that various aspects of the diatom flora must be considered, such as the changes in numbers of species, numbers of individuals and kinds of species. As a result of over 100 analyses of diatom floras from natural areas of rivers in eastern and southern United States, our laboratoryhas found that the numbers of species making up the diatom flora are quite similar. By natural areas are meant areas which so far as we could ascertain from state and federal agencies as well as from information concerning chemical and bacteriological analyses were not adversely effected by effluents entering the river. Indiscriminately selected results from such studies showed the following numbers of species to be present: Marsh Creek, Pa. - 46; Tionesta Creek, Pa. - 42; North Fork of the Holston, Va. - 52, Savannah River, South Carolina and Georgia - 80, 69; Flint River, Ga. - 40; Escambia River, Fla. - 55, 54; Sabine River, Texas - 46, 55; Guadalupe River, Texas - 42; Neches River, Texas - 65.

Part of the variation shown here in the numbers of species present in the various studies is due to the fact that the studies on Marsh Creek, Tionesta Creek, Flint River, Sabine River and Guadalupe River were made in a somewhat different manner than those on the other creeks and rivers.

When one considers the kinds of species a very different picture is presented. In a study of natural river areas soon to be published, 354 species from ten rivers were considered. Of this number only one was found in all ten rivers. Two hundred and two were found in only one river; 74 in two; 30 in three; 20 in four; 9 in five; 5 in six; 9 in seven; 3 in eight and 1 in nine. This clearly shows the difficulty of using single species as indicators. On the other hand, if one considers a group of species as indicating a condition a reliable methodology can be developed. For instance, most of the species of Eunotia and all of the North American species of Actinella are found in soft, usually somewhat acid waters. The presence of an association of any of the species of these genera will indicate these conditions. Likewise, the presence of well developed populations of Synedra affinis or S. pulchella or Navicula pygmea will indicate the presence of brackish water. As a general rule, species which form the largest populations should be selected as indicators. In other words diatoms most truly indicate those physiological conditions which enable them to multiply most rapidly over a period of time.

An analysis of the histograms given by Patrick (1949) shows that the diatom flora responds to the severe effects of deleterious effluents in a manner similar to that of fish and insects. Approximately 200 analyses of sections of rivers and estuaries which have been made by the Limnology Department of the Academy of Natural Sciences of Philadelphia subsequently support these conclusions.

Diatoms are in some cases more sensitive to small changes in the chemistry of the water than are some of the larger forms, as was pointed out by Liebmann according to Fjerdingstad (1950). However, the response of diatoms to lethal concentrations are usually similar to those of fish.

Analyses of results of 50 bioassay tests run on wastes of chemical, steel, gas and electric industries show that the concentrations which produced 50 per cent reduction in growth of diatoms and 50 per cent kill in fish were very similar (Table 1), This work was carried out by Dr. John Cairns, Jr. and Dr. Arthur Scheier. In 75 per cent of the cases one concentration was never more than twice that of the other, and in most cases there was very little difference. Since the concentration recommended as biologically safe is less than a third of the above concentration, it is evident that results from either test will bring about very similar recommendations for safe discharge of wastes. In only eight per cent of the cases was the diatom found to be less sensitive than the fish, These results clearly show the

Table 1

Comparison of effects of industrial wastes on fish and diatoms (Figures in column headed "Diatom" represent concentration, in per cent, of effluent causing 50 per cent reduction in division rate in five days; those under "Fish" show concentrations causing death to 50 per cent of the fish in 24 hours and in 48 hours.)

Effluent	Diatom	Fish 24 hours	Fish 48 hours
1	5.3	10	9
2	2.78	2.8	2.8
3	3.65	3.2	2.7
4	0.001	0.18	0.155
5	1 16	3.25	2.87
6	2.3	7.6	6.0
7	no reduction	70.0	60.0
8	49.3	no deaths	90.0
9	2.34	10.0	9.1
10	0.78	0.57	0.52
11	2.15	3,2	3.2
12	18.7	26.5	21.7
13	4.4	75.0	75.0
14	32,8	62.0	52.0
15	65.0	no deaths	no deaths
16	35.0	no deaths	no deaths
17	44.5	1.6	1.6
18	no reduction	12.7	9.0
19	0.74	no deaths	no deaths
20	no reduction	no deaths	no deaths
21	no reduction	no deaths	no deaths
22	56 0	no deaths	no deaths
23	18.0	no deaths	no deaths
24	no reduction	no deaths	no deaths
25	no reduction	no deaths	no deaths
26	no reduction	no deaths	no deaths
27	98.0	no deaths	no deaths
28	no reduction	no deaths	no deaths
29	no reduction	no deaths	
30	no reduction	no deaths	

"No death" or "No reduction" means that 100% concentration of the effluent was not deleterious.

Table 1 (Continued)

Effluent	Diatom	Fish 24 hours	Fish 48 hours
31	no reduction	no deaths	
32	no reduction	no deaths	
33	no reduction	no deaths	
34	56.0	no deaths	
35	18.0	no deaths	
36	no reduction	no deaths	
37	no reduction	no deaths	
38	no reduction	no deaths	
39	98.0	no deaths	
40	no reduction	no deaths	
41	no reduction	no deaths	
42	60.0	no deaths	
43	no reduction	no deaths	
44	6.0	6.9	
45	10.0	20.5	
46	0.77	0.44	
47	16.2	42.0	
48	no reduction	no deaths	
49	15.0	29.5	
50	no reduction	no deaths	

value of diatoms as indicators of conditions essential for or inimical to fish life.

Results of work done by the Limnology Department under grants from the U.S. Public Health Service indicate that when pure chemicals are tested singly, responses of fish and diatoms are more varied. More study is needed before an explanation can be given for the more variable responsesto pure chemicals than to industrial effluents. The differences were not due to procedure since the same methods were used in both types of tests.

From the many studies that have been made it is apparent that diatoms can be used as a group to indicate the ability of a water to support aquatic life. Collections must be prepared correctly and the studies based only on specimens living at the time collections are made. Furthermore, collections should be taken from populations which are attached or definitely living in the habitat and not from plankton forms.

Diatoms are a desirable group to use for indicating stream conditions for several reasons:

1. They need no special treatment for preservation because the cell wall, on which the identification is based, is composed of silica.

2. The diatom flora of a normal stream is made up of a great many species and a great many specimens. Thus the group lends itself to statistical treatment.

3. Diatoms vary greatly as to their sensitivity to chemical and physical conditions of water. Some species are able to tolerate a wide variety of environments. Therefore, some diatoms can be found in any aquatic habitat inhabitated by plants and animals (excluding bacteria). However, each of these species has a range of conditions in which it achieves best development. Other species of diatoms have a very narrow range of tolerance. Thus we have *in* the diatoms enough different kinds of species so that they can be used as an indication group in most of the possible types of aquatic environments found in rivers and estuaries.

4. A considerable amount of information is already available as to the type of environment in which many species are found.

In order to use any group of organisms as indicators one must have a method of collecting and studying them which will be comparable for different types of water. A suitable methodology for diatoms has been set forth by Patrick, Hohn and Wallace (1954) by the use of the Catherwood Diatometer.

The Diatometer 'Fig 1) is an instrument which floats in the water and provides a substrate, ordinary glass slides, which seems to be non-selective for diatom growth These slides are left in the water for a long enough period of time to allow a considerable amount of growth to develop on the slide. In many cases this length of time has been about two weeks. From the studies we have made, it appears that dead diatoms do not remain attached, but slough off the slides. Studies based on these slides thus include only live diatoms which are actually living in a given area

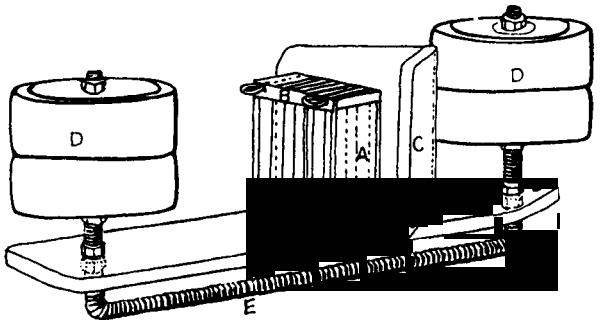
When the slides are prepared for study the diatoms are scraped off and cleaned by the acid method A small aliquot which is representative of the material is then placed on a slide and mounted in Hyrax.

In these studies all species observed are identified and the number of indivduals of each is recorded. Enough specimens have to be identified in order to construct a truncated normal curve according to the method set forth by Preston (1948) If results are to be comparable the modes of the curves should be in the same interval. Therefore, varying numbers of specimens may be counted. This study usually takes 3 to 4 days to complete.

In such rivers as the Savannah where little change occurs, the structure of the curve remains very similar from season to season and from year to year. However, any serious change in the quality of water is readily seen by a change in the structure of the curve (Figs. 2, 3, 4). In interpreting these curves one must take into consideration the height and position of the mode, the dispersion factor, the number of species found, the total theoretical population, the kinds of species and the number of specimens used in constructing the curve.

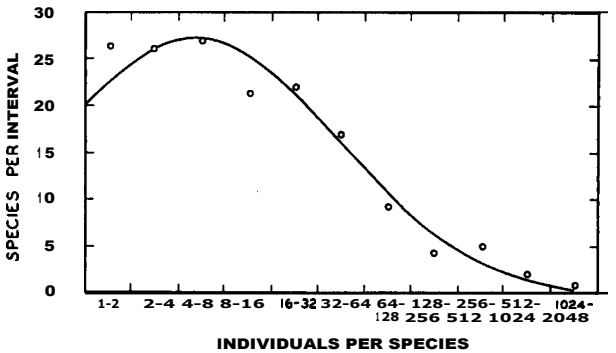
It can be seen that this method avoids many of the pitfalls which have been encountered by trying to use diatoms as indicators of pollution. It provides a uniform method for collecting and studying diatoms. Thus the results are comparable. This method is based on the study of the live species in a given area which are growing and dividing. It is mainly concerned with the number of species and number of individuals of each of the species. It also considers the kinds of species. Thus its basis is a shift in pattern of the whole flora rather than the behavior of a few indicator species

Of course, it must be remembered that any conclusion based on only one group of organisms or kind of analyses should only be considered as giving an indication of conditions. This applies not only to biological analyses but physical and chemical analyses as well. If one wants a complete picture of river or estuary conditions then all groups of organisms as well as chemical and physical analyses must be included.



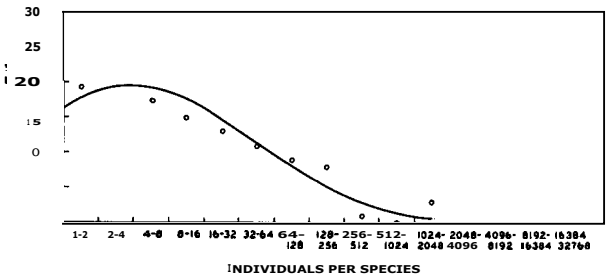
The Diatometer.

Fig. I



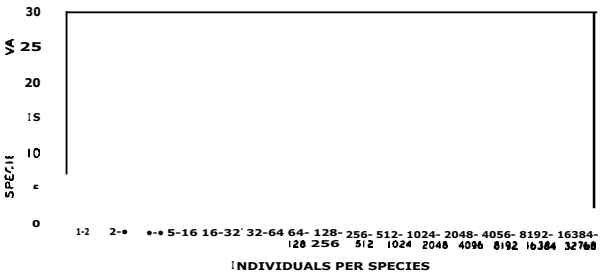
Graph of the Diatom Population from a River Not Adversely Affected by Pollution.

Fig. 2



Graph of a Diatom Population from a River Showing Mild Effects of Pollution.

Fig. 3



Graph of a Diatom Population from a River Showing Severe Effects of Pollution.

Fig. 4

T[illegible]

A[illegible]

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USE AND VALUE OF FUNGI AS BIOLOGICAL INDICATORS OF POLLUTION

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A person who has spent more time studying North American forest ttan the streams which flow through them thinks of indicators in terms of trees. The presence of subalpine fir in Paradise Valley on Mount Rainier iridicates that one is in the spurce-fir zone near the upper limit of trees on that type of mountain in that geographical location; the presence of Joshua trees in southeastern California indicates that one is in one phase of the Mojave Desert; the presence of sycamores and American elms in a shallow valley indicates that one is near a stream in the vast eastern deciduous or mixed mesophytic forest.

But these trees all indicate that one is on relatively undisturbed land. Our immediate concern is based on whether or not fungi can be used as indicators of pollution. It has been said (Suter and Moore, 1922) that the Saprolegniaceae can be used as indicators of pollution. It has been said (Suter and Moore, 1922; Butcher, 1932; Cooke, 1954) that Leptomitus lacteus is an indicator of pollution. The filamentous bacterium, Sphaerotilus in its several forms, and the stalked protozoa including Carchesium have been referred to as fungi which indicate pollution (Butcher, 1932; Cooke, 1954). That little interest has been shown in analysing the components of "sewage fungus" may be seen in Wilson's paper on the microbiota of sewage published in 1944 Here, "fungi indet." is simply one category among the many types of organisms listed according to genus. The number and types of fungi found in polluted water and sewage to date have only indicated that certain strains of a number of common soil fungi have become adapted to or are able to tolerate this different habitat.

Materials from several types of fungus studies will be drawn on in an attempt to determine something of the relationship between fungi and their habitats with special emphasis on pollution.

From the beginning of life, various members of the populations of the orld have been leaving their bodies or the remains of their metabolic processes on the land or in the water to continue the process of decay so that the organic materials of which they were composed may be used and reused. One type of intermediate organism in the waters of the world is the group commonly called "sewage fungus". These organisms developed a specialized type of metabolism in which certain forms of carbon and nitrogen compounds can be utilized but not others. As the numbers of man increased, and as his physical requirements increased, the waters of the world have increasingly carried the waste products resulting from this development, and organisms

adapted to the special nutrients found therein have thrived and multiplied. In this way, we may suppose, such organisms as Sphaerotilus and Leptomitus have developed, multiplied, and filled their special ecological niches. Leptomitus has developed to the point that it uses fatty acids rather than sugars, and amino acids rather than ammonia (Cantino, 1955).

There are at least three sources of pollution by which food materials usable by fungi are placed in streams. The first may be considered natural pollution. Pieces of decaying vegetable or animal materials may inadvertently reach streams through run-off, passive falling into the water, or other means. We are not concerned with this type of pollution. Many of the true water molds or aquatic fungi - Saprolegniales and related fungi - are found only under these conditions. They require something to attach themselves to during their vegetative phases. Such fragments of materials may serve as food as well as anchoring places for these water molds. Other water molds, belonging to the Chytridiales, do not always live freely in the water but may be parasitic upon various species of algae and in various kinds of pollen which float on the water usually in the spring. When their sources of nutrition become exhausted, these fungi produce resting spores and cells ("gemmae"

The second source of pollution is fecal material and other materials such as ground garbage added to the stream in domestic sewage. Such materials are high in organic content and may furnish large amounts of foods to fungi that have become adapted in one way or another to life in the water or in the stream bed. Under restricted conditions, with certain carbohydrate, nitrogenous, and as yet undetermined additives, and under certain conditions of substrate and bottom materials resulting in special pH and mineral content conditions, certain Saprolegniaceae, Leptomitus lacteus, Sphaerotilus natans, and various soil fungi, such as Fusarium aquaeductuum, Geotrichum candidum, Penicillium lilacium, and others, may thrive. Under these conditions, such organisms appearing in large numbers will indicate the presence of polluting substances, substances which make the waters of the stream unfit for human and most industrial uses.

The third source of pollution is various types of wastes from industry. Different types of industrial pollutants can produce varying conditions of growth in the vicinity of the point of discharge of the pollutant. Such pollutants will become diluted downstream. It is possible that, at the immediate outfall, oxygen supplies may be too poor and certain substances may be too toxic to support growth, but as oxygen increases and the toxic substances become diluted by increased flow, precipitation, or other factors, the toxic effect will be weakened and growth of various fungi and fungus-like organisms may be encouraged or enhanced. When the pollutant is organic, Leptomitus lacteus and the filamentous bacterium Sphaerotilus natans may thrive under such conditions; and at least Sphaerotilus will present several growth habits, mistaken as species, genera, or varieties in the literature, as the concentrated waste becomes more dilute. At Lytle Creek (Cooke, 1954c), a number of species of fungi have been found in isolations from water, sediment from polls and riffles, and apparently septic bank soil, at the outfall of primary settled sewage.

Both Leptomitus and Sphaerotilus have been cultured in the laboratory with little difficulty after the proper combination of nutrients has been determined. Such combinations indicate only that these organisms have special requirements for sugars and compounds containing nitrogen. In nature such substances are present largely under conditions of pollution by organic wastes from the home or factory; in the wilderness, such substances may be present following death and during decay of plants or animals. It is quite possible that these organisms may be common around watering holes and other places where animals have congregated, where their remains or droppings may have produced local natural pollution.

Another type of fungus may be thought to be more nearly indicative of pollution, especially of the domestic type. In the forest we find these coprophilous fungi associated with dung of wild animals, rarely of man. In many cases these fungi are restricted to specific types of dung. Mucor ramanianus is found on frog droppings; Pilobolus species are usually found on horse droppings Other types of these fungi are found on the dung of any animal that uses plants as food. The spores of these fungi are so produced and discharged that they become attached to or glued to leaves of plants Lsed as food by grazing or browsing animals. After the spore has passed through the alimentary canal of an animal, it is able to germinate, use the dung as food., and fruit quickly after deposit of the dung. Ephemeral mushrooms like the inky cap and the common mushroom are edible, but since man cooks the mushrooms he eats, their spores are killed before ingestion Few if any coprophilous fungi are found associated with human feces, especially where this material accumulates as night soil or is carried away in sewers to rivers or sewage treatment plants. While Sordaria humana occurs on other types of dung, the specific name indicates that man also can ingest such fungus spores and that under certain conditions these fungi can be recovered from human wastes.

Three types of fungi which may be placed in this physiological class have been found in sewage polluted water and on trickling filters. A species of Pilobolus (Harvey, 1952) was found at one station on Lytle Creek by use of hemp seed as an isolating medium. It was found again on the Glendale, Ohio, trickling filter. Intensive biochemical nutrition research (Hesseltine, et al , 1953) has shown that this fungus can be propagated in the laboratory without ingestion and alimentation by an animal.but the technique has not been tried at Cincinnati. Ascodesmis microscopica was found only twice (Seaver, 1928) in the world prior to 1955. One of these growths was in Europe on tiger dung, the other at the Bronx Park Zoo on the dung of a raccoon dog. In 1955 the species was found on a trickling filter in the Dayton, Ohio, sewage treatment plant. It requires no special techniques or media but will grow on any medium used in culturing the so-called sugar fungi.

In 1953, Hesseltine, studying the fungi of trickling filters at Pearl River, New York, found a fungus (Subbaromyces splendens) which in pure culture grows only on lima bean agar. This has since been found on

trickling filters at Dayton, Ohio, and Pullman, Washington (Becker and Shaw, 1955). It is possible that this fungus may grow in other habitats with similar environments, such as on stones of streams polluted with domestic wastes and on other trickling filter beds.

An organism can become adapted to one set of environmental conditions or another. In some cases the adaptation•may be complete, so that the organism cannot tolerate any other conditions, but more often various degrees of adaptation may be attained by an organism. Moser (1949), studying the adaptation of certain fungi, mosses, and seed plants to areas in the tyrolean forests which had been burned by fires ranging from large forest fires to small camp fires, developed the following set of terms to describe the degree of adaptation of the organisms to burned areas: anthrocobiont species were found only on burned soils and are not known to occur elsewhere; anthracophilous species are found more commonly on burned areas; anthracoxenous species occur more commonly on unburned areas but will tolerate burned areas; while anthracophobous species will not grow on burned areas. This set of suffixes used other habitat requirements of the same or different species. Since "copro-" has been used commonly in the combination "coprophilous" for a fungus growing on dung, it might be confusing to use in the terms we want. The Greek work "lyma" means "filth". Then, lymabiont species will grow only on or in the presence of fecal materials; lymaphilous species will grow commonly on such material but will also grow on other materials, lymaxenous species will grow commonly on other materials but will tolerate fecal matter; while lymaphobous species will not grow on or in the presence of fecal material. In this sense, a coprophilous species may be either a lymabiont or a lymaphile. A lymaxene could be an organism which on occasion will colonize the substrate material, while a lymaphobe will not grow on such materials. The emphasis here is on nutrient requirements or tolerances, rather than on habitat types or tolerances as is the emphasis in the Kolkwitz (1950) system.

To equate this set of terms with those of Kolkwitz, the following parallels are suggested. Polysaprobes would include both lymabionts and lymaphiles. For use in connection with fungi, polysaprobe would be confusing since it implies the ability to live saprobically on any substrate. Alpha (or strong) mesosaprobes would include lymaphiles primarily, but lymabionts and lymaxenes could be expected to be found. Beta (or weak) mesosaprobes would include lymaphiles, but lymaxenes c=be found more frequently than in the preceding categories. Oligosaprobes would include the lymaphobes and probably some lymaxenes and lymaphiles

Indicators of fecal pollution will thus be found in the first two categories, lymabionts and lymaphiles, rather than restricted to the first category. Coprophilous species connote only those species of fungi or other organisms growing on dung at the time of observation, so the term is not useful for our purposes. Of the lymaphilous species, one must be careful that the habitat is described in more than one way. For instance, the fungus Fusarium aquaeductuum was first described from wooden water pipes which under certain conditions could be plugged with its massive growth. At present, wooden pipes are used infrequently, pure water systems have been rarely

checked for the fungus, and it is abundant on trickling filters throughout the country as well as in polluted streams.

In intensively cultivated fields there is strong competition for nutrient materials among the various species, varieties, strains, and clones of fungi present. Such competition has given rise to species that have become adapted to the utilization of special nutrients (Garrett, 1956). A strictly saprobic species will attack almost any dead organic material. Certain species are able to attack youngseedlings, produce seedling death, and then use the dead tissues as nutrient sources for primary colonization of new substrata. Other species can penetrate the roots of older plants, grow through their vascular tissues, and produce the type of disease referred to as vascular wilts. Such fungi in the soil may be considered ecological obligate parasites, for although they can be readily cultured on any agar medium on which sugar fungi develop, in nature they cannot compete with those fungi that colonize dead tissue, but must create their own dead tissue by killing the host As the host plant dies, the fungus can utilize its tissues as food until forced into retirement by competition from other fungi or depletion of nutrient materials.

These vascular wilt fungi belong to the genera Fusarium and Verticillium, among others. Three species of the wilt-producing fungi, F. oxysporum, F. roseum, and F. solani, have been isolated occasionally from sewage and polluted water. The source of these fungi in the samples that have been studied is unknown, but the species are widely distributed agricultural pests. Nothing is known of their activities away from the soils in which they are of greatest importance as plant disease producing fungi, except that they are capable of causing deterioration of cellulose. In preliminary experiments in dilution water it was apparent that a strain of F. oxysporum is capable of using hydrocarbons as its sole source of carbon. That these organisms are isolated occasionally or even commonly from sewage and polluted water indicates that they are capable of competing with other fungi and other organisms for whatever nutrients are offered. In contrast to the soil, where only occasionally does dead organic matter become available to the fungi of a specific unit of soil, or where only occasionally does a plant root become available for colonization even in a field where the crop is relatively densely planted, polluted water presents to the fungus a continuous source of nutrient supply. Such a supply makes it unnecessary for the fungus to assume a resting phase upon the depletion of one source of nutrient while waiting for a new source.

Nutrition requirements of organisms found in industrial types of pollution are more difficult to define. Albritton (1955) is very general on the point of fungus nutrient requirements. Fecal material is at a minimum in such wastes, while sugars and possibly organic nitrogen sources are of greater importance. Here a somewhat different type of organism will be ascendant, although many of the same species will occur commonly in both types of pollution . Again, where a waste contains metallic ions usually inhibitory to the growth of organisms, certain species may become adapted to life in the presence of those substances. For instance, at least one species of Penicillium, P. ochrochloron,can tolerate high concentrations of copper, another, manganese. Some fungus contaminants can be found, commonly in

acids thought to be toxic to any growth. In general, such fungi fall into the broad classification recently proposed: "sugar fungi". These fungi are able to utilize simple sugars but in many cases are not able to develop an enzyme system for the degradation of more complex carbohydrates, or other carbon sources.

A further difficulty with the definition of a pollution indicator among the fungi is the inability of the investigator to identify most of the fungus growth in any one sample on sight or on preliminary microscopic examination. Most soil fungi growing in an aqueous habitat do not produce spores but form an extensive mycelial mat. The mat may be formed by the interweaving of a number of mycelia of many species. The orange color produced by Fusarium aquaeductuum is a result of large numbers of spores piled together or produced concurrently. The white color produced by Geotrichum candidum on surface films of trickling filters is quickly masked by algae or other fungi, or the filamentous bacterium Sphaerotilus natans which produces a grey color; or, it may be confused with the occasionally produced white mat of Leptomitus lacteus when that is able to appear. In some cases, species of Penicillium may be observed in fruiting condition, but it is rare that a typical fruiting structure will be produced; the phialides producing the spores may be found singly on the hypha or in atypical clusters, rather than clustered in the typical penicillate brush at the tip of the spore-producing hypha. It is virtually impossible to study the several genera of the white yeasts, the red yeast, and the black yeast by direct observation of scrapings from trickling filter stone or stones in creeks or rivers subjected to continual pollution loads Such studies must be made by using cultural techniques and studying the colonies that develop directly on properly prepared dilution plates or later in pure culture.

Techniques used most successfully so far in the isolation of fungi from polluted water and sewage have been described by Cooke (1954b). For isolation of aquatic fungi the technique described by Harvey (1952) is adequate. For help in studying the fungi that have been isolated, several books are available. The various media used in the study and identification of yeasts are described by Lodder and Kreger-van Rij (1952). The genera Aspergillus and Penicillium are best studied by techniques defined respectively by Thom and Raper (1945) and by Raper and Thom (1949). When species obtained by baiting or using hemp seed according to Harvey's method are obtained, they can be studied with the help of Coker (1923) or Coker and Matthews (1937). If species of Pythium appear following use of this technique, Middleton (1943) is useful. Parasites of plankton, pollen grains, and similar substrata can be studied with the help of Sparrow (1943). Of other fungi, that have not been studied consistently on one medium or do not belong in the categories mentioned above, some groups cannot be satisfactorily studied at present. These include primitive Ascomycetes, mycelial Basidiomycetes ;sterile mycelia, members of the Sphaeropsidales, and such genera as Chaetomium. For none of these groups are there adequate taxonomic treatments, and cultures must be sent to specialists. Of the Sphaeropsidales, most known species are studied with reference to a specific host plant. Cultures from water, sewage, or soil, therefore, cannot be identified satisfactorily without

screening the potential host plant population of the region. To aid in the identification of a genus of fungus. the worker can consult Clements and Shear (1932), whose keys are lengthy and cumbersome, or he can study Barnett (1955), who has illustrated nearly one-third of the genera including those that are more common For more specific information, Gilman's (1945) compilation is useful, and Smith (1954) gives considerable help with the commer species found in industry and the laboratory. Finally, Hughes ('1953) points toward newer concepts in developing a workable system of classification of the so-called mold fungi

A technique for obtaining samples of growth from trickling filters, modified from similar techniques whose origin dates back to 1905, has been developed in which, rather than scraping ths stone, glass slides are placed in mounts in contact with the growth on the stone and continually irrigated by the settled sewage spray applied to the filter beds. Growth on the slides can be removed by scraping with a rubber policeman, the algae counted, the protozoans studied, and the fungi and bacteria plated. If the material to be studied for development of fungi is broken up in a Waring Blendor, an approximation of the importance of each fungal species can be obtained by counting the number of colonies of each species or of all species Using this technique, it appears that 10 to 15 species of soil fungi form the largest part of the fungal portion of the slimes on trickling filters.

Annotated Bibliography

Albritton, E C 1955. Standard Values in Nutrition and Metabolism. xiii, 380 Philadelphia, W. B. Saunders Co.

A tabular presentation of the nutrient requirements of the principal groups of organisms, of certain specific groups and of certain species. Tables also present food and feed values of certain species of organisms, and metabolic products of various organisms used in industry.

Barnett. H. L., 1955. Illustrated Genera of Imperfect Fungi. i, 218. Minneapolis, Burgess Publishing Co.

The commoner genera of mold fungi and plant pathogens are illustrated with line drawings Habitat descriptions and references are given for each species illustrated.

Becker. J. G. and C. G. Shaw, 1955. Fungi in domestic sewage treatment plants Applied Microbiology 3 173-180

A study of fungi found in the sewage treitment plants at Pullman, Washington, and Moscow Idaho, based on studies of effluents from various process points in the two plants which treat domestic sewage.

Butcher, R W., 1932 Contribution to our knowledge of sewage fungus. Trans Brit Myc Soc. 17: 112-124

A systematic study of the various organisms which are associated with polluted water and sewage treatment plants and which have been termed "sewage fungus"

Cantino, E. C., 1955. Physiology and phylogeny in the water molds - A reevaluation. Quart. Rev. Biol. 30: 138-149.

A study based on the writer's personal experience with some of the groups of organisms listed as well as on a thorough knowledge of the literature, in which an attempt is made to base a phylogeny of the water molds on what is known of their physiology.

Clements, F. E. and C. L. Shear, 1931. The Genera of Fungi. iv, 496. New York, The H. W. Wilson Co.

A compendium designed to give a key to all genera of fungi known up to 1930 and whose species are listed in Saccardo's Sylloge Fungorum. This manual is unwieldy, has some errors based on increasing knowledge of some of the greater groups, and has many incomplete or uncritical key characters.

Coker, W. C., 1923. The Saprolegniaceae. 201. Chapel Hill, The University of North Carolina Press.

A well illustrated monographic study of the canmonest or easiest isolated family of water molds, the Saprolegniaceae.

Cooker, W. C. and V. D. Matthews, 1937. Saprolegniales. North American Flora 2: 15-67.

The definitive rrionograph of the Saprolegniales as known at present. In this work certain concepts presented in the preceding reference are clarified, but it is not illustrated and the two should be used together.

Cooke, W. B., 1954a. Fungi in polluted water and sewage. I. Literature Review. Sewage and Industrial Wastes 26: 539-549.

A review of the papers available at the time on fungi in polluted water and sewage.

Cooke, W. B., 1954b. Fungi in polluted water and sewage. II. Isolation technique. Sewage and Industrial Wastes 26: 661-674.

A description of isolation techniques and a discuss n of the various media used in the isolation of fungi from polluted water and sewage.

Cooke, W. B., 1954c. Fungi in polluted water and sewage. III. Fungi in a small polluted stream. Sewage and Industrial Wastes 26:

A quantitative study of the fungi found at eight stations on Lytle Creek.

Garrett, S. D., 1956. Biology of Root-Infecting Fungi. xi, 293. New York, Cambridge University Press.

A number of older concepts are clarified and some new concepts given concerning the biology of root-infecting fungi and their occurrence in the soil.

Gilman, J. C., 1945. A Manual of Soil Fungi. xi, 392. Ames, Iowa State State College Press.

A compilation listing and describing all fungi known to occur in the soil. Since the book is based on available descriptions and not on laboratory experience of the writer, it indicates areas where additional work is required. For instance, in certain larger genera one species may be described as it appears on one medium, another from a different medium.

Harvey, J. V., 1952. Relationship **of aquatic fungi to water pollution. Sewage and Industrial Wastes 24: 1159-1164.**

A study based on the isolation of water molds from polluted streams in the Little Miami Basin over the period of a year. It appears that few, if any, aquatic fungi are definitely associated with polluted water.

Hesseltine, C. W., 1953. Study of trickling filter fungi. Bull. **Torr. Bot. Cl. 80: 507-514.**

Geotrichum **candidum, Fusarium aquaeductuum and Subbaromyces splendens are considered to be the fungi of most importance on trickling filters treating pharmaceutical and chemical wastes at Lederle Laboratories, Pearl River, New York. Other fungi appearing in isolations were considered contaminants or only incidentally present.**

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Hughes, S. J., 1953. Conidiophores, conidia and classification. Canadian Journ. Bot. 31: 577-659.

A description of a new system of classification for the Fungi Imperfecti based on methods of spore production.

Kolkwitz, R., 1950. Oekologie der Saprobien. Uber die Beziehungen der Wasserorganismen zur Umwelt. **Schrift.** ver. **Wasser.-, Boden-, und Lufthygiene. 4:1-64.**

An annotated list of species of organisms **found in the several zones of a stream identified in the Kolkwitz and Marsson system as: polysaprobic, alpha-mesosaprobic, beta-masosaprobic and oligosaprobic.**

Lodder, J. and N. J. W. Kreger-van Rij, 1952. The Yeasts. A Taxonomic Study. xi, 713. New York, Interscience Publishers, Inc.

The definitive manual of yeasts. Classification into genera, families and orders is based on morphology, into species and varieties is based on reactions in culture to a number of standardized morphological and chemical tests.

Middleton, J. T., 1943. The taxonomy, host range and geographic distribution of the genus Pythium. Mem. Torr. Bot. Cl. 20(1): 1-171.

The definitive manual for identification of the difficult **genus Pythium which occurs in soils and water. It is isolated occasionally on hemp seed but without knowledge of the species of plant with which the parent strain was associated it is difficult to make an identification.**

Moser, M., 1949. Untersuchungen Uber den Einfluss von Waldbranden auf die Pilzvegetation I. Sydowia 3: 336-383.

A study of burned areas in the Austrian Tyrol near Innsbruck in which the concept of four degrees of relationship of a fungus or other organism to its environment is introduced.

Raper, K. B. and C. Thom, 1949. A Manual of the Penicillia. ci, 875. Baltimore, The Williams and Wilkins Co.

The definitive manual for the genus Penicillium. This is most useful in our work since of the 150 species described on standardized media, about half have been isolated during the course of our work.

Seaver, F. J., 1928. The North American Cup Fungi (Operculates). pp. 284. New York, Published by the author.

A manual for the study of the Operculate Discomycetes, cup fungi.

Smith, G., 1954. An Introduction to Industrial Mycology. Fourth Edition. xiv. 378. London, Edward Arnold (Publishers) Ltd.

A manual for workers and students of industrial mycology. In this book the commoner species used in industry and the commoner contaminants of the laboratory and of industry are described. There are valuable chapters on various phases of the biology and use of fungi.

Sparrow, F. K., 1943. Aquatic Phycomycetes, exclusive of the Saprolegniaceae and Pythium. xix, 785. Ann Arbor, The University of Michigan Press.

A manual for the study of various groups of aquatic fungi. This will be of help to one who isolates fungi from waters especially if they are associated with plankton, pollen, and other materials in the water. It does not overlap the work of Coker or Middleton mentioned above.

Suter, R. and E. Moore, 1922. Stream pollution studies. State of New York Conservation Commission, Albany. pp. 1-8, pl. 9.

This little pamphlet indicates that certain aquatic fungi can be used as indicators of pollution but identifications, and therefore groups mentioned appear to be in error,

Thom, C. and K. B. Raper, 1945. A Manual of the Aspergilli. ix, 373. Baltimore, The Williams and Wilkins Co

The definitive manual for the identification of Aspergillus. Species in most of the subgeneric groups have been isolated from polluted water and sewage and use of this manual makes their identification relatively easy.

Wilson, J. N., 1949. Microbiota of sewage treatment plants and polluted streams pp. 1-15, A .A .A .S . symposium: Limno logical Aspects Water Supply and Sewage Disposal.

This paper presents certain basic concepts on the composition of the slimes on trickling filters. While admitting the presence of fungi, no atter is made to determine the comparative role of any one or another species and of bacteria in the formation of the basic slime.

USE AND VALUE OF BIOLOGICAL INDICATORS OF POLLUTION:

FRESH WATER CLAMS AND SNAILS

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I. INTRODUCTION

In discussing fresh water clams and snails (mollusks), not enough is known yet about molluscan ecology to name any species a pollution indicator, There are mollusks tolerant to certain effects of pollutants such as septicity, but even these are not pollution indicators, Species that are found associated with domestic sewage in septic reaches of water, as Musculium transversum, Pisidium idahoensis, Physa integra, and Physa heterostropha, are also found in high dissolved oxygen areas of lakes and streams unpolluted by domestic sewage or putrescible industrial wastes.

On the other hand certain mollusks, such as the Unionidae*/, are not associated with near-septic water resulting from pollution. These have an index value in that their presence typically indicates good dissolved oxygen and attendant physical and chemical conditions associated with unpolluted water. Such mollusks can be called clean water index organisms

Apart from systematic morphological studies, it is not realistic to isolate a single group of organisms such as mollusks from other animals and plants that are associates under similar ecological conditions in clean or polluted water. It is the study of the total biota which tells one most about water conditions.

*/ Members of the family Unionidae have had various common names applied to them: Mussels, fresh water clams, and naiads.

I [illegible], [illegible]-[illegible],
E [illegible]; [illegible], C [illegible]; [illegible],
Tubifex [illegible]; [illegible], C [illegible]; [illegible],
Physa integra; [illegible]-[illegible], M [illegible], [illegible]
[illegible] U [illegible], [illegible], [illegible], [illegible], [illegible]
[illegible]. T [illegible]
[illegible]. T [illegible]
[illegible]

I [illegible] [illegible]. [illegible] A [illegible]
[illegible]

T [illegible] U [illegible] S [illegible]
T [illegible], [illegible] S [illegible]
[illegible]. L [illegible] information
[illegible].

T [illegible] working [illegible]
[illegible]-[illegible]. C [illegible]:[illegible];
[illegible];[illegible];[illegible]

II. DISCUSSION OF SELECTED REFERENCES

Selected references that may be readily available to those working in sanitary sciences are cited here that especially deal with waterways of the United States. No attempt is made to present a complete literature review covering pollution and its effects on mollusks. Many of the included references should point out to those studying bottom organisms the importance of recording chemical and physical data that can be analyzed in relation to tolerances of specific mollusks to pollutants,

Available literature relating mollusks to water chemistry is woefully lacking. When the word "pollution" is used, the general inference is to domestic sewage. Except in a few specific studies of industrial wastes, cognizance often is not taken of the effects of such wastes in association with domestic sewage, even though they may have been related to the presence or absence of mollusks.

In order to make data concerning the effects of pollution on mollusks comparable, pollution should be defined both chemically and physically. It is also necessary to identify mollusks to species, if pollution-tolerant ones are to be exactingly separated from intolerant ones. Specific identification is particularly important to those who hope to find indicators of degrees of pollution.

If work, under field conditions, on the relationship of mollusks to physical and chemical factors is contemplated, Boycott (1936) should be consulted early in the planning stages. Even though relatively few of the species he deals with are found in North America, the information he presents associating mollusks with water chemistry should provide valuable background information for North American studies.

(1) References Relating Mollusks to Pollution in General

The following references relate mollusks to pollution in general without consideration of chemical and physical data. Such papers are valuable, in that they contain references to mollusks already identified to genera or to species by outstanding authorities in Conchology. By being aware of such references aquatic biologists working on water pollution problems have mollusk names available from certain areas, that may give them a lead to identification of current collections.

I[illegible] P[illegible], Ortman (1909) [illegible] Unionidae [illegible] F[illegible], [illegible] Pleurocera, Goniobasis, [illegible] Anculosa [illegible] Unionidae [illegible] A[illegible] R[illegible] Venango C[illegible], P[illegible]. T[illegible] Lymnaea, Physa, [illegible] Planorbis [illegible] Physa [illegible] "... [illegible] B[illegible] [illegible], [illegible] Physa [illegible]."

B[illegible] (1911), [illegible] F[illegible] Sphaerium, Pisidium, [illegible] Planorbis [illegible] Lymnaea. B[illegible] (1911) [illegible] R[illegible], N[illegible] Y[illegible], [illegible] Genessee R[illegible] "... [illegible] ... [illegible] ... [illegible] Galba catascopium [illegible] Planorbis trivolvis [illegible] T[illegible] [illegible]." I[illegible] B[illegible] Genessee R[illegible] "...[illegible] a [illegible]."

I[illegible] Genessee R[illegible] R[illegible], N[illegible] Y[illegible], B[illegible] (1922) [illegible] 27 [illegible] 1892 [illegible] 1919. H[illegible] "..[illegible] ... [illegible], " [illegible] "[illegible], [illegible], [illegible].. M[illegible] 1892, [illegible] 9 [illegible]: Musculium transversum, M. partumeium, Bythinia tentaculata, Planorbis trivolvis, Physa gyrina, P. sayii, P. [illegible], G[illegible], [illegible] G. [illegible]. I[illegible] 1907 [illegible] species of M[illegible] B[illegible], [illegible] P[illegible], P[illegible], [illegible] G[illegible] [illegible]. I[illegible] 1910 [illegible] 1910 [illegible] 1913. B[illegible] (1922) [illegible] G. C. W[illegible] [illegible] 1912 [illegible]. H[illegible] W[illegible] [illegible] 5 [illegible] 41 [illegible] A[illegible].

On one day in this month saturation did not exceed 5 per cent in a 3 mile reach from the surface to bottom in a depth of about 26 feet. He states that in 1917 a large part of the Rochester sewage, 32 million gallons a day, was diverted from the river to a sewage treatment plant, the effluent of which was discharged into deep water of Lake Ontario. In 1919 Baker reports the following mollusks occupying the reach of stream that had become devoid of them before sewage treatment was installed: Musculium transver sum, Bythinia tentaculata, Galba catascopium, Planorbis trivolvis, Physa integra, and P. oneida.

According to the study by Wilson and Clark (1912) on the mussel fauna of the Kankakee Basin in relation to destruction by dredging operations, "The most fatal condition is the constant movement of the fine sand and silt along the bottom of dredged channels." They further state, "Portions of the basin which were dredged 15 or 20 years ago show no signs of restocking with mussels, though there are thousands of them close at hand in old channels."

Considering the effects of pollution on the mussel fauna of the Big Vermilion River and its tributaries in Illinois Baker (1922) states that, "sewage pollution has killed all clean water life for a distance of fourteen miles below Urbana and has made the stream an unfavorable environment for a distance of twenty miles. Below this point the fauna is normal and is not affected by sewage pollution." He observed that of that large species of Unionidae, Amblema undulata and Lasmigona complanata,resisted pollution conditions better than others, In a preliminary paper to this report, Baker and Smith (1919) also wrote on the same subject,

Baker (1928), in the pelecypod part of his monograph on Wisconsin fresh water snails and clams, mentions that stream pollution by sewage and manufacturing wastes produces unfavorable conditions for mollusks. In reference to industrial wastes, he writes that coal tars and oils in particular quickly make a stream totally unfit for any kind of animal life.

In his work on the mollusca of Michigan Goodrich (1932) states that Lymnaea stagnalis appressa are being reduced by drainage enterprises and pollution. He adds that this gastropod probably disappeared from great areas in a few years because waterways were used for logging purposes and sawdust disposal.

Van der Schalie (1936) notes that over the years domestic sewage and industrial wastes in general, along with other factors, have had a detrimental effect on the naiad fauna of the St. Joseph River Drainage in southwestern Michigan. Effects of pollution on specific mollusks are not discussed.

Van der Schalie (1936a), when discussing factors contributing to the depletion of naiades in the eastern United States in relation to the fauna of the Mississippi River, states, "Pollution, particularly below the several large cities located on the river and its important tributaries, was responsible for a heavy mortality in glochidia which were attached by bacteria and infusoria. " In discussing Ellis' (1931) field investigations relating to the effects of silt on the fisheries of the Mississippi River, he further states that "The river is practically devoid of mussels from the region of St. Louis, Missouri, to its mouth, a condition accounted for by the tons of silt carried to the stream and deposited in it by the waters of the Missouri River which enters the Mississippi near St. Louis.... . " Writing about the "South Atlantic Costal drainages" he mentions that mine wastes from coal mines in the headwaters of the James River have damaged the fauna of this stream. With reference to the mussel fauna of the Great Lakes Drainage rivers in Michigan he comments that industrial wastes and sewage are particularly damaging to mussels in the Saginaw drainage, St. Joseph, the Kalamazoo, the Grand, and Rouge Rivers; wastes from beet sugar refineries are felt to be responsible for "unproductive" areas in the Raisin and Pine Rivers. In reference to the above drainage area he states, "In many localities action has been taken by the State to curtail such destructive influences (pollution), though usually much irreparable damage has been done before preventive action has become effective. " Thus, in relation to the effects of factors affecting mussels of the eastern United States van der Schalie writes, "... silting, pollution by sewage, mine and industrial wastes, power-dam developments and unrestricted mussel gathering for the pearl button industry, have resulted in the critical depletion of the formerly abundant Naiad fauna. "

In writing about the depletion of the mussel resources of Michigan van der Schalie (1938a) mentions that among the outstanding factors are pollution in streams by sewage and industrial wastes and the extensive program of power dam developments. He further comments that mussels are among the most sensitive of organisms to pollution and are among the first to perish where pollution is in evidence.

Van der Schalie (1938b) points out that the Cahaba River in northern Alabama, as of 1938, was unusually productive conchologically, but that there are several potential dangers to Cahaba mussels:

the possibility of acid pollution from the Cahaba coal field, industrial waste concentrations and sewage from Birmingham, and dam construction. He writes, "In view of these possible changes, studies of the fauna under natural conditions are highly desirable."

Goodrich (1939) mentions that the lumber industry has affected mollusks by fire, sawdust and rafting. However, he does not assess damages.

From mussel studies of the Grand and Muskegon Rivers in Michigan van der Schalie (1941) writes that many factors are causing the depletion of the indigenous fauna. Thus, it becomes increasingly important to gather ecological and distributional data about them before damage becomes too severe. Factors in addition to dredging, that have damaged mussel populations in the Grand River, are many kinds of industrial wastes and sewage in reaches below Jackson, Lansing, •and Grand Rapids. Gathering mussels with apparatus injurious to mussel beds is also mentioned. In relation to the Muskegon River, he lists power dams and pollution as being detrimental to mussels and hindering their distribution through obstructing fish, the "..... carrying agents for fresh-water mussels."

Van Horn (1949) associates the air-breathing snails Physa and Planorbis with a zone of recent pollution, and states, "In this zone also, as the oxygen concentration is decreased, one may find sewage fungi . . . such as Sphaerotilus, Leptomitus, Thiothrix, and others."

According to van der Schalie and van der Schalie (1950), reporting on the mussels of the Mississippi River, "The several surveys of the Mississippi emphasize that the changes brought about in this drainage through adverse conditions, such as silting, pollution, intensive exploitation of the mussel fauna, power dam construction, etc., are all tending to alter decidedly, as well as to reduce, the original fauna."

Beck (1954), in his ecological classification of the streams of Florida relating organisms to water pollution surveys, refers mollusks of the genera Physa and Ferrissia and the Sphaeriidae to a "Class III" which includes organisms which have been found in heavily polluted areas. He comments that no organisms in this class may be considered indicative of pollution, since organisms contained therein may be found in clean, moderately polluted, or grossly polluted water. Goniobasis *spp.* is placed in a "Class I" containing organisms that have been found to tolerate no appreciable organic pollution, "...the more sensitive forms." He states that, The presence of class I organisms is considered to indicate that the water in which they are found is clean."

Carlander (1954) writes about the general effects of pollution, including silt, on the mussels of the upper Mississippi River using material published by the U. S. Fish and Wildlife Service as a basis for her discussion.

In presenting his list of mollusks (Table-1) that can survive "....at least to some degre..." in zones of degradation and recovery, Wurtz (1956) points out that "....we are woefully lacking in knowledge on this subject." He further writes that the exact tolerance limits of the mollusks he lists are not known, and that as far as he can ascertain no mollusks are able to withstand protracted gross pollution. From among the 34 species and subspecies of mollusks he has considered, he states that Physa heterostropha is the most tolerant species that has been found.

TABLE I

Mollusks Reported to Survive "at least some degree" in Zones of Degradation and Recovery

GILL-BREATHING SNAILS
(Ctenobranchiata)

Family - Viviparidae
1. Campeloma integrum
2. C. rufum

Family Amnicolidae
1. Bulimus tentaculatus

LUNG -BREATHING
(Pulmonata)

Family - Lymnaeidae
1. Lymnaea cape rata
2. L. humilis
3. L. obrussa
4. L. polustris
5. L. stagnalis
6. L. auricularia
7. Pseudosuccinae columella

Family - Physidae
1. Physa gyrina
2. P. heterostropha
3. P. integra
4. Aplexa hypnorum

Family - Planorbidae
1. Helisoma anceps
2. H. trivolvis
3. Gyralus arcticus
4. Menetus dilatatus

Family - Ancylidae
1. Ferrissia fusca
2. F. tarda

FRESH WATER CLAMS

Family - Sphaeriidae
1. Sphaerium rhornboideum
2. S. corneum
3. S. striatinum
4. S. sulcatum
5. S. (Musculium) securis
6. S. (Musculium) tranversum
7. Pisidium amnicum
8. P. casertanum
9. P. compressum
10. P. fallax
11. P. henslorvanum
12. P. subtruncatum

Family - Dresisseniidae
1. Mytilopsis leucophaeatus

Family - Mactridae
1. Rangia cuneata

* Mollusks of Wurtz (1956) that can survive "... at least to some degree ..." in zones of degradation and recovery. Wurtz's data have been organized to form this table by W. M. Ingram.

Only one species in each family in the United States; the former with a range from Maryland to Florida, and the latter with a range from Alabama to Texas.

(2) **References Relating Mollusks to Specific Aspects of Water Quality Associated with Pollution.**

The following investigators have presented varying amounts of physical and chemical data that relate mollusks to certain aspects of water quality.

Juday (1908 and 1921) reports a Sphaeriid clam, Corneocyclas [Pisidum] idahoensis, from septic water in Lake Mendota, Wisconsin; however he does not mention specific pollutional conditions. From laboratory observations he concluded that this clam may remain quiescent with its valves closed for as long as three months each summer in the septic bottom ooze of the lake. He found that clams surviving in septic water with their valves tightly closed became active when placed in aerated water. Shelford (1913) has listed Juday's (1908) work relating to survival of Pisidium idahoensis under anaerobic conditions.

Weston and Turner (1917) found Physa heterostropha, Helisoma trivolvis, Segmentina armigera, and Unio complanata living in a stream below a sewage treatment plant outfall where average monthly dissolved oxygen values in October, June, July, and August were between 1 and 2 p.p.m. These writers do not present D. O. figures other than in monthly averages. The gastropod Campeloma decisum was not taken in any stream reaches where the average monthly D. O. was below 5 p. p. m.

Jewell (1920) discusses mollusks and other organisms associated with clean water and polluted water reaches of the Sangamon River, Illinois. This author mentions that there is some domestic sewage pollution from the effluent of a septic tank in the town of Monticello entering the clean water area, but such pollution only affects the water in a restricted area. Chemical conditions of clean water reaches are stated to be, "... alkalinity to methyl orange of 222 p. p. m. and a pH of 8... transparent to a depth of a foot or more." Bottom materials are said to consist of gravel. Close to shore it was observed that a "...layer of organic deposit covered the bottom... ." Mollusks associated with pools in such water were the Unionidae species, Lampsilis luteola, Quadrula pustulosa, Q. undulata, Q. rubiginosa, Tritigonia tuberculata, Symphynota costata, Strophitus edentulus, and Anodonta grandis, unnamed species of Sphaeriidae, unnamed species of Pleuroceridae, and Campeloma subsolidum.

Below the reach of the Sangamon described above, sewage of Decatur is discharged. Bottom animals were stated to disappear immediately and "...not until Noantic is reached, about 30 miles below

[Decatur], do the first animals appear." No mollusks are listed in Jewell's tables for 75 miles below Decatur. In this distance figures are listed showing variations in chemical and physical conditions: D. O. from 0 to 4.1 p.p.m. alkalinity from 260 to 348 p. p. m., pH 7 to 8, odor from septic and putrid to not pronounced, appearance from sewage to inky to milky to grey to turbid to green-grey. At the 75-mile station where mollusks are first noted to reappear, chemical and physical conditions show: D. O. of 7.8 p. p. m. ; an alkalinity of 287 p. p. m., a green appearance, and no odor. The mollusks occuring at this station were Unionidae, Quadrula undulata, Q. lachramosa, Lampsilis alata, L. luteola, L. anadontoides, Unio gibbosus, Anodonta grandis, Tritigonia tuberculata, Strophitus edentulus, Ala smodonta costata, unidentified "Sphaerium," and the gastropods Pleurocera elevatum and Campeloma subsolidum. Unionidae and gill-breathing snails at various stations are associated with disolved oxygen conditions that were not less than 7. 5 p. p.

Richardson published a number of papers relating general pollution of the Illinois River to bottom fauna, among which his 1921, 1925, 1925a, and 1928 papers are cited in the bibliography as being of special interest to those studying mollusks. Richardson's 1925 paper lends itself to an attempt by the writer to generally correlate dissolved oxygen-mollusk relationships at certain stations in the reach from Chillicothe into Spring Bay Narrows, from the few dissolved oxygen figures that he presents under the title, "Bottom Dissolved Oxygen Mid Channel." Three species of Sphaeriidae, Musculium transversum, M. truncatum, and Pisidium complanatum, can be associated with a minimum D. O. of 0.2 p. p. in. The gill-breathing snail, Valvata tricarinata can be associated with a minimum D. O. of 1.4 p. p. m. The gill-breathing snail, Campeloma subsolidum, which Richardson stated as being a survivor under poor dissolved oxygen conditions, can be associated with dissolved oxygen figures between 0. 5 to 3.0 p. p. m.

Baker (1926) presents a review of Richardson's 1925 papers on the effects of pollution on bottom organisms in the Illinois River. Data from the review state, "It is shown prior to 1915 there have disappeared from Peoria Lake (a widening of the Illinois River) about forty species of river mussels (only three or four species are left), all of the small snails belonging to the water-breathing [sic. = gill-breathing] family Amnicolidae, all but one species of the large water snails belonging to the Viviparidae, the remaining species being recorded as Campeloma subsolidum, and a varied weed fauna. There remains certain species which appear to be more tolerant of pollutional conditions. These are Musculium transversum,

Pisidium compressum, P. paupereulum crystalense, Campeloma subsolidum, and Sphaerium stamineum. Several species less tolerant to sewage conditions were observed in peculiarly favorable conditions, usually in strong current in midchannel or elsewhere where oxygen conditions were good. These included Anodonta imbecillis, an insect Corixa, a caddis-fly larva (Leptocerid - obasis livescens, Pleuroc era elevatum lewisii, Quadrula plicata (= peruviana) Hyalella, a sponge, and a Hydropsyche. pecies, however, were observed to vary in presence during different years."

Suter and Moore(1922) state that Physa heterostropha may be found in septic regions and list it with Pisidium abditum, Goniobasis virginica, and Campeloma decisum as an organism tolerant of pollution; they do not mention that the latter three species can survive septicity.

Turner (1927) lists Physa heterostropha as found in septic water and Planorbis panus from stagnant water and the vicinity of sewer outlets. Campeloma decisum is described as being more sensitive than the other species since ". . . . it seems to thrive under clean water conditions. "

Wiebe (1928), in his paper on the effects of pollution on the upper Mississippi River, collected Helisoma trivolvis, Musculium transversum and Campeloma integrum on August 27 at his Red Wing station with the D. O. at 2. 83 p. p. m. ; of 22 D. O. measurements made in August the average at this station was 2.25 p. p. m. with a minimum of 1. 12 p. p. m. and a high of 4. 01 p. p. m. Individuals of Musculium (near) transversum were collected at his Jackson Street station on August 17 with the D. O. at 0. 87 p. p. m. ; of 22 D. O. measurements made in August the average at this station was 0. 87 p. p.m. with a range from 0 to 2. 52 p. p. m. His data on tables 4 and 5 indicate that mussels (not identified) were taken in September at the Jackson Street station when the D. O. was 5.73 p. p. m. with the range of 20 readings for September varying from 0. 44 to 8. 14 p. p. m. He records collections of Anodonta imbecillis on September 17 at the Red Wing Station with the D. O. on this date being 4. 39 and a range of 21 readings for September varying from 2.89 to 6.44 p. p. m. Campeloma rufum was taken at two stations where the D. O. was always above 5 p. p. m. for 61 measurements; Wiebe nevertheless comments in relation to this species, ". . . . very likely it is one of the more tolerant forms;" this conclusion is based on the fact that 1, 600 specimens per square yard of bottom were taken some 50 yards below a sewage outfall at one station and were associated with 15, 120 Tubificidae and 54 Sphaerium notatum per square yard. Pleurocera acuta is reported from two stations where the D. O. was always above 4. 30 p. p. m. for 65 measurements.

Purdy (1930), in summarizing pollution data on organisms other than plankton in his Illinois River work, states that the Sphaeriidae are often very numerous in moderately polluted water. He further writes that these mollusks cannot stand "extreme" conditions that Tubificid worms can and that they will die when oxygen becomes largely depleted. He states that, "Apparently their Sphaeriidae large numbers in places where water is polluted is a question of their abundant food supply of microscopic organisms normally found there." Purdy's data for the Illinois River, as correlated by Ingram with those of Hoskins et. al. (1927), show that unidentified Sphaeriidae were collected at Chillicothe where the dissolved oxygen was recorded as low as 1.23 p. p.m. in August. The highest D. O. at this station was 7.79 p. p. m. in February. Unidentified air-breathing snails collected by Purdy (1930) at Lockport, as correlated with Hoskins's et. al. (1927) data, *were* taken at this station where septicity existed in August and the D. O. was 9.11 in February. The pollution in the Illinois River at the time the above data were collected was from numerous industrial wastes and domestic sewage.

Ellis (1931a) writes that juvenile and young mussels are quite sensitive to oxygen reduction and that adult mussels "... usually become inactive when the oxygen tension of the water is reduced to 20 per cent saturation or less." He emphasizes the detrimental effect of erosion silt on clams. In relation to the general effect of industrial wastes on mussels, he writes, "Whenever concentrated industrial wastes are poured into the streams the fresh-water mussels suffer because of their inability to change location quickly and because of the ease with which the blood of fresh-water mussels takes up the various substances in the surrounding waters...."

Van der Schalie (1938) has presented chemical and physical data associated with unpolluted water and water polluted by domestic sewage in relation to the distribution of Unionidae in the Huron River, Michigan. Referring to the effect of pollution on mussels, he states, "Below Ann Arbor, sewage has been very detrimental to the fauna. It is true that sewage may, if not too concentrated, increase the productivity of sections of a stream by increasing the dissolved organic compounds, but below Ann Arbor and as far as the backwaters of Geddes dam, pollution is so concentrated that it has killed all Naiades. Furthermore, there is such a heavy deposit of sludge in this zone that it will be many years before bottom conditions will permit the re-establishment of a fauna even though the discharge of sewage into the river be discontinued.

Goodrich (1945), in his monograph on the gilled-snail Goniobasis livescens in Michigan, associates water chemistry with its occurrence and with Pleuroceridae in general. He writes that this species has disappeared from the heavily polluted Huron River. He associates massive kills of Pleuroceridae in western Lake Erie with exhaustion of oxygen under ice cover. G. livescens has been found in streams with the alkalinity ranging from 87 to 233 p. p. m. He states that a drop in pH much below 7.0 does not permit survival of pleurocerid life judging by studies made by C. S. Shoup in the Obey River in 1939. Streams with a pH above 7.0 were extensively colonized, while these mollusks were absent in water with a pH of 6.1. Members of the genus Goniobasis in general endure periodic silting of streams that accompany freshets. Certain ones, G. virginica for example, may live in tidal reaches of streams where the salinity is 50 per cent of that of sea water.

Whipple, Fair, and Whipple (1949) present a list of mollusk species that they have associated with the pollution zones of Kolkwitz and Marsson (Table 2). They list no mollusks in the polysaprobic zone that they partially characterize by the presence of black sludge accumulations on the bottom and a lack of oxygen. They note that life in the mesosaprobic zone is commonly tolerant of dilute or imperfectly purified sewage and its products of decomposition, and state that "Many bacteria are still present." Pertaining to the oligosaprobic zone they write, "This is a zone of cleaner water in which mineralization has been completed ..... The water is practically saturated with oxygen, sometimes even supersaturated." It should be noted from Table 2 that many mollusks associated with the mesosaprobic zone are also associated with the oligosaprobic. One Unionid clam and certain gill-breathing snails of the genera Campeloma, Viviparous, and Valvata that commonly inhabit clean water are associated with the mesosaprobic zone.

Patrick (1949) associated Physa heterostropha with "polluted water" conditions at a station 105 in Lititz Run, Pennsylvania. Chemical data are presented in tables, separated from biological data; such information presented for August, a month indicated to be "low water" for this station, shows a pH of 7.3; D. O., 8.00 p.p.m. ; turbidity, 10.8 p. p. m. ; Nitrogen (N as NH_3), 0.015; Nitrogen (N as NO_2), 0.0260; Nitrogen (N as NO_3), 3.120; etc.

TABLE 2

Mollusks Related by Whipple, Fair, and Whipple to Pollution Zones of Kolkwitz and Mar sson*

Mollusks	Pollut	onal Zones of	Kolkwitz and Mars son				
	Oligo-sap-robic	Poly-sap-robic	Mesosaprobic				
			No sub-design-nation	Alpha	Beta		
FAMILY - SPHAERIIDAE						CLA S	
Pisidium amnicum	x						
" compre ssum			x				
" fossarinum	x						
" pauper culum			x				
Sphaerium corneum				x	x		
" moenanum	x				x		
" vivicolurn	x				x		
" stamineum			x				
" striatinum			x				
Musculium transversum			x				
" truncatum			x				
FAMILY - UNIONIDAE							
Unio batavus	x						
" pictorum	x (indi	ferent)					
" tumidus					x		
Anodonta mutabilis	x(indi	ferent)					
FAMILY - MARGARITANIDAE							
Mararitana margaritifera	x						
FAMILY - PHYSIDAE						Lung Breathing Snails	SNAILS
Aplexa hypnorum	X				x		
Physa acuta	x				x		
" fontinalis	x				x		
FAMILY - LYMNAEIDAE							
Lymnaea auricularia					x		
" ovata					x		
" palustris	x				x		
" peregra	x				x		
" stagnalis	x						
FAMILY - HELISOMIDAE							
Planorbis carinatus	x						
" corneus	x				x		
" marginatus	x						
FAMILY - ANCYLIDAE							
Ancylus lacustris	x				x		
" fluviatilis	x				x		
FAMILY - VALVATIDAE						Gill Breath-ing Snails	
Valvata piscinalis					x		
" tricarinata	x						
FAMILY - VIVIPARIDAE							
Viviparus contectus				x	x		
" fasciatus					x		
Campeloma subsolidum				x	x		

* Not all species are presented here (arranged by W. M. Ingram)

Gaufin and Tarzwell (1952) reported Physa integra from all stations on Lytle Creek, Ohio, during studies conducted in May and August, and list it as abundant at a station where D. O. was recorded as low as 0.2 p. p. m. Sphaerium solidulum, as reported, was not collected at stations where the D. O. was less than 4.5 p. p. m. as based on diurnal sampling.

On the basis of collections from Lytle Creek Ingram, Ballinger, and Gaufin (1953) report Sphaerium solidulum intolerant of pollution from domestic sewage including septicity and sludge deposits, and intolerant of bottom areas covered with zoogleal organisms. Certain literature relating to the tolerance of the sphaeriidae to pollution is discussed.

Gaufin and Tarzwell (1955) show that during January and February of 1952 Ferris sia rivularis, Sphaerium solidulum, and Pisidium casertanum were not taken in Lytle C[illegible] A[illegible] 1951, [illegible] D. O. was [illegible] 7.0 [illegible] T[illegible] O[illegible] 1951 [illegible] F[illegible], M[illegible], P[illegible], [illegible] Lymnaea [illegible] a septic [illegible] A[illegible] 1951. A [illegible] Sphaerium solidulum were collected from a [illegible] A[illegible] record.

(3) R[illegible] R[illegible] M[illegible] W[illegible] S[illegible].

I[illegible] O[illegible]

F[illegible].

I[illegible] C[illegible] (1930) [illegible] T[illegible] C[illegible], N[illegible] Y[illegible], [illegible] Campeloma [illegible]. In addition, the following mollusks are listed in a table as being associated with "a pulpy bottom 8 inches thick ... ": Amnicola limosa, Planorbis antrosus, Calyculina hirsutus, Lymnaea decidiosa and Sphaerium fabale. Sphaerium striatinum is [illegible] C[illegible] L[illegible] E[illegible]; [illegible] pulp. A[illegible] L[illegible] E[illegible], [illegible] pulp [illegible]: V[illegible], A[illegible],

Bythinia tentaculata, Calyculina securis, Planorbis campanulatus, Planorbis hirsutus, Lymr . Acella) haldemanni, Lymnaea decidiosa, Sphaerium stiratinum, and Sphaeriumfabale. No consideration was given to possible effects of toxicity on mollusks.

Henderson (1949) has observed that mollusks once killed by wastes from certain viscose operations do not re-inhabit sections of streams rapidly. Through bioassays, he found that quantities of zinc proportional to quantities in the Shenandoah River from a viscose operation proved fatal to snails, daphnia, and bass fry.

Bartsch and Churchill (1949), in studying the effects of waste sulphite liquor on the biota of the Flambeau River, Wisconsin, made obervations relating such a waste to mollusks. They state that Campeloma integrum, with three unnamed snail species, apparently resist high concentrations of waste sulphite liquor, although they are not found immediately below the industrial sewer outfall. The sphaeriid clam, Sphaerium rhomboideum, was likewise noted to be absent from such an area.

Neel (1953), in pollution studies relating to oil refineries of the North Platte River below Casper, Wyoming, writes that absence of larger benthic forms below "...the refinery area may be laid to periodic releases of large quantities of toxic wastes. " He comments that a dearth of bottom organisms, including unnamed snails, is probably to be associated with blackish oil rather than to any lethal phenol concentrations. Conducted bioassay tests showed oil to be deadly to benthic snails.

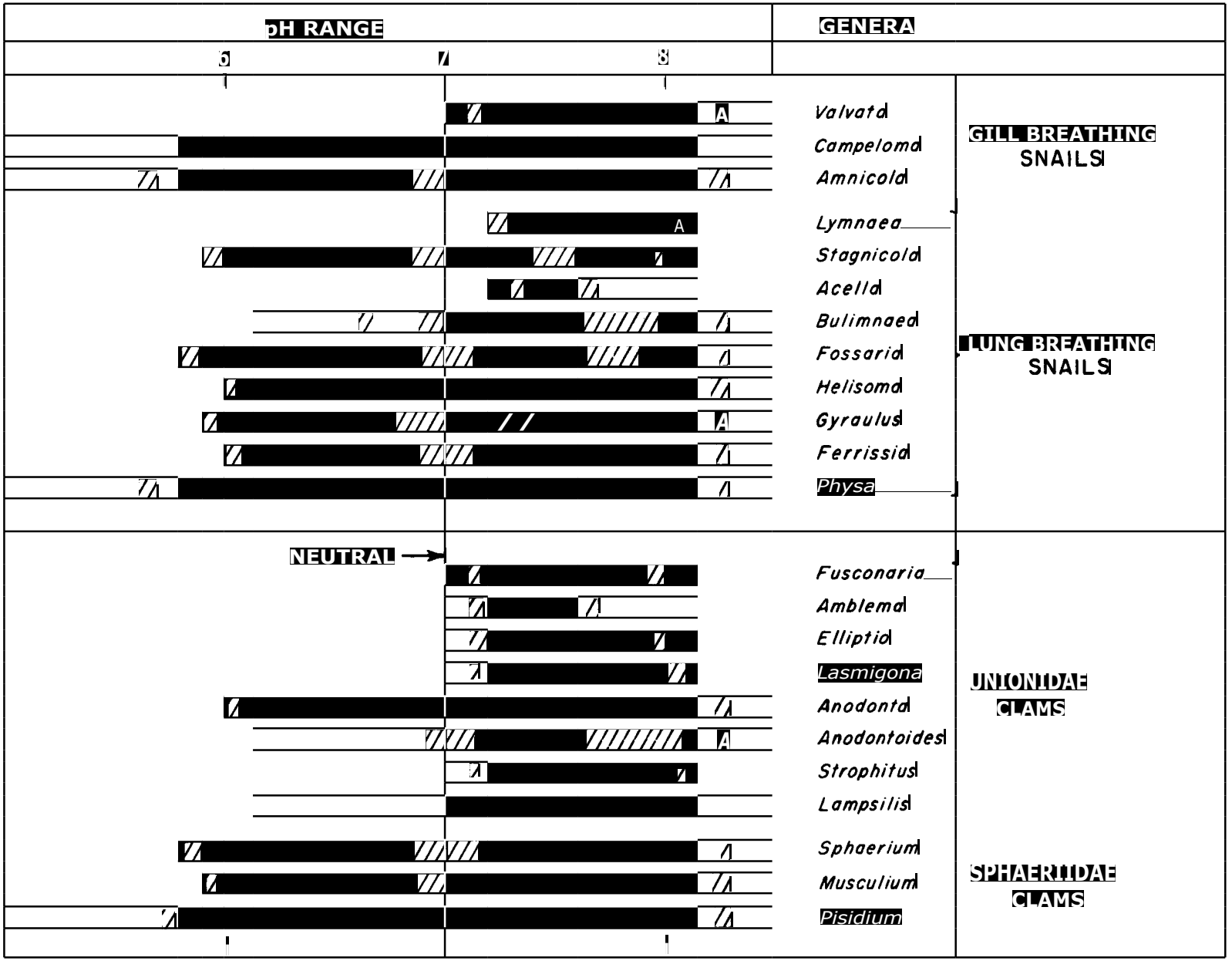
44) Low Hydrogen Ion Concentration not Associated with Pollution, Under Which Mollusks Have Been Reported Living

Mollusks have been reported living in natural waters where low pH values have not been associated with pollution. Data are presented to illustrate low pH values that many might not suspect mollusks could tolerate.

Based on Morrison's (1932) Wisconsin studies that do not associate with pollution the low pH values recorded therein, various mollusks are shown to live in natural waters with a pH as low as 5. 6 (Table 3). Figures indicate that many mollusks live in waters with a pH as high as 8. 3; no figure is cited above this pH value, Hydrogen ion figures taken from this work and shown in Table 3, are ones cited under each species in Morrison (1932).

Table 3

pH RANGES ASSOCIATED WITH SELECTED MOLLUSK GENERA (AFTER MORRISON 1932)



Such a range of pH values tolerated by mollusks is within that which is listed by Doudoroff and Katz (1950) tolerable to most fresh water fish without lethal effects. These writers state, "It appears that, under otherwise favorable conditions, pH values above 5. 0 and ranging upward to pH 9. 0 at least are not lethal for most fully developed fresh-water fishes." In addition, these investigators write, concerning fish survival in relation to pH, "Much more extreme pH values, perhaps below 4, 0 and well above 10.0, also can be tolerated indefinitely by resistant species. However, regardless of the nature of acid or alkaline wastes responsible, such extreme conditions, associated with industrial pollution, are evidently undesirable and hazardous for fish life in waters which are naturally so acid." Most species of Unionidae, as reported by Morrison (1932) and dependent upon fish for the completion of their life-cycles, were found in a pH range upward from 6. 9. However, one member of the genus Anodonta, A. marginata, was reported from water having a pH of 6. 3.

Jewell (1922) has reported mollusks living in the Big Muddy River, Illinois, a stream characterized as a "... naturally acid stream. ", with a pH range stated to be from 5.8 to 7.2, Jewell presents species identifications of nine Unionidae from the stream. In relation to snails found in this river she writes, "Abundant as were mussels in the Big Muddy River, only two snails (individuals) were found: one a living Pleurocera elevatum taken opposit Benton. . . , the other a Canweloma subsolidum taken near Murphysboro. No dead shells were found to indicate that snails had ever been present. " This writer states that fish were"... everywhere abundant. ", and that dogfish, sunfish and native carp were taken with hook and line, while large numbers of minnows, cat fingerlings (at one time a school of several hundred), and unidentified fry were seen in pools and riffles. There were also numerous swarms of top minnows, while gar, fifteen to eighteen inches in length, were seen in ever-increasing numbers toward the mouth of the river."

The writer has found Physa integra in sections of the Mahoning River, Ohio, where the pH range over several days varied from 4. 1 to 7. 3.

(5) Mollusks Associated with Sewage Treatment Installations

The information included here deals with species of Physa snails in trickling filter beds of sewage treatment installations treating domestic sewage. A number of persons talking withthe writer have stated that they have seen snails in trickling filter beds; however, very little data are available in the literature to describe conditions under

WHICH SNAILS LIVE IN SUCH SITUATIONS , STUDY OF SNAILS IN TRICKLING FILTERS OFFERS READY ACCESS TO THOSE WHO WISH TO OBTAIN DATA RELATING SNAILS TO WATER NOT COMPLETELY PURIFIED FROM THE EFFECTS OF POLLUTION . CERTAIN CHEMICAL AND PHYSICAL TESTS , KEPT ROUTINELY AT MANY SECONDARY SEWAGE TREATMENT PLANTS EMPLOYING TRICKLING FILTERS , CAN BE USED TO RELATE SNAILS LIVING IN FILTERS TO SPECIFIC RANGES OF WATER QUALITY .

Brown (1937) STUDIED PHYSA ANATINA LIVING IN SPRINKLING FILTERS IN A SEWAGE TREATMENT PLANT AT URBANA CHAMPAIGN , ILLINOIS , DURING PARTS OF THE YEARS 1932-35. BROWN WRITES THAT THE PLANT HAS IMHOFF TANKS AND SPRINKLING FILTERS , "... WHERE JETS OF THE SEWAGE ARE FORCED INTO THE AIR FOR AERATION .", AND A SECONDARY SETTLING TANK . PART OF THE FINAL EFFLUENT IS DIVERTED INTO AN EXPERIMENTAL LAGOON AND PART INTO THE SALINE DRAINAGE DITCH , A TRIBUTARY OF THE BIG VERMILION RIVER . EXCEPT FOR BACTERIAL NUMBERS NO OPERATIONAL DATA ARE PRESENTED IN THE PAPER . I REFERENCE TO BACTERIA IT STATES , "A THE TIME CRUDE SEWAGE ENTERS THE PLANT IT CONTAINS 2, 100, 000 BACTERIA PER CUBIC CENTIMETER , BUT WHEN FINALLY TREATED , THE NUMBER HAS BEEN REDUCED TO 700 PER CUBIC CENTIMETER , " BROWN (1937) BELIEVES THAT SNAILS PLAY A PART IN THE REDUCTION OF NUMBERS OF BACTERIA I ADDITION TO COLLECTING PHYSA ANATINA IN THE ROCK BEDS OF SPRINKLING FILTERS , INDIVIDUALS WERE TAKEN FROM THE SECONDARY SETTLING TANK AND FROM THE SALINE DRAINAGE DITCH AND EXPERIMENTAL LAGOON RECEIVING THE FINAL EFFLUENT . DURING THE COURSE OF THE STUDY , IN ADDITION TO PHYSA , 8 INDIVIDUALS OF THE SNAIL FOSSARIA MODICELLA WERE REPORTED FROM THE secondary SETTLING TANK , BUT FROM NO OTHER STRUCTURES ,

I IS MENTIONED THAT IN MAINTENANCE OPERATIONS FROM 25 TO 30 BUSHELS OF EMPTY SHELLS ARE REMOVED EACH YEAR IN JULY AND NOVEMBER FROM A CONDUIT OF THE SECONDARY SETTLING tank. BROWN BELIEVES THAT THE MAJORITY OF SNAILS IN THE SPRINKLING FILTER BEDS DIE EACH winter. REPORTED OBSERVATIONS BASED ON SHELL SIZE PRESENT EVIDENCE TO INDICATE THAT LIFE CYCLES OF SNAILS ARE COMPLETED IN THE SPRINKLING FILTER beds. WHETHER SNAILS OCCUR THROUGH THE DEPTH OF THE BEDS IS NOT INDICATED .

A 1929 ANNUAL REPORT OF THE URBANA -CHAMPAIGN SANITARY DISTRICT , AS QUOTED by BROWN (1937), MENTIONS THAT PASSAGE OF SNAILS FROM THE SPRINKLING FILTERS INTO THE SECONDARY SETTLING TANK , "...PROVES BEYOND DOUBT THE PRESENCE OF A HIGH AMOUNT OF DISSOLVED OXYGEN IN THE LOWER PART OF THE FILTERS ." ASSOCIATED WITH THIS QUOTATION IS THE STATEMENT BY BROWN THAT PHYSA ANATINA BREATHES ATMOSPHERIC OXYGEN . REFERRING TO OTHER RECORDS OF SNAILS REPORTED FROM SEWAGE TREATMENT PLANTS BROWN MENTIONS THAT PHYSA HALEI HAS BEEN REPORTED FROM A FORT WORTH , TEXAS , installation.

Lchmeyer (1955) [illegible] P[illegible] [illegible] U[illegible] Flcrida's [illegible] G[illegible]. I[illegible] M[illegible] 1956 [illegible] [illegible]; [illegible] W. J. C[illegible] [illegible] M[illegible] C[illegible] Z[illegible] H[illegible] U[illegible], [illegible] P[illegible], [illegible] F[illegible] W[illegible] I[illegible] L[illegible] [illegible] filter. H[illegible] [illegible] 3 [illegible]. M[illegible] [illegible] P[illegible] [illegible], [illegible]

I[illegible] M[illegible] 1955, [illegible] P[illegible] [illegible] D[illegible], O[illegible], [illegible]. E[illegible] [illegible] S[illegible] [illegible]. T[illegible] [illegible] ontc[illegible]: B, O.D. 5,9 [illegible] 131 [illegible]; [illegible] 24.4 [illegible] 24 8 [illegible] p. [illegible]; [illegible] 13 [illegible] 17. 9 [illegible], [illegible] 122 [illegible] 128 p. [illegible], [illegible] D. O., O.O p. m. T[illegible] [illegible] 2.7 [illegible] 4. 9 p. [illegible]. H[illegible] [illegible] A[illegible] 7. l

III. SOME STRUCTURAL AND LIFE CYCLE VARIATIONS RELATING TO MOLLUSCAN SURVIVAL ABILITY IN ASSOCIATION WITH DOMESTIC SEWAGE POLLUTION

S[illegible] [illegible]. B[illegible] [illegible].

(1) Survival of Lung-Breathing Versus Gill-Breathizig Snails

Snails possessing lungs can generally be expected to survive under low dissolved oxygen or septic conditions because they typically rely on atmospheric oxygen in breathing. It is important for the aquatic biologist to become familiar with snail morphological characteristics in order to separate readily gill-breathing from air-breathing snails. An obvious character revealing whether the gastropod being dealt with is a gill-breather or a lung-breather is the presence of an operculum in the aperture of the former. Families and common genera of gill-breathing fresh water snails are listed in Table 4 for convenience in separating them from those that typically breathe atmospheric oxygen.

Table 4

FAMILIES AND GENERA OF GILL-BREATHING SNAILS	
Family	**Genera**
Amnicolidae*	**Amnicola** **Pyrgulopsis** **Hydrobia** **Somatogyrus** **Lyogy sus** **Bulimus (Bithynia)**
Pleuroceridae	**Pleurocera** **Goniobasis**
Valvatida e	**Valvata**
Viviparidae	**Vivipara** **Campeloma**

*** Amnicolidae genera listed from Berry, 1943; other families and genera from Baker, 1902.**

It is important to point out to those conducting investigations in lakes or deep rivers that the presence of a lung in a snail does not necessarily mean that atmospheric oxygen will serve as the only source for respiration in all seasons. Periodic movement of lung-

breathing snail, from shallow to deep water, may be induced by a lowering of water temperature to 10°C. as fall merges into winter and may be an annual occurrence as reported by Cheatum (1934) in Douglas Lake, Michigan. A change in temperature from 10°C. to 21°C is accompanied by an ascent from deeper water to marginal lake areas. Cheatum presents data indicating that when pulmonate snails are submerged, the lung functions as a gill in taking dissolved oxygen from water. Lung-breathing snails may remain submerged for three or four months out of every twelve in areas where winter months are climatically similar to Michigan.

Experimental work conducted by Cheatum indicates that lung snails are not completely dormant when submerged, but rather are in a state of suspended animation, and that they actively respire using dissolved oxygen. Individuals of the following seven species and subspecies of lung-breathing snails are noted by Cheatum to breathe dissolved rather than atmospheric oxygen for a part of each year when submerged in Douglas Lake, Michigan: Lymnaea stagnalis appressa, Lymnaea emarginata angulata, Helisoma campanulatum, Helisoma smithii, Helisoma antrosum percarinatum, Physa sayii crassa, Physa parkeri.

In submerged experimental cages individuals of each of the species named above survived for 65 days with the water temperature varying between 16.8 to 26.6°C. Submergence experiments indicated that mortality was greatest among individuals whose oxygen requirements were the highest. The presented data does not indicate that submerged pulmonate snails can live under conditions of septicity.

In writing about the completion of life cycles, by certain pulmonate snails, when submerged Cheatum (1934) states, "In all probability, many individuals of the species H. campanulatum smithii, H. antrosum percarinatum, L. emarginata angulata, and P. sayii crassa complete their life cycles and reproduce normally witEout emerging forair, "

Shelford (1913) writes that pulmonate snails of the genus Ancylus, ". . . are said to take water into their lung and thus do not need to come to the surface for air. "

It is especially pertinent to point out literature, like the above, concerning lung-breathing snails that can obtain oxygen from either the atmosphere or from water, because in deep water during submerged living conditions in winter certain pulmonates would be killed by oxygen-consuming pollutants that could lower oxygen to asphyxial levels.

Thus, it may not be advisable to always include pulmonate snails with sludge worms, certain blood worms, rat-tailed maggots, and household or sewage mosquitoes as being tolerant to pollutional conditions involving septicity.

Of all snail genera, members of the genus Physa, especially, may occur in great abundance in septic zones of streams. Two species, Physa interga and Physa anatina, are commonly associated with septic zones in shallow streams in the mid-west during summer and fall months.

The writer has not collected any of the gill-breathing snails in Polluted water where the dissolved oxygen, as measured during daylight hours, was less than 2 p. p. m. Even though these mollusks possess an operculum which, if tightly sealed, should enable them to close themselves away from low dissolved oxygen waters, the fact that such snails are not reported from septic or near septic water would indicate that low dissolved oxygen may be one of several factors denying such water to them.

(2) Survival Relating to Shell Closure in the Sphaeriidae and Unionidae

The fact that certain of the Sphaeriidae can survive low dissolved oxygen conditions and a shifting bottom of sludge, as related to domestic sewage, and that the Unionidae do not poirts somewhat speculatively to the ability of certain fingernail clams to close the shell and survive until stream conditions improve,

Allen (1923), in studying reactions of -certain Unionidae under low dissolved oxygen conditions writes, "When under conditions of deficient oxygen not only to the siphons widen to bring in more water, but also additional spaces between the mantle edges are thrown open. " He does not mention whether all of the Unionidae that he studied opened the valves as indicated under the stated circumstances. The following Unionidae are listed as being used in general experiments: Anodonta grandis, Lampsilis luteolus, L. ligamentinus, L. altus, Quadrula heros, Q. pustulosa, Q. lachrymosa, Q. undulata, Unio U. ibbosus, Plagiola elegans, "and otEers. " If the Unionidae, in general, have a response to open their valves under low dissolved oxygen conditions resulting from pollution by domestic sewage and industrial wastes, they are most vulnerable to destruction. If they do open their valves as described by Allen, their bodies are vulnerable to any number of substances in polluted water that may be toxic enough to destroy them. Also, in an open position they could be covered by settable solids.

It is known that the Sphaeriidae can live under septic conditions and on sludge-covered bottoms as discussed earlier. Apparently, when living under conditions of septicity the valves remain tightly closed. Thus, the Sphaeriidae would not be subjected to toxic materials as they would if they lived under such conditions with their valves opened. Juday (1908) has written about the behavior of Corneocyclas [Pisidium] idahoensis under laboratory conditions in water containing and devoid of dissolved oxygen, and has related such data to field conditions. In water without dissolved oxygen, individuals remained quiescent with their valves tightly closed without activity being observed in the mud of the experimental jars. When individuals were placed in aerated water they became active. He states that his experiments seemingly indicate that this mollusk remains quiescent or dormant in Lake Mendota, Wisconsin, when the muddy ooze at the bottom of the lake contains no dissolved oxygen, a period of about three months each summer. Juday (1921), in further studying Pisidium idahoensis in lake Mendota, writes that there is no free oxygen below a depth of 20 meters from about the middle of July until early October, and again in March for two or three weeks in some years. He mentions that organisms living under such conditions must be "... facultative anaerobes.", and includes in this category, in addition to Pisidium idahoensis, worms of the genera Tubifex and Limnodrilus, and three dipterous larvae: Corethra punctipennis, Chironomus tentans, and Protenthes choreus.

Baker (1928) writes about Sphaeriidae being able to live in the mud bottom of pools where the water has dried up, and Ingram (1941) has reported Pisidium abditum living out of water on the beach of a lake from at least June 15 to September 1.

(3) Survival Relating to Weight of Shell

The entombment effect of heavy sludge or silt pollution may relate to the absence of heavy Unionidae and presence of certain light Sphaeridae, other factors being favorable. In Dawley's (1947) study of the distribution of aquatic mollusks in Minnesota, he comments on the survival of mollusks on varying substrates, "A mussel [Unionidae] is more exacting in its requirements than a snail, being heavier and less motile. The bottom in which it lives may be sand, gravel, or mud, but not rock or soft muck because its foot cannot penetrate rock and it sinks too far into the muck and is smothered. " Based upon commonly finding certain Sphaeriidae on a sludge bottom, such a physical substrate may not deter the existence of certain species of this family. General observations, based upon reconnaissance of flocculent bottoms in rivers and streams polluted by domestic sewage, indicate that the Uniomdae do not seem to favor such areas.

In studies of erosion silt as a pollutant under laboratory conditions, Ellis (1936) found that certain mussels were unable to maintain themselves, in either sand or gravel bottoms, when a layer of silt from one-fourth to an inch in depth was allowed to accumulate over such, other conditions being favorable to survival. The yellow sand-shell, Lampsilis [illegible], a sand species, most readily succumbed; the species least readily killed were: Obliquaria [illegible], Q [illegible] and Q. metanevra.

Coker et. al. (1922) compiled data of various investigators on types of bottoms on which mussels were reported to be living. From his analysis of such data he concluded, "It appears that the preferred bottom for the majority of species is mud (but not deep, soft mud, to which type of bottom few species are adapted) and gravel, including sand and gravel. Sand ranks next and sandy clay last; but few species of mussels exhibit a preference for sand or sandy clay, and only two are recorded (by one observer) as finding the most favorable environment in a bottom of clay mixed with sand." Baker (1928), in writing about fresh-water clams of Wisconsin, discusses types of bottoms that mussels prefer: gravel, sand, mud, and clay; he says that they are common or abundant in the first three and rare in the latter. A shifting bottom, whether it consists of mud or sand, is stated to be usually devoid of mussels. Fine silt bottoms are always avoided by mussels, and Baker (1928) doubts if mussels could live in such a bottom environment. He states that mussels are usually absent or rare where great quantities of silt are carried into streams. Ingram (1948) reported Anodonta wahlamatensis by the thousands in the soft mud bottom of Stow Lake, San Francisco, California.

Many fresh-water snails are heavy enough to sink into the sludge covering stream bottoms to become buried and suffocate. The writer has observed areas of streams where sludge deposits were two to three feet in thickness, such as reaches of the M [illegible] below Warren and Youngstown, Ohio, where Physa [illegible] used higher aquatic plants as a substrate rather than the flocculent sludge deposits. In sludge filled sections of streams without higher aquatic plants, snails may be found on rock islands protruding from the sludge, and may be absent or rarely occur on sludge. In weight, adult fresh-water snails are much more comparable to the S [illegible] than to the U [illegible].

(4) Survival Relating to Type of Life Cycle

Of clams, the U [illegible] are especially vulnerable to pollution which may eliminate species by affecting larval stages. It is well known that after being released from the female the immature [illegible] stage of the U [illegible] must parasitize various fish in order to assure life cycle completion, L [illegible] and Curtis (1912), Coker et. al. (1922), van der S [illegible] (1938), and Jones (1950).

After spending from 10 to 14 days as an external fish parasite, the glochidium drops off the fish and continues its life as a free living form. If a fish is not parashtized, the glochidium dies. No information is available on the direct effects of pollution on the glochidium or on sperm cells which pass freely in water from male to female clam.

Because it is necessary that a part of any Unionid's life cycle be spent as a fish parasite, there is a direct relationship between the effects of pollution on fish and perpetuation of succeeding generations of Unionidae in any stream. If adult Unionidae are more resistant to various pollutional affects than fish, they may survive to die of old age, without succeeding generations developing to replace them. If glochidia-carrying fish are denied areas of streams by pollutants, expanded distribution of the Unionidae is hindered. A number of fish have been reported in the literature as carrying glochidia of various Unionidae, Coker et. al. (1922), Danglade (1922), Murphy (1942), Ingram (1948), Jones (1950). The following fish are examples of some that have been associated with glochidia, and are noted so that those working in water pollution might be aware of them if it is ever desired to correlate mussel-fish relationships relative to pollution: black bullhead, common bullhead, bowfin, eel, sheepshead, gizzard shad, mooneye, pike, spotted catfish, yellow catfish, long and short-nosed gars, red-ear sunfish, orange-spotted sunfish, blue-gill sunfish, small-mouth black bass, largemouth black bass, striped bass, river herring, yellow perch, white crappie, black crappie, sand sturgeon, madtom, sauger, and drum.

The Sphaeriid's sex cells are not subjected to any possible pollutional effect outside of the adult's body. They are hermaphrodites, fertilization is internal, and the young may be carried in the adult for as long as a year, Goodrich and van der Schalie (1944). The growth stage that leaves the parent to fend for itself is a small mirror-image of the adult. Such protected reproduction and shielding of the very young, when compared with the haphazard early life cycle stages of the Unionidae, should enhance survival of fingernail clams over mussels.

Gastropods that one would encounter in water pollution investigations copulate with resulting internal fertilization. Most lay eggs that are attached to submerged objects and, on occasion, to each other's shells; the Viviparidae are ovoviviparous. Thus, the eggs and very young stages of most are exposed to external changing environmental conditions at all times.

IV. NATURAL VARIATIONS IN DISTRIBUTION OF MOLLUSKS NOT RELATED TO POLLUTION

In studying the effects of pollution on bottom organisms, with

emphasis on mollusks, cognizance should be taken of natural phenomena affecting distribution not related to pollution. Normal variations in kinds, size, and abundance of mollusks, unrelated to pollution, make inventories of species of little value in pollution studies unless those interested in delineating indicator organisms include chemical, physical, and bacteriological descriptions of water quality so as to establish tolerances of mollusks to pollutants.

It has been shown by Baker (1918) that in lakes the numbers of molluscan species decrease with depth. In further writings about the increase of mollusk abundance in relation to depth, with reference only to mussels, Baker (1928) states that "The great majority of naiades live in comparatively shallow water from a foot to six feet in depth. More rarely they descendto depths as great as 25 feet. Records of fresh water mussels from greater depths than 25 feet are to be viewed with suspicion." Thus, in studying the effects of pollution on benthonic organisms in a lake, one should always be aware that paucity of a variety of mollusks may naturally be related to water depth and not to pollutional effects. In such studies chemical, physical, and bacteriological tests could be most important in presenting data to indicate whether a reduction of molluscan variety was a natural phenomenon of depth or whether it could be attributed to pollution.

In streams, it is known that Unionidae and Gastropods tend to increase in numbers of species from headwaters to the stream mouth, Goodrich and van der Schalie (1944), Baker (1928). For example, Baker (1928) lists on increase of Unionidae from three species upstream to 28 downstream in a 27 mile reach of the Big Vermilion River, Illinois. Certain pollution sources on the headwaters of a stream may be suspect in relation to a dearth of mollusks such as the Unionidae; however, a small number of species may represent a natural condition rather than a relationship to pollution.

There may be a greater number of species and individual gastropods living in stream areas where higher aquatic plants are present and usuable as a substrate in addition to the stream or lake bed. Thus, it is important to select stations to include sampling of higher aquatic plants in studies designed to provide data on indicator organisms. For example, in certain reaches of the Mahoning River, Ohio, in 1952, the writer made collections of bottom organisms in sludge deposits two to three feet in thickness and found no mollusks. In those reaches the river had a water temperature of 96 °F., pH of 4.1, and D. O. of 0.2 An initial conclusion from these meager chemical and physical analyses could have been that mollusks were unable to stand such conditions in this stream. However, Physa integra was present by the hundreds in various growth stages as well as in egg masses, using higher aquatic plants as a

substrate. Thus, some data were collected to show certain conditions under which P[illegible] can survive and carry out its life cycle. If higher plants present had not been searched for organisms, one might not have associated this snail at all with such a low pH or high temperature. On the basis of collections limited to the stream bottom, this [illegible] snail would have been associated only with waters having more favorable pH and temperature and with but little sludge.

V. IDENTIFICATION SOURCES FOR FRESH WATER MOLLUSKS

To assist those interested in the relationship of mollusks to water pollution, certain publications which may serve as examples of aids to their identification are cited. Also, certain museums having collections available for comparison of species or personnel that can assist in identification of specimens are presented. Much additional information relative to identification can be obtained by literature searches, or by consulting State and municipal museums and natural history societies.

A great deal of information concerning fresh water mollusks is contained in various numbers of "The Nautilus, " a quarterly journal devoted to the interests of [illegible]. This journal is edited by Dr. H. B. Baker of the University of Pennsylvania's Zoological laboratories, Philadelphia, Pennsylvania.

The foremost [illegible] housing collections of fresh water mollusks are: the United States National Museum, Washington, D. C., with Drs. Harald R[illegible] as Curator of Mollusks and Dr. J. P. E. Morrison as Associate Curator; the Academy of Natural Sciences of Philadelphia, Pennsylvania, with Dr. Henry A. P[illegible] as Curator of Mollusks and Dr. R. Tucker Abbott as Curator of the P[illegible] Chair of M[illegible]; Museum of Comparative Zoology of Harvard University, Cambridge, Massachusetts, with William J. Clench as Curator of Mollusks; Chicago Museum of Natural History, Chicago, Illinois, with Dr. Fritz Haas as Curator of Mollusks; Museum of Zoology, University of Michigan, Ann Arbor, Michigan, with Dr. Henry van der S[illegible] as Curator of Mollusks; California Academy of Sciences, San Francisco, California, with Dr. G. Dallas Hanna as Curator of Mollusks and Dr. Leo George H[illegible] as Associate Curator; and the Carnegie Museum, Pittsburgh, Pennsylvania. Smith's (1943) directory of [illegible] can be useful to those interested in having mollusks identified, because it lists in alphabetical order, [illegible] who are specialists in mollusk identifications.

The following are cited as examples of keys and faunal lists developed from studies limited geographically that can serve to provide species names as a base for specific identification in future studies of fresh water [illegible]: Baker (1922) on mollusks of the

Big Vermilion River, Illinois; Eddy and Hodson (1950) on mollusks of the North Central states; Goodrich and van der Schalie (1939) on mollusks of the upper peninsula of Michigan; Morrison (1932) on mollusks of the northeastern Wisconsin lake area; Strecker (1931) on the Unionidae of Texas; and a series of papers by van der Schalie on the Unionidae bearing the following dates from the stated geographical areas (1936) St. Joseph River, southwest Michigan; (1938b) Cahaba River in northern Alabama and (1938) Huron River in southeastern Michigan; (1940) Chipola River in northwestern Florida; (1948) commercially valuable mussels of the Grand River, Michigan, and (1950) of the Mississippi River.

Examples of regional fresh water mollusk identification guides are: Baker (1898) on clams of the Chicago area and (1902) on gastropods of the same area; Baker (1928) on clams and snails of Wisconsin; Chamberlin and Jones (1929) on the Mollusca of Utah; Goodrich (1932) on mollusca of Michigan; Goodrich and van der Schalie (1944) on mollusca of Indiana; Henderson (1924) (1936) on mollusca of Colorado, Utah, Montana, Idaho and Wyoming; Henderson (1929 and 1936a) on mollusca of Oregon and Washington; Ingram (1948) on the larger fresh water clams of California, Oregon and Washington; and Strecker (1935) on the mollusca of Texas.

The following are monographs, papers, and catalogues of varing degrees of comprehensiveness that deal with fresh water molluscan classification: Baker (1911) on the Lymnaeidae of North and Middle America, Berry (1943) on the Amnicolidae of Michigan; Brooks and Herrington (1944) on a preliminary survey of the Sphaeriidae; Ortman (1919), a monograph of the naiades of Pennsylvania; Pennak (1953), a key to the families and genera of North American fresh water mollusks; Sterki (1916), a catalogue of North American Sphaeriidae, and Walker (1918), a synopsis of fresh water mollusca of North America north of Mexico and (1918), keys to fresh water mollusca of the United States.

VI. SUMMARY

1. Presented literature affirms that little specific information is available relating one species of mollusk to a specific or over-all set of well-defined water quality conditions, and another species to different degrees of water quality.

No information is available to be used in selecting from mollusks any species that may be called a pollution indicator. Several species are tolerant to low dissolved oxygen or septic conditions resulting from pollution of water by domestic sewage. However, such species cannot be considered to indicate pollution, as related to septicity, for they are also found in umpolluted streams and lakes.

2. Most specific data available relating mollusks to certain effects of pollution on water quality deal with survival under septicity. Other such data relate to survival in relation to blanketing bottom deposits and acid water.

a. In relation to survival under conditions of septicity resulting from domestic sewage pollution, general conclusions that have been apparent for many years, can be reaffirmed here: certain Sphaeriidae are collected under such conditions while Unionidae are not; and certain pulmonate snails are commonly found in septic water, while gill-breathers typically are not.

b. In relation to survival on bottoms blanketed by sludge from domestic sewage and by silt, certain Sphaeriidae and Physidae are most often collected.

3. Information on hydrogen ion concentration is presented indicating that a number of genera of mollusks may live naturally in acid water conditions not associated with pollutants.

4. Variations in molluscan structure and types of life cycle are discussed, as such variations may result in enhancing survival under pollutional conditions relating to dissolved oxygen, sludge and silt deposits.

5. Natural variations in the distribution of mollusks in streams and lakes are considered in order to make those working in water pollution aware that variations in numbers of species may be a natural phenomenon not related to pollution.

6. Selected museums and personnel as well as mollusk guides and lists are presented to assist those who are not authorities on molluscan taxonomy with identification.

VII. ACKNOWLEDGMENTS

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THE USE AND VALUE OF AQUATIC INSECTS AS INDICATORS OF ORGANIC ENRICHMENT

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A knowledge of the ecological requirements of aquatic organisms is of outstanding importance in judging the extent of pollution due to organic enrichment in our streams. The species composition of the aquatic population in a given area is determined by the environmental conditions which have prevailed during the developmental period of the organisms involved. If at any time during its development, environmental conditions become lethal for a given organism, that organism will be eliminated even though the unfavorable conditions are of very short duration. The aquatic population which occurs in a given area is, therefore, a representation or indicator of environmental conditions which have prevailed during the life history of the organisms comprising the population.

It is this property of indicating past environmental conditions, especially the extreme conditions of brief duration, that make macro-invertebrates such valuable indicators of pollution. Most representatives of the group have longer life histories than the micro-benthic fauna and are thus better fitted for indicating past conditions than are the latter organisms. In addition, the larger size and the more distinctive morphological characteristics of the macro-invertebrates make them easier to identify under field conditions.

The interpretation of stream conditions based on the biota present has been used for many years. Kolkwitz and Marsson (1908-1909) first proposed the use of aquatic organisms as indicators of the ecological conditions under which they exist. They classified organisms as oligosaprobic, mesosaprobic, and polysaprobic, depending on the concentration of decomposable organic matter in the streams under consideration. Richardson, (1921, 1928), based upon his studies of the Illinois River, developed a classification of bottom organisms using seven groups of species. These included a pollutional group, three sub-pollutional groups, an air breathing group, current loving species other than pulmonate snails and air breathing insects and clean water species. Patrick (1949) presented a comprehensive method involving the use of histograms to show the response of certain groups of aquatic organisms to given environmental conditions. This method was modified and made more applicable to macro-invertebrates by Wurtz (1955).

A number of writers have published shorter works cortaining many valuable ideas and suggestions Bartsch (1948) presented information on the response of the biota to various degrees of pollution. Gaufin and Tarzwell (1952) employed both indicator species and associations in utilizing aquatic invertebrates as indicators of pollution. Schiffman (1953) presented a very useful method of cataloging stream bottom organisms found in Illinois

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with respect to their pollutional tolerance. Beck (1954) contributed a valuable survey method in his simplified ecological classification of aquatic invertebrates found in Florida streams.

In satisfactorily using macro-invertebrates as indicators of organic enrichment and its effects, certain criteria should always be kept in mind. First, several factors other than the presence of a pollutant may limit the distribution of certain species, as for example, the type of bottom, speed of current, lack of certain nutrients, scouring floods, excessive turbidity, and the flight range. Second, the mode of occurrence of the forms considered is just as important as their presence or absence in a given area. For example, most of the macro-invertebrates which characteristically occur in large numbers in heavily enriched water may also be found in limited numbers in cleaner situations. Such forms as the mosquito, Culex pipiens, leech, Macrobdella, and sludgeworms, Tubifex and Limnodrilus often occur in the quieter confines of clean water streams in limited numbers, but they reach far greater numbers in waters polluted by organic wastes. When conditions are favorable for those organisms which can adapt to such conditions, they thrive and build large populations In some instances of organic pollution very often the important factor in determining the occurrence of certain forms is the abundant food supply which favors their growth and numerical increase rather than a deficiency of some material such as dissolved oxygen. Similarly those forms which are most characteristic of clean water conditions, such as mayflies, stoneflies, or caddis flies are occasionally found during winter in stream sections which are highly polluted or septic in summer. When these insects drift into such a stream from nearby tributaries they may live for considerable periods of time because the septic zone of summer often has an adequate dissolved oxygen supply during winter. Thus, before such isolated examples are taken as evidence that the forms involved are tolerant of such environmental conditions, an investigation as to their source and abundance in the area is advisable.

In arriving at a better definition of the habitat preference and indicator significance of the various groups of macro-invertebrates consideration of the structural and physiological adaptations of the organisms is very important.

While there are exceptions, in general, an association of mayflies, stoneflies, and caddis flies in a stream is indicative of clean water conditions, and their absence often denotes a super abundance of organic wastes and/or a low oxygen supply if the physical nature of the habitat is otherwise suitable. Usually the presence or absence of representatives of other orders of aquatic insects which breathe by gills, and which are, therefore, dependent upon oxygen dissolved in the water for their respiratory needs, has similar indicator significance. For example, while most aquatic beetles can renew their oxygen supply directly frorn the atmosphere and are thus unaffected by oxygen-depleting wastes, the larvae, pupae, and adults of those species which are entirely aquatic, are dependent upon dissolved oxygen and are restricted to clean water streams which are well aerated.

In Ohio several species of riffle beetles such as S[illegible] and S[illegible] sexlineata, are found only in the cleanest streams. Their distribution in such a habitat indicates that the family, E[illegible], to which they belong, is a member of the clean water association.

While most gill bearing aquatic insects are limited in their distribution by low dissolved oxygen supplies, some forms which have more than one means of respiration, such as the dragonflies and damselflies, display considerable tolerance to low levels of dissolved oxygen. Their greater adaptability to environments low in dissolved oxygen is made possible by the possession of respiratory structures which are the most highly developed of the various gill systems. These insects can carry on respiration by means of four different structures; namely, (1) caudal tracheal gills; (2) rectal folds; (3) the integument; and (4) spiracles. Since all four organs may function at the same time and many of the stream forms occur in either riffles or shallow marginal areas, the group is remarkably well adapted to withstand the oxygen depleting effects of organic pollution. As a result of this adaptability, the nymphs of both dragonflies and damselflies were often taken by the author in Lytle Creek, Ohio, in sections of the recovery zone where the dissolved oxygen supply in summer was as low as 1.0 [illegible] for a short time during the night or in the early morning hours.

In streams receiving large amounts of organic wastes insects of the orders H[illegible], C[illegible], and D[illegible] have the most varied representation, are the most widespread in their distribution and are least affected by dissolved oxygen concentration. Representatives of these orders may be found in all stream habitats representing all degrees of environmental modification and stream recovery. Some species from each group may be found fairly widely distributed throughout the stream; others while not restricted to either a clean water or "polluted" area, may show by their abundance a strong preference for one or the other type of habitat. Still other species, particularly among the D[illegible], may be restricted to clean water or to water rich in organic materials.

Of the three orders, the H[illegible] and C[illegible] are the poorest indicators of organic enrichment and oxygen depletion in the stream. With the exception of the E[illegible], or riffle beetles, other species of beetles and all of the species of water bugs may be found throughout a stream usually occurring most abundantly in the "polluted" areas where they may find an abundant food supply. The ability of members of these two orders to withstand the oxygen-depleting effects of organic pollution is due to special modifications of their tracheal system. These modifications serve to increase the internal air capacity of the tracheal system, supplement tracheal diffusion by ventilation movements when the insects come to the surface for air, and provide supplementary external air stores. Common to all of these forms are the modification of the body surface for breaking the water surface film, and changes in the wings and body surface for capturing and holding stores of air, and in the tracheal system for surface ventilation and connection with the external air stores. In oxygen-

deficient waters members of these two group have only to increase the frequency of their visits to the surface to cope with decreasing oxygen supplies.

The efficiency of these modifications for obtaining and storing atmospheric oxygen is well illustrated by Dytiscus, one of the diving beetles. K D. Roeder (1953) reports that this beetle can remain submerged for 36 hours without coming to the surface to renew its oxygen supply. Dytiscus, to obtain a supply of oxygen, breaks the surface periodically with hydrofuge hairs and ventilates violently by means of accessory respiratory muscles. The fore wings, elytra, have a locking mechanism to trap the atmospheric air and the abdominal and thoracic spiracles are displaced so as to open into this respiratory air store.

Aquatic Diptera may be found in a str.2am in many different ecological niches in both the clean water and other life zones. However, with the exception of only a few species, representatives of this order are highly selective in their choice of habitat. A number of species such as Diamesa nivoriunda Cricotopus absurdus, and Calopsectra neoflavella, are found only in the cleanest, most highly aerated sections of a stream, while others such as the mosquito, Culex pipiens, and rattail maggot, Eristalis bastardi, while found in limited numbers in water areas, show a decide ference for the organically enriched sections The variability in their choice of habitat and in their range of distribution is determined largely by the food-getting and respiratory requirements and adaptations of the different individual species. The larvae and pupae of the mosquito and rattail maggot, w'ith their special respiratory tubes, are unaffected by low oxygen supplies as evidenced by the extremely large number of each that may be taken in the most septic areas Certain redblooded Chironomids, such as Chironomus riparius, also demonstrate a remarkable ability to thrive in the septic and recovery zones, Walshe (1950) has shown that the hemoglobin possessed by midge larvae such as Chironomus riparius, Chironomus plumosus, and closely related sPecies apparently acts in both the transportation and storage of oxygen. Its greatest transport role is during anaerobiosis, when it permits the larva to continue filter feeding in low oxygen tensions and thereby increases the rate of recovery from exposure to such conditions.

As in the case of the insects, the other groups of macro-invertebrates show considerable variation in their distribution and adaptability to varying environmental conditions due to the breakdown of organic matter. Certain groups such as the sludge worms, Tubificidae, may be found in very large numbers in bottom sludges of high organic content in the lower end of the septic zone and the upper end of the recovery zone. Their numbers decrease rapidly as the nature of the bottom sediments change. The ability of two genera of these worms, Tubifex and Limnodrilus, to utilize the rich supply of organic mater al under practically anaerobic conditions, make them important and conspicuous members of one of the most easily recognized communities ci-aracteristic of streams receiving organic wastes.

Summary

In order to utilize macro-invertebrates as indicators of environmental conditions in streams, it is essential to have a knowledge of the species composition and abundance of the various organisms in the populations involved, under the various ecologic conditions which prevail in clean and organically enriched waters. Clean waters, with some exceptions, are characterized by a great variety of invertebrates consisting of herbivores, carnivores, and omnivores, prey and predators, lung, tracheal tube, and gill breathers. In general a population containing numerous gill breathing forms as mayflies, stoneflies , and caddis flies is indicative of clean water conditions and their absence denotes the super abundence of organic materials and/or low oxygen.

By contrast, associations engaged in the utilization of excess organic materials are characterized by few species but large numbers of individuals. The association of organisms normally present under the most septic conditions consists largely of scavengers with few plant and animal eaters. The macro-invertebrates most characteristic of septic zones are those which can exist under conditions of very low oxygen or have adaptations for breathing atmospheric oxygen.

Thus by reference to the qualitative and quantitative composition of an aquatic population as an index of water quality, it is possible to delineate the life zones in a polluted stream.

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BIOLOGICAL INDICES OF WATER POLLUTION, WITH SPECIAL REFERENCE TO FISH POPULATIONS[1]

P[illegible] D[illegible]
U. S. P[illegible] H[illegible] S[illegible]

C[illegible] E. W[illegible]
D[illegible] F[illegible] G[illegible] M[illegible]
O[illegible] S[illegible] C[illegible], C[illegible], O[illegible]

A[illegible] (1) (2) (7) (13) (14) (15) (16) (26) (27) (28) (29) (30) (36) (38). F[illegible] (12) [illegible] E[illegible]. T[illegible] "[illegible]" [illegible] (12). B[illegible] P[illegible] (26) (27) (28) (29) (30) [illegible] U[illegible] S[illegible].

A[illegible] I[illegible] "[illegible]". I[illegible] "[illegible]". S[illegible] "[illegible]". I[illegible]

1/ M[illegible] P[illegible] N[illegible]. 31, O[illegible] A[illegible] E[illegible] S[illegible].

it is that the indicators can be expected to indicate. Too often this has not been done, or the ideas and definitions presented have not been carefully developed and appear to be unsound from a practical standpoint.

Should the mere change (physical, chemical, or biological) of some aquatic environment resulting from waste disposal be regarded as pollution even when ordinary human use and enjoyment of the water and of associated natural resources have not been affected adversely? When there is evidence of environmental change, is this always reliable evidence of damage to a valuable natural resource? May not certain beneficial uses of water be sometimes seriously interfered with by the introduction of wastes which may cause little or no detectable alteration of biological communities? Have there been any studies which have conclusively demonstrated a useable fixed relation between the biological indices of pollution and the actual fate or change in value of aquatic resources which are subject to damage by pollution? If water pollution can be the result of introduction of any of a great variety of substances, organic and inorganic, is it proper to refer to those biotic responses which are only known to occur in the presence of putrescible organic wastes (i. e. to organic enrichment of water) as "indices of pollution"? Can there be any general biological solution for all problems of detection and measurement of water pollution, or is effort being wasted in a search for such a general solution? Are broad limnological investigations being undertaken where intensive study and appraisal of supposedly damaged natural resources of obvious value to man would be more profitable? Is immediate practical value of research results being claimed improperly in an effort to justify fundamental limnological studies for which no such justification should be necessary? These are questions which all biologists interested in water pollution should perhaps ask themselves. Many of these questions have no categorical answer, but it is hoped that the following discussion will prove thought-provoking. It may not only call attention to certain inconsistencies in claims made and terminology used, but may also indicate the need for revision of objectives or a change of emphasis in pertinent future investigations.

Biological investigation now is an integral part of water pollution detection and control, and biologists have become increasingly aware of their opportunities for contributing to progress in this field of work. Their ideas have been solicited and have been well received by other specialists. In trying to aid the advancement of their science, biologists owe it to their profession to seek thorough understanding of the practical problems of water pollution control. Understanding the complexity of these problems will make apparent the need for thorough and critical testing of new ideas previous to their widespread practical application.

First, it is necessary to consider the meaning of the term "pollution". The introduction of any foreign substance which merely alters the natural quality of water without materially interfering with any likely use of the water cannot be said in a practical sense to constitute pollution. Virtually every stream and lake in any inhabited region receives at least a trace of something which measurably or not measurably alters the natural quality of the water. What is significant or important from a practical standpoint is not the mere presence of the added material, but its influence upon the economic and esthetic value of the water, or on human welfare in a broad sense. It appears that most authorities in the field of water pollution control and abatement agree in defining water pollution as an impairment of the suitability of water for any beneficial human use, actual or potential, by any foreign material added thereto.

This definition agrees with repeatedly expressed judicial opinion, that is, with definitions of "pollution" and of "clean water" established by courts of law. The following legal definition, cited on page 100 of "Water Quality Criteria", a publication of the California State Water Pollution Control Board (4) is typical: "For the purposes of this case, the word 'pollution' means an impairment, with attendant injury, to the use of water that plaintiffs are entitled to make. Unless the introduction of extraneous matter so unfavorably affects such use, the condition created is short of pollution. In reality, the thing forbidden is the injury. The quantity introduced is immaterial. " Other definitions cited agree essentially with this one.

In accordance with the above definition of the word pollution, a demonstrable change of some components of the biota of a stream clearly caused by the discharge of some waste into the water is not invariably evidence of pollution, any more than is a demonstrable chemical change. If it cannot be reasonably asserted that a hazard to human health or interference with some beneficial use of the stream, such as fishing, must accompany a particular alteration of the biota, the change cannot correctly be said to indicate pollution. Even the discharge of a waste which eliminates virtually all organisms initially present in a very small or temporary stream capable of supporting no aquatic life of any value to man is not necessarily pollution. Oxygen-depleting organic wastes maybe thoroughly mineralized in such streams through natural self-purification processes, so that only harmless substances and beneficial plant nutrients may reach larger watercourses to which these streams are tributary.

In agreement with the definition offered above, Beck (1) has defined pollution broadly as "the alteration of any body of water, by man, to such a degree that said body of water loses any of its value as a natural resource. "

Patrick (28), on the other hand, has proposed a distinctly different, strictly biological definition. This author defines pollution as "any thing which brings about a reduction in the diversity of aquatic life and eventually destroys the balance of life in a stream. " By way of explanation, it is further stated that "As conservationists interested in using rivers today - but not abusing them so that they are damaged in the future - this is the basis on which pollution should be judged. For it is by preserving the biodynamic cycle that the ability of a river to rejuvenate itself is maintained.

Unfortunately it is not clear just what is to be regarded as pollution according to the definition given by Patrick. Is any reduction in the diversity of aquatic life evidence of pollution which will eventually destroy the "balance of life", or only such a severe reduction of the diversity of life that the ability of the stream to "rejuvenate itself" is indeed destroyed? A reduction of species numbers is not always necessarily followed by the eventual destruction of the "balance of life" in a stream and of the ability of the stream to "rejuvenate itself" (i. e., to undergo natural self-purification). Patrick (28) has pointed out that the so-called "food chain" in aquatic environments "consists of many series of interlocking links so that if one series is broken another can take over so that the chain is not destroyed." It is well known, also, that in certain "zones" of streams heavily and continually enriched with organic wastes relatively few animal and plant species are present, as a rule, yet natural purification proceeds at a very rapid rate. Here, as in an efficient trickling filter, an ideally adapted and obviously vigorous, healthy, and in certain respects very well balanced biota of limited variety can exist, and the organic waste is mineralized far more rapidly and efficiently than it could possibly be in a previously uncontaminated stream with its original, primitive biota. The ability of the stream to "rejuvenate itself" certainly cannot be said to have been destroyed, or even impaired.

Thus, a stream can be seriously polluted, in any usual sense of the word, without lasting destruction of the "balance of life" and of self-purification capacity (which balance hardly can be permanent anyway, in any unstable environment). On the other hand, mere reduction of the diversity of aquatic life without impairment of any important "food chain" (i. e., the food supply of valuable fishes, etc.), or interference with existing stream uses, does not necessarily have anything to do with the conservation of natural resources. It appears, therefore, that the last-mentioned definition of pollution is unsatisfactory, from a practical standpoint, no matter how it was meant to be interpreted.

Careful consideration of the other pertinent writings of Patrick and of the proposed method of judging stream conditions leads to the conclusion that probably this author regards any marked reduction of the diversity of aquatic life as evidence of pollution.

B[illegible] (1) [illegible] "P[illegible]" [illegible] -[illegible]," [illegible] "[illegible] -[illegible] P[illegible]." A[illegible] P[illegible] "[illegible]" [illegible] primitive [illegible]. A[illegible] -[illegible] ", [illegible]. T[illegible] P[illegible] [illegible]. I[illegible], [illegible] [illegible]. I[illegible] P[illegible] [illegible], [illegible] [illegible]. P[illegible] [illegible].

O[illegible] [illegible]. T[illegible] [illegible]. A[illegible], [illegible] [illegible]. T[illegible] [illegible], [illegible] [illegible]. N[illegible] [illegible]. O[illegible] [illegible] (i.e., [illegible]). T[illegible] [illegible]. I[illegible] [illegible] (i.e., [illegible], [illegible]) [illegible] valuable [illegible]. T[illegible] [illegible].

A [illegible] T [illegible] Y [illegible] W [illegible]

I [illegible] reveal [illegible] pollutional [illegible] water. W [illegible] O [illegible] I [illegible] and [illegible] pollutional [illegible] A [illegible] I [illegible] A [illegible] applicable [illegible] ?

B [illegible] E [illegible] (23) (24) (31) [illegible] pollution [illegible] I [illegible] "[illegible]" [illegible] justifiable. U [illegible]

was by far the most important and perhaps the only well known and generally recognized water pollutant. Its discharge into public waters in amounts sufficient to bring about appreciable biotic changes being usually a hazard to human health, it was and is almost always pollution in any ordinary sense of the word. Today, the importance of pollutants other than domestic sewage is generally recognized. Yet, many authors still speak of "pollution indicators" when they actually are referring only to indicators of organic enrichment of water with organic wastes, which may or may not involve demonstrable damage to natural resources. Some readers are known to have been misled by this terminology, believing that the same biological indices are useful in detecting every kind of pollution.

G and T (13), when reporting their studies of stream pollution with domestic sewage, obviously were considering the effects on aquatic life of an oxygen-depleting organic waste only. Nevertheless, such unqualified and seemingly general statements as their conclusion that "P associations are characterized by few species but large numbers of individuals" can be misleading. As the quoted authors well know, the numbers of many organisms initially present are reduced and the numbers of none are markedly increased in some waters polluted with toxic wastes, suspended solids such as silt, or even oxygen-depleting organic wastes discharged intermittently. These authors undoubtedly did not intend the conclusion in question to be a very broad generalization from their observational results having to do with one kind of pollution only. Their use of the expression " associations" for designating associations found in waters polluted with domestic sewage, or in waters enriched with organic matter, can be excused on the that no term that is more appropriate than the term " " has come into general use in the biological literature. Yet, this lack of a more precise terminology is not any less deplorable because the use of inappropriate terms, and terms which are not sufficiently specific, has become prevalent.

Beck (1) (2) explicitly confines his discuss the subject of "organic pollution". He has proposed the use of a numerical "biotic index", which is said to be "indicative of the cleanliness (with regard to organic pollution) of a portion of a stream or lake" (2). He recognizes that his methods are "confined to fresh waters and encroaching salinity has a marked effect on the fauna of a stream. " Inasmuch as many different pollutants, including toxic constituents of some organic wastes, likewise can have a marked effect on the fauna of a stream, it is apparent that Beck's methods may have only very limited applicability. It may be usable only in connection with the investigation and description of waters known in advance to contain no pollutants other than non-toxic organic matter.

Patrick (26) (27) (28), recognizing the importance of a variety of pollutants, apparently has attempted to devise a general procedure for the reliable biological detection and measurement of the different kinds of pollution. For reasons already indicated, however, this desirable objective appears to be attainable only when one defines pollution as "any thing which brings about a reduction in the diversity of aquatic life", which is not a generally acceptable definition.

Wurtz (381, while evidently realizing the existence and importance of a large variety of pollutants, seems to overlook completely the important differences of biotic responses to the different pollutants. Thus, his Figure 1 suggests that the same pollutional zones, including a "degradation zone" extending from the point of mixing of an effluent with the water of a stream to a "polluted zone" located some distance downstream, can be expected to occur in any heavily polluted stream, regardless of the nature of the pollutant (i. e., whether it be "organic", "toxic", or "physical"). Furthermore, he speaks of "pollution tolerant species" and of "non-tolerant organisms", suggesting that organisms are consistentely tolerant or consistently non-tolerant with respect to all pollutants. Nowhere does he specify that he has in mind resistance to putrescible organic pollutants only, and there is considerable evidence that he has in mind all pollutants. In large degree, Wurtz seems to have adopted methods similar to Patrick's, but one of his innovations seems to require the probably impossible classification of all or nearly all aquatic organisms as "tolerant" and "non-tolerant" to all kinds of pollution, including the various toxicants, etc. Unfortunately, Wurtz does not include in his paper a list of all organisms considered by him to be tolerant and all those thought to be non-tolerant.

There can be no doubt that some of the so-called "pollution-tolerant" organisms, which actually are simply forms known to thrive in waters markedly enriched with organic wastes, are less tolerant with respect to some other water pollutants than a number of the species known as "clean-water" forms. For example, a species of Physa, a genus of snails generally believed to be resistant to organic pollution (1) has been found to be extremely susceptible to dissolved copper. Certain fish (e.g., centrarchids), may fly nymphs, etc., thought to be more susceptible than Physa to the effects of organic pollution, proved much more resistant to copper. An aquatic environment in which "clean-water" organisms are predominant might possibly be more seriously polluted than one with a decidedly "pollutional" biota. The biological terminiology evidently needs revision, so that the word pollution would not be used synonymously with organic enrichment.

It appears that, in general, very broad significance of the various biological indices of water quality and the severity of pollution has been only assumed and not actually demonstrated. This is well exemplified by the following quotation from the summary of one of Patrick's papers

(27): "On the premise that the balanced physiological activities of aquatic life in surface waters are essential for the maintenance of healthy water conditions, it may be assumed that the most direct measure of this biodynamic cycle will indicate the condition of the water." It will be noted that we have here an assumption based upon a rather nebulous premise. Most writers have failed to supply entirely satisfactory, clear definitions of terms used (e.g., "pollution", "health", etc.) to show precisely what it is that they believe they can detect or measure biologically. Others have failed to use defined terms in a manner entirely consistent with their own definitions. The need for demonstration of the validity of some of the most fundamental assumptions concerning the reliability of pollution indices designed for general application has not been satisfied. Some authors seem to be of the opinion that the proof is unnecessary. It must be admitted that investigations designed to provide such proof would be extremely complex and difficult, and it is not likely that the search for this proof would be very rewarding, for there can hardly be a simple, general solution for the problem of pollution detection and measurement. Like a panacea, a general test for all kinds of pollutional damage is something for which biologists and engineers alike probably would be wise not to seek.

The value of fish as indicators of environment conditions and the importance of fish population studies in connection with the estimation of the intensity of water pollution now can be considered. Doubtless there is much more published information on the environmental requirements of fish than on the requirements of species of any other group of aquatic organisms excepting perhaps a few invertebrate species of outstanding economic importance. The vast quantity of published data relating to the water quality requirements of fish is partly revealed by a few recently prepared compilations and summaries of some of this information (4) (5) (8) (9) (10) (11) (17) (33). The resistance of many fish species to extreme temperatures, to unusual concentrations of dissolved oxygen and other dissolved gases, to variations of water salinity, and to extremes of pH, their susceptibility to the harmful effects of a great variety of toxic substances and of suspended solids of importance as water pollutants, the influence of some of these environmental factors upon embryonic development, growth, and activity, and so forth, have all been studied intensively. There exists also a voluminous literature on the food of fishes, their life history and reproductive requirements, their habitat preferences, movements, avoidance of adverse environmental conditions, and so on.

While it is evident that more is known of the environmental requirements of many fish than is known of the requirements of most, if not all, of the other aquatic organisms often considered as indicators of environmental conditions, the use of fish as indicators has received considerably less attention than has the use of other major groups, plant and animal, microscopic and macroscopic. Fisheries workers recognize

the difficulty of adequately sampling fish populations even in bodies of water of moderate size, and this, along with the mobility of fishes, has been advanced as a reason for the unsuitability of fish as indicators of environmental conditions. But, other aquatic groups are difficult to sample too, as Needham and U [illegible] (25) have demonstrated in the case of the invertebrate [illegible] of a riffle. The difficulty of sampling and the mobility of fishes may not be the chief reasons why fish have not been given more consideration as indicators. The taxonomic groups which have received the most attention no doubt have reflected to some extent the special interests of investigators who happened to be working in the field of water pollution. Fish being the usual economic and recreational yield of stream productivity, their study has obvious applied value and so has required no additional justification. Further, the status of a fish population may indicate suitable or unsuitable environmental conditions, but when knowledge of this population is the end or aim of an investigation, the population status is not regarded as an index of anything else. The value of fish as indicators of the suitability of water for uses other than fishing has not been clearly demonstrated. Whatever the reasons may be, the emphasis in most discussions of the "biological indices" has been on groups other than fish, even though very little is known of the environmental requirements of the species of many of these groups.

The value of knowledge of fish populations in connection with the classification of aquatic environments has not been entirely overlooked. Ricker (32) made important use of the brook trout (S [illegible]) and the Centrarchidae and E [illegible] as a basis for his ecological classification of certain Ontario streams. Fisheries workers frequently use such expressions as "trout waters" or "bass waters", thus conveniently classifying waters according to the fish species for which the waters are well suited. European workers have made more formal use of such a system of stream classification (34) (37). B [illegible] and K [illegible] (3) have classified waters and named various [illegible] "zones" of streams in the Ohio River drainage basin according to the kinds of fish populations found therein. As has been done with other animals [illegible], some species of fish have been classified as to their "[illegible]" preferences by a few authors (22) (24) (19) (35). The basis for such classification of fish is highly questionable. Patrick (26) (27) includes fish among the groups considered in her "biological measure" of stream conditions. D [illegible] (7) and G [illegible] and T [illegible] (14) have emphasized the need for thorough fish population studies in connection with water pollution investigations and the determination of the [illegible] status of waters.

Studies of fish populations in variously polluted waters, which reveal varying susceptibility of different fish species to [illegible] conditions in their natural habitats, have been reported by a number of investigators (3) (6) (11) (20). However, sufficiently intensive sampling of fish populations has not often been undertaken in connection with routine pollution

surveys and investigations, the sampling of other aquatic life having been probably more often emphasized when the scope of the biological studies has had to be limited. Inasmuch as it is not often possible adequately to study all of the aquatic biota, including the fish, the practical value of information to be obtained by concentrating attention on fish populations must be carefully weighed against that of information to be derived from equally intensive study of some of the other aquatic organisms, and from comparatively superficial study of the entire biota.

The absence or extreme scarcity of some fish in a stream below the point of entry of a waste, and not above the point of entry, strongly suggests that the waste is somehow detrimental to these fish. If valuable food and game fish species are among those believed to be adversely affected, pollution is indicated. Neither the presence nor the absence of fish is a reliable indication of suitability or unsuitability of water for domestic, agricultural, and industrial uses and for recreational uses other than fishing. Nevertheless, because of the great economic and recreational value of many fish species, this information is essential to sound classification of waters according to their pollutional status.

The presence of fish does not necessarily show that their environment has been suitable for them for a very long time, nor that the species found can survive indefinitely and complete their life cycles under the existing environmental conditions. However, the presence of thriving populations of non-migratory species, including numerous representatives of different age classes whose growth rates have not been subnormal, is significant. It suggests strongly that pollution which is highly detrimental to these fishes and to migratory species whose habitat preferences, natural food, and water quality requirements are quite similar has not occurred recently. Far example, the presence of numerous cottids in Northwestern salmon and trout streams which receive organic wastes is believed to indicate that dissolved oxygen concentrations have been adequate for some time and other environmental conditions probably have been suitable not only for the cottids, but also for migratory salmon and trout. There is now no sound reason for believing that the presence of any invertebrate form is a more reliable and appropriate biological indicator of the suitability of past environmental conditions for the migratory salmonids than is the presence of cottids.

The value of waters used for fishing, and of the fisheries which they support, bears no fixed, direct relation to the number of fish species to be found therein, just as it bears no such relation to the number of species of other organisms present. Some 35 species of fish were collected in the Midwestern warm-water stream studied by Katz and Gaufin (20). Because of the scarcity of valuable food and game fishes, this small, polluted stream is not regarded as a valuable fishing stream. On the other hand, many cool, pure streams which are highly valued as trout and salmon streams contain very few fish species other than the salmonids. Indeed, the invasion of valuable trout waters by other fish

[illegible]

S[illegible] (18). Warm, [illegible] ".

S[illegible] P[illegible] (26) (27) [illegible] C[illegible] R[illegible] B[illegible] P[illegible] "T[illegible] present." I[illegible] based. I[illegible] P[illegible] "[illegible]". I[illegible] collected [illegible]. I[illegible] ("[illegible]") [illegible] D[illegible] (e.g., [illegible] No. 152, [illegible]) [illegible] "[illegible]" [illegible] examined. I[illegible] (e.g., [illegible] No. 152, [illegible]) [illegible] "[illegible]" [illegible] etc. O[illegible] "healthy" [illegible] BOD, [illegible] CO_2 [illegible]. T[illegible].

W[illegible] arying [illegible] organisms, [illegible]. I[illegible] discharge [illegible] (21), [illegible] (20). A[illegible]. I[illegible]

[illegible]

[illegible] determined. [illegible] pollutional [illegible]

[illegible] 1952, [illegible] discharge [illegible] 293 [illegible] 822 [illegible] 0 [illegible] 22.3 [illegible] 5- [illegible] 60 [illegible] 575 [illegible]

T[illegible] T[illegible] seasons[illegible]. S[illegible] studied, [illegible] only [illegible] phytoplankton, zooplankton, [illegible], [illegible] physical, [illegible], [illegible] river. T[illegible] [illegible].

T[illegible] T[illegible] R[illegible] realized, [illegible], [illegible] instance, had [illegible] T[illegible], [illegible] zooplankton, [illegible] ? O[illegible] fisheries, [illegible], T[illegible]. T[illegible], [illegible] [illegible].

T[illegible] T[illegible] R[illegible] of [illegible] "pollutional" [illegible] and [illegible], B[illegible], [illegible] "clean-water" [illegible] summer, [illegible] outfall, [illegible]. A [illegible] place, [illegible], W[illegible] pollution, [illegible] -S[illegible] 10 [illegible] 12 [illegible]. T[illegible] wastes, [illegible].

C[illegible] (Lepomis macrochirus) [illegible] S[illegible] O-[illegible]. T[illegible] 1-year [illegible], T[illegible] O-[illegible] -[illegible],

W[illegible] interesting, [illegible] Tuolumne [illegible]. S[illegible]

of adult chinook salmon (Oncorhynchus tshawytscha), though the downstream migrant young were presumably unaffected, being apparently absent from the Tuolumne by the time of critical summer river flows and waste discriarges. Juvenile shad may perhaps have been affected also. Had the principal objective of the Tuolumne River investigation been an evaluation of damage to fisheries resources by pollution, the study could not have been deemed complete in the absence of conclusive evidence that interference with salmon migrations and other possible damage to valuable fish populations had or had not occurred. None of the proposed "biological measures" of pollution intensity could have revealed the degree of such interference or damage. In order to obtain the crucial evidence required, it would have been necessary to emphasize the fisheries phase of the investigation.

It is not the purpose of this paper to discourage limnological research pertinent to water pollution problems, nor is it intended to deny the value of all biological indicators of pollution. There can be no doubt that a drastic modification of any natural aquatic biota, attributable to a change of water quality, can have highly undesirable aspects or consequences. Such changes presumably are detrimental to human use and enjoyment of natural waters more often than they are not. Many a readily demonstrable effect of wastes upon aquatic life in a valuable stream is suggestive of probable existing or incipient pollution which deserves close attention and investigation. Even before valuable fish populations have been materially affected by some potentially harmful pollutant, an observed detrimental effect upon other organisms which are somewhat more susceptible than fish may give warning of possible future damage to fisheries by continued or additional waste discharges. The nature and the source of existing or incipient pollution also may be revealed by appropriate biological indices. Finally, inasmuch as some of the organisms considered to be indicators of pollution are organisms which can directly interfere with human use or enjoyment of waters (e. g., unsightly slime-forming organisms such as Sphaerotilus, odor-producing algae, etc.), their unusual abundance may not be disregarded in evaluating over-all damage caused by pollution.

CONCLUSIONS

It must be concluded that every change or peculiarity of the flora and fauna of a stream which has been referred to as an index or measure of pollution is in reality only an index of environmental disturbance or environmental anomaly. The disturbance or anomaly indicated may or may not be pollutional in the sense that stream uses are interfered with

Pollution (i.e., interference with stream uses) can be negligible when the effect on the aquatic biota as a whole is great, and it can be severe when most of the aquatic life is unaffected. Gross pollution often can be demonstrated without any biological investigation. When biological investigation may be necessary, [illegible] damage to valuable aquatic organisms can probably best be determined by concentrating attention upon these particular organisms. Yet, since all aquatic life forms are more or less sensitive to changes of water quality, the fate of any of them theoretically can be instructive, revealing something about the nature and magnitude of these changes that may not be obvious nor easily determined otherwise

A genuine contribution to water pollution science can be made whenever the presence or relative abundance of living organisms of any kind can be shown to be a reliable index of something tangible that one may need to know in order fully to ascertain and understand the [illegible] status of an aquatic environment. When proposing and describing the use of such biological indices, one should state specifically what it is that each is believed to indicate, carefully avoiding such general, vague, or abstract terms as "pollution" and "stream health", which may be variously understood. Does it indicate, for example, continual presence of dissolved oxygen in certain concentrations believed to be adequate for sensitive fish species? Does it indicate organic enrichment likely to interfere in some way other than through oxygen depletion with certain specific uses of water? Or does it indicate that particular toxic substances have not recently been present in concentrations likely to be injurious to fish, to man, or to certain crops? No simple biological indicator and no one measure of stream conditions can indicate all of these things. But any species can become a biological indicator of environmental conditions of possible interest as soon as its nutritional and other environmental requirements, its relative resistance to various toxic substances, etc., become known. Widely distributed sessile or sedentary organisms should be the most useful indicators of past conditions. Unfortunately, the water quality requirements of most of the "indicator organisms" have never been thoroughly investigated, so that there is no real knowledge of specific factors which limit their distribution and abundance. Probably nobody now knows just why any of the so-called clean-water organisms begin to disappear from waters subject to progressively increasing organic enrichment Here is a field for future research which is far more promising than is, for example, the questionable classification of all aquatic organisms as "[illegible]", "clean-water", or "facultative". If there are common sedentary organisms whose water quality requirements can be shown to correspond closely with those of valuable fish species, they are potentially useful indicators. At the present time, however, excepting instances of gross pollution, only fish themselves can be said to indicate reliably environmental conditions generally suitable or unsuitable for their own existence.

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Many, if not most of our productive warm-water lakes have low-oxygen conditions existing in them through at least a part of the summer. At this time, conditions prevail that closely approach conditions caused artificially by domestic or industrial pollution. In the eutrophic "chironomus" lakes of Connecticut described by Deevy (1941), one part per million of oxygen or less prevailed from late June until early November, but zero oxygen was not recorded.

The fact that aquatic earthworms of the family Tubificidae (Limnodrilus and Tubifex in this report) and such red midgefly larvae as Tendipes decorus, Tendipes tentans, Tendipes plumosus and Tendipes riparius are able to live in water of very low oxygen content is well known. Lindeman (1941) demonstrated that "chironomus" larvae could withstand complete lack of oxygen for four months at 10°C, but Deevy (1941) pointed out that much of the case for existence of these organisms under anerobic conditions rests on analyses made with either the unmodified Winkler method or the Rideal-Stewart modification. Recently, Ruttner (1953) cautioned that Alsterberg's modification of the Winkler method should be used for oxygen determinations otherwise values are lower due to the presence of reducing substances.

This report stemmed from a study of American papers dealing with the quantitative abundance of aquatic earthworms in both lakes and streams. It was concluded last year in a report to the Midwest Benthological Society that in large unpolluted lakes, such as Lake Michigan, Lake Nipigon, Douglas Lake (Michigan), etc., the average number of tubificids per square foot is usually less than 50, but that in lakes in good agricultural areas numbers averaged higher, 100 to about 300 per square foot. At that time, the writer overlooked a paper by Wright and Tidd (1933) which classified the bottom areas of western Lake Erie as clean, lightly-polluted moderately-polluted, and heavily-polluted. Clean bottom was defined as having less than 9.3 (100 per square meter) tubificids and more than 9.3

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H[illegible] (B[illegible]) [illegible], [illegible] 9.4-93 [illegible]; [illegible] 94-456 [illegible]; [illegible] 456 [illegible].

I[illegible], [illegible]-[illegible], [illegible]-[illegible]. E[illegible], [illegible], [illegible] (B.O.D.), [illegible], [illegible]., [illegible].

W[illegible] W[illegible] L[illegible] S[illegible] B[illegible], [illegible] 6 6 [illegible] E[illegible], [illegible] N[illegible]. 30 [illegible].

M[illegible] [illegible]. W[illegible], [illegible] 15 [illegible]. I[illegible] [illegible]-[illegible]. I[illegible] [illegible].

S[illegible] W[illegible] L[illegible] M[illegible] C[illegible], M[illegible], [illegible] 8 [illegible] 17 [illegible] 60 [illegible]; M[illegible] L[illegible] 6 [illegible] 8 [illegible] 61 [illegible]; L[illegible] C[illegible]-[illegible] B[illegible] C[illegible] 3 [illegible] 9 [illegible] 44 [illegible], [illegible] M[illegible] (1) [illegible] 100 [illegible], (2) [illegible]-[illegible], (3) [illegible], [illegible].

This "collective look" brought regrets **that more samples were not taken** in many places, but the presentation of the data collected may be of interest and some value in establishing norms and methods of sampling in the future which may lead to quantitative numerical values or norms above or below which damage to bottom animal life is more apparent.

Manistee Lake may be cited as an example of a lake in which even tubificid worms could not live in the deeper waters. In this lake, 17 of 24 samples collected September 22, 1954, were entirely without bottom animals of any kind. This lake has been severely polluted with paper mill and other wastes.

The deeper areas of Fremont Lake, likewise, were apparently severely damaged by organic pollution exceeding the quantity the lake could assimilate, and many of the samples were without tubificid worms and other species normally present. On September 16, 1952, 4 of 14 samples were entirely without animals. A neighboring unpolluted lake, Pickerel Lake, sampled September 15, 1952, of similar depth and without oxygen in its deepest waters had three times as many bottom animals. There were about three times as many Tendipes plumosus and ten times and many Chaoborus in the bottom samples .

In Saginaw Bay, the key area of Lake Huron from a fisheries standpoint, samples were taken at widely scattered points, but it has now developed that these were not collected at the best season; collection apparently followed the emergence of Michigan "caddis" Hexagenia limbata and the "bloodworm" Tendipes plumosus, the young of which occurred in several samples.

Saginaw Bay receives pollution from the Saginaw River which carries industrial and municipal wastes from Midland, Saginaw and Bay City. Minor contributions of pollution are made to the bay by the Kawkawlin and Pinconning Rivers. A beet sugar plant at Sebewaing introduces wastes seasonally into the bay. There is also a milk plant located at Sebewaing which discharges treated wastes into Sebewaing Bay. The City of Saginaw began operating a new sewage treatment plant before the 1954 survey. Pollutants peculiar to the operation of a large chemical plant at Midland include brine and phenolic wastes. The latter have been reduced considerably in recent years. Chlorides ranged from about 25-50 parts per million in the bay.

Data on Green Bay, the key area of Lake Michigan, have been included for comparison with Saginaw Bay, although sampling there occurred at a more favorable time, prior to June 1. The area of Green Bay referred to as the "Lower Bay" in Table I is closest to the polluted Fox River which has many paper mills located on its banks between Green Bay and Appleton, Wisconsin.

The series of samples in the Oconto line probably represent relatively unpolluted conditions in spite of the fact that the lower Oconto River is heavily polluted by paper mill wastes.

White Lake, at Whitehall and Montague, Michigan, may be classed as virtually unpolluted. The surveys made of it both preceded and followed the construction of a large chemical plant which provided adequate waste treatment facilities.

Ellsworth Lake, Antrim County, Michigan, a lake in a chain of connected lakes receives canning wastes seasonally. When it was sampled on October 8, 1953, most of the lake had 02 part per million or less of dissolved oxygen. Because of the shortage of oxygen, crayfish, which were abundant in the lake at the time, had migrated from the deeper water to the very margin and some even out of the water in an effort to survive low oxygen conditions. Many of them died at the water's edge. Freshly dead midgefly larvae of Tanytarsus nigricans were found in only two bottom stations, but many had risen to the water surface where they were fed upon by gulls. Larvae of the biting midge Palpomyia tibialis[1] were numerous in these bottom samples in spite of the low oxygen, and apparently many of them left the bottom temporarily for only 55 percent of the samples contained them on October 8, 1953. When the lake was revisited February 10, 1954, they were twice as abundant as during the sampling of October 8, 1953, and 91 percent of the samples contained them.

Tendipes plumosus larvae occurred in 64 percent of the Ellsworth Lake samples on October 8, and in 55 percent of the samples collected from under ice cover February 10, 1954, indicating that most of them were hardy enough to survive the temporary period of low oxygen in the lake.

A series of three stations across the lower end of St. Clair Lake located in the Intermediate River chain of lakes immediately above Ellsworth Lake porvided a small amount of data from an unpolluted area to compare with

[1] Identified for the writer by Dr. Willis Wirth, U.S. National Museum.

data from Ellsworth Lake. One of the principal differences was low average number of tubificids, average 9.3 per square foot in St. Clair Lake compared to 52.4 in Ellsworth Lake on October 8, 1953, and an average of 8 tubificids in St. Clair Lake compared with 63 per square foot in Ellsworth Lake on February 10, 1954. Larvae of Calopsectra dives were present in St.Clair Lake samples and absent from Ellsworth Lake.

Paper mill wastes enter Muskegon Lake on the south shore, and in that vicinity tubificid worms were particularly numerous. They were nearly ten times as numerous as they were in the control area on the north side of the lake. Midgefly larvae of Procladius and Tendipes plumosus were present in small numbers in at least half of the samples on both sides of the lake.

Lake Charlevoix at Boyne City is polluted by wastes from a large tannery and by the untreated wastes of the city. An extensive area of the lake bottom near Boyne City contains hair (originating in the tannery wastes). The bottom sampled was roughly divided into two areas. The area of maximum pollution adjacent to the tannery and city had deposits of hair ranging from 2,000-6,950 pounds per acre on a dry weight basis. The area of minimum pollution coincided with hair deposits of less than 2,000 pounds per acre. Tubificids were most abundant in the zone of greatest hair deposits. Midges of Procladius were also most abundant in this zone.

Hart Lake is an artificial impoundment on the South Branch of the Pentwater River. It receives cannery wastes seasonally, and the samples collected there were taken at a time (July 22, 1953) when cherry canning had been in progress for some time. It receives untreated domestic sewage from a city of about 2,200 population. Probably the point of greatest interest in this study was the abundance of Chaoborus punctipennis in the samples collected below the sources of pollution. They ranged in number from 16-504 per square foot, with three samples containing 296, 324, and 504 per square foot. Larvae of Tendipes tentans-plumosus and Procladius were numerous in some samples.

There are many details of description and- notes on abundance of species that might have been noted in this report; however, the relatively small number of samples taken in most cases does not justify the drawing of many conclusions at present.

The following quantitative data are presented primarily to show trends of abundance of species in polluted areas. Tables 1 and 2 show average number of species in each area; total number of animals in all samples of each area; average number of animals per square foot, both with and without tubificid worms; average number of tubificids per square foot, abundance of several species of midgefly larvae, phantom midges, and fingernail clams.

Tables 3 and 4 show the frequency of occurrence of the above kinds of bottom animals in all samples in percentages of the number of samples taken.

CONCLUSIONS

A survey of the lake reports showed that an abundance of tubificids in excess of 100 per square foot apparently truly represented polluted habitats. Severe pollutions such as occurred in a depression in Fremont and in virtually all of Manistee Lake precludes all forms of animal life. Such data must be approached with care when statistical analyses are considered because considerable pollution may cause increased abundance in some areas and severe pollution may eliminate or reduce numbers of even the hardy forms in other areas.

The data show that Procladius species are usually present with tubificids in polluted areas, but in severe pollution only the tubificids are able to survive.

Palpomyia tibialis was found to be a very hardy species in one lake where conditions brough about the destruction of Tanytarsus nigricans.

The fingernail clam Pisidium sp. was more prevalent in polluted areas than Sphaerium or Musculium, and judging from the abundance of dead shells in grossly polluted areas, these small mollusks are not able to survive the adverse conditions tolerated by tubificids

Cryptochironomus digitatus and Calopsectra dives are common associates of Tendipes plumosus or Tendipes tentans, tubificids and Procladius in the deeper waters of the polluted areas sampled. It is presumed that they are hardy, although their hardiness is apparently less than either tubificids or Procladius. The greater average abundance of Procladius in polluted habitats is presumed to be due in part to the fact that they feed upon tubificids.

Chaoborus punctipennis may frequent rather heavily-polluted areas and become abundant there. Although their swimming ability and habit of migrating to the surface at night permits them to leave polluted areas in which tubificids

either die or thrive, there is some evidence, as in the comparison of Pickerel Lake (an unpolluted lake) and Fremont Lake (a polluted lake) and the north and south sides of Muskegon Lake, that Chaoborus can not survive in large numbers in a polluted lake where low oxygen conditions have apparently prevailed for an excessive period of time.

When average numbers of bottom animals per square foot including tubificids are compared with average numbers less tubificids, most populations reveal the large contribution of the latter numerically. The difference may also reflect the contribution of organic wastes as a fertilizer in lakes as it did in lower Hart Lake.

Number of species in lakes as well as streams appears to be the most reliable criterion of pollution. Unpolluted Michigan lakes from these limited observations appear capable of supporting a number of species even to depths of 60 feet.

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TABLE 1. AVERAGE NUMBERS OF BOTTOM ANIMALS PER SQUARE FOOT IN POLLUTED MICHIGAN LAKES

	Saginaw Bay July 25-26 1953	Saginaw Bay July 16-17 1954	Green Bay (Wis.) Lower Bay May 27, 1952	Green Bay Oconto line May 26, 1952	White Lake May26,27 1954	White Lake Aug 19 1952	Ellsworth Lake Oct. 8, 1953	St. Clair Lake Oct. 8, 1953	Ellsworth Lake Feb. 10, 1954	St. Clair Lake Feb. 10, 1954
Number of Samples	8	14	20	7	22	16	11	3	11	3
Average number of species	5 .8	9.1	9.7	6.3	12	7.4	6.1	15	5.6	12.7
Total number of animals (all samples)	1,908	3,339	25,332	1,212	10,504	4,163	1,672	824	1,868	629
Average number per square foot	239	239	1,267	173	478	260	152	275	170	210
A. Aquatic earthworms										
Average number of tubificid worms	162	130	1,084	35	98	60	52	9	63	8
Average number less tubificids	77	109	183	138	380	200	100	266	107	202
B. Midges										
Av. number Procladius sp.	7.8	5.1	652	44.0	53.8	46.0	23.3	44	36	60
Av. number Cryptochironomus digitatus	6.0	2.8	72	2.9	53	5.3	0.4	0.0	0.0	4.0
" " Tendipes tentans-plumosus	6.0	22.3	16.0	14.8	46.8	54.3	5.1	0.0	7.6	2.7
" " Calopsectra dives	6.3	9.0	162	32.6	26.1	12	0.0	12.0	0.0	20.0
" " Pelopia stellatus	0.0	0.0	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. Phantom midges										
Av. number Chaoborus punctipennis	0.0	0.0	0.0	0.0	602	10.8	24.4	10.7	19.3	6.7
D. Punkies										
Av. number Palpomyia sp.	0.0	0.1	0.0	0.6	4.8	0.0	13.5	8.0	32.0	4.0
E. Fingernail clams										
Av. number Pisidium	2.0	3.1	41.3	26.8	14.1	8.9	13	2.7	0.7	4.0
" " Sphaerium-Musculium	0.0	1.1	8.3	4.0	1.9	0.8	0.7	0.0	0.3	0.0

TABLE 2. AVERAGE NUMBERS OF BOTTOM ANIMALS PER SQUARE FOOT IN POLLUTED MICHIGAN LAKES

		Muskegon Lake. Northside Sept. 15, 1954	Muskegon Lake Pol- luted Area Sept. 15, 1954	L. Charle- voix. Zone of least pollution May 19, 1954	L. Charle- voix. Zone of max. pollution May 19, 1954	Pickerel Lake Sept. 15 1952	Fremont Lake Sept. 16 1952	Manistee Lake Sept. 15 1953	Manistee Lake Sept. 22 1954	Hart Lake above pollution July 22, 1953	Hart Lake below pollution July 22, 1953
	Number of samples	4	12	11	12	8	14	24	24	2	8
	Average number of species	8.2	5.8	5.7	7.3	3.6	1.5	0..95	0.8	11.5	4.8
	Total number of animals (all samples)	2,396	26,659	1,060	5,839	368	228	720	556	504	5,314
	Average numbers per square foot	599	2,222	96	487	46	16	30	23	252	664
A.	Aquatic earthworms										
	Average number of tubificid worms	227	2,092	19	344	1.0	5.0	18	16	76	373
	Average number less tubificids	372	130	77	143	45.0	11 .0	12	7	176	291
B.	Midges										
	Av. number Procladius sp.	9.0	10.7	39.3	60.3	2.5	2.0	0.2	1.2	6.0	37.5
	" " Cryptochironomus digitatus	0.0	2 .3	2 .5	1.7	0.0	0.1	0.2	0.2	1.7	12.0
	" " Tendipes tentans-plumosus	9.0	8..3	0.7	6.0	9.5	3.4	0..0	0.0	10.0	71.7
	" " Calopsectra dives	1.0	0.0	3.3	0.7	0.0	0.0	0.2	0.0	2.0	0.0
	" " Pelopia stellatus	0 .0	0.0	0.0	0.0	0.0	0 .0	0 .0	0.0	0 .0	1.5
C.	Phantom midges										
	Av. number Chaoborus puncti-pennis	46.0	2.3	0.0	0.0	16.0	0.6	0.0	0.0	2.0	163
D.	Punkies										
	Av. number Palpomyia sp.	0 .0	0 .0	0.0	0.0	0 .5	0.0	0.0	0.0	4.0	0 .0
E.	Fingernail clams										
	Av. number Pisidium	15.0	14.7	6.9	12.6	1.0	0.0	0.5	0.8	40.0	3.5
	" " Sphaerium-Musculium	7.0	9.0	0.4	10.0	0.0	0.0	02	0.0	22.0	12.5

TABLE 3. FREQUENCY OF OCCURRENCE OF VARIOUS SPECIES IN LAKE SAMPLES EXPRESSED AS THE PERCENTAGE OF THE TOTAL SAMPLES TAKEN

	Saginaw Bay-July 25,26 1953	Saginaw Bay-July 16. 17 1954	Lower Green Bay, May 27 1952	GreenBay Oconto line May 26, 1952	White L. May 1954	White L. Aug. 19, 1952	Ellsworth Lake Oct. 8, 1953	St. Clair Lake Oct 8, 1953	Ellsworth Lake Feb. 10, 1954	St. Clair Lake Feb. 10, 1954
Tubificidae	100	100	100	71	95	88	82	67	91	67
Procladius	63	71	95	100	100	94	73	100	82	67
Cryptochironomus digitatus	63	71	80	80	64	50	9.1	0	0	67
Tendipes tentans-plumosus	13	79	70	43	55	69	64	0	55	33
Chaoborus punctipennis	0	0	0	0	64	56	91	33	64	33
Calopsectra dives	50	43	45	86	86	19	0	100	0	67
Pelopia stellatus	0	0	15	0	0	0	0	0	0	0
Palpomyia	0	14	0	14	36	0	55	67	91	67
Pisidium	25	50	65	86	86	44	18	33	9	33
Sphaerium and Musculium	0	21	40	43	23	6	9.1	0	18	0

TABLE 4. FREQUENCY OF OCCURRENCE OF VARIOUS SPECIES IN LAKE SAMPLES EXPRESSED AS THE PERCENTAGE OF THE TOTAL SAMPLES TAKEN

	Muskegon Lake North Side Sept. 15, 1954	Muskegon Lake polluted area Sept. 15, 1954	Lake Charlevoix. Zone of least pollution May 19, 1954	L. Charlevoix . Zone of greatest pollution May 19, 1954	Pickerel Lake Sept. 15, 1952	Fremont Lake Sept. 16, 1952	Manistee L. Sept. 15, 1953	Manistee Lake Sept. 22, 1954	Hart Lake above pollution source July 22, 1953	Hart Lake below pollution sources July 22, 1953
Tubificidae	100	92	91	100	13	29	17	21	100	100
Procladius	75	75	100	100	25	21	4	8	100	88
Cryptochironomus digitatus	0	8.3	45	33	0	7	4	4	100	25
Tendipes tentans-plumosus	75	50	18	42	75	36	0	0	100	72
Chaoborus punctipennis	50	25	0	0	75	7	0	0	50	100
Calopsectra dives	25	0	27	17	0	0	4	0	50	0
Pelopia stellatus	0	0	0	0	0	0	0	0	0	25
Palpomyia	0	0	0	0	13	0	0	0	50	0
Pisidium	75	25	18	42	25	0	4	4	100	25
Sphaerium and Musculium	50	33	45	17	0	0	4	0	100	50

THE USE AND ABUSE OF INDICATOR ORGANISMS

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In the foregoing discussions, several methods for the use of indicator organisms for many taxonomic groups have been presented. The discussions have been perhaps more enlightening than the formal papers. As last speaker, I have the advantage of having heard all the previous papers and discussions. From all we have heard, it would appear that several points remain to be discussed.

Throughout the foregoing presentations, the term "biologifal indicators of pollution" has been widely - albeit loosely - used. Most of the speakers agreed that individual species as indicators of pollution do not exist, yet the entire day was devoted to indicator organisms.

The indicator organism program which we use in Florida is based on organisms which are indicative of the absence of organic pollution rather than on its presence. Such a program has served our needs very well. Separate approaches or classifications are needed in the case of chemical or physical pollutants. These are developed as the need arises.

There appear to be many opinions as to just what an indicator organism program is for. Our indicator organism program in Florida is but a part of the overall stream sanitation program. The amount we can learn from the distributions of the stream inhabitants is great. From some of the questions which have been asked here today, it appears obviousstthat at least a few people came here seeking a simple indicator organism program which would solve all their problems. It should be obvious that no such classification of organisms exists.

The question naturally arises, then, as to just what is to be expected from an indicator organism program. Such a program may prove an excellent tool, with the limitations of a tool in that it may, when handled properly, do some jobs well while proving useless for others.

Perhaps a review of the use of our program in Florida will clarify the generalities stated above. As has been pointed out in literature (Beck, 1954) the more widely distributed stream organisms have been classified with regard to organic pollution in what, historically at least, would appear to be a backward manner. Instead of indicating pollution, our methods are set up with regard to absence of pollution. The former concept was found to be untenable - as most of the previous speakers have indicated.

In general stream surveying the indicator organism method is used in conjunction with the normal chemical, physical and bacteriological methods.

The biological program is thus supplemental to the more widely established methods. From it we obtain the evidence of yet one more discipline. The fauna of a stream is the result of the combined chemical, biochemical, physical, biotic, climatic and geologic factors of the area in which the stream occurs. Chemical or physical damage to a stream may be a rather ephemeral thing, but biotic evidence of such damage may persist for a significant period. Herein lies one of the main values of an indicator organism program, that of, if I may risk approaching the ridiculous, "faunal memory".

The use of indicator organisms in making a quick check of the condition of a stream is of particular value. In the Biotic Index paper (Beck, 1955: 1196) figure 3 was included to show that the results of surveys made one year apart were quite similar when conditions remained constant. Results since then are most interesting. All data are for station 2 which is located about two miles below the source of waste.

Year	Biotic Index
1953	5
1954	2
1955	16
1956	31

It would appear that station 2 was in poor condition in 1953 and in slightly worse condition in 1954. However, a quick check of the stream in 1955 showed evidence of a significant biotic change indicative of less pollutional effect. A visit to the industrial plant revealed that major changes had been made which had already helped the river. Actual work on the stream which revealed this change took less than an hour. The 1956 result reveals that the stream has completely recovered.

The disappearance of a snail (Goniobasis sp.) was the first evidence we had of a new source of pollution in another river. No single classification of organisms will suffice for all aspects of stream pollution. The pollution of two rivers with wastes high in fluoride ion content necessitated a new classification of indicator organisms. Some species which were quite tolerant of organic pollution proved incapable of surviving even moderate concentrations of the fluoride ion. One of the most fluoride-resistant insects is normally a clean water species.

I believe that it has been stated here that indicator organisms seemed to be indicative of dissolved oxygen concentration. We found it impossible to classify stream invertebrates in Florida with regard to dissolved oxygen content of the water. The presence of a great many springs along such rivers as the Suwannee and the St. Johns greatly alters the oxygen picture without damage to the fauna. As a result we find the more sensitive species of invertebrates tolerating surprisingly low oxygen tensions.

It should be stated that the methods we use in Florida are, we feel, useful. They are subject to revision any time better methods are suggested. They are essentially beginning methods and are being studied, subjected to testing and revised almost constantly.

In conclusion it should be stated that nothing has been written or spoken which has in any way shaken my belief in the usefulness of a properly developed indicator organism program as long as the user realizes that any system, no matter how carefully developed, has its limitations. No such system will ever be a substitute for a knowledge of the behavior of streams and of the inhabitants of streams.

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CURRENT INVESTIGATIONS IN WATER POLLUTION BIOLOGY

Current Water Pollution Investigations and Problems in Wisconsin

by

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The Wisconsin statues define the term "pollution" as the "contamination or rendering unclean or impure the waters of the state, or making the same injurious to public health, harmful for commercial or recreational use, or deleterious to fish, bird, animal, or plant life".

The major problems in stream pollution concern themselves principally with domestic wastes and industrial wastes. At the present time, about 94. 8% of the population served by sewers is connected to treatment plants. In 1949, 62 communities still discharged untreated sewage. As of January 1, 1956, this number has been reduced to 29, and of these, 5 have plants under construction. There are a total of 319 treatment plants serving 406 communities.

The 35 pulp and paper making firms in Wisconsin operate 45 paper mills, 16 sulphite pulp mills, 5 kraft pulp mills, and 14 groundwood mills. There are 1, 766 dairy plants which include some 1, 064 cheese factories. In addition, there are 151 canning plants as well as a full complement of other industries. The total sources of pollution in Wisconsin at the present time, number 1, 086.

The history of water pollution control in Wisconsin dates back to about 1925. Prior to 1925, there was a limited amount of pollution control through the application of statutes granting certain powers and duties to the State Board of Health, and conservation statutes prohibiting the discharge of certain specified materials to streams.

In 1925, the legislature appropriated $10, 000 from conservation funds for water pollution control. This was expended in cooperation with the State Board of Health in a study of major problems.

The legislature in 1927 created the Committee on Water Pollution, consisting of the State Chief Engineer, a member or representative of the Public Service Commission designated by the Commission, a Conservation Commissioner or an employee designated by the Conservation

Commission, the State Health Officer or a member of the Board of Health, and the State Sanitary Engineer or other engineer appointed by the Board of Health. The State Board of Health was designated as the administration agency of the Committee.

The revision of the 1927 act by the 1949 legislature recognized the need for a full-time water pollution control program and provided for a full-time director and authorized an appropriation which made possible the employment of a full-time staff. The present staff consists of the Director, an industrial wastes engineer, an engineer in charge of coordinating stream surveys, a biologist, a chemist, and four field engineers.

The Committee's basic responsibility is to exercise general supervision over the administration and enforcement of all laws relating to the pollution of the surface waters of the state, and to study and investigate all problems connected with the pollution of surface waters of the state and its control, and to make reports and recommendations thereon. It further has the authority:

> "To conduct scientific experiments, investigations, and research to discover economical and practicable methods for the elimination, disposal, or treatment of industrial wastes to control pollution of surface waters of the state. To this end, the Committee may cooperate with any public or private agency when requested by such an agency in the conduct of such experiments, investigations, and research, and may receive on behalf of the state any moneys which any such agency may contribute as its share of the cost under such cooperative arrangements.
>
> "To supervise chemical treatment of waters for the suppression of algae, aquatic weeds, swimmers' itch, and other nuisance-producing plants and organisms.
>
> "To issue general orders and adopt rules and regulations applicable throughout the state for the installation, use, and operation of practicable and available systems, methods, and means for controlling the pollution of the surface waters of the state through industrial wastes, refuse, and other wastes.
>
> "To issue special orders directing particular owners to secure such operating results toward the control of pollution of the surface waters as the Committee may prescribe within a specified time.
>
> "To make investigations and inspections to insure compliance with any general or special orders, rules and regulations which it may issue.

"To enter into agreements with the responsible authorities of other states subject to approval by the Governor relative to methods, means, and measures to be employed to control pollution of any inter-state streams and other waters, and to carry out such agreements by appropriate special and general orders.

"In addition to all other powers and duties of the Committee on Water Pollution, it shall have the power and it shall be its duty to hold a public hearing relative to alleged water pollution upon the verified complaint of six or more citizens filed with the Committee"

During the course of development, cooperative programs of investigation were established with the Sulphite Pulp Manufacturers' Research League, the Pulp and Paper Advisory Committee on Waste Disposal, the National and Wisconsin Canners' Associations, and recently cooperative biological surveys have been conducted with the Institute of Paper Chemistry.

The Sulphite Pulp Manufacturers' Research League has been continuously engaged in research on waste disposal problems of the industry since its formation in 1939. Spent sulphite liquor is produced when wood is cooked for production of the pulp which is used in the manufacture of paper. For each ton of pulp produced, about one ton of solids is contained in the spent liquor which was formerly discharged directly to the streams. One of the first methods developed for the utilization of waste sulphite liquor was that of aerobic fermentation leading to yeast production. Today there are two full-scale yeast plants, one at Rhinelander, Wisconsin producing 14,000 pounds of yeast per day, and one at Green Bay, Wisconsin designed to produce 28,000 pounds of yeast per day. Other processes upon which much work has been done include evaporation and burning of which there are three installations in Wisconsin, the production of vanillin, the use of waste sulphite liquor in roadbinding of which about 60 million gallons is used per year, soil filtration, and others of less potential.

In 1949 when the water pollution control statutes were revised, it was recognized that some of our streams were still being adversely affected by raw sewage and certain industrial wastes. At that time, the state was divided into work areas comprising the 28 major drainage basins, and a field engineer was assigned to each basin and instructed to conduct surveys to determine the condition of the streams and the sources of pollution discharging to the streams. An industrial waste census survey and the data on stream conditions above and below sources of pollution compiled into a comprehensive report, including biological data taken above and

below the various sources, then became available for each basin studied. This report, used as an exhibit at a public hearing, provided information of value to the Committee in deciding on the types of orders to be issued in order to eliminate or reduce pollution. The Committee has over a period of years issued 1, 086 orders of which 316 have been fully satisfied as of January 1, 1956.

The role of a biologist in the program follows two principal lines or activity. The Committee is charged with the supervision of all chemicals placed in the public waters of the state for the control of algae, weeds, swimmers' itch, or other nuisance-producing organisms. The administration and supervision of the aquatic nuisance control program thus becomes the responsibility of the biologist. During the summer of 1955, for example, some 37, 000 pounds of copper sulphate was used for algae control in 15 state lakes. In addition, on an experimental basis, 60 gallons of Cutrine, 90 gallons of Delrad, and 400 pounds of Phygon were used for algae control. The submergent aquatic vegetation control program entailed the use of some 18, 800 gallons of commercial sodium arsenite solution in 34 state lakes. In addition, a small quantity of 2, 4-D, Dalapon, and methoxone chlorax was used. The control of snails harboring the organism producing swimmers itch on some of the bathing areas of the state involved the use of 1740 pounds of copper sulphate, 550 pounds of copper carbonate, and 320 pounds of lime.

The other phase of principal biological activity lies in stream survey work. In the past, the stream study program has consisted of biological samples, principally those of bottom organisms, bracketing the sources of pollution in the 28 drainage basins. From 1949 to the present time, some 37 such surveys have been completed. The entire state has now been covered and the initial phase of the pollution investigation program completed.

The current and future biological program is aimed at more intensive studies in more localized areas on the major waterways of the state. Biologists of the Institute of Paper Chemistry at Appleton, Wisconsin have also initiated a program of intensive biological study on some of the rivers affected by the pulp and paper industry.

A recent cooperative biological study with the Institute of Paper Chemistry has been completed on the lower Fox River in the central portion of the state. The river is 39 miles long, has a fall of 4. 25

feet per mile, and contains 14 dams. Effluent from 19 paper making processes enters the river at various points and includes the effluent from 5 de-inked mills, 2 rag pulp mills, 1 kraft mill, 4 sulphite mills, and 2 groundwood mills. In addition, there is the effect from algae as tests have indicated an excess of 130 tons per day (dry weight) of algae flowing into the Fox River from Lake Winnebago.

Another biological study of the more intensive type was recently completed on a 200-mile stretch of the Wisconsin River. This section receives effluent from pulping processes of 5 sulphite mills, 3 kraft mills, 2 semi-chemical pulp mills, 1 rag pulp mill, and 3 groundwood mills. The combined pulp production is some 1480 tons of pulp per day, and the combined daily paper production is some 2300 tons. A follow-up partial survey in the late winter of some of the clean-water, summer-time stations indicated quite drastic changes and severe conditions. It is apparent that nearly as much attention must be given to the study of a stream in winter as well as under summer-time conditions.

The Botany Department of the University of Wisconsin has been continuing its efforts in the field of nutritional requirements of blue-green algae. At the same time, a continuous study on the removal of nutrients from sewage effluent by the use of algae is conducted. The study involves nutrient balances, biological examinations, and methods of algal harvesting. Three ponds with a combined area of one-half acre, and with controlled physical factors (flow, depth, and recirculation), are being used in this investigation.

INVESTIGATIONS AND PROBLEMS IN ONTARIO

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In Canada, relatively few biologists are working exclusively in the field of pollution control and water supply. Consequently, it is necessary that the studies include most phases of pollution biology as is evident from the following discussion of "Investigations and problems in Ontario."

Pollution control in Ontario is generally directed by the Pollution Control Board, which is composed of members from various interested government departments. The actual investigations and control are the responsibility of the Sanitary Engineering Division of the Department of Health and the Fish and Wildlife Division of the Department of Lands and Forests. The Sanitary Engineering Division maintains a laboratory from which both field work and analytical work is done.

Recently, increased emphasis has been placed on education, in order that excessive pollution caused by a lack of understanding of the harmful effects of municipal and trade wastes to natural waters would be recognized. Courses are now provided for sewage disposal plant operators and sanitary inspectors, and included in their course of instruction is a section on the life in streams and the effect of pollution. In addition to these courses, the Pollution Control Board of Ontario has sponsored an Industrial Wastes Conference for the past two years. Technical papers are presented on waste problems and treatment, and each year one or more papers have been devoted to pollution biology.

Probably the most intensive combined biological and chemical investigation made in Ontario concerned the pollution of the Spanish River. This study was promptedby litigation and an injunction obtained by riparian landowners against a Kraft paper mill. The injunction was dissolved by an act of legislature in 1950 pending the investigation. The study was made over the period of one year, during which time continuous observations were made on biological conditions and samples were regularly taken for chemical analysis. A quantitative study was made of the bottom organisms from above the mill to the mouth of the river, a distance of about thirty-two miles. The graphic pattern of numbers and species of bottom dwellers clearly demonstrated the deleterious effect of the mill effluent to the river.

Nets were fished more or less continuously over the year, and observations were made on the species and relative abundance of fishes inhabiting the river. It is interesting to note that while fish could traverse the river until stopped by the mill dam, they did not remain in numbers in the section extending for twenty miles below the mill. During the period of investigation, a spill of rosin acid soaps occurred in the mill and passed to

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R[illegible], [illegible] [illegible]. T[illegible] [illegible] H[illegible]. T[illegible] [illegible]. T[illegible] [illegible]. N[illegible] [illegible] S[illegible] [illegible]. T[illegible] [illegible]-[illegible].

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The study was divided into three phases:

(1) A study of the biological and chemical conditions in the lake.

(2) A study of control measures.

(3) A study of the toxins produced by the algae.

The following are a few of the observations and findings of this experiment.

The plankton were found to grow in quantity at most times of the year, with a definite preponderance of blue-greens during the summer months. The blue-green counts during the four summers that the experiment was conducted reached peaks between 800,000 and 1,200,000 units per litre. A number of chemical determinations were made, including total and soluble phosphorous, various nitrogen analyses, etc. The high productivity of the lake is connected with the fertility, and phosphorous is believed to be of prime importance.

In considering control, the one important source where excess plant nutrients might be removed was the sewage disposal plant for the town of Lindsay. A study was made of the fertilizing quality of the effluent, and a laboratory investigation was begun on methods of removing phosphorous from sewage. Alum, lime, ferric chloride and other chemicals and combinations were tested. Ultimately a choice of alum and activated silica was made because of the strength and settling characteristics of the floc. In addition, the presence of activated silica appeared to enhance the ability of alum to absorb phosphorous. Continuous treatment was started on a trial basis in 1954. In May of 1955, treatment began and extended until the end of September.

The Lindsay disposal plant provides primary clarification only and has a dry weather flow of 1,200,000 Imperial gallons. While there were no special facilities for building floc other than the turbulence within the flow, a reasonably good coagulation was obtained and a total of 81% of the total phosphorous was removed. Soluble phosphates were virtually eliminated, and we are reasonably sure that most of the 19% remaining in the effluent was lost with a portion of the alum floc which did not settle. It was estimated that during the five months' treatment last summer, a total of 8487 pounds of phosphorous, as "P", was removed. If this figure is converted to tons of fertilizer using superphosphate as an example, it is equivalent to 54 tons. Other improvements noted were: a 71% reduction in **BOD,** a 63% removal of suspended solids and a clear effluent.

The average addition of alum was 94 ppm and silica was 3.4 ppm. The total expenditure for chemicals was $5006.00, a cost of $25.75 per million gallons.

It would appear that with proper facilities for mixing and settling, virtually all the phosphorous could be removed and saving would undoubtedly

be made on chemicals. This is believed to be the first use of an alum activated silica treatment and one of the first experiments on continuous phosphorous removal from an operating disposal plant.

While a review of the findings has not yet been completed, a considerable drop was noted in the concentration of phosphorous in the river that receives the effluent. The soluble phosphorous in the lake was very low and remained lower than any of the previous years. The total phosphorous was approximately the same as in previous years and while the blue-green algae built up rapidly under favourable weather conditions that occurred all summer, they levelled off at a peak a little less than would have been expected from previous observations and generally remained at that level for the duration of the season. While it is unlikely that a definite correlation will be shown, it is felt that if treatment was continued on a permanent basis, the level of plant nutrients could be lowered sufficiently to maintain a balanced algal population and a reduction of the incidence of nuisance blooms.

The investigation into the nature of the toxin developed at the time of blooms of blue-green algae has been done by the Defence Chemical Laboratories. While not much information is available yet, toxicity to mice has been demonstrated in unialgal cultures. Previous investigations into the nature of the toxin have shown that it does not belong to any of the known poison groups. It is hoped that the development of pure cultures, which is proceeding at present, will lead to the identification of this toxin.

The work done by the Sanitary Engineering Division is primarily routine analysis and regulatory in nature in the fields of water supply and water pollution. There are, however, projects such as have been previously mentioned that the Division is called upon to study or direct. Undoubtedly, this function will increase in the future as the population and industry grows.

The people of Ontario are justly proud of the abundance of desirable species of fish in their waters and care is being .taken to conserve this valuable resource.

REPORT ON POLLUTION STUDIES CONDUCTED IN WESTERN CANADA

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I. Industrial Pollution

Emphasis in pollution studies in British Columbia has been on the industrial development of forest product industries. There are at present eight pulp mills on the B. C. coast, four of which are undergoing expansion. One new pulp mill is being constructed, and at least two new pulp mills are in the embryonic stages of planning. Characteristically large water users, pulp mills can be a major source of water pollution. The research work has been closely coordinated with the supervisory branch of the Department of Fisheries which receives the requests for approval of plans for waste discharge from industry. The policy that "prevention is more effective than abatement" has been continued, and pollution problems are carefully assessed before they occur. In most instances an effort is made to draw on existing data and knowledge in these studies, inasmuch as it is not desirable to hamper industrial development by delays of long-term research. Where additional survey data or bioassay information are required, these are taken as facilities and manpower permit.

From the point of view of economy and lower pollution, new pulp mills are installing equipment for production of Kraft (sulphate) pulp as opposed to the sulphite process. The Kraft process permits reclamation of the salts in the "black liquor" by evaporation and burning of the organic material. ("Black liquor" is the dark brown fluid which results from the cooking of wood chips in an alkaline solution at about 330°F under pressure in a digestor). Any of the liquor which escapes in the wash from the screens is generally too dilute for economical recovery. It is this wash water which constitutes the major pollution hazard from a Kraft pulp mill, Sulphite digestion is predominantly used in the older pulp mills. An economical recovery process for reclamation of the salts in sulphite waste liquors has not yet been perfected for wide use. In general, effluent from a given tonnage of sulphite pulp produces about 10 times as great a pollution hazard as that from an equal tonnage of Kraft pulp.

Newsprint mills contribute a relatively small pollution. The mechanical grinding of wood in groundwood mills results in a discharge of wastes contaminated only by sap, bark extracts, and fibres from the wood. Most of the pollution stems from the oxygen demand of the organic constituents during decomposition.

The major factors which are responsible for the harmful aspects of pulp mill wastes to fish are:

(1) Biochemical oxygen demand

(2) Direct toxicity

(3) Destruction of food organisms

Analysis of pollution problems with respect to Kraft mill effluent has shown that where dilution is sufficient to combat the B. 0. D. (biochemical oxygen demand) of the waste, direct toxicity is only secondary. Bioassay studies conducted at this Station show that concentrations greater than 4.0% of Kraft mill effluent are toxic to young sockeye in sea water for periods greater than one week (Brett and Alderdice, 1954). No research has been pursued on the effect of Kraft mill effluent on fish food organisms. There has been no evidence of a severe reduction in plankton and benthic fauna in regions of British Columbia where pulp mill wastes have been discharged.

There have been three basic types of marine systems on the B. C. coast where pulp mill pollution conditions have had to be studied.

A. Inlet-type - Numerous pulp mills in British Columbia are located at the head of an inlet, often adjacent to an estuary. The choice of such a locality stems from the availability of good forests nearby, ease of transportation of logs from inlet logging regions, fresh water supply, hydroelectric power, and favourable harbour facilities. As a hazard to anadromous fishes these pulp mills require the greatest precaution in effluent disposal. Wastes passing into estuarial waters come directly into the path of migrating salmon. A severe case of pollution might completely wipe out a salmon run.

Evaluation of the capacity of an inlet system to receive wastes according to oxygen supply and dilution has been based on the study of Tully (1949) on Alberni Inlet. Waters from runoff undergo a continuous displacement seaward. The use of the fresh water as a tracer to determine the movement and mixing of effluents has permitted prediction of pollution with varying pulp production. Being of about the same density as fresh water, pulp mill effluent mixes only in the surface brackish layer. Thus the most effective control on the extent of pollution in an inlet-type condition is (1) controlled discharge of fresh water above a certain minimum flow, (2) release of the effluent at the surface and (3) maintenance of the effluent in the jet stream of the surface flow.

The problem of pulp mill effluent discharge in volumes above the safe capacity of natural receiving waters was met in the expansion plans of the Port Alberni pulp mill. Present production of 230 tons of unbleached Kraft pulp, established on the basis of Tully's (1949) work, does not impose an excessive pollution load on the inlet. The effluent can be flushed effectively from the inlet by the natural tidal conditions and Somass River discharge. But the expansion to roughly 500 tons of Kraft pulp and about 500 tons of newsprint per day would impose an oxygen demand on the inlet system in excess of the natural supply. This is particularly true with the reduced river flow during the late summer.. A stream which normally fluctuates from about

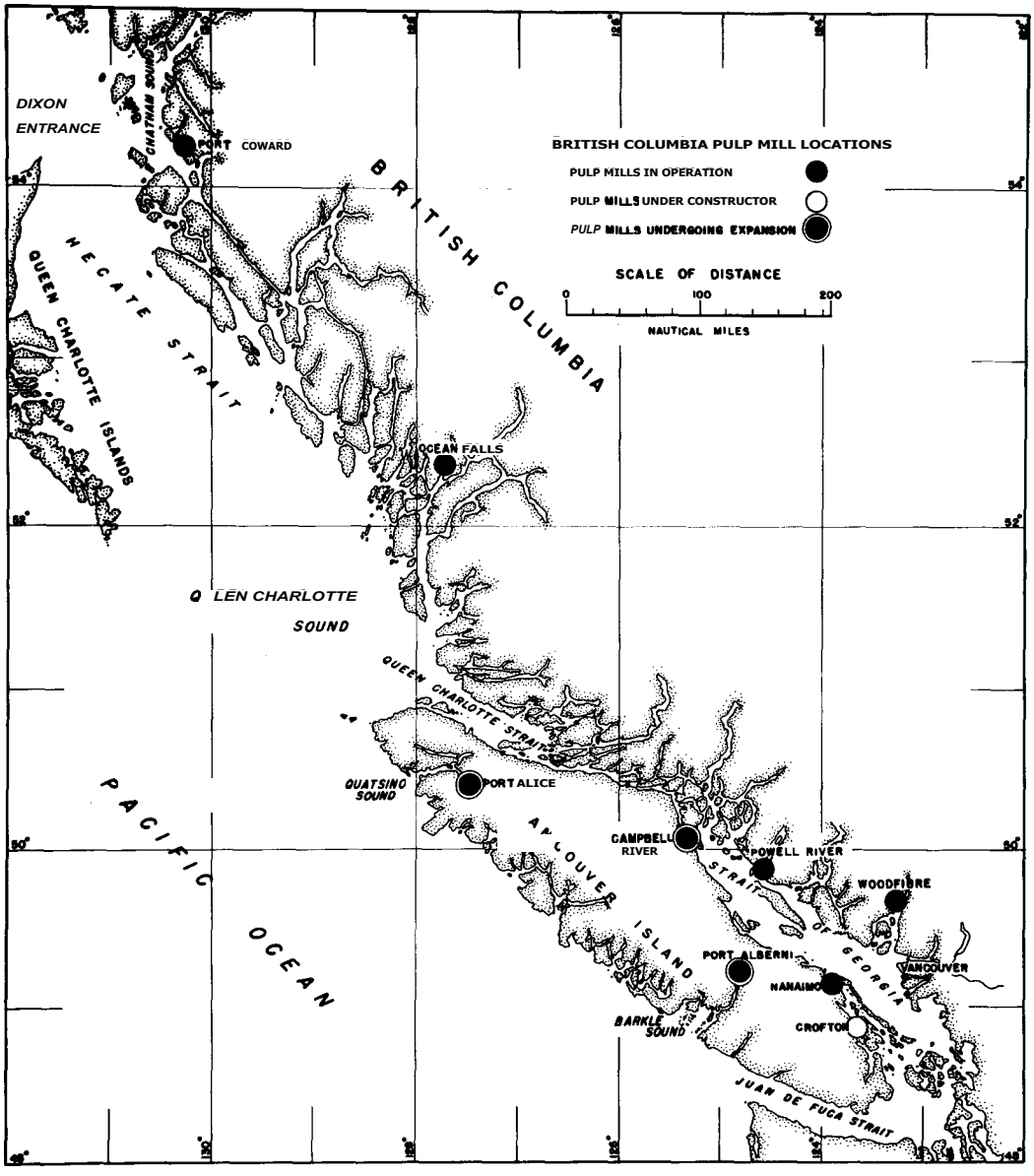

Figure 1. Chart of the British Columbia coast showing existing and proposed pulp mill locations.

300 c.f.s. in September to peaks of 30,000 c.f.s. in November, the Somass River must be maintained at a discharge of 1,000 c.f.s. or greater to overcome any serious pollution from the proposed mill.

B. Coastal Seaway type - In this type of location, effluent is discharged directly into a seaway, use being made of tidal currents and mixing to remove and disperse the wastes rapidly. There is seldom any estuary adjacent to such pulp mill locations so that fisheries of salmon or trout are not likely to be endagered. At the Harmac (Nanaimo) pulp mill, effluent from a daily production of 600 tons of fully bleached Kraft pulp is discharged into Northumberland Channel. Tidal currents here are predominantly southbound (ebb) reaching a knot on certain stages of the tide. The geography is such that little of the effluent reaches the vicinity of Nanaimo Harbour, but is rapidly funnelled into the turbulent waters of Dodds Narrows. With currents commonly over 5 knots strength, pulp mill effluent is completely mixed into the sea water in Dodds Narrows, no traces being detectable below the narrows.

Other examples of this type of effluent discharge can be seen at the Duncan Bay (Campbell River) pulp mill and at Powell River. Duncan Bay, located on Discovery Passage, is relieved of its effluent by the strong tidal currents (up to 7 knots) just south of Seymour Narrows. Powell River discharges effluent into more quiescent waters of Malaspina Strait and Algerine Passage. No evidence of pollution has been reported in either region. Popular sport fishing for salmon is especially renowned at Campbell River.

Any pollution which could arise in these cases in future expansion would probably result from an adverse effect on migrating juvenile salmon. In Discovery Passage large schools of young salmon seek the shelter of the bays and inlets in their journey to the sea. It is hoped that observations can be made on the effect of expansion of the Duncan Bay pulp mill on the young salmon passing through the bay during the summer.

C. Restricted Embayment type - Being intermediate between the inlet-type condition and the coastal seaway, the restricted embayment does permit some flushing by virtue of its openness to an adjoining channel. Generally, however, it possesses a circulation all its own independent of that in adjoining channels. Hence a certain amount of stagnation results.

The pulp mill at Port Edward near Prince Rupert is an example of this type of situation. Shallow, restricted conditions in Wainwright Basin and Porpoise Harbour hinder the flushing of the effluent into Chatham Sound. Large concentrations of mill wastes have been particularly evident during low tides. Mixed reports have been received on the effect of the wastes on the biotic environment. Some observers have reiterated that all marine life in that vicinity is being destroyed. But observations carried out by biologists of the Department of Fisheries (May, 1953) claim that these reports are exaggerated, and that both fish and bottom fauna appear to prevail in normal health. A further investigation of the region is planned in the near future from the Biological Station, Nanaimo.

The proposed pulp mill at Crofton will create another sample of a pulp mill in a restricted embayment. Osborn Bay, into which the effluent would be normally discharged, is relatively sheltered from Stuart Channel; its flushing and circulation are sluggish. The bay is fringed, particularly at the northern end, by oyster-growing leases. Living primarily in the intertidal zone, oysters are vulnerable to any harmful wastes found in the surface waters. In order to prevent the lost of oysters entirely, action would have to be taken to discharge the effluent outside the oyster-growing area or render it innocuous by treatment. The most satisfactory solution to industry economically was the piping of mill wastes into deep water (10 fathoms) in Stuart Channel beyond the Shoal Islands protecting Osborn Bay. Recommendations have been made to this effect and have been met with agreement by both the Provinical Department of Fisheries, Federal Department of Fisheries and Industry.

II. Domestic Pollution

A limited amount of work has been conducted on pollution from domestic wastes in coastal communities of British Columbia.

A. Vancouver Sewage Disposal

Study of the effects of sewage disposal from Vancouver was undertaken as a cooperative effort by the Pacific Oceanographic Group, National Research Council, Institute of Oceanography of the University of British Columbia, Tidal Branch of the Hydrographic Service of Canada, and Air Surveys Branch of the British Columbia Department of Lands and Forests at the request of the Vancouver and Districts Joint Sewerage and Drainage Board. Oceanographic data were collected seasonally in the Fraser River estuary and in Vancouver Harbour over a period from September, 1949 to March, 1951.

Preliminary analyses of these data along with tidal current information and aerial photographic surveys have been used for establishing the circulation in Vancouver Harbour and at the Fraser River estuary. These studies showed how the tides and discharge from the Fraser River combine to produce a characteristic circulation in Burrard Inlet.

Off Point Grey, there is a predominent northward set of the current into English Bay on all stages of the tide. Off Point Atkinson, at the northern shore of the entrance to Burrard Inlet, there is a prevailing westward current out of English Bay. A counterclockwise main eddy system with numerous smaller eddies and stagnation points superimposed on the principal circulation were noted to exist in English Bay.

The results of the oceanographic studies have permitted the evaluation of different outfall points for their suitability in sewage disposal. Final choice of discharge locations was based largely on the effectiveness with which the sewage would be dispersed and removed by the currents.

B. Nanaimo Harbour Sewage Disposal

An oceanographic study of currents in Departure Bay and Nanaimo Harbour was conducted as a cooperative venture between the Pacific Oceanographic Group and community groups of the city of Nanaimo. A large measure of assistance was given by the Nanaimo Yacht Club with their boats, and much volunteer help came from the Nanaimo city employees. Close cooperation was maintained with the local branch (Central Vancouver Island Health Unit) of the Provincial Department of Health and Welfare. The prime concern in the survey was the determination of existing pollution from a health standpoint and further contamination of beaches to be expected from additional sewage discharge.

Results of the survey indicated that Nanaimo Harbour is already polluted by existing sewer outfalls and that beaches within the harbour are not fit to be used for bathing. Departure Bay beach is still free from contamination, but is threatened by any sewage discharged at Brechin Point (located at the northern end of Newcastle Island Channel between Nanaimo Harbour and Departure Bay). Surface currents in both bays are largely wind-driven. The prevailing southeasterly winds would drive any increased sewage output into Departure Bay and gradually wipe out the remaining beach recreational area in the district.

Recommendations were submitted to the City Council of Nanaimo that either the sewage be totally treated before discharge, or it should be piped beyond the barrier islands (Newcastle and Protection Islands). These recommendations are being acted on at present and further survey work is awaited to determine the most appropriate discharge points outside the islands.

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The Relationship of the Polychaetous Annelid Capitella capitata (Fabricius) to Waste Discharges of Biological Origin

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INTRODUCTION

Biologists have long recognized the shortcomings of chemical and physical measurements of water quality, and thus have searched for organisms which could serve as indicators of different degrees of pollution. Organisms, particularly those that are attached or bottom inhabitants, are favored by many since they reflect the water conditions not only at the time of sampling but for some time previously.

The use of organisms as indicators of pollution in marine waters has lagged considerably behind that of fresh water studies. Wilhelmi (1916) mentioned that the polychaete Capitella capitata played a similar role in marine waters as Tubifex does in the fresh waters of Germany. Blegvad (1932) studied the bottom fauna in the vicinity of domestic outfall sewers in Copenhagen Harbor, Denmark. He was able to divide the region surrounding one outfall into three zones; an inner region lacking animals and with the substrate characterized by a sulfide odor, an intermediate zone containing a few animal species and with the substrate possessing a sulfide odor, and a third zone showing no measureable effects of the discharge. A second domestic outfall lacked the intermediate or marginal zone. Filice (1954), working in the Castro Creek area of San Francisco Bay, also separated the bottom fauna into three zones: (1) a healthy zone unaffected by waste discharges, (2) a marginal zone characterized by a few tolerant species, notably the polychaetes Capitella capitata, Neanthes succinea (Frey and Leuckart), and Streblospio Webster, and molluscans Mya arenaria Linnaeus and Macoma inconspicua (Broderip and Sowerby) and (3) a zone essentially lacking in animals. Recently the author (1955) published the general results of three quantitative bottom surveys conducted in the Los Angeles -- Long Beach Harbors. This area, which receives waste discharges of domestic and industrial origin (Anon, 1952), was divided into five zones on the basis of bottom conditions. Capitella capitata was found to be particularly abundant in regions receiving effluents of biological origin. This organism was the characteristic animal in what was termed the polluted bottom zone. The present report discusses some of the results of bottom surveys made in the Los Angeles-Long Beach Harbor and in some other marine waters of southern California. Emphasis has been placed upon the occurrence of C. capitata and its possible role as an indicator of pollution of biological origin. Biological wastes are herein defined as discharges from fish canneries, domestic sewage, and garbage.

OBSERVATIONS

Los Angeles -- Long Beach Harbors (Figure 1). The bottom of the Los Angeles -- Long Beach Harbor has been sampled five times during the past five years. Ihree of the samplings were made in 1954. The distribution of C. capitata for the June 1954 Survey is shown in Figure 1. Slip 5 of Los Angeles Inner Harbor receives waste discharges of domestic and fishery origin. The bottom of the inner portion of the slip was covered with fish scales. Capitella capitata was found in the area in large numbers along with a few other species of invertebrates. Domestic, fishery, and non-biological industrial wastes are emptied into Slip 2 of Long Beach Harbor. Here this worm was found with one other species of polychaete. The region of fish harbor in the Los Angeles Harbor receives some of the effluents from the fish canneries and the bottom was covered with fish scales. Capitella capitata was collected either alone or with one or two other species of polychaetes.

Following primary treatment the effluent of the terminal Island sewage treatment plant is discharged into the Los Angeles Outer Harbor. Three outfalls from the fish harbor canneries are located nearby (Figure 2). The samples taken in 1954 at the sewage outfall either contained C. capitata or lacked animals. Additional stations were sampled in DecembeF 1955 (Figure 2) to determine whether or not an intermediate assemblage of animals existed between the polluted zone possessing C. capitata and the healthy zones typical of the outer harbors (Reish, 1955; see also Hartman, 1955, Stations 29 and 44b). No animals were taken from the samples near the sewage or fish cannery outfalls. The substrate possessed a strong odor of domestic sewage. Capitella capitata was found in the stations a short distance from these outfalls. The farther the stations were located from the outfall the more varied the fauna became. There was no indication of an intermediate zone between the polluted bottom containing C. capitata and the healthy area.

Alamitos Bay. This bay, which is located at the southern boundary of Los Angeles County, is essentially a clean body of water used primarily for recreation (Reish and Winter, 1954). Capitella capitata was found at three stations in the vicinity of a public dump where garbage was pushed into the bay. This species was present at two other widely separated stations where there was no known pollution. There two stations were the only ones sampled where C. capitata had been collected where there was no pollution of biological origin discharged nearby.

Lower San Gabriel River. The tidal portion of this river is separated from Alamitos Bay by a jetty. This region was sampled in 1952 and again in 1954 (Reish, 1956). No animals were found in the bottom materials in 1952, but animals were encountered at some of the stations in 1954. A portion of the river was diked and dredged about four to five feet deeper in 1952 after the survey of that year. This dredging activity removed the accumulated sludge from the river bottom. A total of 12 different species was collected in 1954, of which C. capitata was the most common. This

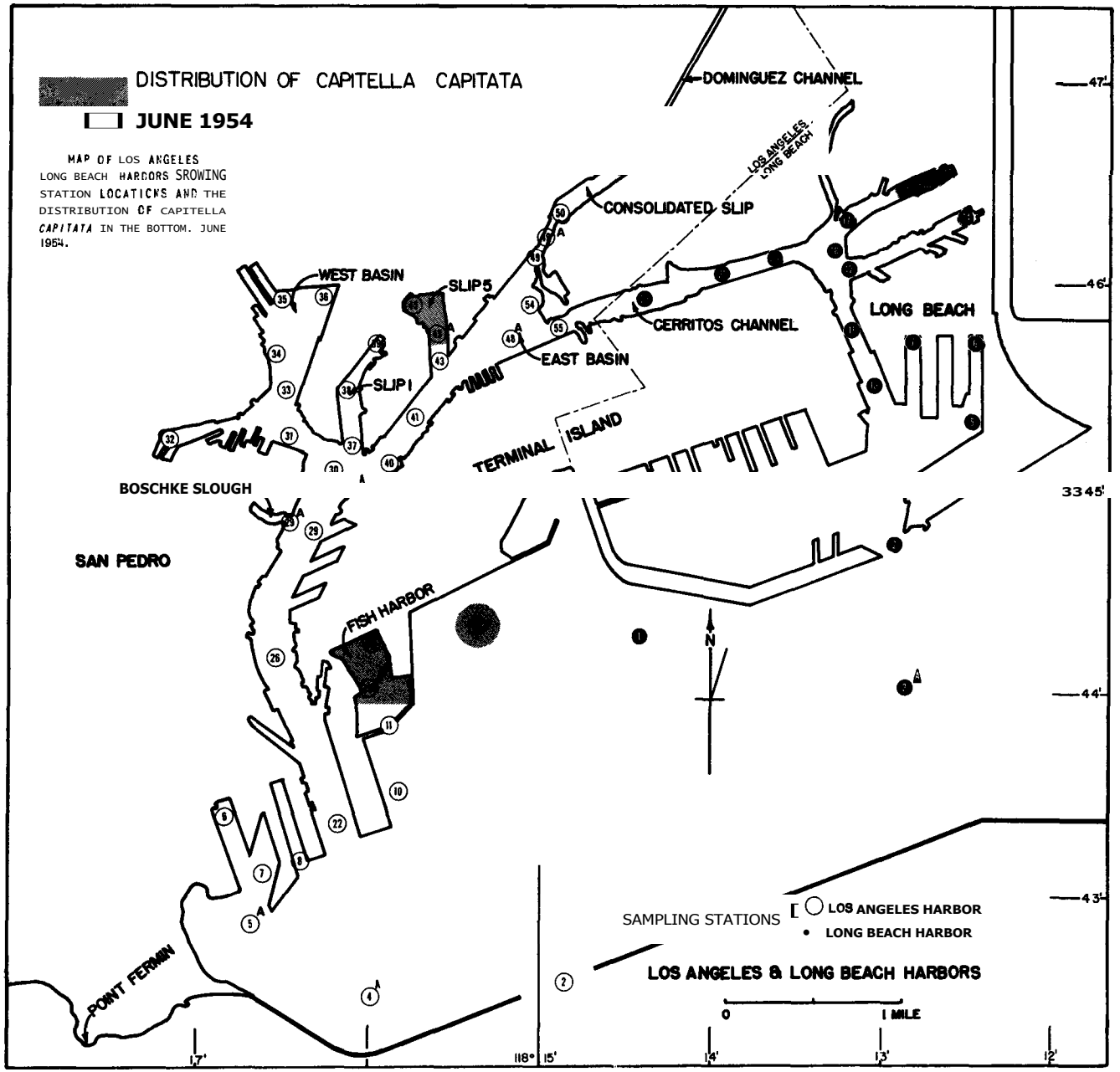
DISTRIBUTION OF CAPITELLA CAPITATA
JUNE 1954
MAP OF LOS ANGELES LONG BEACH HARBORS SROWING STATION LOCATICNS ANC THE DISTRIBUTION OF CAPITELLA CAPITATA IN THE BOTTOM. JUNE 1954.
DOMINGUEZ CHANNEL
LOS ANGELES LONG BEACH
CONSOLIDATED SLIP
WEST BASIN
SLIP 5
SLIP 1
EAST BASIN
CERRITOS CHANNEL
LONG BEACH
TERMINAL ISLAND
BOSCHKE SLOUGH
SAN PEDRO
FISH HARBOR
POINT FERMIN
N
SAMPLING STATIONS
LOS ANGELES HARBOR
LONG BEACH HARBOR
LOS ANGELES & LONG BEACH HARBORS
0
1 MILE
47'
46'
44'
43'
33'45'
118°
15'
14'
13'
12'
17'

FIGURE I

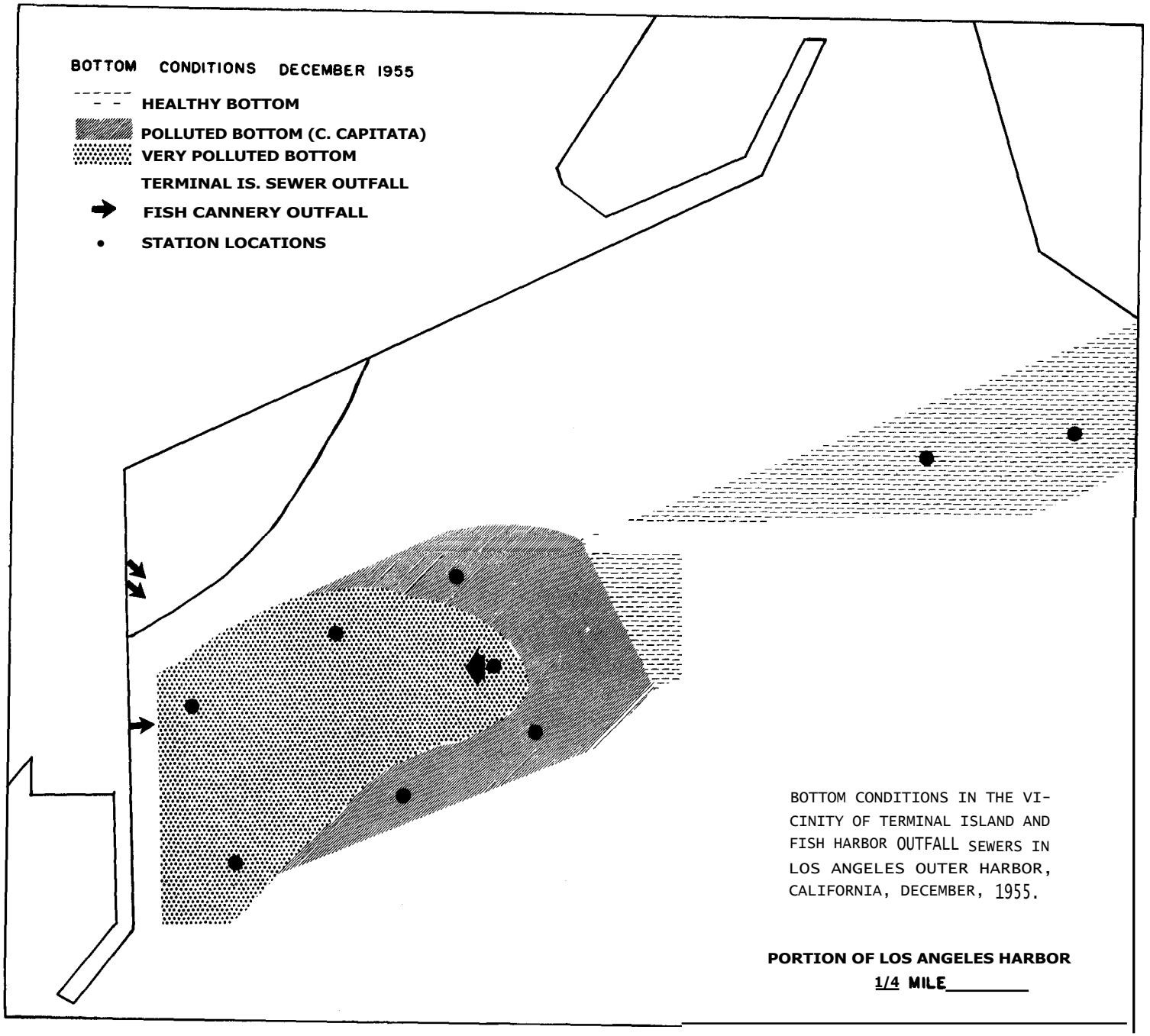
BOTTOM CONDITIONS DECEMBER 1955
HEALTHY BOTTOM
POLLUTED BOTTOM (C. CAPITATA)
VERY POLLUTED BOTTOM
TERMINAL IS. SEWER OUTFALL
FISH CANNERY OUTFALL
STATION LOCATIONS
BOTTOM CONDITIONS IN THE VICINITY OF TERMINAL ISLAND AND FISH HARBOR OUTFALL SEWERS IN LOS ANGELES OUTER HARBOR, CALIFORNIA, DECEMBER, 1955.
PORTION OF LOS ANGELES HARBOR
1/4 MILE

FIGURE 2

polychaete was found near the outfall sewers of the City of Seal Beach and downstream from the Los Alamitos Naval Air Station.

Newport Bay. This body of water is also essentially clean and used primarily for recreation. However, C. capitata was taken along with fish scales at one station in 1951 and again in 1954. This station was situated at the end of a small arm of the bay in the proximity of fish canneries.

DISCUSSION

Gaufin and Tarzwell (1952), while concerned with fresh water pollution, included four points that should be considered when testing possible indicators of pollution. These criteria are: (1) large number of individuals, (2) few species in the fauna, (3) principally scavenger feeding habits, and (4) either a toleration for low dissolved oxygen or possess some adaptation to a low dissolved oxygen environment. At least some of these qualifications are fulfilled by C. capitata. It has been encountered in large numbers, as many as 400 having been taken in a sample covering a surface area of 100 square inches. Sometimes it was the only species taken. More frequently there were a few additional forms present. In those samples containing organisms, only three or four species other than C. capitata were observed at the stations nearest the Terminal Island sewage treatment plant and the fish cannery outfalls. In contrast, there were 15 or more species present at each station throughout much of Los Angeles -- Long Beach Outer Harbors. Capitella capitata burrows into the substrate and engulfs substrate in much the same manner as an earthworm. The oxygen requirements of this worm have not been studied but it has been encountered in bottoms in Los Angeles Inner Harbor where the overlying water lacked oxygen at the time of sampling. However, this was exceptional, Amore typical situation was 3.5 ppm oxygen at the stations where C. capitata was taken.

It is not known whether or not a relationship exists between the number of C. capitata present in a sample and the degree of the biological pollution. Field data and laboratory observations indicate that C. capitata has a short life history as it reaches sexual maturity in about a month. It is capable of reproducing throughout the year in southern Californian waters. It is not known whether or not any reproductive peaks occur during the year. This speices is cosmopolitan in its distribution, but, with the exception of the statement by Wilhelmi (1916), the ecology of C. capitata has not been studied in the other geographical areas.

SUMMARY

1. The use of the polychaete Capitella capitata has been discussed as a possible indicator of pollution of biological origin in marine waters.

2. This species has been encountered' near outfalls discharging biological wastes in Los Angeles -- Long Beach Harbors, Alamitos Bay, Lower San Gabriel River, and Newport Bay.

3. A special study made in the vicinity of sewage and fish cannery outfalls in Los Angeles Outer Harbor showed no intermediate assemblage of animals between the polluted C. capitata zone and the healthy zone characteristic of much of the outer harbor.

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COOPERATIVE RESEARCH AT OREGON STATE COLLEGE IN THE BIOLOGICAL ASPECTS OF WATER POLLUTION 1/

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Water pollution problems are essentially limnological or oceanographic field problems. Yet, the variability and complex interactions characteristic of real situations make the understanding of that which is observed in the field so difficult that a considerable part of water pollution investigation has had to be carried on in the laboratory. Idealization of the laboratory experiment greatly facilitates the analysis of results, but the idealized experiment iarely approximates reality. Field problems being often too complex for thorough analysis, and laboratory models being usually too simple to be truly representative of natural situations, neither field investigation nor experimentation in the laboratory alone is sufficient for the solution of many water pollution problems. The best information that can be obtained by each approach is needed, and it is highly desirable to bridge the wide gap between ordinary field observations and pertinent idealized experiments. The more nearly laboratory experiments can be designed to model actual situations under study and still retain the advantageous feature of comparative simplicity, and the more nearly a field study can be made to resemble an idealized experiment through the control of variables, the more efficient will be the investigation and the more reliable the interpretation of the findings. Many current problems having to do with the biological aspects of water pollution can be solved only by utilizing these different approaches in an entirely complementary manner.

The cooperative program of investigation in water pollution biology being conducted at Oregon State College by the Department of Fish and Game Management and the R. A Taft Sanitary Engineering Center of the U. S. Public Health Service includes such complementary field and laboratory research. Personnel of these two organizations, with the aid of a number of graduate students, are participating in a research program designed to provide fundamental information pertaining to present and future biological problems of fresh-water and marine pollution. Close cooperation with various state regulatory agencies and with industry is helpful in keeping this research pertinent to present-day problems and also facilitates anticipation of future problems.

Current field studies and those soon to be initiated at Oregon State College fall into two general categories. The first of these categories includes broad studit!.s of the physics, chemistry and biology of rivers and streams variously affected by pollution. Through these studies it is hoped to increase available information on the influence of domestic and industrial wastes on fresh waters,

1/ Miscellaneous Paper No. 29, Oregon Agricultural Experiment Station

to shed more light on the value of biological indicators, and to furnish ideas and direction for the over-all research program. The second category includes those studies which are more nearly field experiments than they are field surveys. Small streams which can be purposely subjected to controlled experimental pollution and variously modified as necessary, and rivers where appropriate experimental designs of a statistical nature can furnish a mathematical control are to be utilized for these studies.

Plans have been made to undertake research on a small stream in which the desired dilutions of added chemicals or wastes can be maintained. This field study will aid in extending information resulting from laboratory studies in which a system of artificial streams discussed below is employed. Field observations and experiments under the somewhat controlled conditions attainable in a small stream will make possible detailed investigation into the influences of wastes on the productivity of such streams at the plant, herbivore, and carnivore levels of production, the last level including game fish.

The results of short-term laboratory experiments designed to determine what conditions are rapidly lethal for aquatic organisms held in glass vessels, though informative, are of limited practical value. In order to infer from such ordinary bioassay results what changes of water quality can be tolerated for long periods by an organism in its native environment, it must be assumed that the manner of action of the lethal agent under consideration is the same at rapidly fatal and at slowly fatal concentration or intensity levels, and the same in the natural environment as in the aquarium. This assumption is by no means always a valid one. Furthermore, though environmental conditions may not be so adverse as to be demonstrably fatal to aquatic organisms, their effect on a population of these organisms may still be thoroughly destructive if they interfere with the reproduction, development, feeding, growth, normal activity, or migratory movements of individuals of that population. Chronic injury to fish populations, due to pollution, may well be much more common and important than spectacular mortalities of fishes caused by acutely harmful pollutional conditions of relatively brief duration, which may or may not have a serious and lasting adverse effect on fisheries. Therefore, some laboratory experiments should be designed so that their conditions approximate selected features of the natural environment. Some of these experiments should be of prolonged duration and should measure the effects of the tested conditions on some of the essential life processes and over-all well-being of organisms, attention being given to the most susceptible stages of the life-history of the organisms. Knowledge of the concentration of a toxic waste, or of dissolved oxygen, which can be barely tolerated for a short period of time, or when the organisms may be relatively resistant to adverse conditions, may be necessary, but is not sufficient.

Encouraging results have been obtained at Oregon State College by using wooden troughs with various current and bottom conditions as artificial streams for the purpose of evaluating the effects of exposure to relatively low waste concentrations for periods as long as a month on different aquatic organisms. In this way, it has been possible not only to determine long-term lethal concentrations of waste, but also to note the effects of the waste on

the feeding and growth of fish, on the development, habits, and emergence of insects and on other life processes of aquatic organisms, such as ecdysis in young crayfish. Changing bottom conditions such as the excessive production of periphyton can be closely observed, as can be the influence of the periphyton on bottom-dwelling forms. These experiments not only make possible a determination of the concentrations of a waste having marked effects on the organisms under artificial stream conditions, but may suggest some of the reasons for these effects.

Thus, when stonefly and caddisfly larvae were held in troughs receiving pulp-mill wastes in various concentrations, it was possible to observe closely the environmental conditions under which mortality occurred and the condition and behavior of the animals before death. The abundant growth of periphyton over the rocks placed on the bottom, and also on some of the experimental animals, the upward movement of the insects from the undersides of rocks where they are usually found (which, in nature, could make them more subject to predation), and the changes of dissolved oxygen concentration among the rocks and beneath the blanket of periphyton all could be readily noted or measured. Furthermore, in seeking to determine the causes of distress and mortality of the insects (which may be referable to toxicity of the wastes, to oxygen deficiency, to some mechanical effect of the periphyton, or to their combined influence) it has been possible to evaluate the role of a single environmental factor by modifying the artificial environment with respect to that factor. For example, by introducing oxygen into the water before it is mixed with waste and enters the troughs, relatively high dissolved oxygen concentrations have been maintained, compensating for the oxygen demand of the waste. In this way, and through additiohal experiments on the influence of dissolved oxygen concentration and current velocity on the survival of insects in cages inserted in glass tubes, which also have been undertaken, the part played by dissolved oxygen reduction in causing the observed pollutional damage to these aquatic animals can be determined.

Long-term experiments with complex industrial wastes inevitably present many problems. One of these is the variability among the several waste samples or batches necessary for completing a single experiment, large amounts of waste being required. The same volumetric dilutions of different waste samples from the same source often differ greatly in toxicity, so that the analysis of test results obtained without .standardization of waste toxicity would be difficult, if not impossible. Frequently, the toxic components of complex wastes are unknown, or there are no chemical or physical means for measuring and standardizing the lethal factors. Biological standardization of waste samples has been accomplished with apparently good success by determining for each sample the 24-hour median tolerance limit of one of the test animals (a fish) being used in the long-term experiment. Some constant percentage of this median tolerance limit is then the strength of diluted waste maintained in each experimental trough during the entire course of the experiment, the dilution used thus being adjusted to the relative acute toxicity of the individual samples. This procedure has two distinct advantages. First, the toxicity of the trough dilution, at least for the control species, should remain constant from sample to sample if the .short-term

and long-term effects of the waste do not vary independently. Secondly, acute lethality data are provided for later comparison with the results of long-term tests of lower concentrations.

Once the relationships between concentrations lethal to a representative fish in a short period of time (median tolerance limits) and concentrations harmful to a variety of stream organisms over a long period are known, it may be possible to predict the long-term harmful concentrations on the basis of short-term test results. Sufficient investigation of the toxicity of a particular industrial waste usually should make possible the determination of dilution factors which, when applied to the short-term median tolerance limit, would yield a reliable estimate of the maximum safe concentration of that waste in the environment of fish and other organisms of importance as fish food. An industry, when supplied with these bioassay application factors, could control waste discharges through routine bioassays of the effluent. Such bioassays would be no more difficult than many chemical and physical determinations now routinely used in the control of industrial effluents. They would, however, provide much greater assurance that the aquatic resources supposedly protected by the waste-control measures are in fact being protected. Only too frequently, ineffective, though complex, chemical tests are being used to evaluate the potential toxicity of industrial wastes to aquatic organisms.

Some of the species which have been used in these experiments for standardization of wastes are believed to vary in their tolerance to certain adverse conditions with such variables as size, age, season, source, time in captivity, and diet. Consequently, the standardization procedure may result sometimes in adjustments to variation in the standard animal rather than adjustments to variability among the waste samples. Needless to say, variations in the tolerance of the fish, as well as variations in the toxicity of the waste, are of considerable interest in connection with practical application of the results. In order to make possible their separation and study, as well as to provide a dependable standardization procedure, guppies are now being raised under very constant conditions to furnish an animal more standard than the wild fish. Genetic strain, age and state of sexual development of the guppies at the time of use, as well as the conditions under which they are reared, such as light, temperature, and diet, can be kept fairly constant; and this will result, it is believed, in sufficient uniformity of the test animals. Standardization and control experiments with the guppy will not replace experiments with species of economic importance (e.g., juvenile salmon) but will supplement these experiments. Only in this way can variability in the valuable native fish species be distinguished from variability in the waste.

A rather broad study entitled "The Influence of Dissolved Oxygen upon the Survival, Development, Growth, Activity, and Movements of Fresh-Water Fishes" is now being carried on at Oregon State College. The survival of fish at low concentrations of dissolved oxygen in different waters has been studied intensively, while the temperature, carbon dioxide content, alkalinity,

[illegible]

[illegible] column. A [illegible] days. [illegible]

[illegible]

[illegible]

[illegible] and [illegible]

[illegible]

are tolerable. It is known that riot all harmful conditions are readily avoided by fish, and tolerable conditions apparently can be repellent. Avoidance reactions of fishes to reduced dissolved oxygen concentrations, as well as to varying dilutions of industrial wastes, are being investigated at Oregon State College, chiefly through laboratory studies. The application of the results of the laboratory tests to problems encountered in the field presents serious difficulties, the circumstances within the confines of even a large laboratory apparatus being a very poor model of conditions in stream environments of much greater area, but the tests can nevertheless be instructive.

In the Pacific Northwest, as in other parts of the country, suitable industrial sites with adequate process and waste-disposal waters are becoming increasingly scarce. Many industries are now selecting locations adjacent to marine or estuarine waters. The aquatic resources of many of these areas are of tremendous commercial and recreational value. Yet, we now know even less about the basic water-quality requirements of marine organisms and their relative resistance to harmful pollutants than we do about the requirements of fresh-water forms. At the marine laboratory of the Department of Fish and Game Management, studies of the water-quality requirements of a number of marine forms have been initiated and are to be greatly expanded in an effort to fill to some extent this serious gap in our knowledge. These studies are of both short and long duration and include an attempt to reproduce marine environments in the laboratory. Sufficient field work will be carried on to assure pertinence to present and future practical problems.

A research program such as that considered above requires certain facilities and a location where different species are readily available and where the desired field studies are possible. The Department of Fish and Game Management has a fisheries research laboratory on Mary's River at Corvallis, and a marine research laboratory near Newport on Yaquina Bay. These laboratories are equipped and operated jointly with the U. S. Public Health Service. The Corvallis laboratory is housed in five buildings, has a supply of river water, and six 250-gallon tanks provided with running water for holding stocks of fish for experimental purposes. Two constant-temperature rooms (one very large and one small) are available at the Corvallis laboratory for standing-water experiments. These have both heating and cooling units. The Yaquina Bay laboratory has both fresh-water and salt-water systems, a spring furnishing a good flow of fresh water for experiments requiring water of considerable purity. Dock and live-box facilities are adequate. There is one large constant-temperature room available at this laboratory. Yaquina Bay probably has a greater variety of commercial and non-commercial fish and shellfish and other marine invertebrates than any other Oregon bay. Corvallis is situated on the Willamette River. This river system, with its varied stream environments, its wide representation of the cold-water and warm-water fish species of North America, and its examples of certain of the effects of domestic and industrial waste disposal, offers excellent opportunities for field study and is a good source of experimental material.

Specialized apparatus has been requisite to much of the work outlined above. Preliminary experiments with artificial streams as a means of studying the influences of wastes on stream ecology have proved very encouraging. The most recently installed apparatus provides a system of six artificial streams. Each stream consists of two 10-foot troughs, one having the water recirculated by a $\frac{1}{2}$-horsepower pump so as to provide riffle-like currents, the other representing a pool-like environment. The water in the troughs is continually renewed, flowing river water. Appropriate bottom materials and lighting result in what is believed to be a rather good model of a stream environment. After the desired plant and animal communities have been established, the wastes to be studied can be introduced by means of chemical pumps in different amounts into the six streams, so as to determine effects of different waste concentrations.

Another apparatus now in use in the laboratory was devised to make possible the study of the effects of 'low concentrations of dissolved oxygen and other water quality conditions on fish swimming against currents with velocities up to 1 foot per second. This apparatus consists of a glass pipe of 4-inch diameter through which the water is recirculated by means of a centrifugal pump. Water quality in the glass pipe is controlled by exchange at a rate of about 1 liter per minute, the exchange water having its characteristics such as temperature and dissolved gas content adjusted by other appropriate components of the apparatus. An apparatus of this kind now being constructed should make possible the study of the influence of critical water conditions on fish resisting currents of relatively high velocities.

The avoidance reactions of fish to water having various characteristics have been studied in a 2 by 9 foot tank with one-third of its length subdivided by partitions into four channels. Each channel is equipped with an adjustable water input and an adjustable drain which, with proper balancing of flows, result in quite sharp boundaries between waters of different quality at the channel openings. Other apparatus has made possible the study over short or long periods of time of the lethal and other effects of water having temperature, oxygen concentrations, carbon dioxide concentrations, total alkalinity and waste concentration controlled. A number of such constant-flow experimental units, each consisting of about five test vessels (usually of 12-gallon capacity) and such other components as are necessary for adjusting the dissolved gas content or other properties of the water in each container independently, are available at the Corvallis and Yaquina Bay laboratories for studies of the influence of water quality variations upon the survival and growth of fish and the development of their eggs. A laboratory shop and the cooperation of research apparatus specialists have greatly facilitated the development of these and other pieces of equipment necessary for pursuing the problems under investigation.

The provision of these facilities has been possible only through the pooling of resources by the Department of Fish and Game Management and the U. S. Public Health Service in the joint undertaking of all of these investigations. Other departments of Oregon State College and industrial repre-

sentatives participate in many of these investigations. Considerable support is given these research projects through grants by Federal agencies and by industry, notably The National Council for Stream Improvement.

Much of this research is accomplished with the aid of graduate research assistants and fellows who are employed to work on problems suitable for graduate theses. Master of Science and Doctor of Philosophy degrees may be pursued with majors in fisheries and minors in desired fields. Several of these assistantships and fellowships are available each year. The research program is complemented by an appropriate instructional program designed to prepare specialists for employment in the water pollution field. College and Public Health Service personnel cooperate in the instructional program.

SOME ASPECTS OF WATER POLLUTION IN THE MISSOURI BASIN

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DESCRIPTION

The Missouri Basin (Figure 1) comprises the larger part of arid and semi-arid regions contributory to the Mississippi Drainage System. Precipitation declines from about 40 inches per year in the eastern part of the basin in Missouri to 10 to 15 inches per year along its western boundary in Montana, Wyoming, and Colorado. The middle basin (the Dakotas, Nebraska, and Kansas) has annual precipitation ranging from 15 to 30 inches, Long dry seasons are prevalent over most of the basin; and the river normally exhibits only two high stages each year-- one coming from melting snow on the praires in March and April, and the other arising from prairie rains and snow melt in mountainous headwater areas in June.

The main stem and many tributaries are undergoing development for irrigation, power production, water supply, navigation, flood control, and waterfowl refuges. The main stem reservoir system, which will consist of three huge one large, and two small impoundments, now lacks only Oahe and Big Bend Reservoirs. Construction of Oahe is in progress. Total storage capacity for this reservoir system exceeds 70,000,000 acre feet. Reservoirs now in operation (Ft. Peck, Garrison, Ft. Randall, and Gavins Point) have permitted significant flow regulation and are retaining a large amount of the suspended sediment carried in the traditionally muddy river.

Tributary developments are, at present, more concerned with irrigation than the main stem impoundments; although the latter will figure in development of new irrigation areas. Irrigation water use may involve natural flow diversion as now practiced in the Yellowstone Valley, reservoir storage as exists on the North Platte River, or ground water pumping from river valleys as is customary along the lower Platte River. Some irrigation reservoirs also furnish hydro-electric power and municipal water supplies. Impoundments used solely as waterfowl refuges compose a very small percentage of impounded waters in the basin. Fish and game interests, however, receive due consideration in the operation of larger multiple purpose reservoirs, some of which also serve as water fowl refuges.

SOURCES OF POLLUTION

The Missouri Basin in primarily an agricultural region, and major industries are concerned with processing agricultural products. Meat packing houses are scattered over the basin but their greatest concentration, and greatest waste contribution is along the Missouri River from Sioux City, Iowa, to Kansas City, Missouri. Wastes from these establishments reach the river in a raw state.

T[illegible] P[illegible], B[illegible] H[illegible], B[illegible] F[illegible], [illegible] Y[illegible] R[illegible]. O[illegible] M[illegible] B[illegible]. T[illegible] K[illegible] C[illegible], [illegible] N[illegible] P[illegible], Y[illegible], [illegible] O[illegible] wastes--steel [illegible]—[illegible]. S[illegible] S[illegible] H[illegible], S[illegible], [illegible] S[illegible] R[illegible] K[illegible] B[illegible].

M[illegible] B[illegible] M[illegible] M[illegible] M[illegible] J[illegible] R[illegible], [illegible]. Some [illegible] wastes [illegible]. B[illegible], M[illegible], [illegible]—[illegible]--[illegible].

B[illegible], [illegible] Missouri B[illegible] T[illegible] paucity of [illegible] B[illegible] Horn, P[illegible], Y[illegible], L[illegible] M[illegible], [illegible] M[illegible] Rivers. B[illegible] M[illegible], [illegible] "B[illegible] Muddy." M[illegible].

T[illegible] I[illegible],

POLLUTIONAL EFFECTS

Pollutional [illegible] factors. R[illegible].

For example, oil refinery wastes generally eliminate most aquatic animals for varying stream distances; organic wastes normally change the character of the bottom and its biota, and eventually stimulate plankton and other algal growth; and release of both types of waste from one locality frequently results in alteration of the usual effects of each, In several Missouri Basin streams, waste effects are further influenced by irrigation practices.

The North Platte River in Wyoming and Nebraska has been utilized for irrigation since 1850. Development of the reservoir system began in 1909 and has continued to date. Operation of this river consists of storing runoff during seasons of greatest snow melt and later releasing it for irrigation during dry periods. Discharges from upstream reservoirs, frequently used for power generation, are caught in lower reservoirs, whose releases are directly concerned with supply of irrigation demands. Drawdown of lower reservoirs provides capacity for storage of power releases in nonirrigation seasons. The operation envisions maximum power generation consistent with necessary conservation of water for irrigation. With the exception of relatively insignificant amounts of ground water inflow, discharge is wholly controlled by reservoir releases. Reservoirs have been noted to increase ground water discharge in areas just below dams, Dams without power generators are usually cut off completely at the end of the irrigation season; and the stream below must subsist on limited ground water inflow. Below such dams sudden transitions from high-to-low or low-to-high water stages are the rule, and they involve overnight changes from big river to headwater conditions, and vice versa.

Raw municipal sewage and oil refinery wastes enter the North Platte at Casper, Wyoming, about 50 river miles below Alcova Reservoir. This reservoir has power generation planned for the future, but at present operates solely to supply irrigation water, and has restricted or no releases at other seasons. In 1950, Alcova discharges varied from 0 to more than 5, 000 cfs. During the period of high discharges, roughly May to mid-October, biotic influences of the Casper pollutional load consisted largely of marked plankton suppression by oil refinery discharges, followed at some interval by stimulation from nutrients added in municipal sewage. The plankton population developed in Alcova Reservoir, and its concentration gradually declined in the stream until affected by waste components. When Alcova releases ceased in October, the plankton algae were replaced by benthic growths in shallow areas below Casper.

These algae attained very dense development in the rich medium (discharge was then less than 200 cfs) but were intermittently eradicated for short periods of time by releases of phenols and crude oils. Their photosynthesis promoted supersaturated oxygen concentrations for many miles downstream. The oil refinery discharges, with periodic releases of crude oil and caustics, had eradicated all animal life in 114 miles of stream below Casper, except for certain fishes that led a short-lived existence near the mouths of some tributaries. Organic sludge deposits were completely untenanted by sludge worms and other characteristic organisms. This sludge and that arising from soda-lime water softening in refineries built up concentrations during low flow periods that were scattered downstream for about 100 miles during later flow increases.

Irrigation degradation of the river became progressively greater with downstream distance. Its most obvious influences were accelerated hardness, alkalinity, and turbidity increases. Silt accumulations suppressed benthic organisms, and suspended sediment reduced plankton concentration.

The sugar beet industry discharged processing wastes to the lower North Platte during fall and early winter. Cold water at that time slowed decay of beet particles and other organic matter, and oxygen was not completely exhausted. Flume water containing beet washings added considerably to the river's turbidity. Irrigation ended before the beet processing campaign began; but high river stages were maintained by return of ground water surcharges contributed by irrigation. Except for regions below beet factory discharges, the lower river was generally clear in fall and winter and promoted benthic algal growth. Lime slurry from beet processing added to alkalinity and hardness, and formed unsightly bottom deposits inimical to benthic fauna. Many solids contributed by the beet industry were caught in Kingsley Reservoir. Their decomposition near the bottom of this lake provided nutrients that stimulated algal growth in the river below. Discharges largely consisted of bottom waters that were generally excluded from plankton productivity in the impoundment. Storage in this reservoir reduced hardness and alkalinity in 1950, and it discharged better quality water than it received from upstream.

Prairie streams lacking the benefits of mountain snow melt permit much more limited water use than occurs along the North Platte. The James River, arising in the prairies of central North Dakota and joining the Missouri below Yankton, South Dakota, has been described as the longest non-navigable river on earth.

It is fed by prairie snow melt and limited summer rains, and normally dries or partially dries over long reaches in early autumn. The main flow at such seasons comes from municipal sewage and water discharges in reaches below Huron, South Dakota. The stream bed has a very flat gradient, and tributary inflows have been observed to run upstream during seasons of high runoff. Several low head dams have been constructed to conserve water for municipal usage, stock watering, waterfowl refuges, and other purposes. The river meanders extensively--requiring 710 channel miles to traverse a distance of about 350 miles. Channel restrictions induce extensive inundation of the flood plain in early spring.

The James drains an area of rich soils and has a naturally high biological productivity. Pollution consists largely of municipal sewage treatment plant effluents. Oily discharges from railroad yards and artifical gas plants affect some reaches; and waterfowl have marked effects in and below some impoundments. The usual effect of sewage plant effluents and waterfowl wastes is excessive stimulation of plankton algae, which frequently occasion tastes and odors in water supplies drawn from the river. Effluent from one overloaded sewage plant promotes anaerobiasis in the river--producing an oxygenless zone along one side for a few miles that is followed by a region of dense plankton growth. Tars contributed by a gas plant at that locality have eradicated bottom organisms; and the oxygenless zone is, with the exception of fungi, a lifeless area. Benthic organisms, especially midge larvae and water mites, attain very dense development in areas influenced by concentrations of waterfowl. The Sand Lake Migratory Waterfowl Refuge northeast of Aberdeen, South Dakota, has had transient populations of more than one-half million waterfowl, mostly geese. Due to the number of impoundments, all waste influences are largely localized; and plankton growth usually result's in very effective treatment of wastes in each impounded stretch.

The Yellowstone River, although supporting considerable areas of irrigated lands, lacks impoundments. Alleviation of irrigation effects by reimpoundment of water is, therefore, not possible. Thus, water quality deteriorates with downstream distance in lower reaches when irrigation is practiced.

The Yellowstone is also polluted by ,sugar beet factories, oil refineries, packing houses, and treated and untreated municipal sewage Oils and tars originating in petroleum refineries have almost completely eradicated bottom fauna in some reaches. Municipal wastes have been noted to lower oxygen and stimulate algal growths.

Periodic taste and odor problems in water supplies withdrawn below Billings result directly and indirectly from waste discharges.

An unusual fish kill affecting long reaches of the Yellowstone was indirectly occasioned by aerial spraying with DDT to control spruce budworm in headwater forest areas. The application was followed by heavy local rains that carried insecticide into the upper Yellowstone system. The DDT was applied in July 1955, and a great mortality of fishes occurred in October and November. Autopsies of fishes indicated starvation as the cause of death. Examination of the streams disclosed a paucity of food organisms, mainly acquatic insects, whose widespread scarcity was then traced back to insecticidal operations in July.

Another unusual case of pollution resulted from practices at a trout hatchery that permitted excess fish food and wastes to reach Rapid Creek in western South Dakota. These materials caused deleterious algal blooms in water supplies taken from the creek, necessitating revision of fish rearing procedures at the hatchery. Fish protection is normally considered an objective of water pollution control, but here fish actively contributed to water pollution.

The above examples are offered to illustrate some pollutional problems related to geography and human practices in the Missouri Basin. Space does not permit reference to various other pollution cases that have been encountered and water quality relationships involved in the main stem reservoir system. The heavy waste load discharged to the lower main stem promotes taste and odor problems in water supplies during low winter discharges, particularly if flow is reduced by ice formation. It also maintains a high concentration of coliform-type bacteria over the reach from Sioux City, Iowa, to below Jefferson City, Missouri. Effects upon the biota in the lower reach are incompletely known. The more desirable fishes., walleyed pike, channel catfish, etc., are most concentrated in the river above Sioux City, while carp form the bulk of the fish catch in polluted reaches below that point.

Water pollution in large areas of the Missouri Basin may appear quantitatively insignificant when compared to that contributed in more heavily industrialized regions. However, limited water supply over such areas results in serious effects from amounts of pollution that would not occasion critical conditions in some areas of great rainfall.

CURRENT INVESTIGATIONS IN WATER POLLUTION BIOLOGY

Investigations and Problems in Ohio

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Prior to white man, it is reported that there was an approximate population of 10,000 Indians whose lives were, generally speaking, based on the factors of self-preservation and actual needs. By 1800, there were some 45,000 whites who introduced the factors of trade and commerce. By 1950, 150 years later, the human population had increased about 176 times the 1800 population to a number of almost 8 million. It is estimated that by 1975, the population will have increased to 10 million.

Prior to the coming of the whites, practically 90 percent of the land was covered by hardwood forests. It may be said that deforestation had begun about the year 1788. By 1880, the entire state was settled, and some land had already been abandoned.

Ohio has turned from an agricultural to an industrial economy. By 1959 or shortly after, the Great Lakes – St. Lawrence waterway is expected to be open, which will have an indeterminable, but probably considerable, impact on the sociological-economic status of the state..

During the early 1900's, water treatment and purification plants were built to safeguard human health from effects of sanitary wastes. By the 1930's, the situation had progressed to the stage at which it was recognized that action was needed to control degradation of the water courses. World War II checked progress temporarily, after which construction of waste treatment plants again began to pick up. In 1951, the Ohio Water Pollution Control Act became a law giving further impetus to corrective steps being taken.

Under this act, now on the statutes as Chapter 6111.01 to 6111.08 inclusive, a Water Pollution Control Board was established in the State Department of Health with the Director of Health as chairman, the other members being the Director of the Department of Commerce, the Director of the Department of Natural Resources, a representative of industry, and a representative of municipal government. The Board administers the pollution abatement program of the State

This Board is vested with a number of duties and powers. Briefly:

1. To develop programs for prevention and control of new and existing pollution. (Pollution means placing of any noxious of deleterious substances in any waters of the state which renders such water harmful or inimical to the public health, or to animal or aquatic life, or to the use of such waters for domestic water supply, or industrial or agricultural purposes, or for recreation.)

2. To advise and consult with other agencies in furtherance of the program.

3. To encourage and conduct studies concerning pollution.

4. To collect and disseminate information concerning pollution.

5. To prohibit or abate pollution by issued orders dependent on certain specified limitations.

6. To issue permits based on compliance with specifications.

7. To institute legal proceedings to compel compliance with the statutes.

Although the Water Pollution Control Board is nominally the pollution control agency, it is still an infant organization which is cautiously developing its program with an eye towards continually improved effectiveness rather than sudden blundering moves which might cause rejection of its purpose. "Industrial wastes and acid mine drainage are exempt from the provisions of Section 6111.04 of the Revised General Code of Ohio until the Water Pollution Control Board after a hearing determines that a practical means for the removal of the polluting properties of such wastes". "Removal" is interpreted in Administrative Resolution No. 1, of the Water Pollution Control Board, to mean any procedure applied to a waste which will effect a reduction in the polluting quality of that waste. The program operates under a permit system which generally does not specify the amount or characteristics of discharges.

The Board deals with 140 cities (actually 118, since 22 are suburbs), of which 39 do not have treatment works, but construction, planning, or investigations are under way to correct this status.

There are 767 villages, 380 of which have been exempted as having no pollution problems to date; 105 have been postponed because of insignificant pollution or difficulty in financing treatment works; 22 are still to be investigated. Of the 114 which have been found to need installation of treatment works, all are under construction or planning operations.

The Board deals only with industries which discharge wastes directly into waters of the State. Of roughly 13,000 listed industries in the State, the Board is dealing with only 646. Of this number 283 are "currently acceptable", 181 need improvement, and 182 are now constructing, planning, or making preliminary studies. Those industries which discharge wastes into municipal sewers are a primary problem of the city but are also under the supervision of the State Health Department.

At first, the Board began bringing industry into the program by compulsory hearings. Following several such hearings, industrial representatives suggested that this implied that industry was not willing to join in the abatement effort and was as a result receiving unwarranted bad publicity. Date

lines were set and industires were allowed to voluntarily apply within that period. All the industries asked into the program under this system are reported to have complied.

The bulk of the Board's information concerning the polluted status of waters is obtained from the Ohio Department of Health. This data generally consists of B. O. D., coliform index, acidity, tastes and odors (phenol tests particularly), suspended and dissolved solids, temperature, and chemical determinations for specific elements. These are generally directed towards human health maintenance. Surveys have been made of the Mahoning, Miami, Maumee, Muskingum and Cuyahoga Rivers.

The Division of Wildlife, Ohio Department of Natural Resources is responsible by statute for the protection and preservation of wild animals. Because of the preponderance of observable effects result in fish kills, the majority of the investigations are carried out by fish managers and game protectors.

The variability of conditions attendant to pollution incidents, of course, necessitates adaptation of procedures to fit the circumstances. The basic procedure is for the county wildlife management agent (known also as the game protector) to notify the district office that a kill has occurred and then to proceed to determine the extent and source until the fisheries technician arrives. Together, an effort is made to pinpoint the source of pollution, to take samples indicative of conditions, such as, pH, dissolved oxygen, temperature, and turbidity tests, and to make counts of the observable dead wild animals.

This report is submitted to the Division's Office of Pollution Abatement which is also notified of the occurrence of the incident at the initiation of the investigation. This office analyzes the information obtained in the field and recommends the action to be taken. A claim for damages is first presented to the offender, and if settlement cannot be reached within 30 days the claim is certified to the Ohio Attorney General for collection.

In 1955, 111 incidents were reported. Sixty-two_were reported as harmful, and forty-nine as threatening situations. A break-down of the information concerning the sixty-two harmful incidents shows the following data in rounded figures:

Forty percent reported counts or expressed kill figures ranging from 5 to 10, 150 fishes. The highest monetary value was reported as $36. 22 for an estimated kill of 2, 289 fish.

Thirty-one percent of the reports showed no counts, because of such matters as too lengthy a period between occurrence and discovery of the kill, non-feasibility of producing creditable estimates because of stream conditions (flooding, natural swiftness, etc.).

Nineteen percent reported damage to habitat, but as yet no basis for monetary value has been developed for collection of these damages.

Five percent of the kills reported were "natural" kills. White bass, Lepibema chrysops, died in great numbers with no determinable cause in Lake Erie. A severe water temperature change is believed to have caused the death of numbers of smelt, Osmerus mordax. A small kill was reported ina lake in which no pollutional discharges could be found.

Three percent reported that the fish had disappeared by the time of the investigation.

Two percent reported no identifiable pollution, although a kill had been reported.

Information obtained from an investigation by the Division is forwarded to the Water Pollution Control Board.

Because it is obvious that present procedures are not entirely adequate, the Division is constantly attempting to improve on its effectiveness. An intensive study of acid-mine drainage effects on Raccoon Creek was made to determine the effect of mining operations on a stream and the possibility of reclaiming the watershed; investigation of feasible corrective measures are now under way. An intensive study of the effect of land use improvements is being conducted on the Little Miami River. Statewide pollution investigators whose duties are to supplement and augment the work of regular pollution investigations and to carry out special investigations have been added to the staff of the Office of Pollution Abatement. The Fish Management Section in Wildlife District Two has carried out an investigation showing the improvement on stream fish life after the installation of a sewage treatment plant; data showed a species number increase from one to a more normal population of 18 species in a period of three years. As an initial program an Aquatic Biology Laboratory is being developed in order to provide more adequate information concerning the effects of pollution on fish life.

Other investigators and their investigations of which I am aware are:

Professor Charles Riley, Kent State University, is investigating for the Ohio Reclamation Association, the reclamation of coal mined areas by the formation of ponds in abandonned cuts and plantings on spoil banks.

Dr. W. D. Sheets, Ohio State University, Waste Treatment Laboratory, using a moderate sized pilot plant for the studies, is investigating the effects of plating metals on sewage treatment installations.

Dr. George E. Barnes, Case Institute of Technology, is directing the investigation of the effects of industrial wastes on the biota of sewage systems in an effort to determine the effects of industrial wastes introduced into a municipal system.

The Ohio River Valley Water Sanitation Commission, headquartered in Cincinnati, is an eight state organization of the Ohio River basin. Its role is to abate and prevent pollution of this great river.

Our problems are quite similar to those concerning other states:

1. A rapidly growing population together with ever increasing industrialization. The topography of Ohio is such that, except perhaps for some of the area in the southeastern hilly region, there is little area which can be exempted from development.

2. A need is felt for developing more effective methods of demonstrating harmful effects of pollution on wildlife.

3. A revision of the status has made the interpretation of their meaning and precedence of one section over another more difficult.

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BIOLOGY AND WATER POLLUTION IN GREAT BRITAIN

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In a general way I think it is true to say that the trends of biological studies in problems of water pollution in Britain have been largely influenced by the legislation controlling river pollution. This is not surprising as most of the applied work in this field has been carried out by government agencies. It means, though that a brief review of the legal aspects of pollution is helpful to the understanding of the biological work that has been and is being done in Great Britain in connection with it.

The problem of river pollution, like many other social problems, arose with and out of the industrial revolution. By the middle of the nineteenth century it had become serious in the industrialized central belts of England and southern Scotland. A series of Royal Commissions were set up to study and report on the situation and the last of these remained in being until the early 1920's. These Royal Commissions carried out a great deal of applied research on river pollution and much of the basic work in this field - including I believe the development of the five day B. O. D. test - was done by them. As a result of these studies they suggested standards for effluent purity depending on available dilution, but their recommendations were not carried through to legislation to any great extent.

In 1876, before most of the Royal Commissions' studies had been made, a River Pollution Prevention Act was passed, but it was really an administrative rather than a technical Act. In effect it made any sort of pollution illegal, but, as the complete prevention of pollution and the existance of industry and urban living were incompatible, the Act provided legal loopholes that were so large that the whole Act proved almost useless as a means of preventing pollution.

This remained the legal situation until 1951 when two River Pollution Prevention Acts, one for Scotland and one for England, became law. These Acts were very similar technically, the differences being chiefly in the bodies charged with the administration of the Acts. For the first time in Great Britain these Acts accepted the principle of allowing a controlled amount of pollution and authorized the setting up of standards of purity to which effluents must comply. As these standards necessarily vary from one river to another and even between different parts of the same river, provision was made for them to be enacted by means of by-laws of limited local operation only.

Apart from the early work carried out by the Royal Commissioners, the Ministry of Agriculture and Fisheries employed a team consisting of Southgate,

Pentelow and Butcher to carry out stream surveys in the 1920's and 1930's. These surveys, as one would expect from the later history of the men employed on them, were very thorough and provided a valuable basis for biological survey work in connection with river pollution.

One of the recommendations of the Royal Commission on Sewage Disposal that came to fruition was the setting up of a permanent research laboratory to work on water pollution problems, namely the Water Pollution Research Laboratory (9) of which Dr. Southgate has been Director for many years.

Particularly since the second world war the study of pollution problems in Great Britain has very largely centered on this laboratory and in collaboration with the Fisheries Inspectorate of the Ministry of Agriculture and Fisheries, of which Pentelow is Chief Inspector, a considerable amount of research is at present in progress.

The programes of research have been shaped very largely to meet the requirements of the 1951 legislation already referred to, and particularly to enable standards of purity to be established. The approach to this problem has been four-fold. First, one team which includes Herbert, Merkins, and Kathleen Downing has been carrying out fundamental researches on the toxicity to fish of various chemicals, in particular cyanide, ammonia and carbon dioxide (2) (4) (5) (6) (7) (8). For this work a very fine piece of apparatus for controlled flow experiments has been developed and used.

Another team including Herbert, Alabaster and Allan has been working on the field aspects of the problem by studying rivers in various degrees of pollution and also by conducting field experiments with fish kept under pollutional conditions:W. Alabasters, in collaboration with other workers has also been engaged in the development of a standard method for measurement of toxicity and although I am not aware that he has yet published his results, he has accumulated a considerable amount of data.

Apart from these purely biological researches, some of the chemists and physical chemists - particularly Downing, Gameson, Knowles and Truesdale - have been working on the problems of re-oxygenation, de-oxygenation, oxygen sag curve and other physical and chemical problems that are very closely related to the biological aspects of river pollution([3]).

There has been a great deal of discussion at this seminar on the problem of bioassays and toxicity tests. It may be of interest for me to give, as far as I know it, the British approach to the problem([5]).

First there seems to me to be a difference in the goals of the workers in Britain and the United States of America. In Britain it has been assumed that toxicity tests will be carried out by a specialist agency, so that the aim has been to develop a practical and reproduceable test that can be handled by a laboratory specially equipped and staffed for the purpose. In the United States the aim seems to be to develop a test that can be carried out by an industrial control laboratory that is probably not specially equipped or staffed for the purpose.

The British workers have not favoured the method which has come to be known as the Doudoroff, Katz method of retaining fish in solutions of poison through which air is bubbled to maintain oxygen concentrations and measuring 50% tolerance limits, because they felt that the shortcomings of the method, which were of course known and stated by its originators, were as difficult to overcome as the problems posed by some of the more complicated experimental procedures. The shortcomings to which I refer are, of course, the removal of volatile materials by aeration and the difficulty of prescribing safe limits from 50% tolerance limits. Instead the approach of the British workers has been, first by means of the fundamental work of Herbert, Merkins and Downing and the field work already mentioned to try to learn sufficient of the mechanism of toxicity to enable safe limits for toxicity to be calculated from comparatively short term tests, probably measuring median survival times rather than 50% tolerance limits. Secondly, attempts have been made to develop an experimental technique that will enable these measurements to be made without aeration. Controlled flow seems to be the most practical line of enquiry and I believe that current work is taking place along these lines.

Apart from the work being carried out by the Water Pollution Research Laboratory in conjunction with the Ministry of Agriculture and Fisheries, there are other workers in Great Britain interested in freshwater biology who may from time to time carry out work having a bearing on pollution problems but, as far as I know, none of them specialize in this work. The Freshwater Biological Association of the British Empire, which has its headquarters on Lake Windemere does a great deal of valuable research of a rather more fundamental character than the Water Pollution Research Laboratory. The Scottish Home Department has a Brown Trout Research Laboratory at Pitlochry, Perthshire, but it is concerned more with fishery improvement than pollution. Some of the universities have freshwater biology stations; for example: Aberystwyth, where Kathleen Carpenter carried out much of her early pioneer work on freshwater ecology, Liverpool, Glasgow and Wessex - formerly University College Southampton - to mention a few that happen to be known to me personally.

I should like to make it clear that this statement does not purport to be a comprehensive review of the biological work in connection with river pollution in Great Britain. It is merely the observations of one person who has been connected with this work for many years and has tried, often vainly I fear, to keep in touch with the work that is being done in this field. I trust that no one will be hurt by my omissions but will attribute them to my ignorance of their work, not to any disparagement of it.

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THE TRAINING OF AQUATIC SANITARY BIOLOGISTS

THE TRAINING OF AQUATIC SANITARY BIOLOGISTS

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INTRODUCTION

Our subject for discussion this evening is certainly not the least in importance among the six panel discussions that comprise this Seminar on Biological Problems in Water Pollution. The success of the training programs of today will be reflected in the solutions of tomorrow's problems

As new problems arise in science and industry, new or revised training programs are born. There is nothing unespected about that, rather it is a natural educational trend. There was a time, not too long ago, when a sanitary engineer as a separate species, did not exist. Today, many of our larger universities have separate departments of Sanitary Engineering.

But when it comes to sanitary biology the problem is not so simple. Let us consider for a moment two somewhat analagous training problems arising from the expansion of two fields of study--first, Conservation and second, Operation Analysis or what the British call Operational Research.

Dwindling national resources accentuated the need for conservation. Advocates of one school of thought favored treating conservation as a more or less discrete entity to be presented or taught as a separate subject or series of subjects. A second viewpoint advocated the teaching of conservation as an integral part of practically all other courses of instruction. After much debate, this latter viewpoint has received widest acceptance.

On a basis of the diversity of subject matter presented during the past four days it is evident that aquatic sanitary biology is indeed a broad field, requiring basic training in mathematics, chemistry, and physics as well as a number of branches of biology.

I propose, as a basis for discussion by this panel, that a sanitary biology curriculum be composed of strong basic courses in each of the aforementioned subjects followed by one or possibly two courses designed particularly for the sanitary biologist. Incorporated in these latter courses should be the history of advances in this field, the biological contributions responsible for research accomplishments to date, the status of today's research in this field and the future trends indicated by current investigations. Also, these courses should set forth the problem needs of the engineer and attempt to bridge any existing gap between these respective disciplines.

What we might term a second reference field is Operation Analysis. It is of course a very much newer field than conservation -- an outgrowth of military research experience during World War II.

Operations Analysis may be defined as a discipline for planning actions most effectively for some purpose. Optimization of achievement of purpose is stressed. It may involve a problem in reducing traffic casualties or diagnosing the source of pollution in a river. In every case, it involves an action taken for a purpose and involving expenditure of effort.

We may decide, let us say, to allocate a certain amount of effort to accomplish a mission. First, we must know the real specific purpose of the operation. We must establish a problem model. The term model is not used here in the sense of a small scale structure but rather as a thinking device with which to analyse a situation. It is important to devise effective odels in approaching a pollution problem or any other kind. Such models are, of course, approximations. Their upper limits or permissible boundaries must be specified.

Thus, from operations research, we learn to construct a problem model, to analyse it, to find out how sensitive our conclusions are to this model.

Presently certain colleges are grouping courses to form special curricula for training students in Operations Analysis.

One highly significant outgrowth of experience to date in Operation Analysis is that investigators representing unrelated disciplines have attacked problems and solved them although these problems have been entirely new and most unique. This is attributed to a fresh and unprejudiced viewpoint provided by the unindoctrinated researcher. To preserve this "take nothing for granted atmosphere", curriculum designers have an understandable hesitancy about prescribing special courses in Operation Analysis.

We are here this evening to consider problems similar to those faced by curriculum makers in the fields of conservation and operation research. Among other things:

(1) Shall we train aquatic sanitary biologists by introducing special courses?

(2) by modifying existing course?

or (3) shall we simply regroup unmodified existing courses? and come up with a cross fertilization that may meet the need.

If we aim to give our undergraduate major and masters degree work as a prerequisite to taking a position in the field, what training courses and fields should be included?

What additions should be made for students continuing for the doctorate?

Are our students to be trained strictly as applied researchers or should we make the program flexible?

What provision can be made for enabling the student to get one or two semesters of actual field experience in sanitary biology before completing his training?

BASIC CONSIDERATIONS

Having dealt with a number of general considerations and alternative approaches to the problem of adequate training of aquatic sanitary biologists, we need now to formulate specifically our problem. Let us consider the objectives of the training program. It has been said that the Case Institute is successful because it turns out what industry wants.

What does the sanitary engineer want from a sanitary biologist? I would like, Mr. Chairman to hear this question discussed.

Pm sure he would, among other things want a biologist capable of recognizing the central problem, of evaluating the techniques most likely to be effective in solving it, and then capable of applying those techniques.

The biologist must recognize alternative courses of action, he must recognize different measures of effectiveness, let us say, in respect to alternative control measures, he must be able to analyse and evaluate the variables involved, he must be capable of careful theoretical analysis and most important be able to subject his data to adequate statistical analysis.

We should perhaps at this point expell the notion that our task here is unique. The correct answer, if there is one, to this training problem may be unique but the problem itself certainly does not stand alone.

In common with chemists, physiologists and teachers of other sciences, we aim to graduate scientists first. Our student product must have those earmarks of a trained mind, one capable of clear and logical thinking for which there is no substitute in the evolution of mankind.

Ernst A. Hauser of M. I. T. states[1] it well "The true purpose of education should not be to make living textbooks, so to speak, rather we must change our curricula so that more emphasis is placed onthe disciplines that teach proficiency in doing and thinking."

I believe therefore that the objective in our training program should be to train students to think and do as scientists first and then as sanitary biologists.

Engineers often use public moneys. They need to know what represents the best bet. They will ask what are the best probabilities in selecting an alternative.

I have heard it said that during the war a statistician undertook to evaluate how effective British bombing missions were in helping Russia. Number of

casualties was the objective. They started by determining the effect in England of German raids. It was found that 5000 tons of bombs were dropped on England yielding casualties of 0.8 persons per ton. Due to various factors the German equivalent was estimated to be 0.4 persons per ton and later this figure was reduced to 02. In other words 400 civilians were killed per month. Then it was realized that, since 40 British bombers each with 5 trained men were lost per month, it therefore required a loss of 200 trained personnel to produce 200 civilian casualties. Not a very encouraging result. Further after the war it was learned that they had been using a wrong measure of effectiveness. Spread-out bombing operations would have been more effective than concentrating on particular centers as was usually done.

This story illustrates, I think, the importance of defining one's problem, evaluating the effectiveness of alternative methods of action or control.

In grouping of training courses to comprise a sanitary biology curriculum, I would attach primary emphasis on the basic sciences prerequisite to aquatic sanitary biology--physics, chemistry, bacteriology, biometry and the calculus accompanied by a number of biology courses which I have elected to leave for discussion by others on this panel better able to present this part of the subject.

Allow me to venture the opinion that too many of our institutions have gone overboard in the offering of specialized courses. Not that I have anything against the refinement of departmental offerings. They, of course, have a place. That definitely is not to serve as a substitute for courses that are more effective in terms of the principal purpose of the program in question. In other words first courses should come first. Thinking capacity--has priority, over textbook mastery.

Again, I would like to hear our panel discuss this thesis: Are we training our students adequately to solve problems? I say emphatically, no. We complain that our current graduates of high schools are steering away from science. If this is true, there doubtless are a number of causative factors. I think the problem approach could well be stressed. Further I believe curriculum makers could well mold their offerings in such a way as to get our graduates to recognize and solve problems. Ability to use the tools of basic science, to focus them on a practical problem, such as how to design a more effective or efficient oxidation lagoon, requires more than routine classroom work in a variety of academic courses. First it presupposes some experience in the practices of thinking or reasoning. A problem such as the lagoon one mentioned, here, is no respecter of courses. Varying disciplines are represented.

In fact, we are in this panel, faced with a problem in operation research. We must define our training problem--our training objective, or purpose, examine alternative means of meeting the objective, undertake to express or state the anticipated effectiveness of the one or more alternatives.

Advances in sanitary biology will depend upon having the best brains and upon having a large corps of dedicated investigators to enlarge the advances. Paraphrasing the words of Paul Weiss ([2]) of the University of Chicago: "All it takes to join these forces is: some aspirations, to point the goal; some inspiration, to point the way; and a lot of perspiration, to get the job done."

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THE JOHNS HOPKINS UNIVERSITY
SCHOOL OF ENGINEERING

Department of Sanitary Engineering
and Water Resources

Baltimore 18, Maryland

509 Ames Hall
April 23, 1956

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Dear Tarzie:

I don't see how I can get to the Thursday seminars and back here to keep appointments that have piled up.

I have a few comments on the training of aquatic sanitary biologists that represent my own prejudices in the matter. They grow from limited local experience and from my total ignorance of the requirements and operations of the official agencies.

1. What are our future requirements for such a specialist group?

It would appear that the demand for sanitary biologists specializing in aquatic biology would be determined by employment in state and federal agencies and by a more limited use in industries that have continuing stream pollution problems. I would hazard the guess that federal demands would not exceed 50 men and that the state demands under most active conditions would be about the same level. It is possible that half this many men would be employed by industry in one capacity or another -- a total of 150 men. This can be quite wrong, of course, and the number might be doubled or halved. Some unexpected legislation might, on the other hand, raise the number to a total of 1,500 or 3,000.

In any case, it would appear that aquatic sanitary biology represents a small profession, far less numerous than sanitary engineering or water works operation. The turnover in this total profession determines the need for special training facilities. If we assume the largest likely number, 3,000, and take an annual turnover of 10%, the yearly requirement would be around 300 to be divided up among the various training facilities in this country. The annual requirement, then, is quite small and well within the scope of a comparatively simple special training unit. If the turnover is larger, say 25%, we have demands of another order, and also evidence that the profession is a fairly unsatisfactory one.

2. Why do we need special training?

Special training seems to be required to permit useful integration of biological observations with other criteria of water quality and changes used by sanitary engineers. The sanitary engineering profession has definite ideas of what biologists are and how they should function in a program. The role of biologist is, however, definitely accessory. —The general history of stream pollution investigations in this country has shown a strong movement away from time consuming biological evaluations to the apparently more quantitative evaluations given by sanitary chemistry. In most sanitary engineers' thinking, the biologist's job is to find suitable indicators for water quality. If these correlate well with B. O. D. and other characteristics of the water, he is happy. If they do not, he doesn't know what to do about it and is inclined to drop the matter.

With increase in new industrial wastes, a second major use of biologists has developed -- testing for toxicity and tolerance. Dramatic toxicity results are, of course, big medicine but the ecological evaluation of waste loadings is, as every field biologist knows, much more complicated.

In any case, the special training that is required of biologists appears to be the development of an understanding of sanitary engineering terminology and of the peculiar whims and policies of the "team". This may take some time. Undoubtedly, the best way to establish this rapport is through direct training in the field unit. Classroom training tends to remove itself from current professional uses. Most of our programs that would employ sanitary aquatic biologists grow from special appropriation projects. These are organized from the top down -- the demands on the biologists will be determined by the conditions of the appropriation. Heaven knows what these 'may be, but they need not be wise or far sighted.

3. What is the role of universities in the training of sanitary aquatic biologists?

The general history of biology departments has shown movement away from natural history toward physiological, biochemical, and genetics type departments. There are few universities with strong natural history divisions. While the natural history courses are by nature intriguing, they have not had the professional potential of other branches -- generally speaking, the professional potential of biology majors has been very low. A few years ago more than half of our "professional biologists" were employed in the Department of Agriculture. These are perhaps the most happily used men in our university group.

Industrial uses have been extremely limited and biological courses have not attracted men who expected to enter industry. This restriction is becoming more apparent and the stature of biology generally has suffered.

The value of any special training offered in a university depends first upon the quality of the men who are attraced to it, and secondly, upon the quality of the men who do the teaching and their understanding of the total problem. It may not be flattering to say that we do not get the most brilliant and promising students in public health, conservation, aquatic biology and other courses that might be conditional to sanitary aquatic biology specialities. It is more realistic to admit that the brilliant students simply don't appear in these courses. Our reservoir of talent is severely limited.

With the decline of natural history training, biology departments have lost contact with the field. Although many members of departments serve on meritorious committees and pass large policy statements, comparatively few work directly with either official agencies or with industries. They are not acquainted with the values involved in any realistic way.

4. How can the training of aquatic sanitary biologists be improved?

It would appear that there is little justification for setting up special courses in universities for training biologists to work in official agencies where the law enforcement component dominates. A better use of limited talent is in the prosecution of special investigations and examination of new phenomena. Here the most resourceful people are demanded. My own feeling is that we can do most by making it possible for biology department members to see what the problems in sanitary biology are really like; to study them in their own way, and to reevaluate them in an original fashion. The official point of view is secondary here. In the development of this relationship industry should take a very active part, especially in those states where conflict of recreational and industrial values is likely. The constitution of a special sanitary biological profession perpetuates the concept of public health losses as a level to forward conservation interests) and it is doubtful if these men will be happy in the promulgation of this type of compromise. It is very valuable to universities to have men active in industries and biology departments would gain stature considerably by such contact. We have had numerous instances of men initially involved in pollution studies who were able to make real contributions to the solution of production problems in industry. This transfer of ability increases the respect of industry for biologists.

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WATER QUALITY CRITERIA FOR AQUATIC LIFE

EFFECTS OF TURBIDITY AND SILT ON AQUATIC LIFE

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the Advancement of Science, Washington, D. C.; Dr. Virgil Pratt, University of Idaho, Moscow: and Mr. Philip Pister of the California Fish and Game Department, Garberville, California. We have not made remarkable progress as yet but have set three years as our goal, hoping to have reasonably full coverage by that time, leading toward publication.

My presentation to you should, by rights, be made three years from now when our committee has had an opportunity to complete its work. Nevertheless, we can all profit at this time by a brief discussion of some findings to date. I shall refer to some pertinent activities and publications in the field, including an investigation which I have made with the State of Oregon on effects of gold dredging on an eastern Oregon stream.

Jacob Verduin (1954) at Stone Lab, Put-in-Bay, Ohio, states that turbidity is the major factor in causing poor phytoplankton productivity in Lake Erie.

David Starr Jordan (1889) reported many decades ago on losses of trout and trout spawn owing to turbidity and silt from placer mining operations in Colorado.

Richard Rathbun (1889) in writing of streams in Iowa, tells of changes with development of agriculture causing decrease and deterioration of the better food fishes. With the breakup of original sod of the prairies, rivers which formerly had well-defined, deep, narrow channels have widened and become shallow. They tend to overflow their banks in the rainy season and lose most of their water during succeeding months. Sediment and silt, continually loosened by farming, fill pools and. riffles in streams, thereby causing rapid disappearance of trout. We are presumably to consider the trout which formerly occupied streams in Iowa as passe just as are the buffalo from the western plains.

Higgins (1931) referred to the enormous quantities of erosion silt entering the Mississippi River along with sewage sludge Together these agents smothered bottom life, wiped out mussel beds, and the like.

Emmeline Moore (1937) quotes H. K. Townes in saying that siltation dilutes settling organic debris to such an extent that bottom organisms have a poor food supply. There is a continual "snowing under" of bottom organisms. In fact, induced siltation has been used in irrigation canals to inhibit growth of moss and algae.

Tarzwell, (1937) in the course of stream improvement work in Michigan, evaluated the effectiveness of the structures and came up with some enlightening facts on productivity of stream bottom types.

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4. The presence of some bottom fauna at all stations indicates absence of toxic substances in the mining waste. Principal damages are physical smothering action and interference with light penetration necessary for growth of green plants.

5. Results of fish population studies in the various zones of pollution in Powder River indicated a complete alteration of the population from sport fish, rainbow trout and whitefish,found above all sources of pollution to rough fish in the zone of pollution and recovery.

The sedimentation test which was made on waters of the Powder River downstream from the gold dredge indicated a high degree of reduction in suspended matter in a 24-hour period. During stages of normal to low flow a corresponding progressive decrease in suspended matter was observed downstream from the source of pollution. Although the screening of the light was a significant factor in lowered biological productivity, the abrasive or molar action of the larger particles of sediment and the smothering of fish food organisms and fish spawning beds are considered to be of greater importance.

The more recent investigation showed that remarkable recovery had taken place in the Powder River with flushing of silt from the pools and cleansing of riffles by freshets This signalled the return of a wide variety of bottom fauna in the 15-20 mile reach of the river which had been heavily silted. The Oregon Game Commission has planted trout in this stretch and creel census indicates successful reestablishment of the sport fishery.

This brings us back to our original thesis, namely: The establishment of water quality criteria for silt and turbidity in natural waters. May I be permitted to incorporate here the con sensus of opinions expressed by members of the seminar audience? It has been suggested that rather than to propose arbitrary criteria either for turbidity or for settleable solids, some percentage increase above normal low flow concentrations should be established. This would take into consideration differences in watershed and stream or reservoir characteristics.

In conclusion, the problem of siltation and excessive turbidity stemming from activities of man are widespread and difficult to control. Many question the economic feasibility and practicability of control measures, but a few forward-looking pollution control agencies are undertaking measures of control with varying degrees of success. As a guiding principle in establishment of water quality criteria for permissible concontrations of silt and turbidity in streams, certain percentage increases bove levels at normal low flow in waters is suggested.

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THE EFFECT OF POLLUTION UPON WILDLIFE

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I am pleased to have this opportunity to join you to learn of the many problems facing those working in the field of pollution and to learn of the progress being made in the technical field toward the alleviation of these problems. It goes without saying that the advance of civilization has brought with it many wonders, but at the same time has left in its wake many things, one of the worst being pollution, which are in contradiction to the many fine recorded accomplishments.

As my portion of this discussion, I shall attempt to summarize for you the effect of pollution upon aquatic life as it relates to our objectives and functions in the field of fish and wildlife conservation.

The programs of the Fish and Wildlife Service have the central objective of insuring the conservation of the Nation's wild birds, mammals, fishes, and other forms of wildlife both for their recreational and economic values. In carrying out this objective, which we share with the States and Territories, it is essential to build up these resources, to prevent their destruction or depletion, and to promote the maximum use and enjoyment in consonance with their perpetuation.

Before discussing the Fish and Wildlife Service's interest in the field of pollution as it relates to wildlife, I shall review briefly the functions of the Service, particularly those which are concerned in one way or another with pollution problems.

The conservation of the North American waterfowl and other migratory birds is undertaken in cooperation with the governments of Canada and Mexico. The Service operates 264 refuges to provide for the needs of water fowl and other species as a major part of this program. Our investigations and surveys are the principal basis of the hunting regulations, which are enforced by the Service.

The Service is charged with the administration and enforcement of the laws relating to the commercial fisheries of Alaska. It also conducts biological research on marine species of commercial importance off all coasts of the United States and in waters adjacent to territorial possessions. The fishery research program provides information on the size of the resources, rates of decline or increase, and reaction to various intensities of fishing as a basis of conservation programs. The marine fishery program also includes technological, economic, and statistical research as means of promoting trade and commerce in fishery products, improving processing, distribution, and marketing practices, and effecting more complete utilization of resources.

Our programs for the maintenance of the fresh-water fisheries extend to the conservation and development of commercial fisheries of the Great Lakes and other inland waters, and to cooperation with the States in the maintenance of sport fishing throughout the Nation. The maintenance programs for Federal lands and installations depend upon stocking from the Service's hatcheries and upon our programs for the protection and restoration of habitat. Much attention is given to investigation of the effects of water development projects proposed for construction by the Federal Government or under Federal permit or license. In a number of instances the Service maintains and operates fish screens on Federal irrigation projects.

The Service administers the Federal Aid in Fish and Wildlife Restoration Acts, which authorize grants-in-aid to the States and Territories, including investigations, acquisition of land, and development of fish and wildlife habitat. The Service is authorized to provide assistance to, and cooperate with, Federal, State, and public or private agencies and organizations in the development, protection, rearing, and stocking of all species of wildlife and fish.

Among other important programs of the Service are activities relating to international agreements concerning fish and wildlife, including the Whaling Treaty Act, the Sockeye Salmon Fishery Act, and the North Pacific Halibut Fisheries Act; the management of the North Pacific fur seal herd; the administration and enforcement of the various Federal laws relating to wildlife and fisheries, including the restraints upon interstate transportation contained in the Lacey Act, and the Black Bass Act; and the promotion of domestically produced fishery products in commerce, the development of markets for fishery products of domestic origin, and the conduct of research pertaining to American fisheries.

There is seemingly no limit to the varieties of pollution which directly or indirectly are harmful to fish and wildlife resources. Despite this, there is no Federal authority under which the Service enforces regulations governing pollution.

Since we lack the power of enforcement, we must, when the need arises, seek the aid of State and Federal agencies who are empowered with such authority. Our activities in the pollution field are confined largely to research on special problems and to cooperation with State and other Federal agencies. Without the excellent cooperation received from these agencies in working out solutions to our problems, there is no question that our progress in fish and wildlife conservation would be seriously retarded. To keep abreast with pollution problems, the Service is represented on interagency committees which consider water-development planning. We are also represented on a National Research Council Committee which studies and reports on the effects of radioactive wastes on aquatic life, and on the Interdepartmental Committee on Pest Control.

In our investigations of water-development projects proposed for construction by the Federal Government or by private interests under Federal

permit or license, we attempt to recognize existing pollution problems and consider the possible influence which the project will have on existing pollution conditions and the consequent effects upon fish and wildlife. After consulting the States the Public Health Service, and others, recommendations aimed at avoiding future detrimental effects on these resources are made. In many cases it is difficult to identify a water-development project as a direct cause of pollution, although the operation of these projects would clearly worsen the existing problem. The Roanoke River in North Carolina can be cited as an outstanding example of the need for industry and State and Federal Governments to join forces in conquering a severe pollution problem created by wastes from paper mills and municipal sewage, and aggravated by regulation of flow and by the low oxygen content of water released at the Roanoke Rapids Dam. Among other effects, the striped bass fishery is threatened. Here, the forces have joined together to make studies of the situation, and we are hopeful that the many competing uses for water can be satisfied through an abatement program and modification of reservoir operations.

While on the subject of water-development projects, I should like to point out that some projects threaten fish and wildlife resources in certain coastal areas By changing flow regimen or by re-routing waters, the usefulness of certain marsh areas for waterfowl, other wildlife, and fish may be destroyed as a result of saltwater intrusion into fresh-water areas. In other instances, estuarine environments may become less saline as a result of increased flows of fresh water and seriously affect fish and shellfish production.

A type of pollution that has become one of our most pressing problems is that resulting from the increased use of insecticides, herbicides, and fungicides It is estimated that farmers, home gardeners, and government organizations charged with control of noxious insects and plants now purchase 700 million pounds of these basic materials each year. This represents about 3 billion pounds of finished pesticides that are sprayed or dusted annually on millions of acres of the Nation's crop, forest, range, and marsh lands.

Aquatic organisms, particularly fish, crabs, and insects, are among the most sensitive of all animal life to poisoning by pesticides. Insecticides, especially the chlorinated hydrocarbon group, offer the greatest hazard. Herbicides are generally less toxic, but in addition to direct poisoning they destroy both aquatic and terrestrial food and cover plants. Other groups of pesticides, such as fungicides and rodenticides, present much less danger under conditions of use.

Our wildlife research on the biological effects of pesticides has been confined principally to the effects of insecticides on wildlife species inhabiting marsh areas. Considerable of our attention in these studies has necessarily been devoted to determining effects of these materials on lower forms of life because of the importance of many invertebrates as food for birds and mammals.

For example, fiddler crabs comprise about 90 percent of the summer diet of clapper rails in New Jersey salt marshes. Cooperative investigations conducted with mosquito control agencies in Atlantic coastal marshes have shown that single applications of 0.4 to 0.5 of a pound of DDT in one-half to one gallon of No. 2 fuel oil per acre produced moderate to severe but temporary losses of fish, crabs, and other invertebrates, while applications of 0.2 to 0.3 of a pound of DDT in one-half to one gallon of No. 2 fuel oil per acre repeated several times a summer for several years caused significant, more lasting damage to most of these forms. There was little direct harm to birds and mammals although long-term routine spraying operations appeared to reduce carrying capacity for these higher animals. The studies also revealed that DDT in granules caused less damage than equivalent dosages in oil solution. Of the newer insecticides, dieldrin , aldrin, and lindane were more toxic than DDT to crabs.

Experimental feeding of these chemicals, including DDT and strobane, to quail and pheasants decreased the number of eggs produced and reduced the hatchability and viability of the eggs. The viability of the young was also reduced. These effects were observed when concentrations in the diet were as low as 1 ppm of aldrin, 5 ppm of dieldrin, 50 ppm of strobane, and 100 ppm of DDT. It has been reported that application of 1 pound of these chemicals per acre under agricultural practices would result in concentration of 50 ppm in seeds and vegetation which may be eaten by wildlife.

The Service has recently been requested to participate in an investigation of fish kills in the Upper Yellowstone River area which possibly resulted from DDT spraying operations for the control of the spruce budworm. In 1955, a high mortality of whitefish and a lesser mortality of trout and suckers occurred in the stream several months- after spraying operations in July. Destruction of fish food organisms resulting in fish starvation may be a contributing factor. Although similar spraying in previous years did no apparent damage, this serious incident signals the need for studies which will give us a better understanding of DDT formulations and their effects on fish and wildlife.

Pollution from industrial sources includes a wide variety of substances which are lethal or harmful to fish and wildlife. Among the serious offenders are the wastes from paper and pulp mills, sawmills, chemical manufacturing plants, textile plants, mines, oil fields and processing plants, sugar beet processing plants, meat packing plants, dairy product processing plants, leather processing plants, and many other operations.

The Service has no continuing research program to study the lethal or undesirable characteristics of these wastes upon fish and wildlife, so we look principally to the research studies of others for this type of information. Some of this information is sought by the States under the Federal Aid in Fish Restoration programs which the Service administers. Most of these studies, however, are more in the nature of general pollution surveys.

From about 1941 to 1946, the Service conducted surveys of some of the sources of pollution of this nature and made studies in an effort to determine minimum quantities which fish could withstand. I believe most of you are aware of the difficulties in setting up standards which would apply to a variety of conditions.

This spring, studies by the Service are being initiated in Kentucky in cooperation with the U. S. Public Health Service, the U. S. Geological Survey, and the State of Kentucky to determine, under controlled conditions, the effects of coal strip-mining pollution on stream ecology. Since this pollution has a definite limiting effect on the productivity of the aquatic environment, these studies will serve as a basis for developing recommendations to protect or improve fish and wildlife habitat which may be, or is, affected by such operations.

There are numerous reports of the deleterious effects of mine wastes upon fish and wildlife. Annually, thousands of waterfowl seeking aquatic food and fish fall victim to poisoning. Such a situation occurs regularly in the Coeur d'Alene River in Idaho. The ore deposits in the area are primarily sulfides, chiefly of lead and zinc associated with deposits of silver, cadmium, bismuth, arsenic, antimony, and iron. Sulfides of various heavy metals become even more dangerous when they change over to other compounds. Little progress has been made during the past 30 years in getting the pollutors to remedy these hazards. In addition, mine slimes have created less productive aquatic environment in the river and in Coeur d'Alene Lake. The pollution situation was studied and reported on by the Service in 1932 and we encourage a study which was inaugurated a year ago by the Cooperative Wildlife Research Unit at the University of Idaho.

Oddly enough, even the hunter accounts for a significant amount of lead poisoning in waterfowl. Birds feeding in water areas which have been heavily hunted, swallow enough shotgun pellets to result in quick death.

Oil pollution not only destroys our marine and inland fisheries, but results in a heavy mortality of waterfowl. In many cases the oil pollution is from the discharge of barges, ships, and accidental or careless handling of oil transportation, development, and drilling operations. Waterfowl alighting on oil sump areas or water areas covered with oil are usually rendered flightless. Oil is also a destroyer of aquatic life including foods of both fish and wildlife. Even in minute quantities oil may impart undesirable tastes to the flesh of fish and shellfish, thus rendering them unpalatable as human food. In cases which come to our attention, the Federal and State authorities are contacted. The Corps of Engineers and Coast Guard are often contacted in such cases relating to navigation under the Oil Pollution Act of 1924, which is administered by the Department of the Army. Oil operations on certain of our wildlife refuges are kept under close surveillance to avoid damage to waterfowl.

On many areas where serious pollution hazards of various types occur, it is necessary for our game management agents to keep the waterfowl driven from these areas.

Silt and washings from mining operations are of much concern to us. Dredging and reworking of stream bottoms for gold and other minerals brings indiscriminate destruction to fishery resources and habitat. The turning over of the stream bottoms destroys important spawning and food producing areas, and silt from such operations quickly snuffs out the lives of incubating fish eggs and aquatic organisms. Salmon and trout on the West Coast and in Alaska come in for their share of punishment from these operations. It seems that solutions to some of these problems could best be solved through the withdrawal of public lands along streams to exclude detrimental mining operation. Such a procedure has been attempted, but has met with considerable opposition from mining interests.

In Alaska, fishery resources are not protected by the Alaska Pollution Act in the case of silt from mining operations. The Act specifically states that "the results of activities connected with gravel washing plants and all phases of Placer Mining Operations shall not be considered pollution within the meaning of this Act."

Service hatchery and planting operations are sometimes handicapped by silt from lumbering and other operations. Certain situations occurring on U. S. Forest lands have been remedied by reporting such matters to the Forest Service which took steps to assure that the lumber operator complied with the terms of his contract.

Logging and pulp operations have long presented serious problems to fish and wildlife. This industry is relatively new in Alaska, where the effects are now being felt by the salmon fishing industry. A study will be conducted by the Fisheries Research Institute of the University of Washington in cooperation with the Service to determine the effects of these operations.

Municipal pollution, with particular reference to sewage, brings great concern from the standpoint of health, but it also may seriously affect fish and wildlife. In some instances small amounts of organic pollution may be beneficial to fish, but high concentrations may deplete the oxygen supply and result in fish kills or the loss of food organisms. The Service relies heavily upon the States and the Public Health Service to bring about the abatement or elimination of pollution from such sources. There have been occurrences, however, in which the Service's installations such as refuges have been affected, and the Service has encouraged cities to install treatment plants.

As you can readily see, the Service does encounter many pollution problems affecting its interests, few of which can be resolved without assistance of other agencies. The men who come up with the answers to the technical problems deserve a lot of credit in the total effort aimed at beating pollution.

WATER QUALITY CRITERIA FOR AQUATIC LIFE

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The lakes and rivers of North America have played a very important role in the opening and development of the continent. In our Great Lakes we have a fresh water resource far surpassing any other in the world. Our rivers and hundreds of thousands of inland lakes are outstanding in their many uses and support a diversity of the most valuable fishes to be found anywhere. Perhaps it is because of this great wealth in aquatic resources, which many considered to be inexhaustible, that we have been so remiss in their protection and conservation. Their importance is only now coming to be generally appreciated. The great increases in population since 1900 and the manifold increases in the volume and variety of water uses have shown that in some areas the supply of water is definitely limited. As with other resources we find that value varies directly with demand and inversely with supply. In the western areas which recently suffered from a severe drought it was found that when local drinking water supplies dry up,water will be purchased at any necessary price, however high it may be.

What is true for drinking water is also true in some measure for aquatic life resources. We have already found that as the demand for desirable fishing increases and the supply diminishes, the amount paid for such recreation becomes greater. The development of the country has drastically reduced or eliminated fishing waters in extensive areas. Deforestation, fire, overgrazing, and unwise agricultural practices have increased surface runoff and decreased seepage causing floods, intermittent flow, the drying up of springs, erosion, silting, and the filling of stream beds. Removal of streamside vegetation has promoted bank erosion, the widening of streams, and warming of the water, Industrial and other pollution has blocked fish migration and has rendered many areas unsuitable for fish. These practices which alter or destroy the aquatic habitat are the chief cause of the decline of aquatic life resources. The surest way to eliminate a species or group of species is to destroy their habitat or produce environmental conditions unfavorable for them. The only way to maintain a species is to protect and maintain environmental conditions essential for and conducive to its growth, reproduction, and well being.

Protection and conservation of aquatic life is not a simple task. Some have questioned its value and feasibility. Our fisheries are now an important resource. The commercial fisheries of the United States and Alaska have an annual production of almost five billion pounds (1). This crop represents a high protein resource which can be expected to increase in value, While commercial fisheries utilize a great deal of equipment and employ large numbers of people, their economic worth is only a fraction of that of sport

fishing. In 1955 there were more than 17 million licensed fisherman in the United States. There are several million additional fisherman who fish in the Great Lakes and in marine waters where a license is not required. According to the National Survey of Fishing and Hunting (2), sport fishing in the United States has an annual value of almost two billion dollars. Within the next 100 yeai's the number of fishermen and the value of the fishery will increase severalfold if the resources can be preserved.

In comparing the beneficial uses of a stream, there has often been a tendency to underestimate the wildlife and recreational values and to take a short rather than a long view. When evaluating our fisheries resources we should consider the returns not just for one year but over the centuries because these resources are renewable. The aesthetic, recreational, and health values of our waters are difficult to measure, but they are great. The recreational industry is a large one and is expanding every year. In a few states it is the first ranking industry and in many others it is of considerable importance. In the industrial state of Michigan it is reputed to rank second. As our population increases there will be an ever growing demand for and use of our forests, parks, preserves, wilderness areas, and streams, where people can engage in water sports and "get back" to nature and relax. It is believed that our aquatic life resources and the aesthetic value of our lakes and streams, which are largely inseparable, are well worth our earnest and sincere efforts to preserve them.

The objective of water quality criteria for the protection of aquatic life is to preserve or restore em;ironmental conditions essential for its growth, reproduction, and well being. If these requirements are known and understood, criteria can be set up which will achieve this objective. If habitat requirements are not fully known, criteria can only be based on the best information available and changed whenever the need is indicated by new information.

Under our present state of knowledge a suitable water cannot be defined in chemical terms alone. There are several reasons for this situation. Different species of fishes and the organisms in their food chain vary widely in their sensitivity to dissolved materials. We do not know the effects on aquatic life of various concentrations of many materials individually or in combination. Mixtures of materials often have effects different from those of the individual components. Further, we do not know minimal lethal levels for many materials or their mixtures, nor do we know the most favorable concentrations of materials essential for the organisms. Perhaps the best definition that can be given of a suitable fish habitat is -- "A suitable fish habitat is one which produces a satisfactory fish crop." The adequacy of a fish crop is judged by its quality and the pounds produced per unit of surface area, Commonly, productivity and suitability are judged by catch per unit effort, growth rate, condition factor, quality of the flesh, and the size and species composition of the catch. In fisheries management an effort is made to manipulate the environment so that conditions are made more favorable for the desired species and less fa Torable for those not wanted.

While our knowledge of the habitat requirements of fishes is far from complete, enough information is now available so that some criteria and procedures can be set up which will be of value in the maintenance of a satisfactory environment and production of a suitable crop. However, much research is still needed to obtain all the information essential for the solution of this problem.

The environmental requirements of fishes may be roughly grouped under four main headings, (1) a favorable water supply; (2) suitable spawning facilities; (3) an adequate food supply for all age groups; and (4) good pools and shelter. In the abatement and control of pollution we are chiefly concerned with the first requirement, a favorable water supply. Natural waters have widely varying physical and chemical properties. The suitability of any water for fish life depends on its quantity, permanency, and quality; that is, its temperature, the concentration of dissolved atmospheric gases, salts and other minerals, and suspended solids.

As here used water pollution is the addition of any material or waste to a water in such quantities that it interferes with, lessens, or destroys a beneficial use. In this regard perhaps the simplest definition of pollution is "too much. " For example, if "too much" is not added, the discharge to a stream of organic matter such as sewage has a fertilizing effect which is beneficial to fish production. However, when the capacity of the stream to utilize organic materials is exceeded so that unfavorable environmental conditions are produced and a beneficial use is damaged, such a discharge becomes pollution. It is evident, therefore, that water quality criteria for the protection of aquatic life must entail some quantitative measurements. Before criteria can be set up we must know or have some measure of how much is "too much" for those species we wish to protect at all stages in their life history and in waters of different quality. These criteria must insure environmental conditions favorable to all life activities and to general well being--mere survival is not enough. These environmental factors will be discussed in some detail.

Environmental Factors

Temperature

As a group, aquatic animals of the temperate zone are adapted to fluctuations in temperature between 39° and 90°F. Not all can withstand this range and some can withstand higher and some lower temperatures for a time. The range of temperature which can be tolerated by different species varies considerably as does their ability to withstand sudden changes or to acclimate themselves to unusual temperatures. Each species has a preferred range of temperature within which it does best and a zone above and below this in which it can survive for short periods. Proper acclimatization enables certain species to survive at temperatures which would be fatal under conditions of sudden exposure.

Fish have a rapid rate of adaptation to high temperatures (3) but adaptation to lower temperatures proceeds at a much slower rate. When suddenly exposed to higher temperatures fish can withstand much higher temperatures in summer than in winter. As the summer season develops,, changes in upper lethal temperatures reflect the major changes in water temperature, rising with ascending temperatures and falling as the water cools in the fall. Brett (3) reports that the lethal temperatures for the bullhead rose from 29.1°C. on May 12 to 35.3°C, by July 8. Brett (4) also found that there is a considerable difference in the time required for completion of acclimation with respect to heat tolerance at each level of temperature. It has been shown that some species exhibit geographic differences in their resistance to high temperatures while others do not (5). The writer noted a bass kill in a southern Michigan lake in June 1936 when the water reached 94°F. during an unusually hot period. However, a temperature of 96°F. in Wheeler Reservoir in 1938 appeared to have no lethal effects. The difference may have been due to a different acclimation history. It was observed that a temperature of 108°F. killed all the fish in a pond near Savannah, Georgia, in 1945. Allowable peak temperatures brought about by some unnatural cause may, therefore, be somewhat different in different portions of the country, increasing from north to south.

Members of the family Salmonidae are cold water forms. Brook trout seem to do best in streams, the summer temperatures of which range between 52 and 68°F. (6) (7). While trout can survive much higher temperatures for short periods, streams having such temperatures are not first class trout streams. The writer found brown and rainbow trout surviving a peak temperature of 83°F. in the South Branch of the Pere Marquette River of Michigan in 1930. Brook trout survived peak temperatures of 81° and 82°F. in the East .Branch of the Black River on successive days in July 1931. Fry (8) reported the upper lethal temperature for young brook trout (12 to 14 hours exposure) to be about 77.5°F. Such high temperatures are more favorable for minnows and suckers which increase greatly in numbers and compete for food and space with the trout (9). The result is that trout comprise a very small portion of the total fish population of the stream and supply little fishing even though the overall productivity of the stream may be great.

Four fish population studies made in the East Branch of the Black River of Michigan indicated that trout made upon only 9. 6 percent of the total number of fish taken in the study areas. In the neighboring Pigeon River, another stream having temperatures above 75°F., trout comprised 15 percent of the total fish population in the areas counted (9). In a nearby cold stream, the West Branch of the Sturgeon River, trout represented 96 percent of the total population. In trout streams having high peak summer temperatures, suckers and minnows comprise the bulk of the fish population. Thus, while temperatures higher than the optimum and high temperatures of short duration (75° to 82°F.) may not kill trout, they produce environmental conditions more favorable for the coarse fishes, which increase at the expense of the trout population. This fact must be taken into consideration in

the establishment of temperature criteria for the cold water species. Such criteria must be based on optimum conditions and not on temperatures tolerated by trout. It is believed that for good trout production in streams subject to invasion by coarse species, temperatures should not exceed 68°F.

Since deforestation, overgrazing, unwise land use, removal of streamside shade, and erosion have already caused the warming of streams to such an extent that the amount of trout water has been seriously reduced, and since trout fishing is in highest demand, it is believed that no wastes of significant heat content should be discharged into a trout stream if the stream is to be maintained for trout.

Favorable temperatures are especially important at spawning time for both cold and warm water species. It is well known that bass spawn in the spring when the water temperature exceeds 60°F. If the water is unnaturally warmed to this temperature for a period, spawning may be induced too early in the season. Then if waste discharges are discontinued over a weekend, water temperatures may drop into the 50's with the result that guarding males leave the nests, the eggs are infested with fungi, and no young are produced. Fluctuations of water temperature above and below 60°F. during the spring are detrimental to bass production.

A change in water temperature may affect the aquatic fauna directly or indirectly. While the change may be within the thermal tolerance of the fish, it may so alter environmental conditions that they become unfavorable for essential food organisms and certain life history stages of the fish, or the change may make them more favorable for competitors or predators. Temperature changes will directly affect the metabolic rate, growth, and reproductive processes. They may result in increased or decreased food production, interfere with spawning, or change an important part of the fauna, thereby altering the quantitative makeup of the population.

Although high summer temperatures have been considered of outstanding importance because of their possible lethal effects, it is believed that unnaturally high winter temperatures may be equally important. In the temperate zone the aquatic biota have evolved under conditions of quite wide differences in seasonal temperatures. For example, the eggs of some daphnia have to be chilled or frozen before they hatch. Many other organisms go through resting stages or specific stages of development at certain seasons. Some of the diatoms, for example, are abundant only at temperatures below 50°F. Other forms appear only at certain times of the year and there is a succession of forms with the seasonal changes. At present we have little conception of the changes which might be brought about by permanently elevating stream temperatures. A large portion of the biota might be changed and the whole food chain disrupted. For this reason consideration should be given to upper temperature limits during the winter season. This consideration may require increasing attention as the atomic energy industry develops. Temperature should not be raised to levels that induce spawning at unnatural times if there are periodic drops in temperature, and they should not be such

that they interfere with the development of important organisms in the fish food chain. Considerable study is needed before this problem can be approached intelligently.

Waters of significant heat content should not be discharged into a stream in such a manner that they create a temperature block across the stream. Further, an abrupt change of more than 9°F. may affect fishes adversely even if of short duration.

For a well rounded warm water fish population in the Ohio valley area it is believed that peak summer temperatures should not exceed 93°F. at any time or place. In the south suchpeak temperatures probably should not be above 96°F. This means that in general temperatures will be considerably below these levels. While several species can withstand higher temperatures (100° to 103°F.) for very short periods, 93° and 96°F. represent critical levels for most species in the designated areas. Further, while fish may, through certain adaptations, survive abnormally high temperatures for short periods, they cannot complete their life history at such temperatures. For good production, therefore, temperatures within a favorable range are required.

Settleable Solids and Turbidity

Studies carried out in connection with trout stream improvement investigations in Michigan indicated that sand bottoms are almost barren of benthic organisms and that the addition of sand or silt to rubble or gravel bottom streams greatly decreases stream productivity (9) (10) (11). In fact, shifting sand in quantities so small as to be unnoticed by casual observation can decrease the production of macro-invertebrates by drifting into the spaces between the gravel and thereby decreasing the areas for attachment and cover. It is believed that no inert inorganic, sandy, or other similar wastes should be added to a rubble, gravel bottom stream as such deposition may not only decrease the supply of desirable stream bottom insects but also seriously limit spawning d most nest-building fishes. Studies of the effects of mining wastes in California have shown that salmon select clear water for spawning and that the deposition of silt results in smothering of the eggs (12). Quantitative bottom samples taken in a series of similar streams in California showed that the average number of food organisms was always less in mined areas when inert inorganic materials were discharged to the stream than in nonmined areas (13). On the Scott River, samples from the silted area averaged 36 organisms per square foot, whereas those from the clean stream bottom above the mine averaged 249 per square foot, or 7 times as many. Similar studies (14) have shown that hydraulic mining wastes are detrimental to salmon and trout production. These and wastes from placer mining and from stamp mills and washing operations may completely choke a stream causing it to flow in a shallow sheet over the accumulated deposits. Further, debris dams created by such operations can eliminate the salmon by blocking migration.

From results of studies in various parts of the country, it is apparent that erosion silt is a major stream pollutant and that it produces

[illegible]

[illegible] universally [illegible]

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T[illegible] I[illegible] (15), W[illegible] (15A), [illegible] O[illegible] A[illegible] M[illegible] [illegible] (15) direct [illegible] 20,000 [illegible] 100,000 [illegible] M[illegible] 100,000 [illegible] 175,000 [illegible] 225,000 p.p.m. F[illegible]

I[illegible] O[illegible], B[illegible] (16) [illegible] 1.7 [illegible] 5.5 [illegible] 1954 [illegible] 8 [illegible] 12.8 [illegible] buffalo, [illegible]

S[illegible]

essential on all clear streams and they should be initiated in conjunction with efforts to reduce turbidity and settleable solids through control of soil erosion.

Turbidity standards must be somewhat local in their application as they will depend on the area and type of stream. It is possible to set up relatively simple turbidity standards which can be readily checked for compliance by field tests. Turbidity standards might state that a certain percentage of the incident light at the surface shall reach a stated depthbetween 11:00 A. M. and 1:00 P. M. The depth selected would depend on the depth to which the regulatory agency felt the photosynthetic zone should extend. Different types of water differ in their capacities to absorb light. Water transparency is affected by the suspended matter, including the plankton, and by stain or color. In water of the clarity of usual municipal supplies, 9.5 percent of the solar energy present at the surface reaches a depth of 6 feet. Born (39) states that the limit for growth for the higher aquatic plants lies between 2.5 and 3.5 percent of the total surface energy at bottom depth, but that it rapidly declines below 4 percent where severe etiolation occurs in submerged seed plants. There is some evidence that certain algae can grow at levels of 1 percent of the incident light, but it is not definitely known how much light is required for them to produce more oxygen by photosynthesis than they use in their respiration. While criteria will vary with the area they can be kept relatively simple. For example, a criterion for a particular area might state -- under conditions of brilliant sunlight at or near noon 4 percent of surface incident light shall reach a depth of 6 feet. Incident light and light at any given depth can be readily read by means of a photometer fitted for underwater use.

pH

The pH of a water may exert a direct effect on fish if it is very high or very low due to strong bases or mineral acids. It may have an indirect effect through its influence on the toxicity of certain materials such as HCN, H_2S, ammonia, heavy metals, etc. Longwell and Pentelow (17) found that the toxicity of NaS solutions to brown trout was influenced markedly by variations in pH, the toxicity increasing as the pH became lower. The heavy-metals are considerably more toxic at lower pH levels probably because they are more soluble. Ammonia becomes rapidly more toxic as the pH is raised above 8.2. The toxicity of a number of weak inorganic and organic acids, including hydrocyanic, hypochlorous, hydrosulfuric, carbonic, and tannic, is increased by lowering the pH.

Extreme pH values of 4 and 10 or slightly above have been tolerated by resistant fishes in certain areas. Some levels at which fish have been killed experimentally are: trout, 9.2; bluegills, 10.5; roach, pike, carp, and tench, 10.4 to 10.8. Fish mortality has been observed within a few hours at pH levels of 3.4 to 4. However, certain fish have been acclimated to live for considerable periods at pH levels as low as 4.5 to 4.2.

S[illegible] (18) [illegible], [illegible], [illegible], [illegible] H[illegible] 4.4. E[illegible] (19) [illegible] H[illegible] 7.4 [illegible] 8.5 [illegible]-[illegible] 6.6 [illegible] 9.0, [illegible] 4.0 [illegible] 6.0. H[illegible] 6 5 [illegible] 8.5.

S[illegible] H[illegible]. W[illegible] H[illegible] 9.5, [illegible] H [illegible] 9 [illegible] 9.5 [illegible]. S[illegible] H[illegible] 10 [illegible] 10.5. T[illegible] H[illegible]-[illegible] CO2 [illegible] D. O. [illegible]. H[illegible] H[illegible] (20). A[illegible] 5 [illegible] 9, [illegible] H[illegible]. T[illegible]; [illegible] H[illegible] 4 [illegible] (21) (22). I[illegible], [illegible] H[illegible] 7.0 [illegible] 8.5, [illegible], [illegible], [illegible], [illegible]-[illegible], [illegible], [illegible]. T[illegible] blunt-nose [illegible], N[illegible], [illegible] H 7.0 [illegible] 8.0 (20). S[illegible] H. L[illegible] (21) [illegible] 7.2 [illegible] 9.6, [illegible] 6.6 [illegible] 9.3, [illegible] 7.2 [illegible] 9.6. T[illegible] (21).

I[illegible] 5 [illegible] 9.5, [illegible] H[illegible]. H[illegible], [illegible]. F[illegible], [illegible] H[illegible] 6.5 [illegible] 8.5. A[illegible] H [illegible] minerals [illegible]. T[illegible], [illegible] H[illegible] 5.0 [illegible] 9.5 [illegible], [illegible] H[illegible] 6.5 [illegible] 8.5.

D[illegible] O[illegible]

T[illegible]. I[illegible]. D[illegible]. S[illegible]

of oxygen by the fish or may change or increase their minimum need for oxygen. Among these are temperature, pH, CO_2, and dissolved solids.

Temper9.ture increases within the range favorable to fish are accompanied by a progressively higher.metabolic rate and a continuous increase in the oxygen uptake. Wiebe and Fuller (23) found that at 25°C. the oxygen consumption of largemouth black bass was 282 percent of that at 15°C. At 20°C. it-was 177 percent of the consumption at 15°C. This is in accord with the van't Hoff law which states that for any chemical change the rate of reaction is increased between 2- and 3-fold for every 10°C. increase in temperature. Temperature is of outstanding importance in the determination of environmental requirements since the oxygen consumption increases as temperature rises whereas solubility of oxygen decreases. Because the annual range in temperature of streams of the temperate region may be as much as 28°C., oxygen consumption at peak temperatures may be several fold what it is at minimum temperatures, whereas at peak stream or lake temperatures the water will hold only about half as much oxygen as it does at minimum temperatures. Graham (24) found that for speckled trout the rate of oxygen uptake increased with increasing temperature up to the ultimate upper lethal temperature, if sufficient oxygen were available. Water containing less than 75 percent of the air saturation level of oxygen reduced the activity of speckled trout at all temperatures, and above 20°C. (68°F.) fully saturated water is required to allow the full scope of activities. Several other investigators have also found that the oxygen requirements of fishes become greater with increases in temperature (25) (26) (27).

Temperature also markedly affects dissolved oxygen concentrations with are lethal to various species of fish. Burdick (28) found that smallmouth bass died in 5 to 9 hours at oxygen concentrations of 0.7 p. p. m. to 1.17 p. p. m. at temperatures of 52°F. to 72°F. There was also some variation in the turnover time for different species of fishes. At 55°F. and oxygen concentrations of 1 to 2 p. p. m. the turnover times were as follows: brook trout, 1-3/4 hours; brown trout, 2-1/2 hours; and rainbow trout, 3 hours. At 69°F. to 71°F. these fishes turned over in approximately the same time at oxygen concentrations of 2.3 to 3.4 p. p. m.

Several other environmental factors also interfere with oxygen uptake or increase the oxygen requirements of fishes. High and low pH levels interfere with the ability of fishes to absorb oxygen from the water. High CO_2 concentrations interfere with the utilization of dissolved oxygen. Fry and Black (29) found that the common sucker, with its CO2 sensitive blood, was unable to remove oxygen from water containing CO_2 tension which did not hinder the respiration of bullheads, the latter possessing blood with a very low sensitivity to CO_2. Under pollutional conditions fish generally require more oxygen (45) (46) (20). At low dissolved oxygen levels fish succumb to concentrations of toxic materials which they can tolerate at high dissolved oxygen levels.

Many studies have been made in attempts to determine the lowest D. O. levels tolerated by different species of fish. Gutsell (30) reported that some brook trout could endure, for a short period, an oxygen concentration as low as 1.2 p.p.m. ; however, some asphyxiation occurred at a D. O. content of 2.5 p.p.m. Smallmouth black bass lived for a time at 0.4 p.p.m. D. O. Wiebe (22) found that some fish can withstand sudden wide changes in the concentration of oxygen and that they can live in water supersaturated with oxygen. The increase in D. O. was followed by a slowing down of the respiratory moxrements. Fry (31) states that at 49°F. the ultimate minimal tolerance of brook trout for dissolved oxygen is 0.9 p.p.m. Gardner and King (32) reported the asphyxial level of trout to be 1.1 p.p.m. D. O. at 6.5°C. and 3.4 p. p. m. at 25°C. Thompson (33) on the basis of field studies, reported that carp and buffalo lived in water having 2.2 p.p.m. D. O. However, he found a variety of fishes only when there was over 4 p.p.m. of oxygen and the greatest variety of fishes were present when the D. O. was 9 p.p.m. He found that fish died overnight in water containing less than 2 p.p.m. D. O. Ellis reported (19) that goldfish, perch, catfish, and other species of freshwater fishes when maintained in water of constant flow, composition, and temperature (20° to 25°C,) showed respiratory compensation in both volume and rate when the dissolved oxygen was reduced to slightly below 5 p.p.m.

In addition to those environmental conditions which influence the oxygen requirement, there are several physical, chemical, and physiological conditions which influence the ability of fish to extract oxygen from the water, its need for oxygen, and its ability to resist low oxygen levels. First, it must be realized that ability to extract oxygen from the water and to resist low D. O. levels varies with the species. It is well known that dogfish, carp, and gar can survive at much lower D. O. levels than trout and several other fishes. Some fishes are more efficient in the extraction of oxygen or their blood is not as sensitive to the presence of CO_2.

The amount of oxygen required by fishes is determined in part by activity. It is generally recognized that a man lying in bed does not breathe as deeply or require as much oxygen as one digging a ditch. It has been reported that from two to four times as much oxygen is required by a fish when it is active as when it is quiescent (24) (26) (34). Under actual stream conditions a fish must maintain its position against the current, find, pursue, and catch its food, avoid its enemies, and reproduce. All these activities require oxygen in such amounts that D. O. levels at which the fish can just survive are unsatisfactory. Age, size and season are also of importance. In general, fry and younger fish have a higher metabolic rate and require more oxygen than adults (35) (36). Because of increased activity and their physiological condition fish require more oxygen at the spawning season. Studies carried out in our laboratories indicate that D. O. requirements are different at other times of the year and further, the physical condition of the fish is of outstanding importance in determining requirements and the minimum level tolerated. An actively feeding, rapidly growing fish requires considerable more oxygen than one which feeds very little. Since growth is rapid in the fry to fingerling stage it is expected that for many species D. O. requirements

will be higher at this period. Eggs deposited in bottom materials require higher D. O. concentrations than do adult fish. Since the current through the bottom materials is slow, the amount of water flowing by the eggs per unit of time is small and thus it must contain more D. O. to provide needed requirements.

Through acclimation, resistance to low D. O. levels may be increased. Fry (31) reports that through acclimation the lethal dissolved oxygen level can be reduced to about one-half the corresponding value for trout accustomed to air-saturated water. Lower dissolved oxygen levels can be tolerated for considerable periods through an increase in respiration rate and volume of water pumped, reduced activity and food consumption, and an increase in blood haemoglobin (37) (38). By means of such adaptation fishes may live for considerable periods at reduced oxygen concentrations without apparent harm. This does not mean, however, that they can complete their life cycle at such levels. Further, ability to live more or less indefinitely at low oxygen levels does not mean that some of their physiological processes have not been altered so that their well being and growth are adversely affected. It has been reported (4) that the bullhead is unable to become acclimated to increased temperature when D. O. levels are low whereas it becomes rapidly acclimated at normal D. O. levels. Dissolved oxygen levels adequate for growth, reproduction, normal activities, and well being are considerably higher than levels which can be tolerated for extended periods through acclimation and compensation.

Studies of the oxygen requIrements of fishes fall into two categories: laboratory investigations, where as many as possible of the variables are controlled, the factor under study is -aried, and the effects on fishes directly observed for a relatively short period; and field studies, where the variable in question is measured in different sections of the stream and is related to the fish population in various areas. Both types of study have certain advantages and disadvantages. It is very difficult to relate laboratory results to field conditions, while in the field studies, factors other than the variable in question (dissolved oxygen concentration) might have a bearing upon results. It is believed that the best approach is to carry on both laboratory and field studies so that they supplement each other. In the interpretation of laboratory findings, it must be recognized that fish are usually held under favorable conditions and it is necessary to realize that all findings are not applicable to natural conditions.

The Lytle Creek studies (40) and other field studies have indicated dissolved oxygen concentrations at which fish and their food supply can maintain themselves. Twenty-four hour studies were made on Lytle Creek at all seasons of the year at selected stations to determine D. O., CO_2, pH, and temperature. Such studies or a continuous record of dissolved oxygen are essential for investigations of D. O. requirements as there are great diurnal and seasonal variations in oxygen concentration. Fish populations and growth rate studies were made over a two-year period (41) (42) in order to relate them to environmental conditions and their seasonal variations in

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I[illegible], [illegible] materials [illegible] Š H_2S, NH3, [illegible] CH4 [illegible]. T[illegible] H2S [illegible] B[illegible], T[illegible], [illegible] S[illegible] (43). U[illegible] NH_3 [illegible] NO_3 [illegible] (44). M[illegible] CH_4, [illegible] gas. T[illegible], [illegible].

D[illegible] tasks. A[illegible]. I[illegible], [illegible] results. C[illegible], [illegible] obtained [illegible]. S[illegible]. I[illegible]-[illegible]. I[illegible]. M[illegible] D. O, [illegible]. O[illegible]. B[illegible], [illegible].

Concentration of dissolved oxygen is often expressed as weekly, monthly, or sometimes daily, averages. Such values are not satisfactory as they do npt indicate environmental variations and may actually be misleading from the standpoint of the continued existence of the fish. It is the extreme variations which may become limiting and which are the most important for indicating unfavorable habitats.

Some D. 0. criteria have been set up as percentages of saturation. This procedure is deemed undesirable because over the range of temperature observed in our natural waters, 50 percent of saturation may mean 7.3 p. p. m. oxygen or 3.5 p. p. m. As temperature increases the amount of oxygen which can be held by the water decreases, whereas the amount required by the fish increases. It is believed that criteria for dissolved oxygen should be expressed in parts per million by weight.

Findings in Lytle Creek have indicated that in a stream section in which the oxygen concentration is usually above 5 p.p.m., the occurrence of concentrations below 5 p. p. m., but not below 3 p. p. m. for a few hours, does not have an adverse affect upon a well rounded warm-water fish population. Minnows and other coarse fishes were found in the section where minimum D. 0. levels dropped to 2 p.p.m. or slightly below. On the basis of these studies and other pertinent data it is believed that for a well rounded warm-water fish population, dissolved oxygen concentrations must not be below 5 p. p. m. for more than 8 hours of any 24-hour period and at no time should they be below 3 p. p. m. For the maintenance of a coarse fish population dissolved oxygen concentrations should not be below 5 p. p. m. for more than 8 hours of any 24-hour period, and at no time should they be below 2 p. p. m.

The salmonoid fishes are not usually found in streams where minimum dissolved oxygen concentrations are lower than 4 to 5 p.p.m. For normal feeding and adequate growth at least 5 p. p. m. dissolved oxygen are required. Successful development of eggs and fry require a minimum of 6 p. p. m., while for the full range of activity for brook trout and perhaps for other members of the family, 7.6 p. p.m. are required at 15°C. and full air saturation at 20°C. and above (31). It is believed, therefore, that for good salmonoid production dissolved oxygen concentrations should not be less than 6 p. p.m.

Carbon Dioxide

Carbon dioxide may influence the toxicity of other materials or it may in itself be harmful if present in sufficient quantities. Alabaster and Herbert (48) found that CO_2 was not toxic to rainbow trout within a 12-hour exposure at concentrations up.to 30 p.p. m. but was toxic at 60 p. p.m. and that period of survival decreased as the concentration increased. The presence of CO2 in concentrations from 15 to 60 p. p. m. was found to reduce the toxicity of ammonia. Higher concentrations are toxic; 100 to 200 p.p.m. can be rapidly fatal to moderately susceptible fresh water fishes in well oxygenated water. Fifty to 100 p. p. m. can cause distress and may be lethal. Both marine and fresh water fishes vary greatly in their resistance to CO_2.

Wells (49) reports that resistance of fishes to harmful conditions varies with the species, with age or size and weight, with the condition or physiological state of the individual, and with the season. He found that practically all the fishes with which he worked were least resistant just after the breeding season--June, July, and August--and most resistant before it--March, April, May (50). Powers (51) has shown that the ability of marine fishes to extract oxygen at low concentrations was adversely affected by moderate amounts of CO_2, which lowered the pH. The investigation of Black, Fry, and Black (52) demonstrated the influence of CO2 on the utilization of oxygen by 16 species of fresh water fishes. It was found that oxygen in the respired water at the time of death was higher when the tension of CO2 was increased. The ability to take up oxygen in the presence of CO_2 varied with the species. Powers and co-workers found (53) that fish are able to absorb oxygen at a low oxygen tension through a wider range of CO_2 tension than is found in the natural waters in which they live, Most workers have found that naturally occurring levels of CO_2 are not detrimental to fishes. It is believed that concentrations under 30 p. p.m. in the absence of other adverse factors will have no harmful effects on most species. The majority of investigations indicate that CO_2 becomes rapidly harmful at concentrations of 100 to 200 p. p.m. Surber (54) found that concentrations between 55 p. p. m. and 78.5 p. p. m. in hard water at pH 6.9 to 7.0 caused a decided increase in the loss of eyed eggs and the number of deformed trout fry. Concentrations up to 43 p. p. m. apparently had no harmful effect.

Dissolved Solids

Natural, unpolluted waters of lakes and streams have in solution small amounts of the anions CO_3, Cl SO_4^{--}, smaller quantities of NO_3^-, NH4+, PO_4, and NO_2^-, and traces of many others. The metallic cations are Ca++, Mg^{++}, Na^+, K^+, Fe+++, Mn^{++}, and traces of several others. These materials exert a physiological and osmotic effect to which organisms have become adapted. In fact, these dissolved materials are required by the organisms. Rawson (55) found a positive correlation between the total solids in fresh waters and the average standing crop of plankton and bottom fauna. The type of rock formation and soil largely determines the concentration of dissolved solids in a water but erosion may be of considerable importance. Pollution may also be a factor. During the period from 1906-07 to 1934-43, the average amount of dissolved solids in Lake Erie increased from 133 to 165 p. p.m., whereas those in Lake Superior remained unchanged (56) (57).

Criteria for dissolved solids have little meaning if the purpose of the criteria is the protection of aquatic life, unless the materials to be considered as dissolved solids are specified. It is apparent that salts of Hg, Cu, Ag, Zn, Pb, and Cd will have a much different effect on fishes than will equal concentrations of salts of Ca, Na, Mg, and K. In general when total dissolved solids are referred to in relation to water quality criteria, it is the salts of these relatively nontoxic earth metals which are believed to be under

consideration. Unnatural concentrations of these salts may affect aquatic life in two ways. If the solution of salts is physiologically unbalanced, one of them may exert a direct toxic effect. If they are physiologically balanced, that is, each is present in quantities sufficient to antagonize any toxic effects of one or more of the others, they may occur in such concentrations that they exert an osmotic effect. Wiebe (58) points out that the osmotic pressure that fish can tolerate depends to a large extent on acclimatization. Fish acclimated to the extremely soft waters of East Texas cannot survive when subjected to salinities to which the fish of the Pecos River are continually exposed. Texas rivers (58) range in total dissolved solids from 45 to 4, 810 p. p. m. Wiebe found as much as 28, 000 p. p. m. of chloride in a stream where fresh water fish were supposed to live. However, Young (59) indicates that when dissolved solids reach 11, 000 p.p.m. only certain fish can tolerate them indefinitely. The ability to resist high concentrations of dissolved solids varies with the species. While some fishes move from marine to fresh water or from fresh to sea water, some species have been reported as being unable to resist concentrations above 3, 000 p. p. m. Young reported that Na_2CO_3 in concentrations about 800 p. p.m. was unfavorable for catfish. Huntsman (60) reports that in the Quill lakes of Saskatchewan, which have a total solids content of 16, 550 p.p.m. , there is a resident fish population of somewhat limited extent. It is believed that total dissolved solids in concentrations.up to 3, 000 p. p. m. can be tolerated by most fishes if the materials in solution are the nontoxic earth metals and are physiologically balanced.

Chlorides

The amount of chlorides is often considered as a measure of salinity or of total dissolved solids. When dealing with sea water, which is fairly uniform and the composition of which is known, chlorides can be taken as a measure of salinity or dissolved solids. This does not hold, however, for oil field brines and other wastes. Oil field brines differ drastically from one another and from sea water and many wastes contain large quantities of salts other than chlorides.

The chloride ion as such does not have much significance from the standpoint of toxicity to aquatic life. The cation is so much more important that the chloride anion is not generally considered. This is especially true with chlorides of the heavy metals such as mercury, copper, zinc, etc. Even with salts of the relatively nontoxic earth metals the toxicity of their chlorides is evidently attributable to the specific toxicity of the cations present and not to any toxicity of the chloride ions. Since the cations vary greatly in their toxicity to fish and are governing in the determination of toxicity, it is obvious that the chloride ion content of a mixture of salts is not a reliable index of toxicity.

Physiologically balanced mixed salt solutions such as sea water may be harmful to freshwater organisms because of their excessive over-all salt content and osmotic pressure rather than the specific toxicity of any particular ions present. Provided that the salts and other substances dissolved in water

are balanced against each other so as to exclude any individual toxic effects, certain hardy freshwater fishes can tolerate waters of osmotic pressures equal to those of their own bloods and even higher for extended periods. Other freshwater fishes, however, have been reported unable to tolerate balanced salt solutions with concentrations of 2, 000 to 4,000 p.p.m. It is not known whether a typical freshwater fish can complete a normal life history in water of relatively high osmotic pressure, nor is it known how osmotic pressure affects the life processes of other freshwater organisms. It is, therefore, impossible at present to define the maximal safe osmotic pressure of a freshwater environment. Presumably, the tolerable osmotic pressure entails a salt concentration far higher than the limits imposed by industrial and municipal requirements. When we are dealing only with different concentrations of a particular salt mixture such as sea salt; the composition of which is known and uniform, the chloride content of the solutions (which is easily determined) can be a useful index of osmotic strength. However, when we are dealing with mixed salt solutions of unknown and varying composition (such as oil well brines and other industrial wastes), their chloride ion content is not a reliable index of osmotic strength. For example, an industrial waste brine containing a large amount of sodium sulphate can be much more active osmotically than another brine with a much higher chloride ion content which contains only sodium and calcium chlorides. It is impossible when dealing with mixed wastes to generalize as to the relationship between chloride ion concentration and osmotic, toxic, or over-all pollutional strength. It is believed, therefore, that chloride ion criteria have no practical significance as far as aquatic life is concerned.

Fluorides

Studies at our laboratory in Cincinnati have indicated that fluoride ions do have toxic properties in their own right. Further, they appear to have a cumulative effect. In 10-day tests it was found that the TL_m value for potassium fluoride was 64 p. p. m. It is believed that for good fish production the fluoride content should not exceed 5 p. p. m.

Toxic Materials

There is a great deal of literature dealing with the toxicity of various pure chemicals to fishes. The great majority of investigators have used different approaches and have carried out their studies with a variety of fishes, using different types of water for dilution. Several compilations, reviews, or bibliographies of these studies have been made (38) (61) (62) (63) (64) (67). An examination of these papers clearly indicates that there is great variation in toxic levels reported by various investigators for selected pure chemicals. This variation is especially evident in the California report (61).

The quality of the receiving or dilution water, which is often not reported by some of those investigating toxicity, is of outstanding importance in determining toxicity of a particular material or waste. Several environmental

factors may influence toxicity, such as: temperature, CO_2, D. O., pH, alkalinity, hardness, turbidity, and dissolved materials. Certain dissolved materials may significantly affect the toxicity of a waste through their synergistic or antagonistic action or through complexation, precipitation, or other action. In the case of ferro- and ferricyanide solutions, sunlight is of importance as photo decomposition of these materials occurs with the production of toxic HCN (65). The heavy metals are considerably more toxic at low pH since they are more soluble in acidic solutions. In hard waters at higher pH they are precipitated or changed in other ways to become much less toxic. It has been found that beryllium and uranium are 60 to 80 times more toxic in soft water than in hard water (66). Ellis (38) and other investigators have found that ammonia becomes rapidly more toxic at pH values above 8.0. Calcium antagonizes the toxicity of many of the heavy metals whereas some of them are synergistic with each other and become considerably more toxic when mixed; examples are Cu and Zn, Cu and Cd, and Zn and Ni (67).

The toxicity of many of the metal-cyanide complexes is greatly influenced by pH. Doudoroff (68) has reported that fish can withstand 1,000 times as much nickel cyanide complex at pH 8 as at pH 6.5. Among the metabolites, including weak acids and bases, it is the molecule and not the ion which appears to be toxic. Thus weak acids such as HCN and H_2S become more toxic as the pH is lowered and dissociation depressed, whereas weak bases such as NH_4OH become more toxic as the pH is raised.

In general, materials are more toxic at higher temperatures and at low dissolved oxygen levels. Carbon dioxide may, through its effect on pH, render some materials more toxic or it may serve to make others less toxic. Complexation, precipitation, oxidation, dis so,ciation, recombination, or buffering action must also be considered. The influence of water quality and other environmental factors on the toxicity of various materials has been summarized by Tarzwell (69).

The character of the receiving water can cause wide variations in the toxicity of many materials to fishes. Reference to the literature on the toxicity of specific pure chemicals is of little value for determining the toxicity of a complex waste containing these and other materials. Such an approach neglects water quality, which is of particular importance, as well as synergism and antagonism, oxidation, precipitation, complexation, and other actions which may occur in the stream and may greatly influence the toxicity of a waste in a particular stream. Numerical standards for toxicity of specific pure chemicals have little value and can be misleading. From the standpoint of industry they may be very undesirable. If a regulartory agency sets numerical criteria for the heavy metals and other substances which are to be applied over a wide area, they must be set so low that allowable concentrations are not detrimental to aquatic life under those conditions at which they are most toxic, For example, criteria for nickel cyanide wastes discharged into an acid stream would have to be set so low that the HCN formed from the wastes would not be toxic, whereas in

an alkaline stream the criteria could be much higher as an increase of one and one-half pH units from 6.5 to 8.0 decreases the toxicity of this material over 1000 times. Copper is much less toxic in hard water than it is in soft water with a low pH; variation may be as great as 200 times.

It is believed that the best approach to this problem is to make bio-assays with the total waste, using for dilution, water from the receiving stream at the point where the waste is to be discharged. In this way the many variables which influence the toxicity of that particular waste in that stream are taken into consideration and safe disposal or dilution rates can be determined. With certain exceptions (some of the insecticides and other materials whose toxicity is not influenced by water quality), water quality criteria for toxic materials when applied over wide areas should not be expressed as numerical values. It is believed that a tailor-made approach should be used where toxicity of the waste can be determined for the particular receiving stream at the point of discharge. When the toxicity is determined in this way allowable concentrations for that particular situation can be expressed as p. p.m. or as dilution ratios. Such an approach also permits evaluation and comparison of the toxicity of wastes from different industries along the stream.

Summary and Conclusions

The establishment of water quality criteria for the protection of our valuable aquatic resources is a complicated and difficult problem. Since the basic objective of water quality criteria for the protection of aquatic life is to provide or preserve environmental conditions essential for the survival, normal growth, reproduction, and well being of aquatic organisms a knowledge of the environmental requirements of these organisms is essential for the establishment of such criteria. Many of the activities of man have modified the aquatic environment. Among these are deforestation, unwise agricultural practices, overgrazing, and pollution. Our aquatic resources which produce billions of dollars yearly in revenues from sport and commercial fishing are a renewable resource worthy of our best efforts for preservation.

Siltation due to erosion has been and is a major pollutant. The solution of this problem by means of erosion control must be a cooperative effort among those agencies dealing with water, soil, and other natural resources.

While all the environmental requirements for aquatic life are not now known, application of the data presently available can be used effectively in the setting up of some criteria. As more data become available existing criteria can be modified and others set up in order to meet the problem adequately.

The quality of the receiving water is particularly important in determining the effects of many wastes. Among the factors influencing the toxicity of pollutants in a particular receiving water are temperature, COB, D. O., pH, alkalinity, hardness, and dissolved materials. Other factors which may modify the toxicity of a waste are complexation, precipitation, oxidation, synergy, and antagonism.

In view of the many factors which may influence the effects of pollutants in different streams, it is believed that in most situations numerical criteria when applied over wide areas can be set only for temperature, D. O., and pH. With a few exceptions, such as for insecticides and certain other materials, it is believed a tailor-made approach should be used in setting criteria for toxic substances. This approach would consist of bio-assyas of the waste in question, using for dilution, water from the receiving stream taken from the area into which the waste is to be discharged. Such bio-assays would take into consideration the variables which influence toxicity of that particular waste and can be used to indicate safe concentrations, or the amount of dilution required.

Criteria for settleable solids and turbidity will depend largely on local conditions and will vary with the stream and the area.

When considering large areas pH values should not fall below 5 or exceed 9.5, but for good fish production it is desirable that they be maintained between 6.5 and 8.5.

For a well rounded warm water fish population dissolved oxygen levels should not be below 5 p.p.m. for more than 8 hours in any 24 hour period and at no time should they be below 3 p.p.m. If a coarse fish population only is desired minimum levels may fall to 2 p.p.m. For good production of Salmonoid fishes a minimum of 6 p.p.m. appears to be required. However, trout can live and reproduce in waters where the D. O. content may drop to 4 or 5 p.p.m but the survival of eggs and fry and the production is usually not so great.

It is suggested that in the northern portion of the country peak temperatures for warm water fishes should not exceed 93°F. In the southern portion of the country fish are better acclimated to higher temperatures and can withstand peak temperatures of 96°F. or higher While trout can stand peak temperatures of 80°F. to 83°F. for short periods, best production is attained in streams having summer temperatures of 60°F. to 68°F.

Allowable concentrations of toxic complex wastes for each stream or situation should be determined by means of bio-assavs and safe dilutions estimated by the use of application factors or by other means.

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PHYTOPLANKTON INDICATORS OF ECOLOGICAL CHANGE IN THE EUTROPHYING PAMLICO SOUND SYSTEM, NORTH CAROLINA

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Abstract. Nutrient enrichment and eutrophication of estuarine and coastal waters are accelerating, and there is a need to develop rapidly detectable and quantifiable indicators of these changes. Coastal systems are also impacted by climatic perturbations, including droughts, storms, and floods, the frequencies of which may be increasing. Phytoplankton are excellent indicators of ecological change. They are relatively easy to detect, identify, and quantify; they conduct a large share of primary production; and they are sensitive to diverse environmental stressors. In this study, phytoplankton total biomass, as chlorophyll *a*, and group-specific chemotaxonomic indicators (including chlorophylls and carotenoids) were used to characterize community responses to human (nutrient) and climatic (hydrologic) perturbations in the Neuse River Estuary–Pamlico Sound, North Carolina, USA. This estuarine–coastal continuum is experiencing anthropogenic nutrient enrichment and, since 1996, a rise in hurricane frequency. Freshwater input and flushing strongly interacted with supplies of the limiting nutrient nitrogen (N) to determine the location, magnitude, and composition of phytoplankton biomass along this continuum. Elevated flow (high flushing) following hurricanes favored dominance by the fast-growing chlorophytes and cryptophytes. Diatoms tended to dominate under moderate flow, while dinoflagellates and cyanobacteria increased in dominance when low flow prevailed in winter/spring and summer/fall, respectively. Depending on seasonal hydrologic cycles and episodic (hurricane) conditions, phytoplankton community structure differed substantially. These changes impact eutrophication, food web, biogeochemical (e.g., hypoxia), and habitat conditions in this and other coastal ecosystems currently experiencing changes in nutrient inputs and climatic events. Phytoplankton-based indicators are adaptable to unattended monitoring platforms (e.g., ferries) that can be coupled to remote sensing and modeling efforts, in order to evaluate and help manage ecological change at ecosystem and regional scales.

Key words: climate; estuaries; eutrophication; hydrology; indicators; North Carolina, USA; nutrients; photopigments; phytoplankton; water quality.

Introduction

Nearly three-quarters of the world's human population resides in coastal river basins, which has led to an unprecedented increase of nutrients associated with coastal development (Vitousek et al. 1997). While nutrient over-enrichment and eutrophication of downstream estuarine and coastal ecosystems is accelerating (Nixon 1995, NRC 2000, Boesch et al. 2001), there is a paucity of information on the manner in which the structure and function of aquatic communities mediating productivity and cycling of major elements (i.e., C, N, O, P) in these ecosystems are altered by these activities. Understanding how anthropogenically induced change affects estuarine- and coastal-ecosystem biodiversity, water quality, fisheries habitat, and resources is a major research and management challenge.

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Nutrient (especially nitrogen) over-enrichment is a key driver of coastal ecological change (Nixon 1995, Paerl 1997, Boesch et al. 2001), and nitrogen (N) discharge to coastal waters is directly proportional to human population density of their watersheds (Peierls et al. 1991, Vitousek et al. 1997). Resultant eutrophication has significantly altered coastal productivity, nutrient (C, N, P, Si) cycling, water quality, biodiversity, and overall ecosystem health (Smetacek et al. 1991, Vollenweider 1992, Conley 2000, NRC 2000, Cloern 2001, Rabalais and Turner 2001). Short- and longer-term eutrophication trends have been documented by various indicators, including: sediment paleoecological indices (Brush 1986, Cooper 1995, Bianchi et al. 2000), loss of water clarity (Harding and Perry 1997, Goldman 2000), increased frequencies and expansion of low-oxygen (hypoxic) bottom waters (Officer et al. 1984, Rabalais and Turner 2001) accompanied by a shift from aerobic to anaerobic metabolism (Paerl et al. 1998, Boynton and Kemp 2000), altered stoichiometry and shifts in nutrient limitation (Conley 2000, Rabalais and Turner 2001), and increased harmful algal bloom activity (Paerl 1988, 1997, Hallegraeff 1993, Richardson 1997).

Estuarine and coastal (jointly termed coastal) ecosystems are also affected by climatic change and associated perturbations, including droughts, hurricanes, and floods (Paerl et al. 2001, Peierls et al. 2003). These events appear to be increasing in frequency and magnitude (Goldenberg et al. 2001, Webster et al. 2005). It is suspected that these perturbations affect primary production and higher-trophic-level responses by modifying both hydrologic and nutrient regimes of coastal systems (Odum et al. 1995, Peierls et al. 2003). There is a need for rapid, unequivocal, quantitative detection and evaluation of the ecological impacts of these interactive stressors on coastal-water quality.

Phytoplankton dominate primary production in most estuarine and coastal waters (Nixon 1986, Paerl et al. 1998). They are highly sensitive indicators of nutrient-controlled, and hence nutrient-enhanced, production and physical forcing (i.e., vertical mixing, changes in flushing and residence time, and altered optical properties) of these systems, since their growth is rapid (doubling times as low as 0.5 d^{-1}), growth responses to nutrients occur at nanomole to micromole concentrations, and their photosynthetic responses can be measured by highly sensitive techniques. As such, phytoplankton are relevant, readily detectable indicators of biogeochemical and ecological change.

In recent years, a suite of easily deployed, broadly applicable phytoplankton-based water quality indicators has been developed. These include production, growth rate, and community compositional indicators (Paerl et al. 2002, 2005, 2006). In particular, the use of diagnostic (of various microalgal groups) photopigments as quantitative indicators of phytoplankton taxonomic group biomass has gained popularity and use in water quality monitoring and ecological assessment programs (Millie et al. 1993, Mackey et al. 1996, Pinckney et al. 2001). This technique is complementary to standard fluorescence and spectrophotometric absorbance determinations of chlorophyll *a*, microscopic counts, cell-specific indicators such as microautoradiography and fluorescence in situ hybridization (FISH) (Paerl et al. 2006).

Using these diagnostic indicators, we examined how nutrient supplies interact with climatic (hydrologic) and other physical forcings, including freshwater discharge, temperature, and irradiance, to determine the amount, distribution, and composition of phytoplankton communities in the Neuse River Estuary–Pamlico Sound (NRE–PS). This freshwater–marine continuum is representative of the greater Pamlico Sound system, North America's largest lagoonal estuarine ecosystem and one of its most important fisheries nursery habitats (Copeland and Gray 1991). Previous research has shown N to be the nutrient controlling or limiting primary production throughout the NRE–PS (Rudek et al. 1991, Boyer et al. 1994, Paerl et al. 1995, Piehler et al. 2004). Phytoplankton account for at least 80% of the primary production in this system (Paerl et al. 1998). Hence, N loading and other environmental impacts on the phytoplankton community are likely to have major ramifications for higher-trophic-level, habitat, and ecosystem processes.

Methods and Materials

Site characteristics: The Neuse River Estuary–Pamlico Sound system

The Pamlico Sound (PS) receives input from five watersheds (the Neuse, Pamlico, Chowan, and Roanoke Rivers and the Albemarle Sound) and drains to the Atlantic Ocean via three narrow passages (Oregon, Hatteras, and Ocracoke Inlets; Fig. 1). This results in a relatively long (approximately one-year) water retention time (Pietrafesa et al. 1996). The PS's shallow riverine tributaries also experience comparatively long residence times, e.g., one to over three months for the Neuse River Estuary (Luettich et al. 2000). Consequently, allochthonous and autochthonous inputs of nutrients and other pollutants readily accumulate in this system. These conditions are conducive to effective nutrient retention and cycling (Christian et al. 1991), making the system sensitive and highly responsive to external nutrient loading (Boyer et al. 1994, Paerl et al. 2001, Peierls et al. 2003).

The Neuse River Estuary (NRE) is the largest of the four major riverine tributaries of the PS. It is downstream of rapidly expanding agricultural (hog, poultry, and rowcrop operations), urban (Raleigh-Durham-Research Triangle), and industrial activities in North Carolina coastal watersheds. Excessive nutrient (especially nonpoint source N) discharge associated with such expansion has promoted eutrophication and water quality degradation (Paerl et al. 1990, Rudek et al. 1991, Stow et al. 2001). Symptoms include periodic nuisance algal blooms, hypoxia, anoxia, toxicity, disease, and potentially mass mortalities of finfish and shellfish (Copeland and Gray 1991, Paerl et al. 1995, 1998, Burkholder and Glasgow 1997, Lenihan and Peterson 1998). In response to scientific evidence of N-driven eutrophication and public pressure to reduce its unwanted symptoms, the North Carolina General Assembly mandated a 30% reduction in N loading to the NRE to be in place by 2006 (NCDENR-DWQ 2001). In addition, the U.S. EPA has imposed a total maximum daily load (TMDL) for N (U.S. EPA 1997) for the NRE, using the State's 30% N reduction strategy. Phytoplankton biomass, as chlorophyll *a* (40 μg chl *a*/L is the "acceptable" level) is the criterion for attainment of the TMDL (NCDENR-DWQ 2001). This large-scale N manipulation and earlier (mid-1980s) upstream reductions in P loading have provided opportunities to examine the effects of nutrient management using phytoplankton-based indicators (cf. Paerl et al. 2004).

This system has also been under the influence of natural perturbations, including droughts, hurricanes, and flooding, the frequency and intensity of which have increased markedly since the mid-1990s (Paerl 2005, Paerl et al. 2005). The recent upsurge in hurricanes began in 1996, with the arrival of Hurricane Bertha in July. This relatively low-rainfall storm was followed by

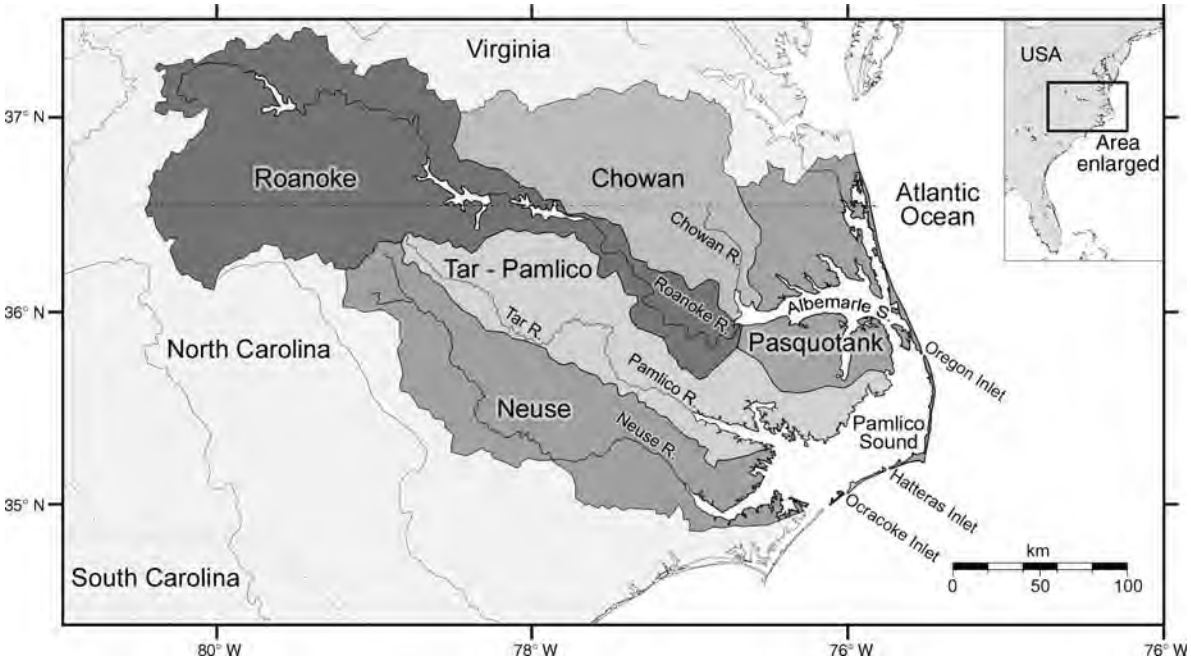

FIG. 1. North Carolina's Pamlico Sound system, including its sub-estuarine tributaries (Chowan, Roanoke, Tar-Pamlico, and Neuse Rivers) and their watersheds.

Fran, a large, category 2 hurricane that delivered >30 cm of rainfall and caused extensive flooding throughout the NRE watershed. Fran's floodwaters delivered very high inorganic-nutrient and organic-matter loads to the NRE, causing severe oxygen depletion and triggering fish kills in the estuary (Paerl et al. 1998). Category 2 Hurricane Bonnie crossed the PS in late August 1998, after moving up the coast from near Wilmington, where it first made landfall. This was a fast-moving, low-rainfall storm. In stark contrast, during the fall of 1999, Hurricanes Dennis, Floyd, and Irene inundated coastal North Carolina with up to 1 m of rainfall, causing extensive flooding throughout the watershed of the PS. During the ensuing six weeks, sediment- and nutrient-laden floodwaters totally freshened the NRE and reduced the PS salinity by 70%. These storms as well as Fran (1996) delivered enough N to equal the annual N load normally entering this N-sensitive system (Paerl et al. 2001) (Fig. 2). Biogeochemical and ecological effects included hypoxic (<4 mg O_2/L) bottom waters, major changes in nutrient cycling, a threefold increase in algal biomass, persistent algal blooms, altered fish distributions and catches, and an increase in fish disease (Paerl et al. 2001, Adams et al. 2003, Peierls et al. 2003, Tester et al. 2003).

Recently, category 2 Hurricane Isabel crossed the Pamlico Sound in mid-September 2003. The storm surges and high waves associated with Isabel caused a breach in the Outer Banks near Cape Hatteras. Despite the violent winds, rainfall amounts from Hurricane Isabel were relatively small (<6 cm in coastal North Carolina; North Carolina Climatology Office, North Carolina State University, Raleigh, North Carolina, USA), in part because it was a fast-moving storm. As a result, freshwater discharge and nutrient (N) load were low for Isabel compared to the 1999 hurricanes and more comparable to effects of localized summer/fall thunderstorms (Fig. 2). Most recently, in September 2005, Hurricane Ophelia grazed the Outer Banks. Ophelia was another relatively dry, low-N-input storm (data not shown).

Like many coastal systems, the PS is strongly influenced by both human (nutrient and other pollutant) and hydrologic perturbations, including water supply diversions and manifestations of climate change (droughts, hurricanes, floods). These interactive perturbations have intensified during the past decade, reflecting a recent rise in Atlantic hurricane activity and increased development in its watershed.

Water column hydrographic measurements

In the NRE, water column hydrographic data have been collected from fixed sampling locations at two-week intervals from 1994 to the present as part of the NRE modeling and monitoring program ModMon (Fig. 3; data *available online*).[2] The numbers and locations of sampling stations have varied slightly through time. In the PS, hydrographic data have been collected from 10 locations since late 1999 (Peierls et al. 2003) (Fig. 3). Vertical profiles of temperature, salinity, dissolved oxygen (DO), conductivity, chlorophyll *a* fluorescence,

[2] ⟨www.marine.unc.edu/neuse/modmon⟩

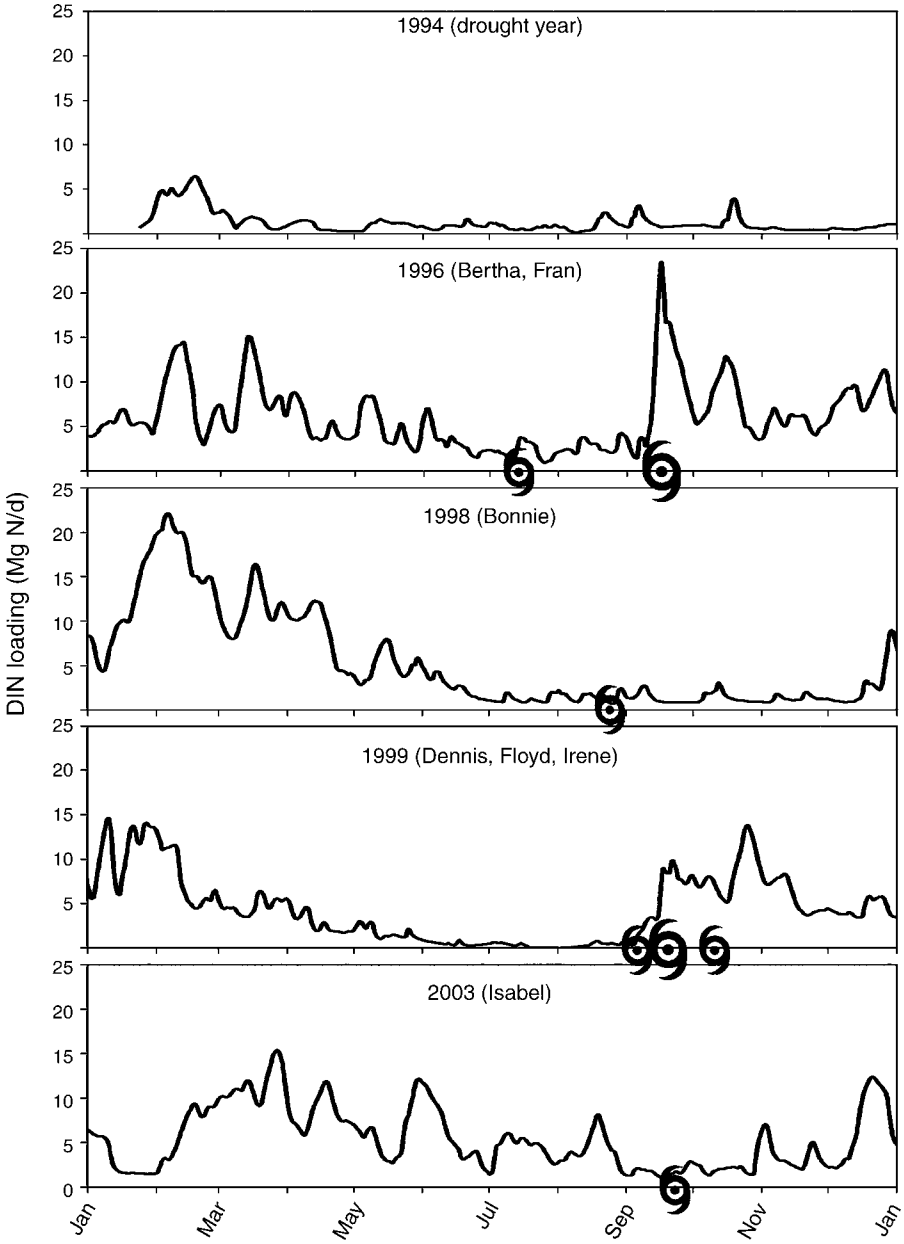

FIG. 2. Dissolved inorganic nitrogen (DIN) loading to the Neuse River Estuary for four hydrologically different years, including those years in which hurricanes (named) struck eastern North Carolina. The DIN loading was calculated by multiplying freshwater discharge from the Neuse River (at the U.S. Geological Service gauging station at Kinston, 30 km upstream from the head of the estuary) by DIN (ammonium, nitrate/nitrite) concentrations. Times of landfall for individual hurricanes are indicated with hurricane symbols.

turbidity, depth, and pH were measured with a YSI 6600 multiparameter water quality monitor coupled to a YSI 650 MDS logger (Yellow Springs Instruments, Yellow Springs, Ohio, USA). Additional hydrographic and water quality data were collected by the ferry-based water quality program FerryMon (data *available online*)[3] and two autonomous vertical profilers (AVPs) located in the NRE (Fig. 3). The North Carolina State Climate Office (Raleigh, North Carolina) provided daily precipitation and tributary discharge data from station Kinston 5 SE located ~30 km upstream from the head of the estuary (35.22° N, 77.53° W).

Nutrient analyses

Nutrient analyses were conducted on water samples collected by the ModMon program at 0.5 m below the

[3] ⟨www.ferrymon.org⟩

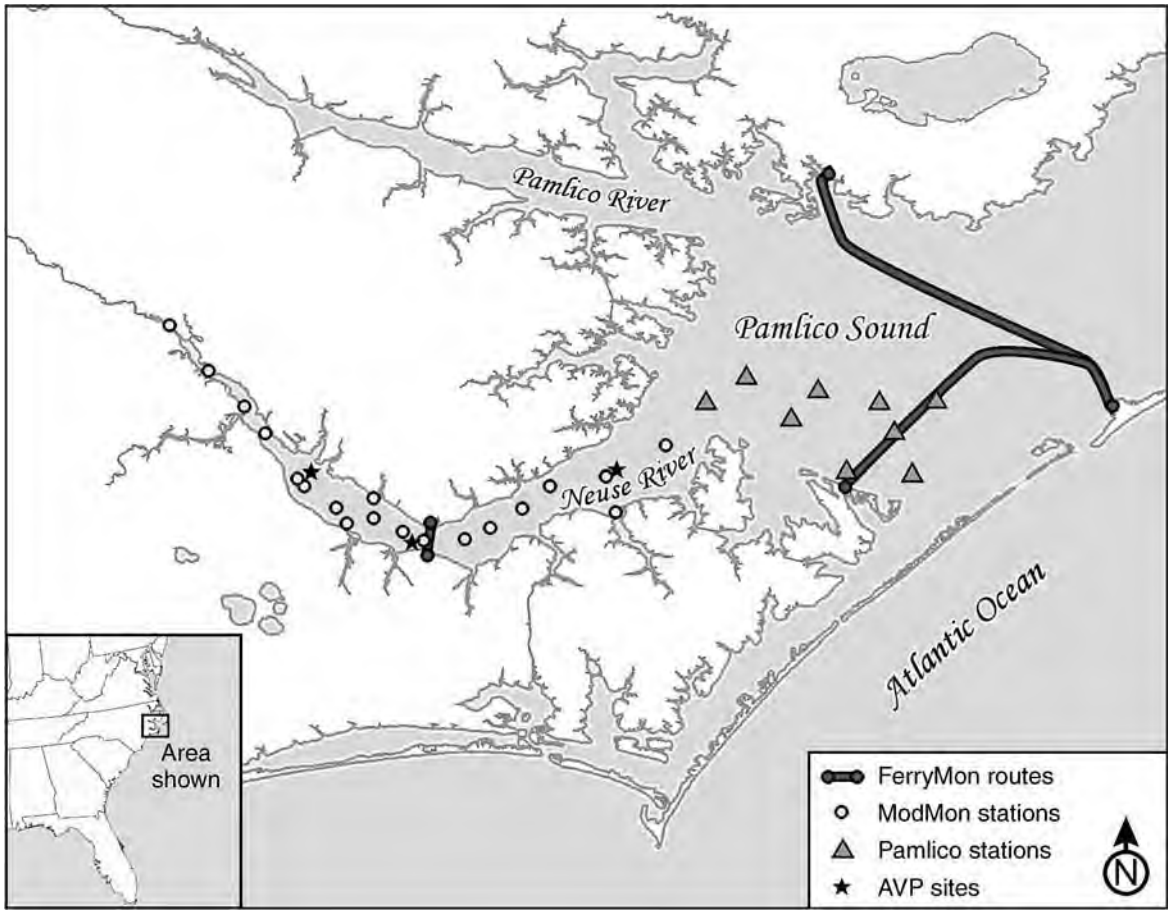

FIG. 3. Map of the Neuse River Estuary and western Pamlico Sound, showing the sampling locations for the Neuse River modeling and monitoring program (ModMon), the ferry-based water quality monitoring program (FerryMon), and autonomous vertical profiler (AVP) locations. These programs were the sources of data for this study.

surface and 0.5 m above the sediments, using a horizontal PVC Van Dorn sampler, and automatically, using a carousel collector at 0.5 below the surface by FerryMon. Water samples for dissolved inorganic-nutrient analyses were gently filtered through 25 mm diameter Whatman GF/F filters and analyzed in the laboratory. Nitrate + nitrite ($NO_3^- + NO_2^-$), ammonium (NH_4^+), orthophosphate (PO_4^{-3}), and silicate (SiO_3^{-2}) concentrations were determined with a Lachat Quick-Chem 8000 auto-analyzer (Lachat, Milwaukee, Wisconsin, USA), using standard protocols (Lachat Quik-Chem methods 31-107-04-1-C, 31-107-06-1-A, and 31-115-01-3-C, respectively).

Phytoplankton biomass

As an indicator of total phytoplankton community biomass, chlorophyll *a* (chl *a*) concentrations have been measured once every two weeks as part of the ModMon transects of stations extending from the upper freshwater region of the NRE to the entrance of PS, daily as part of the FerryMon program, and during specific experimental work since 1994. Near-surface and near-bottom grab samples were analyzed for chl *a* by filtering 100 mL of NRE water onto Whatman glass fiber filters (GFF, 0.7-μm porosity). Filters were sonicated in 90% acetone, extracted overnight, and analyzed fluorometrically for chl *a* (Paerl et al. 1995).

Group-specific diagnostic photopigment analyses

In addition to chl *a*, which accounts for total phytoplankton community biomass, chemosystematic chlorophyll and carotenoid photopigments characteristic of specific taxonomic groups (i.e., diatoms, chlorophytes, dinoflagellates, cyanobacteria, cryptomonads; see Plate 1) have proved useful as measures of taxa-specific biomass (Mackey et al. 1996, Jeffrey et al. 1997). Near-surface and near-bottom samples from biweekly ModMon runs along the NRE–PS continuum were gently filtered (200–500 mL, depending on phytoplankton density) on Whatman GFF filters. Filters were then sonicated in 100% high-performance liquid chromatography (HPLC)-grade acetone and extracted overnight, and the extracts were analyzed by HPLC coupled to

photodiode array spectrophotometry (PDAS) (Wright et al. 1991, Millie et al. 1993).

A statistical procedure, ChemTax (Mackey et al. 1996), which partitions chl *a* into the major algal groups based on diagnostic carotenoids, was used to determine the relative and absolute contributions of each group. The software CHEMTAX was used (Mackey et al. 1998). In the NRE, key photopigment markers include chlorophyll *b* and lutein (chlorophytes), zeaxanthin, myxoxanthophyll and echinenone (cyanobacteria), fucoxanthin (diatoms), peridinin (dinoflagellates), and alloxanthin (cryptomonads) (Paerl et al. 2003). Photopigment composition is generally significantly (linearly) correlated with species cell counts or biovolume estimates (Tester et al. 1995, Wright et al. 1996), although there are some relatively rare "outlier" phytoplankton species that may contain pigments that lie outside of their taxonomic grouping (e.g., euglenoids). Therefore, care was taken to periodically make microscopic checks to ensure that the appropriate phytoplankton groups were identified and quantified (Lewitus et al. 2005). Prior studies have shown that examining phytoplankton community dynamics and successional changes at the taxonomic-group level often provides excellent insight into the environmental controls of shifts in productivity, biogeochemical fluxes, and food web dynamics (Cottingham and Carpenter 1998, Pinckney et al. 2001, Paerl et al. 2003, 2006).

Results and Discussion

Distribution of phytoplankton biomass along the estuarine continuum

Seasonal and multi-annual examinations of phytoplankton biomass, determined as chlorophyll *a* concentrations, indicated that biomass was heterogeneously distributed both with depth and along the NRE–PS continuum. Frequently, chl *a* showed strong vertical gradients during salinity-stratified periods, with highest concentrations appearing near the surface or just above the pycnocline (Paerl et al. 1995, Valdes et al. 2006; N. S. Hall, *unpublished manuscript*). Longitudinally, phytoplankton biomass frequently accumulated as distinct peaks or chl *a* maxima ("chl *a* max"), which most often occurred midway down the NRE, beginning where the river begins to widen and diminishing where the river bends to the northeast (see Fig. 3). The longitudinal distribution of chl *a* concentrations is strongly influenced by the interacting effects of freshwater discharge, nutrient availability, and the morphometry of the NRE. Freshwater discharge controls longitudinal transport and water residence time in the NRE and as such delivers nutrients and simultaneously influences the accumulation and transport of biomass through variable flushing rates. When flow was extremely high, flushing rates exceeded the ability of any phytoplankton groups to accumulate biomass and form blooms in the estuary. This scenario typically occurred during relatively high rainfall during late winter and spring months, when

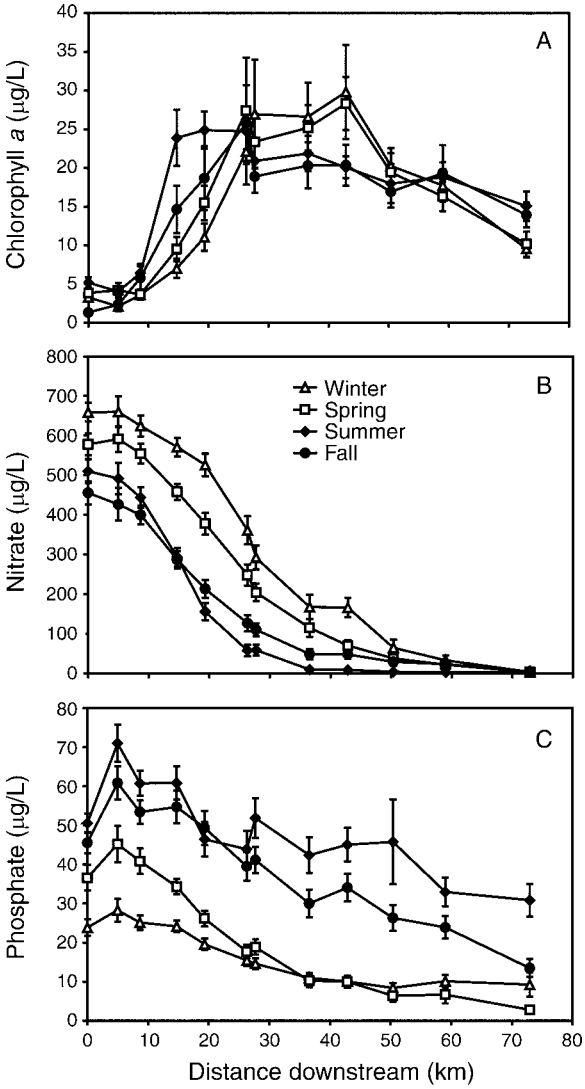

FIG. 4. Seasonal means (±SE) of (A) chlorophyll *a*, (B) nitrate, and (C) phosphate concentrations measured at each sampling station along the length of the Neuse River Estuary for all years from 1994 to 2003. Distance downstream refers to the distance in kilometers of the sampling station from the most upstream sampling location at Streets Ferry Bridge.

elevated runoff prevented accumulation of chl *a* in the upper NRE (Fig. 4A; i.e., flushing greatly exceeded growth rates). During moderate-to-low-flow conditions, which predominated during the summer, chl *a* accumulated further upstream and along the axis of the estuary where the residence time is long enough to allow growth rates to exceed flushing or "washout" (Fig. 4A). Depending on the actual flushing rates and season, specific groups dominated phytoplankton biomass and the chl *a* max.

Typically, the chl *a* max formed where lateral increases (gradients) in residence time coincided with adequate supplies of growth-limiting nutrient(s). These are often correlated in the NRE, because the actively

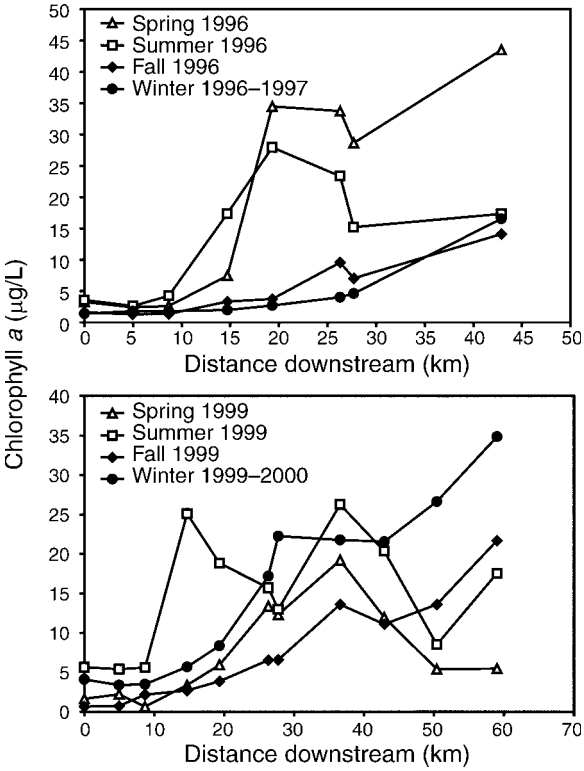

FIG. 5. Seasonal means of chlorophyll *a* concentrations along the length of the Neuse River Estuary during two major hurricane years: 1996 (top panel) and 1999 (bottom panel).

growing phytoplankton populations in the chl *a* max are responsible for removing limiting nutrients, thereby establishing a co-occurring nutrient gradient. This can be demonstrated for the dissolved inorganic forms of the most limiting nutrient nitrogen (N), specifically nitrate, the N form that most directly reflects externally supplied (watershed) or "new" N entering the estuary. This relationship is often most profound in the upstream oligohaline (>10 psu) region of the NRE, where under favorable spring/summer flow rates the chl *a* max coincides with a steep decline in NO_3^- concentrations (Fig. 4B). Ammonium (NH_4^+) concentrations (data not shown) tend to be low throughout the estuary year around, hence no strong gradients are apparent. On occasions, brief spikes in NH_4^+ can at times be seen in the mid-region of the estuary, resulting from periods of hypoxia; however, these spikes rapidly dissipate due to phytoplankton uptake (Paerl et al. 1995, 1998).

Seasonally, the chl *a* max tends to migrate upstream in the summer as dry conditions reduce freshwater discharge (and flushing) and slow the delivery of "new" N (Fig. 4A). This pattern is interrupted by large storm events, including tropical storms and hurricanes, which can suddenly and dramatically increase discharge, flow, and flushing of the estuary. This scenario was observed following Hurricanes Fran (1996) and the three hurricanes of 1999 (Dennis, Floyd, and Irene). In both cases, high flow rates following these high-rainfall events caused the entire NRE to turn fresh and rapid flushing prevented any significant buildup of chl *a* in the estuary. Following the 1999 hurricanes, chl *a* responses to nutrient loads were largely confined to the downstream PS, where longer residence times allow the phytoplankton community to effectively utilize nutrients for growth and biomass accumulation (Peierls et al. 2003) (Fig. 5).

While NO_3^- concentrations show a steep downward gradient concurrent with the chl *a* max, dissolved inorganic phosphorus, or orthophosphate (PO_4^{3-}), does not (Fig. 4B, C). This indicates that P supplies are not limiting, relative to N, and confirms earlier studies that N is the most limiting nutrient for phytoplankton growth in the NRE (Paerl et al. 1990, 1995, Christian et al. 1991, Rudek et al. 1991). Furthermore, the sediments of the NRE are highly enriched in P (Christian et al. 1991, Paerl et al. 1995). Effective sediment–water column recycling of P most likely prevents P limitation in this and other shallow subestuaries of the Pamlico Sound system (Hobbie et al. 1972, Hobbie and Smith 1975). While there is evidence that P reductions have reduced chl *a* concentrations in the P-limited freshwater rivers draining to these estuaries (Paerl et al. 2004), the downstream estuarine waters have remained N-limited. If anything, aggressive upstream P reductions may have exacerbated downstream eutrophication by having reduced the biological filter (chl *a* max) for N removal in the upstream riverine components of these ecosystems.

The location and magnitude of the chl *a* max are useful for assessing the interactive effects and influences of nutrient (largely N) loading and hydrologic discharge on phytoplankton production patterns and trends in the NRE. The size of the chl *a* max can be used as an indicator of the magnitude of new production in response to nutrient loading when more predictable seasonal hydrologic cycles are encountered. Under these conditions, it is a useful tool for assessing long-term effects and benefits of N reduction strategies aimed at reversing eutrophication in this and other seasonally hydrologically variable estuaries. However, when large storms impact this system, the location, magnitude, and longitudinal extent of the chl *a* max are strongly affected. When corrected for and integrated over the volume and length of the estuary, the storm-influenced (displaced downstream) chl *a* max can contain from two to over four times as much phytoplankton biomass than more predictable seasonal peaks. This indicates that elevated N loads associated with storm discharge are effectively utilized and translated into new production along the continuum.

The long-term (1994–2004) phytoplankton biomass and community composition record for the NRE shows highly repeatable seasonal patterns during hurricane-free years as well episodic shifts in composition following large changes in freshwater input. During this time, the NRE was affected by droughts, and since 1996, an increase in the frequency and magnitude of tropical storms and hurricanes. During droughts, like those

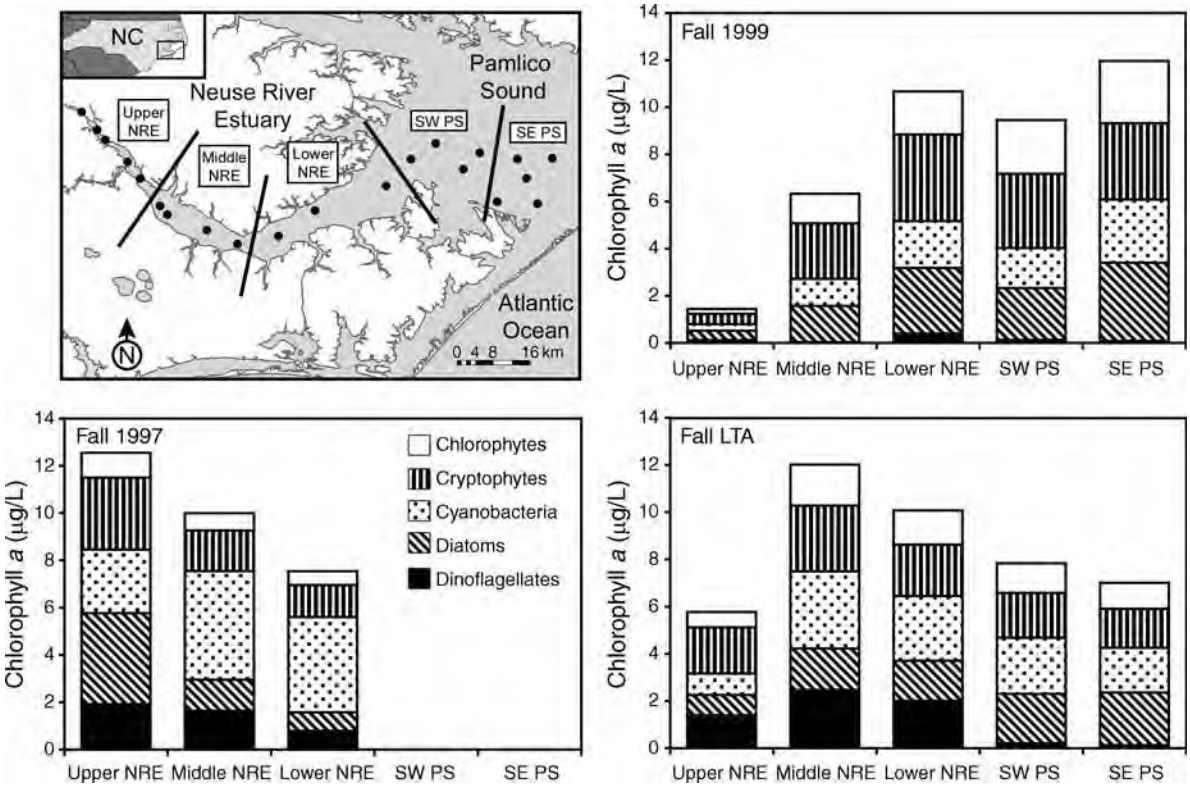

FIG. 6. Mean total and taxonomic group-specific phytoplankton biomass (as chlorophyll *a*) in each of the five regions of the Neuse River Estuary–Pamlico Sound (NRE–PS, including SW [southwestern] and SE [southeastern] PS) system during a drought period (fall 1997) and a period characterized by frequent and intense hurricane activity (fall 1999). These patterns were compared to the long-term average (LTA) phytoplankton community structure during fall months (September through November, 1994–2003) in each of the five regions. Values were determined from CHEMTAX analysis of diagnostic photopigment concentrations measured from surface samples collected at fixed sampling locations (black dots in the uppermost panel). Pamlico Sound regions were not sampled in fall 1997.

observed in summer and fall of 1997 in the NRE, the chl *a* max was consistently observed in the upper NRE region. Dissolved inorganic N (especially NO_3^{-1}) gradients proved to be quite steep in this persistent and stationary drought-driven chl *a* max. This led to strong mid- and downstream N limitation, accompanied by consistently low chl *a* concentrations (Fig. 6). During the 1997 drought period, total phytoplankton biomass (as chl *a*) in the upper NRE was double the fall long-term average (fall LTA) for this region. The opposite pattern was observed during the fall 1999 hurricane period when total phytoplankton biomass was significantly reduced in the upper NRE region (by more than a third when compared to the fall LTA) and incrementally increased along the length of the NRE, remaining elevated within the two PS regions. These results illustrate that drought conditions tend to favor the formation of phytoplankton maxima upstream, while elevated rainfall and freshwater discharge displaced the maxima downstream. The average spatial distribution of total phytoplankton biomass during the fall (fall LTA) illustrates that on average, the greatest abundance of phytoplankton occurs in the middle and lower portions of the NRE (Figs. 5 and 6).

Assessing phytoplankton community composition dynamics along the NRE–PS continuum

When evaluated by HPLC-CHEMTAX, phytoplankton composition was strongly impacted by the changes in hydrology in the NRE–PS. During the fall 1997 drought, diatoms were the most abundant phytoplankton taxonomic group in the upper NRE, while cyanobacteria were dominant in the middle and lower NRE regions (Fig. 6). In contrast, on average (fall LTA), cryptophytes were the most abundant group in the upper NRE, while all five phytoplankton taxonomic groups were on average (fall LTA) present in about equal proportions (~20%) in the middle and lower NRE regions. During the fall 1999 hurricane season, dinoflagellates, which generally comprise ~20% of the phytoplankton in the three NRE regions (Fig. 6), were significantly reduced in abundance during this elevated flushing (reduced water residence time) condition, especially in the upper and middle NRE regions (Fig. 6). Phytoplankton community structure during the fall 1999 hurricane period was primarily comprised of diatoms, chlorophytes, and cryptophytes. Elevated runoff, which continued for at least five months

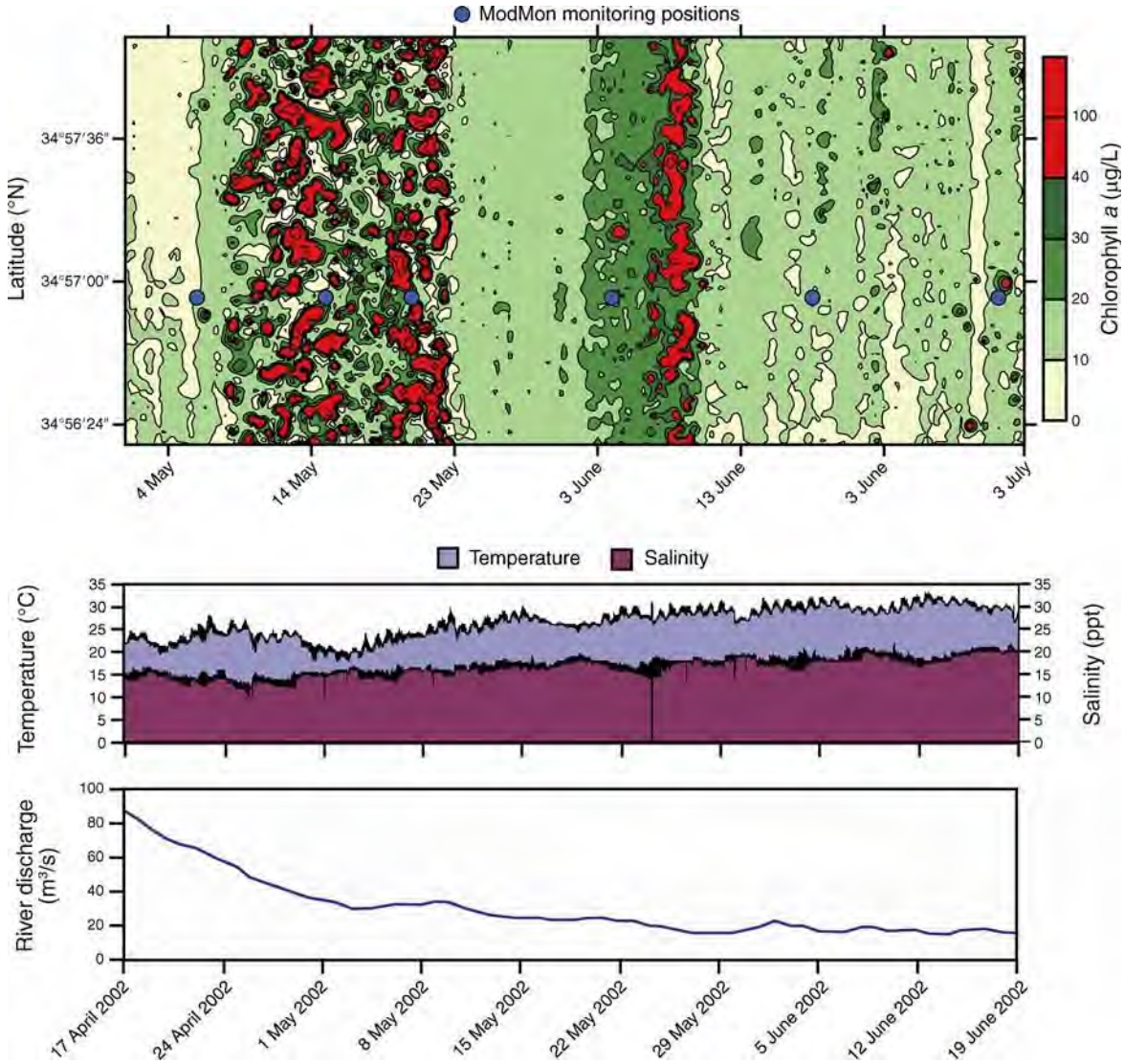

FIG. 7. Example of phytoplankton community dynamics and associated physicochemical "driver" data collected by the FerryMon program. The upper graph shows chlorophyll *a* concentration data (using a fluorescence probe mounted on a YSI 6600 multiparameter sonde, calibrated with extracted/fluorescence measurements [Yellow Springs Instruments, Yellow Springs, Ohio, USA]) collected over a 2.5-month period on the Neuse River Estuary (NRE) crossing. Shown are time (*x*-axis) and GPS-based locations (*y*-axis) of data. Chlorophyll *a* data was based on 20 ferry crossings per day (~15 data points per transect) that the ferry makes between Cherry Point and Minnesott Beach on the NRE over the 2.5-month period. The blue dots show the time and location of ModMon data collection at a site that intersects with the FerryMon crossing. On some occasions ModMon captures data similar to FerryMon, while at other times, ModMon is not able to capture the variability that FerryMon is able to detect. These data indicate that phytoplankton populations occur as distinct patches as they are transported in the estuary. The frequency, magnitude, and location of patches vary with time and space and appear to be related to variable flow regimes (freshwater discharge is shown in the lower frame). They may also be partially controlled by temperature and salinity (middle frame), parameters known to influence phytoplankton community growth rates and structure.

following the 1999 hurricanes, promoted prolonged periods of flushing (short residence time) and reduced salinity conditions throughout the NRE going well into mid-2000. This created conditions favorable for fast-growing, low-salinity-adapted chlorophytes and cryptophytes, which dominated the upper and middle NRE, starting in fall of 1999 and lasting throughout spring and early summer of 2000 (Fig. 6) (Paerl et al. 2005). Conversely, relatively slow-growing dinoflagellates, which regularly form late-winter blooms (e.g., *Heterocapsa triquetra*) in years during which flow is low to moderate (i.e., years free of high-rainfall hurricanes) (Paerl et al. 1995), were absent in winter/spring periods when extensive flooding and freshwater runoff followed the 1999 and 1996 large hurricanes (Valdes et al. 2006). In the hurricane-free decade prior to 1996, these blooms

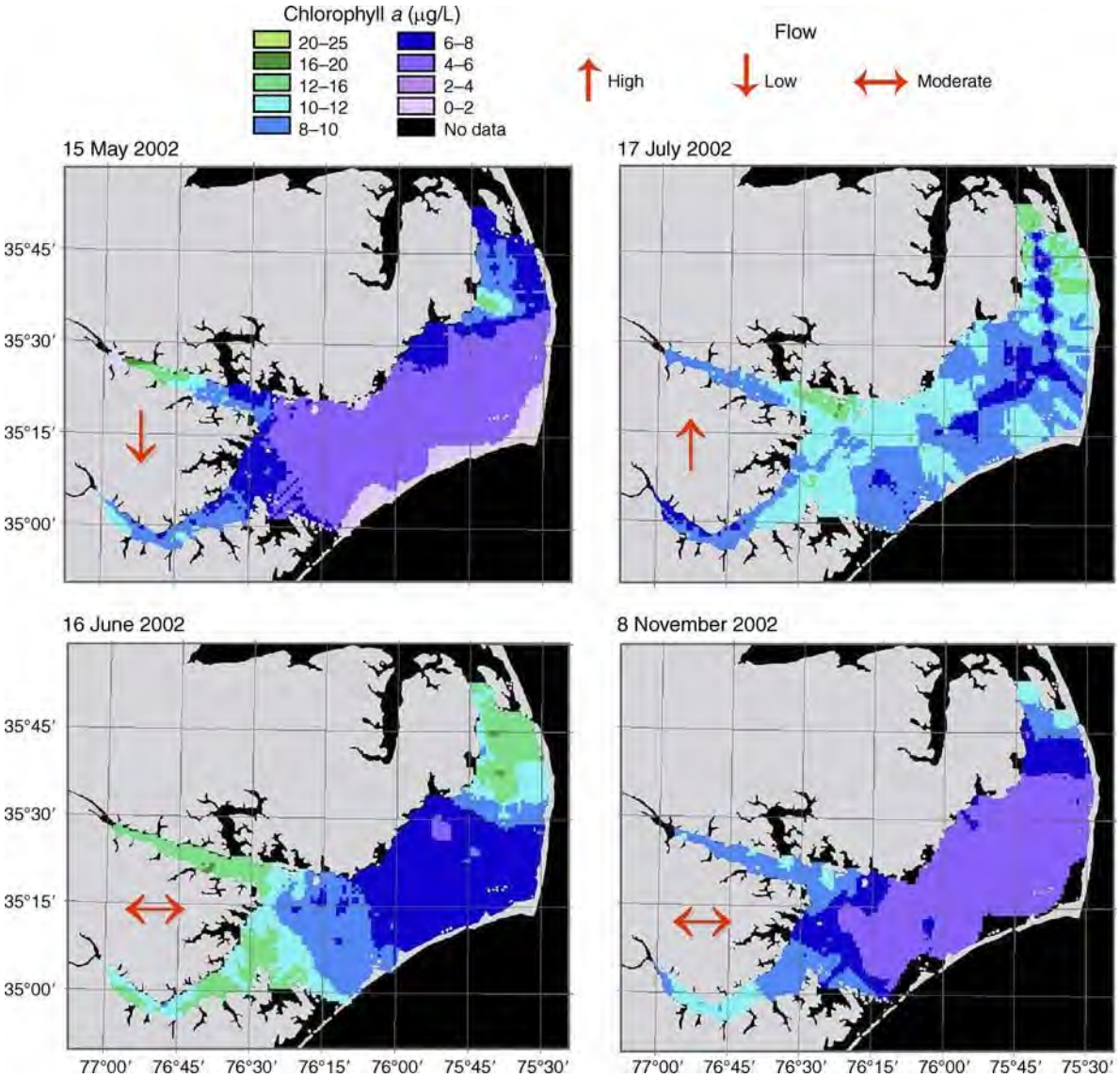

FIG. 8. Spatial relationships between remote-sensed phytoplankton biomass, as chlorophyll *a*, and freshwater discharge (flow arrows) to the Pamlico Sound system. Surface water chlorophyll *a* concentrations were estimated using an aircraft-based sea-viewing wide field-of-view sensor (SeaWiFS) remote sensing system, calibrated by FerryMon-based chlorophyll *a* data. Under relatively low-flow, long residence time conditions, phytoplankton biomass is concentrated in the upstream reaches of the estuaries (i.e., Neuse and Pamlico River Estuaries). Under moderate flow, phytoplankton biomass maxima extend farther downstream. Under high flow (short residence time), phytoplankton biomass maxima are shifted farther downstream into Pamlico Sound.

were a highly predictable feature of the NRE (Paerl et al. 1995). Interestingly, these blooms are now making a comeback since 2000. We speculate that this bloom resurgence is largely a response to the absence of high-rainfall hurricanes in the NRE watershed.

Estuarine phytoplankton taxonomic groups exhibit variable optimal growth rates (doubling times) (Redalje 1993, Pinckney et al. 2001). The net effect of variable growth rates and changeable hydrologic regimes in the NRE–PS continuum is that phytoplankton composition is strongly influenced by large hydrologic perturbations, whether they be droughts or hurricanes. While the most profound examples of this were the shifts observed in response to the lengthy periods of freshwater discharge following the 1996 and 1999 hurricanes, the drought periods in 1997 and the summer preceding the 1999 hurricanes are additional, albeit less spectacular, examples. These periods tended to favor slower growing, relatively high-salinity-tolerant-phytoplankton groups that, by virtue of their slow growth rates are able to grow on lower nutrient supply regimes. These are typified by the picoplanktonic cyanobacteria, which exhibited an increasing and persistent degree of dominance during summer low-flow drought conditions.

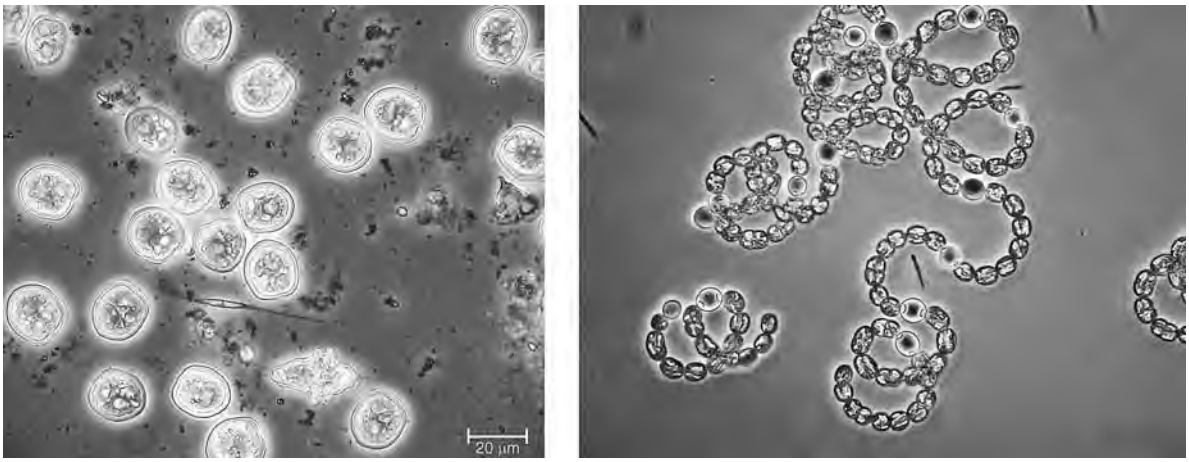

PLATE 1. (Left) A dinoflagellate bloom in the Neuse River Estuary, in response to nutrient inputs associated with runoff from tropical storm Ernesto, which made landfall in eastern North Carolina (USA) in mid-September 2006. The bloom was dominated by *Prorocentrum minimum* (round cells), with another dinoflagellate *Heterocapsa triquetra* ("top" shaped cell on its side) as subdominant. (Right) Filamentous, bloom-forming blue-green algae (cynobacterium) *Anabaena circinalis* from the nutrient-enriched, upstream, freshwater portion of the Neuse River Estuary. This cyanobacterium has specialized cells, called heterocysts (the round, clear cells with bright "polar plugs" at each end of the cell), in which nitrogen fixation takes place. Nitrogen fixation is the biochemical process enabling cyanobacteria like *Anabaena* to convert atmospheric nitrogen gas to the biologically utilizable form of nitrogen, ammonia. This enables *Anabaena* to thrive in nitrogen-depleted waters. Because these organisms supply their own nitrogen, by nitrogen fixation, phosphorus is often the nutrient that controls the growth and expansion of such nuisance blooms. Photo credits: H. Paerl.

CONCLUSIONS AND RECOMMENDATIONS

Photopigment analyses of phytoplankton biomass and community composition have shown that sudden and large changes in hydrologic conditions associated with tropical storms and hurricanes are important, and sometimes overriding, drivers of phytoplankton community structure and function in estuarine and coastal ecosystems. This is especially true for microtidal, relatively long residence time, lagoonal systems like the NRE–PS, where the full sequence of phytoplankton community nutrient utilization, growth, and successional responses falls within the time scales of hydrologic and nutrient-loading drivers. It follows that when formulating nutrient (in this case N) input constraints designed to control eutrophication, hydrologic variability should play an integral role. For example, nutrient eutrophication models that rely on the interactions of nutrient supply and phytoplankton growth kinetics to predict phytoplankton group responses to nutrient-loading events must also take freshwater input, flushing, and water residence time into consideration. Predictability of phytoplankton group successional patterns derived from competition for nutrients in a variable flow environment will strongly rely on incorporating nutrient uptake/utilization kinetics and flushing or "washout," as well as seasonality in models that realistically depict interactive phytoplankton community-structuring features.

Spatiotemporal analysis of phytoplankton community responses to physical and chemical drivers associated with human and climatic perturbations has shown the importance of examining these responses along the entire freshwater–marine continuum. Depending on freshwater runoff associated with seasonal precipitation patterns, acute and sometimes catastrophic storms and droughts, a bulk of the phytoplankton production and successional responses may be confined to either narrow, well-defined chl *a* max zones or more diffuse zones along this continuum. Capturing and quantifying phytoplankton community responses along this continuum requires sensitive process-level indicators and appropriate spatiotemporal sampling strategies. Phytoplankton responses to environmental drivers are often episodic, that is they can form, proliferate, and/or dissipate over a timescale of a few days to several weeks. These responses, as blooms, account for significant fractions of seasonal or annual production. They are also causative agents for hypoxic events, fish kills, and other advanced symptoms of eutrophication, yet may be missed by biweekly or monthly sampling programs, which tend to be the norm for a wide range of estuarine ecosystems (cf. U.S. EPA 1998, 2001, Bricker et al. 1999). As such, there is a widespread need for spatially and temporally intensive sampling and analysis of the causative factors, as well as locations, manifestations, and fates of phytoplankton blooms. Appropriate spatial and temporal coverage and comprehensiveness of the indicators discussed here can be ensured by deploying them on intensive monitoring programs and unattended platforms, including moorings, channel markers, docks, and vessels that cover repeatable routes in estuarine and coastal waters.

One example is the use of the North Carolina Department of Transportation (DOT) ferries for automated water quality monitoring of the NRE–PS system,

FerryMon. FerryMon was initiated as part of the State's response to the flooding, nutrient, and sediment discharge following the three 1999 hurricanes. FerryMon uses three ferries as data-gathering "ships of opportunity" that transit the NRE and PS continuously from 05:00 until midnight daily (see Fig. 3 for routes). Since 2000, FerryMon has provided unattended, near-real-time spatially and temporally intensive data on key environmental parameters pertinent to the control of phytoplankton production and composition. These include physical (temperature, turbidity), chemical (dissolved oxygen, salinity, pH, nutrients), and biological (chl *a* and diagnostic photopigments) data. It is establishing a baseline for assessing the relative impacts of storm-related ecological change on the event and longer term scales. The program provides large data sets (~80 000 data points per year) that can produce maps of chl *a* and algal blooms with a high degree of statistical confidence. This information is used to provide a database for calibrating and verifying process-level and probabilistic models for predicting nutrient productivity–algal bloom relationships (Borsuk et al. 2001, 2003, Bowen and Hieronymous 2003).

Additional examples of FerryMon's recent applications for examining and distinguishing physicochemical drivers of phytoplankton community dynamics include: (1) the use of the Neuse River Ferry data to assess TMDL compliance, based on the State's 40 μg/L acceptable chlorophyll *a* level (Fig. 7), (2) assessing the effects of recent hurricanes such as Isabel (September 2003) and Ophelia (September 2005) on PS water quality (Paerl 2005), and (3) the use of chl *a* data for calibration of aircraft- and satellite-based remote sensing, allowing investigators to enlarge studies of nutrient and hydrologic forcing of phytoplankton production for the entire Pamlico Sound system (Fig. 8).

All three examples illustrate the strong interactive effects of nutrient and hydrologic forcing in this system, based on the use of phytoplankton as an overall response indicator. Current and future nutrient management strategies aimed at controlling eutrophication of coastal systems periodically influenced by tropical storms and hurricanes should develop and adopt criteria and thresholds for gauging success that factor in hydrologic variability, especially on the episodic scale. In North Carolina, this involves adaptive nutrient management strategies that consider the timing of nutrient (fertilizer) applications and wastewater nutrient releases, no-till agricultural practices, improved stormwater and agricultural (animal) waste containment/treatment, and greater coupling and integration of groundwater and atmospheric nutrient sources with estuarine and coastal nutrient budgets and algal bloom dynamics. The overall lesson learned is that, in order to be most meaningful and effective, nutrient productivity and resultant eutrophication management strategies must address the entire airshed/watershed-to-coastal-water continuum.

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Phytoplankton Community Indicators of Short- and Long-term Ecological Change in the Anthropogenically and Climatically Impacted Neuse River Estuary, North Carolina, USA

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Abstract Estuarine and coastal systems represent a challenge when it comes to determining the causes of ecological change because human and natural perturbations often interact. Phytoplankton biomass (chlorophyll *a*) and group-specific photopigment indicators were examined from 1994 to 2007 to assess community responses to nutrient and climatic perturbations in the Neuse River Estuary, NC. This system experienced nutrient enrichment and hydrologic variability, including droughts, and an increase in hurricanes. Freshwater input strongly interacted with supplies of the limiting nutrient nitrogen (N) and temperature to determine the location, magnitude, and composition of phytoplankton biomass. Multi-annual, seasonal, and episodic hydrologic perturbations, including changes in the frequency and intensity of tropical storms, hurricanes and droughts, caused significant shifts in phytoplankton community structure. Climatic oscillations can at times overwhelm anthropogenic nutrient inputs in terms of controlling algal bloom thresholds, duration, and spatial extent. Eutrophication models should incorporate climatically driven changes to better predict phytoplankton community responses to nutrient inputs and other anthropogenic perturbations.

Keywords Phytoplankton · Estuary · Ecological change · Climate · Nutrients · Photopigments

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Introduction

In estuarine and coastal systems worldwide, human activity puts considerable stress on ecosystem functioning. At least half the world's population resides in the coastal zone (Vitousek et al. 1997), and this influence continues to grow. Human development in coastal river basins has greatly increased nutrient, sediment, and other pollutant loads to these waters (Nixon 1995; Paerl 1997; Peierls et al. 1991), resulting in deterioration of water quality, losses of fisheries habitat and resources, and an overall decline in ecological condition (Boesch et al. 2001; Bricker et al. 1999; National Research Council 2000; Scavia and Bricker 2006).

Estuarine and coastal systems represent a formidable challenge when it comes to determining status and trends in water quality and ecological condition. These systems are highly dynamic from hydrologic, nutrient cycling, and biotic resource perspectives. Hydrologically, freshwater runoff interacts with tidal saltwater exchange and variable winds, leading to complex circulation and mixing patterns. These patterns, shaped by climatic forcing features (i.e., temperature, rainfall, winds, etc.) that vary over multiple time and space scales, strongly influence the chemical and biological characteristics and responses of these ecosystems to environmental changes and perturbations. Furthermore, human and natural perturbations often interact. For exam-

ple, accelerating nutrient and sediment inputs can accompany climatic perturbations such as storms and floods, which in the case of tropical storm and hurricane activity, are increasing (Emanuel 2005; Goldenberg et al. 2001; Holland and Webster 2007; Webster et al. 2005).

Given the importance and dynamic nature of estuarine ecosystems, there is an urgent need to develop sensitive and broadly applicable indicators for detecting water quality changes and overall ecological condition and changes therein. Phytoplankton, including prokaryotic cyanobacteria and eukaroytic algal groups, conduct a bulk of primary production supporting food webs and play a central role in carbon, nutrient, and oxygen cycling in estuaries. Because phytoplankton have fast growth rates (i.e., doubling times of a day or less) and can rapidly respond to a wide range of environmental perturbations, they represent a sensitive and important indicator for detecting ecological change in estuaries. Lastly, changes in phytoplankton community structure and activity often precede larger-scale, longer-term changes in ecosystem function, including shifts in nutrient cycles, food webs, and fisheries (Paerl and Peierls 2008).

The Neuse River Estuary (NRE) is a major sub-estuary of the USA's second largest estuarine complex, the Pamlico Sound (North Carolina), a major fisheries nursery for the mid-Atlantic and Southeastern US coasts. The NRE exemplifies an estuary that is increasingly affected by human (nutrient loading) and climatic (hurricanes, droughts, floods) perturbations. We explored the use of diagnostic phytoplankton group-level photopigments to assess the impact of climatic perturbations acting on multiple timescales. Using a long-term (since 1994) data set from the bi-weekly Neuse River Estuary Modeling and Monitoring Program (ModMon), and data from a more recently established (since 2000) ferry-based autonomous monitoring program (FerryMon), we report on the impacts of climate variability spanning multiple timescales on NRE phytoplankton dynamics. Evidence presented here indicates that climatic changes and oscillations can at times overwhelm nutrient inputs in terms of controlling algal bloom thresholds, their duration, and spatial extent. In short, our results indicate that, to fully manage nutrient inputs and eutrophication, parallel (to anthropogenic factors) climatic changes need to be taken into consideration.

Background: Phytoplankton Dynamics of the Neuse River Estuary

Previous work in the NRE has set the stage for this study by demonstrating linkages of nutrient inputs to phytoplankton production and trends in spatio-temporal distributions of major phytoplankton taxa (Paerl et al. 2007; Pinckney et al. 1998, 1999; Valdes-Weaver et al. 2006). These historical data define a baseline from which comparisons can be made to assess trends in phytoplankton community spatial and temporal distributions and abundance in response to human and climatic perturbations and longer-term changes.

Along much of its fresh to saltwater continuum, the NRE is nutrient sensitive (largely N limited) throughout much of the year, with sequential phytoplankton blooms occurring from late winter through fall (Paerl et al. 1995, 2006a; Rudek et al. 1991; Valdes-Weaver et al. 2006). These blooms reflect both nutrient enrichment and favorable hydrologic conditions, i.e., variable freshwater discharge and flushing/residence times suitable for development of blooms (Paerl et al. 2007; Valdes-Weaver et al. 2006). Water residence time varies temporally (depending on freshwater discharge) and spatially along the length of the continuum (Luettich et al. 2000). In the mesohaline segment of the NRE, where the estuary broadens, residence time increases dramatically and persistent summer salinity stratification occurs (Buzzelli et al. 2002; Luettich et al. 2000). It is also most sensitive to symptoms of nutrient over-enrichment, most notably periodic phytoplankton blooms, with chlorophyll *a* (Chl *a*) levels at times exceeding 100 μg l^{-1} (Paerl et al. 1995, 2006a).

Seasonal discharge patterns and episodic pulses of freshwater input strongly influence bloom patterns. The locations of Chl *a* maxima tend to reflect the magnitude of freshwater runoff. For example, the three hurricanes of 1999 delivered the largest amount of discharge to the NRE and Tropical Storm Ernesto proved to also be a very high discharge event. The Chl *a* maxima that formed in the estuary following these events were further downstream than peaks following other wet but not as high discharge periods (e.g., spring–summer 2003) (Fig. 1). During drought periods (such as 2002 and 2007), the Chl *a* maxima tend to be located much further upstream than normal (Fig. 1).

In terms of phytoplankton community composition, the NRE frequently exhibits late winter–early spring dinoflagellate blooms comprised of *Prorocentrum minimum*, *Heterocapsa triquetra*, and *Heterocapsa rotundata* that have been attributed to enhanced N loading (Mallin et al. 1993; Pinckney et al. 1998; Rudek et al. 1991). Dinoflagellate blooms can also occur in fall, especially during sunny, calm periods (Hall et al. 2008). Cryptophyte blooms occur sporadically throughout the year in response to pulses of riverine nutrient loading (Pinckney et al. 1998). Diatoms are most numerous during spring–summer (May to July), but they rarely attain bloom proportions (Pinckney et al. 1998). In the upper, freshwater riverine portion, increases in water residence time in summer, coupled to residual spring N loading, have, in certain years, led to periodic surface-dwelling cyanobacterial (*Microcystis*) blooms (Christian et al. 1986; Paerl 1987). Chlorophytes are a significant but infrequently dominant fraction of the phytoplankton. Occasional blooms occur during the spring and summer (Pinckney et al. 1998), and often "track" nutrient-laden freshets following large storm events (Paerl et al. 2007).

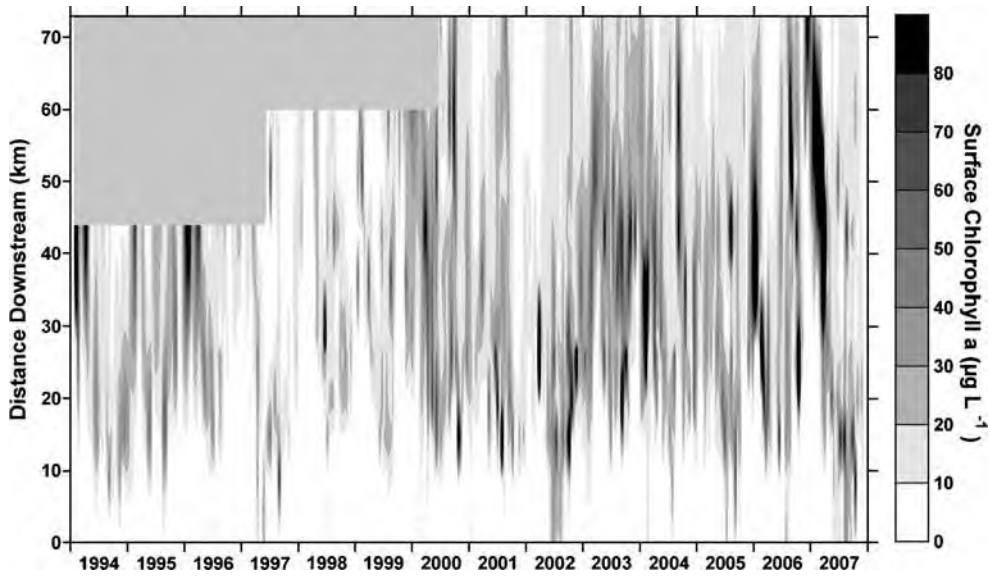

Fig. 1 Distribution of surface water Chl *a* concentrations along the axis of the Neuse River Estuary from 1994 and 2007. Data are from bi-weekly samples collected as part of the ModMon project (see Valdes-Weaver et al. 2006 for details). Contours generated from gridded data using kriging interpolation method. *Hatched area* indicates time and places without data

Materials and Methods

Study Site and Observational Programs

The NRE is a shallow, microtidal sub-estuary of the Pamlico Sound system (Fig. 2; Table 1). The NRE is downstream of rapidly expanding agricultural (animal and row crop operations), urban, and industrial activities in its watershed. Anthropogenic N and P loadings to the NRE have approximately doubled in the past four decades (Stanley 1988; Stow et al. 2001). Urban regions have sprawled in the upper NRE watershed, while industrial-scale hog farms in the coastal plain region have led to an increase in hogs from less than 1 million in 1989 to more than 12 million today. Excessive nutrient discharge (~80% non-point source) associated with expanded human activities has promoted eutrophication in the NRE (Burkholder et al. 2006; North Carolina Department of Environment and Natural Resources 2001; Paerl et al. 1995, 1998).

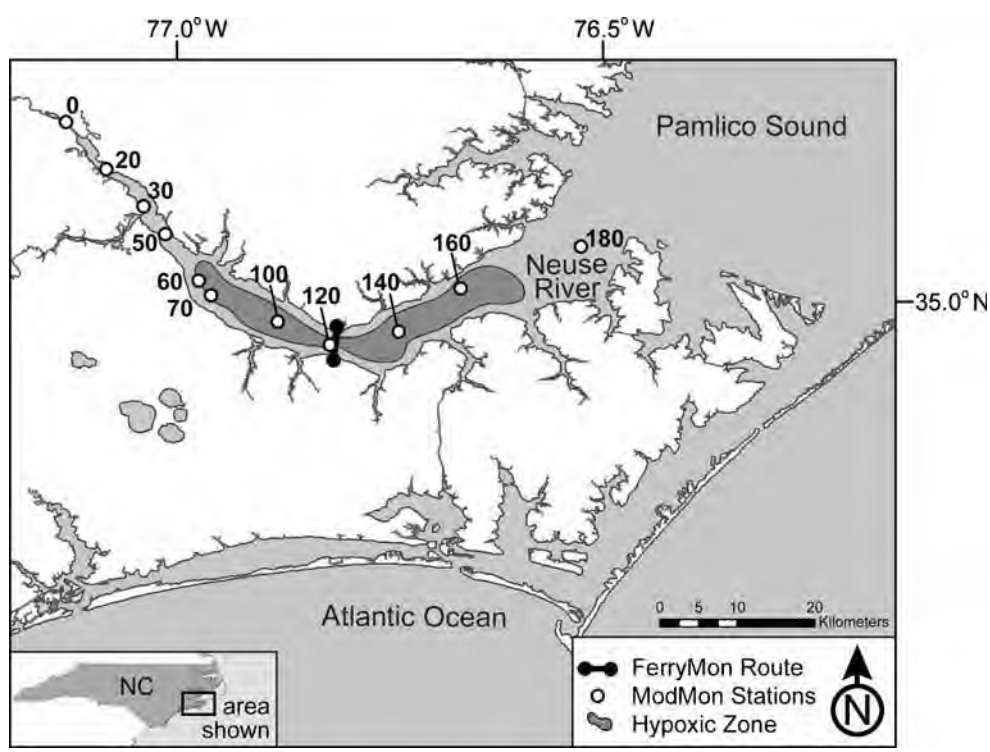

Fig. 2 Map of the Neuse River Estuary and greater Pamlico Sound system, showing ModMon sampling sites, the Neuse River Estuary FerryMon crossing, and the representative zone of summer hypoxia

Table 1 Physical characteristics of the Neuse River Estuary

Characteristic	Neuse River Estuary
Watershed size (km^2)	16,108
Surface area (km^2)	455
Average depth (m)	2.7
Discharge (m^3 s^{-1})	50–1,000
Freshwater flushing time (days)	7–200
Summer hypoxic area (% of bottom)	20–>70

In 1999, the US EPA and the North Carolina Department of Environment and Natural Resources implemented a total maximum daily load (TMDL) for nitrogen inputs to the NRE, which targeted the estuary for a mandated 30% nitrogen load reduction (based on 1990–1995 mean total annual N loads; North Carolina Department of Environment and Natural Resources 2001). However, despite the mandated human nutrient-loading reductions, symptoms of eutrophication persist, in part, due to natural climatic forcing. Since the mid-1990s, increased nutrient loading has accompanied a dramatic increase in hurricanes as well as several extensive rainy periods (Bales 2003; Burkholder et al. 2004; Paerl et al. 1998, 2001, 2006a, b; Peierls et al. 2003). These events have been linked to increases in hypoxic bottom waters (Buzzelli et al. 2002; Paerl et al. 2001, 2006b), altered nutrient inputs and cycling (Peierls et al. 2003), shifts in salinity and nutrient loading (Paerl 2006a, b), with resultant changes in phytoplankton structure and function, as well as impacts at invertebrate and fish consumers levels (Burkholder et al. 2004; Eby and Crowder 2002; Paerl et al. 2006a, b).

Water quality of the NRE has been assessed by two spatially and temporally intensive long-term monitoring programs, ModMon and FerryMon (Fig. 2). These programs include an extensive array of measurements (Table 2). ModMon is a collaborative University–State of North Carolina (North Carolina Department of Environment and Natural Resources, Division of Water Quality; NC DENR–DWQ) program (www.unc.edu/ims/neuse/modmon/index.htm), which has operated since 1994. It consists of bi-weekly visits to 11 mid-river stations in the NRE (Fig. 2; Table 2) for vertical profiling and collection of near-surface and near-bottom water. FerryMon (www.ferrymon.org) consists of NC Department of Transportation ferries equipped with automated water quality monitoring systems (Buzzelli et al. 2003; Ensign and Paerl 2006). The Neuse River ferry makes 34 crossings per day between Cherry Point and Minnesott Beach, a mid-estuarine location in the high productivity, mesohaline region of the NRE (Fig. 2). Measurements are made with the automated systems (Table 2) and data transmitted via the internet to a shore-based data server. In addition, ISCO samplers collect discrete samples at regular programmed intervals. FerryMon serves as a source of space–time intensive information on phytoplankton community biomass (Chl *a*; using a flow-thru YSI sensor that is calibrated by extracted Chl *a* measurements) and composition (HPLC-based Chl *a* and diagnostic photopigments—Paerl et al. 2003). Data from the U.S. Geological Survey (USGS) real time water quality instrumentation (station 0209265810; http://waterdata.usgs.gov/nc/nwis) were used to fill gaps in the FerryMon salinity and temperature data. This station is co-located with ModMon station 120 and situated midway along the ferry transect.

Analytical Methods

For Chl *a* (phytoplankton biomass) and inorganic nitrogen (nitrate+nitrite and ammonium) analyses, ModMon samples were collected from the surface and near the bottom, while FerryMon collected only near-surface samples. All samples were analyzed for Chl *a* by extracting material collected on Whatman GF/F glass fiber filters (0.7 μm nominal pore size) overnight in 90% acetone. Prior to August 1999, extracts were analyzed spectrophotometrically using the trichromatic technique, while a fluorometric technique was used after that date. Concentrations were corrected (Chl $a_{fluor}=1.81\times[\text{Chl } a_{spec}]^{0.935}$) based on an empirically derived relationship between the two analyses and HPLC-analyzed Chl *a* concentrations (see below).

Table 2 Comparison of the sampling programs that serve as data sources for this study

Program	Location	Key measurements	Resolution	
			Spatial	Temporal
ModMon	Along axis (73 km length)	Vertical profiles of temperature, salinity, pH, O_2, chlorophyll fluorescence, and turbidity	2–14 km between sites	Bi-weekly cruises
		Surface and near-bottom inorganic nutrients, Chl *a*, HPLC pigments, preserved phytoplankton, and microzooplankton	0.5 m depth resolution for profiles	
FerryMon	Across channel (5 km width)	Horizontal transects of temperature, salinity, pH, chlorophyll fluorescence, and turbidity	~700 m between data collection	~34 transects day^{-1}
		Surface inorganic nutrients, Chl *a*, HPLC pigments		

Inorganic nitrogen concentrations were measured using flow injection analysis on glass fiber filtrate (Whatman GF/F). For details of these analyses, see Paerl et al. (1998) and Peierls et al. (2003). Instantaneous nitrogen loading was calculated as the product of nitrogen concentration in μg l^{-1} and daily mean flow in m^3 s^{-1} at Kinston, NC (USGS station 02089500 http://waterdata.usgs.gov/nc/nwis).

In addition to Chl *a*, which accounts for total phytoplankton community biomass, photopigments characteristic of the specific taxonomic groups were measured (Jeffrey et al. 1999; Mackey et al. 1996; Millie et al. 1993). Near surface samples from ModMon and from discrete samples collected by FerryMon were gently filtered (200–500 ml, depending on phytoplankton density) on Whatman GF/F filters. For size fractionation purposes, ~300–500 ml of water was first filtered onto 3 μm porosity Nuclepore filters and the resulting filtrate was then filtered onto Whatman GF/F filters. Filters were then sonicated in 100% HPLC grade acetone, extracted overnight in darkness at 4°C, and the extracts analyzed by high performance liquid chromatography (HPLC) photodiode array spectrophotometry (Millie et al. 1993; Van Heukelem et al. 1994; Wright et al. 1991).

Statistical Methods

Trend analyses for nutrient loading and Chl *a* were performed using the non-parametric seasonal Kendall test for uncensored data as implemented in the S-PLUS (Insightful Corp.) version (water.usgs.gov/software/S-PLUS/) of ESTREND (Schertz et al. 1991; Slack et al. 2003). The analyses used 12 seasons per year (i.e., months) and a significance level (α) of 0.05. Concentrations reported as below detection limits were coded as one half the method detection limit. Less than 5% of values were reported as below detection.

Cyanobacterial biomass as estimated by zeaxanthin concentration was regressed on the physical parameters temperature, salinity, and river discharge, and riverine DIN loading using a stepwise multiple linear regression ('stepwisefit', Matlab version 7.0 R14, The Mathworks, Inc. Natick, MA, USA). A square root transformation was applied to zeaxanthin and assumptions of normality and homoscedasticity of the model residuals were confirmed using Lilliefors (Lilliefors 1967) and Breusch–Pagan (Breusch and Pagan 1979) tests, respectively.

Results and Discussion

Hydrologic Variability as a Driver of Nutrient Dynamics

During the study period, the NRE was both chronically and acutely impacted by climatic and subsequent hydrologic variability. The hydrologic variability associated with the various large storms, droughts, and high discharge periods is shown as freshwater discharge at a USGS gauging station (Kinston, NC), ~60 km upstream from the head of the estuary (Fig. 3).

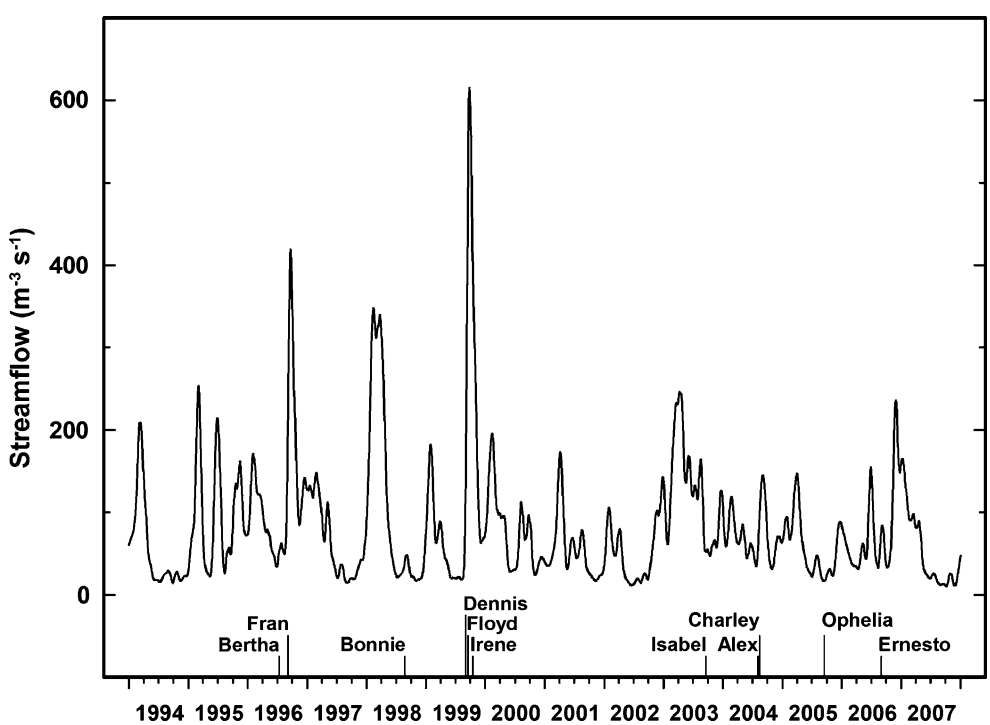

Fig. 3 Neuse River daily mean streamflow data measured at the USGS gauge at Kinston, NC (station 02089500). Plotted is a *smoothed line* through the data using a locally weighted least squared (Loess) regression technique. Major tropical cyclones impacting the system are indicated

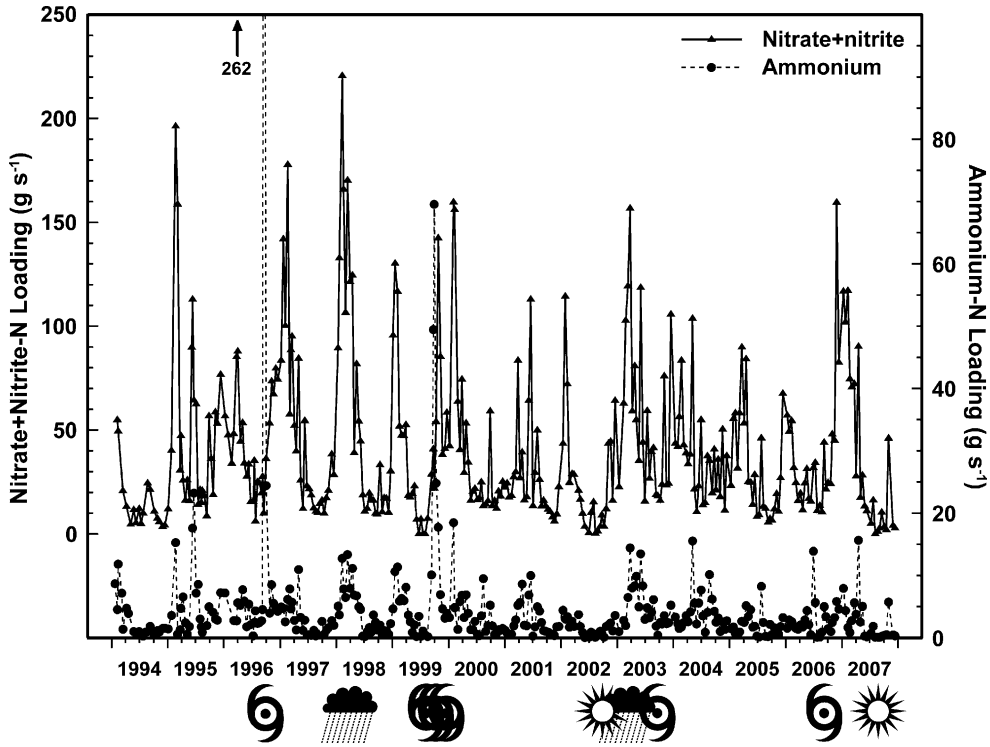

Fig. 4 Instantaneous loading of the dissolved inorganic N forms nitrate+nitrite and ammonium during the 1994–2007 sampling period. Bi-weekly values were calculated as the product of N concentration at station 0 and streamflow on the same date at Kinston, NC. Major storm events, high rainfall periods, and droughts are indicated with *icons*

Hydrologic variability strongly impacted nutrient inputs to the NRE (Fig. 4), shown here as instantaneous loading of dissolved inorganic nitrogen (DIN) at the head of the estuary at the Streets Ferry Bridge location (station 0, Fig. 2). Hurricanes Fran in September 1996, Dennis and Floyd in 1999, Tropical Storm Ernesto as well as the very wet spring–summers of 1998 and 2003 led to increased DIN loading. Conversely, extreme droughts, including the summer of 1999, proved to be periods of lowest discharge and nutrient loading. Externally supplied "new" DIN inputs were dominated by nitrate, which agrees well with previous studies conducted over shorter time intervals (Christian et al. 1991, 2004; Paerl et al. 1995, 2007).

Large spikes in ammonium loading were observed following the two largest (discharge wise) hurricanes, Fran in 1996 and Floyd in 1999. ModMon monitoring of water quality following these storms showed hypoxic (<4 mg O_2 l^{-1}) and anoxic (<2 mg O_2 l^{-1}) water being delivered from the watershed. These reduced conditions helped explain the preponderance of ammonium in the DIN load at these times. Otherwise, ammonium proved to be a relatively minor fraction of the external DIN load. Ammonium concentration spikes have been observed further downstream in the NRE; however, these spikes are strongly correlated to periods of bottom water hypoxia and anoxia indicating that they are internal DIN release events (Christian et al. 1991; Paerl et al. 1995, 2007). Overall, no significant long-term trends in nitrate and ammonium loading were detected (Fig. 4).

Hydrologic Variability as a Driver of Phytoplankton Community Dynamics

Hydrologic variability occurred over a wide range of temporal scales. Multi-annual, seasonal, and event-induced changes in river discharge, and the resulting changes in estuarine flushing and water residence times, affected total phytoplankton biomass and differentially affected specific phytoplankton groups as a function of their contrasting growth characteristics (Figs. 1 and 5). However, there were also indications in the longer-time series of factors other than hydrology exerting controls on the phytoplankton biomass (Figs. 1 and 5). Below, we focus on the impacts of climatic/hydrologic variability on phytoplankton community structure for the NRE.

Multi-year Patterns

Analysis of the 14-year ModMon database has revealed substantial multi-year changes in the NRE phytoplankton community. At a mid-estuarine location, Chl *a* concentration showed no significant long-term trend over the entire period, but large changes in variability did occur (Fig. 5). From 1994 to early 1996, several very large (>80 μg l^{-1} chlorophyll *a*) phytoplankton blooms occurred in this region of the estuary (Fig. 5). However, this was followed by a >6-year period (from mid-1996 to end of 2002) of few large blooms (e.g., only one bloom >80 μg l^{-1} was recorded during this period; Fig. 5) and lower variability.

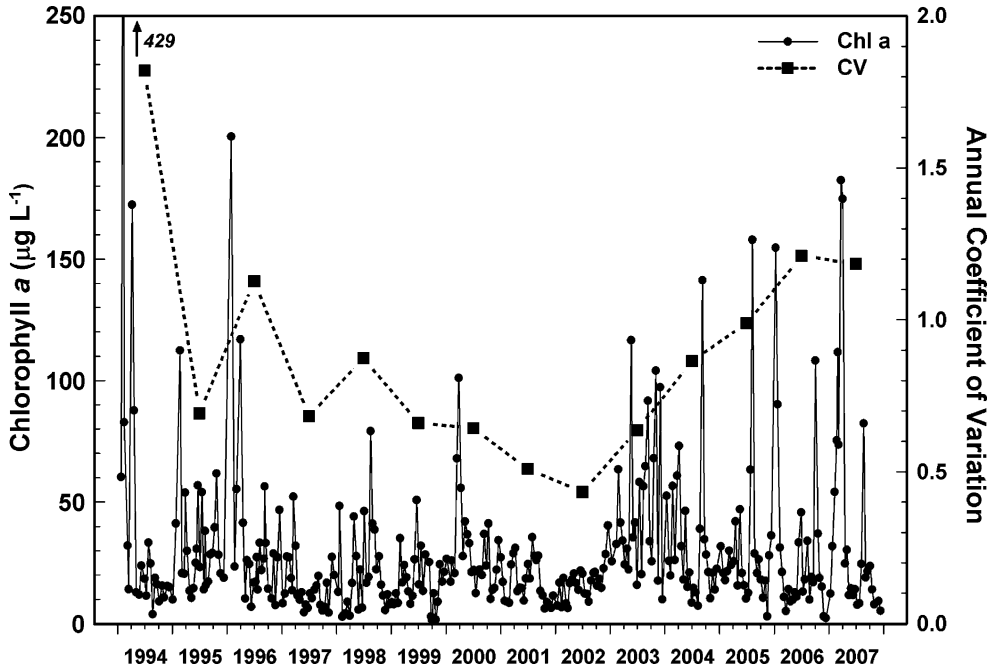

Fig. 5 Surface Chl *a* concentration (µg l^{-1}) at station 120, which is representative of patterns observed at stations 100–160 (see Fig. 2). *Circles* are the concentration values and *squares* indicate the coefficient of variation for each year. Note the increased variability and bloom potentials in phytoplankton from early 2003 onward

It is not clear what led to this prolonged period of relatively stable phytoplankton biomass. Phytoplankton growth and biomass in this region of the estuary is N limited throughout much of the year (Paerl et al. 1995, 2004, 2006c; Rudek et al. 1991), and thus blooms tend to follow freshwater discharge with accompanying "new" N loads. From 1996 to 2002, there were several significant freshwater pulses, and many smaller pulses, to this part of the estuary (Fig. 3). Nonetheless, large blooms did not develop, although the reason for this remains unclear. Through ongoing analysis of archived samples, we are currently exploring the possibility of changes in food web structure (i.e., top-down controls) that may have impacted bloom potentials in this region of the estuary. Beginning in early 2003 at the same site, the NRE again began to experience an increase in phytoplankton bloom frequency and magnitude (indicated by increasing Chl *a* variability) that continues to the present (Fig. 5).

Accompanying the increase in bloom occurrences were shifts in the taxonomic composition of the phytoplankton community, as determined by HPLC pigment analysis samples collected by the FerryMon program. Several chemotaxonomic pigments became more variable, accompanying the increased variability in Chl *a* and bloom frequencies. This was most obvious for peridinin and alloxanthin (representing dinoflagellates and cryptophytes, respectively; Fig. 6), all of which became more variable, and showed increased numbers of blooms. Multiple regression analyses of peridinin and alloxanthin on temperature, salinity, flow, and DIN loading showed that only salinity was significantly related to the two pigments. Both alloxanthin and peridinin were higher under lower salinity conditions. For alloxanthin, the negative relationship with salinity was quite strong (R^2=0.37, $p<1\times10^{-14}$). For peridinin, the relationship with salinity was much weaker (R^2=0.06, $p=3.1\times10^{-3}$). Despite the relationships with salinity, it is clear that salinity alone did not predict the high variability in alloxanthin and peridinin brought on by the large bloom events that began in early 2003 (Fig. 6).

Further analyses are warranted to determine the mechanisms leading to the prolonged multi-year changes in the frequency/magnitude of blooms in the NRE and other estuaries worldwide. While these shifts at particular locations within the estuary do not necessarily imply system-wide increases or decreases in productivity, the spatial extent is still large enough to be of ecological significance. In particular, shifts in bloom potentials may lead to regions of the estuary becoming more/less important in terms of trophic transfer, and may also refocus the sinking flux of phytoplankton-derived organic matter to regions of the estuary that vary in susceptibility to hypoxia formation from the breakdown of phytoplankton blooms (Paerl et al. 1998). Finally, from a water quality assessment perspective, shifts in bloom frequency between different regions of the NRE may change determinations of compliance or failure of the Chl *a*-based TMDL for regions of the estuary where bloom frequency has increased (North Carolina Department of Environment and Natural Resources 1998, 2001).

Seasonal Patterns

Zeaxanthin concentrations, which primarily represent cyanobacteria, exhibited seasonal fluctuations, with minimal

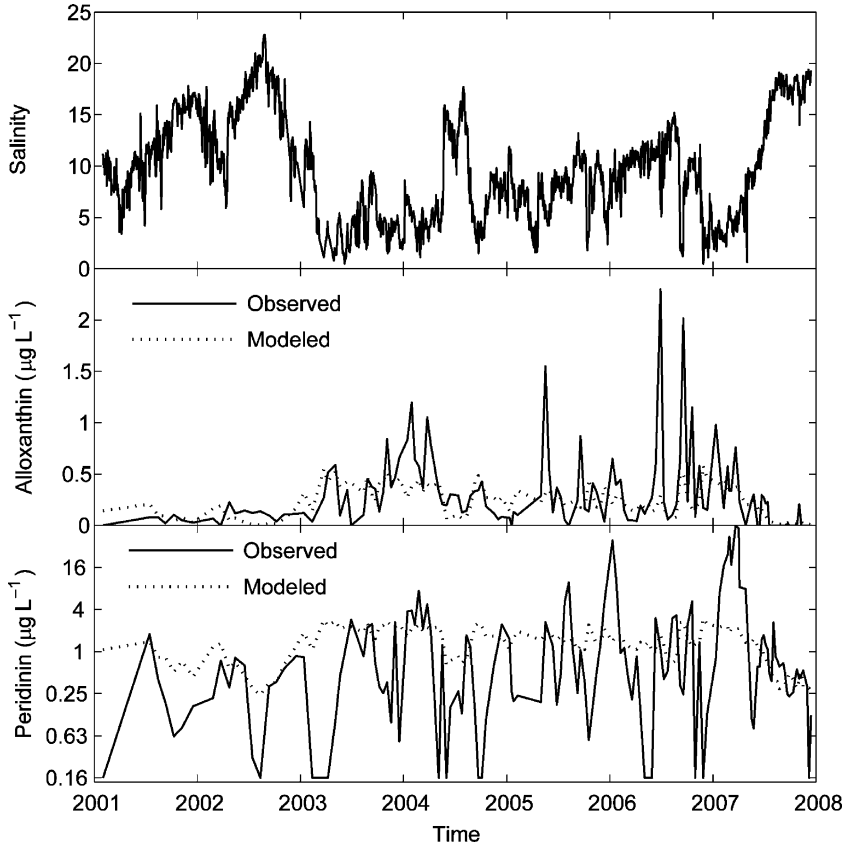

Fig. 6 **a** Daily mean salinity from FerryMon along the Neuse River ferry transect. Salinity data for 10 November 2002 through 22 June 2003 are from the USGS station 0209265810 located midway along the ferry transect. **b** Observed alloxanthin concentration from FerryMon and alloxanthin concentrations modeled by multiple regression of alloxanthin on salinity. Alloxanthin = $[0.78 - 0.036 \times \text{Salinity}]^2$. N= 141. R^2=0.37. F=81.6, $p<1\times10^{-14}$. **c** Observed peridinin concentration from FerryMon and peridinin concentrations modeled by multiple regression of peridinin on salinity. Note the log scale. Peridinin = $[1.75 - 0.063 \times \text{Salinity}]^2$. N= 141. R^2=0.062. F=9.1, p=3.1× 10^{-3}. Pigment data from 18 May 2005 through 3 April 2007 were collected by ModMon at station 120

concentrations during colder winter months and maximal concentrations in warmer months (Fig. 7). A multiple regression analysis of zeaxanthin on temperature, river discharge, salinity, and DIN loading showed a strong positive relationship between zeaxanthin and temperature (p=3.4×10^{-13}) and a weaker negative relationship with riverine discharge (p=4.7×10^{-3}). Neither salinity nor DIN loading was retained as a significant factor by the stepwise regression. Overall, cyanobacteria have relatively slow growth rates and prefer warm waters for optimal growth

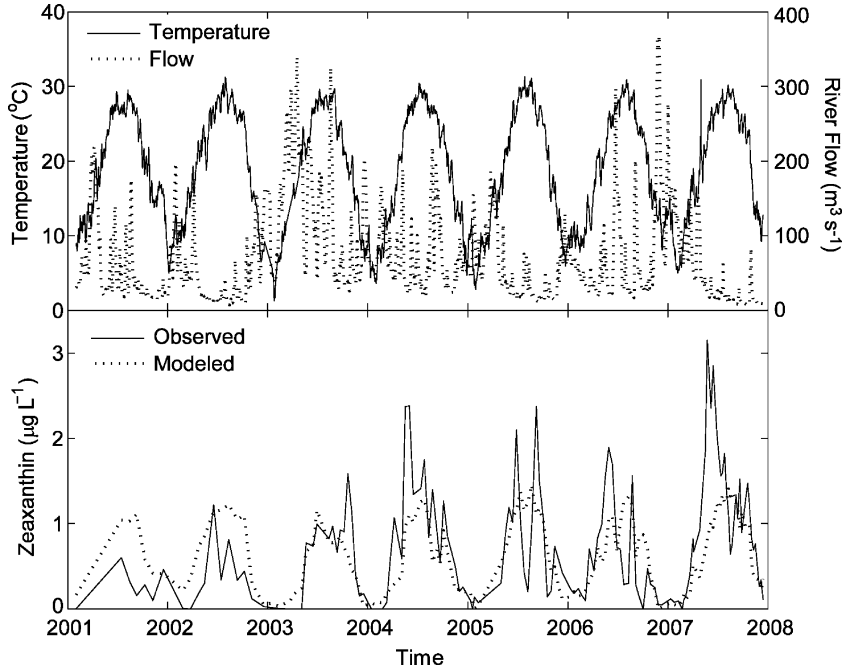

Fig. 7 **a** Daily mean river flow at Kinston, NC and daily mean temperature from FerryMon at the Neuse River Ferry transect. **b** Observed zeaxanthin concentration from FerryMon at the Neuse River Ferry transect and zeaxanthin concentration modeled by multiple regression of zeaxanthin on temperature and river flow. Zeaxanthin = $[0.16 + 0.035 \times \text{Temperature} - 0.0016 \times \text{Flow}]^2$. N=141. R^2= 0.51. F=73.0, $p_{\text{Overall regression}}<1\times10^{-15}$. T tests of significance of individual regression coefficients: $p_{\text{Temperature}}$=3.4×10^{-13}, p_{Flow}=4.7×10^{-3}. Data gaps in pigments, salinity, and temperature were filled as in Fig. 6

(Paerl and Huisman 2008; Reynolds 2006). The negative relationship between cyanobacterial biomass and river discharge is most likely due to the effect of decreasing residence time with increasing flow. Size-fractionated pigment data indicate that nearly all of the zeaxanthin is in the picoplankton (<3 μm) size range (Fig. 8) and microscopic examination of samples from the NRE have revealed many *Synechococcus*-like cells (Gaulke, A., personal observation). These observations confirm earlier studies showing picocyanobacteria to be an important and at times dominant fraction of phytoplankton in estuarine and coastal ecosystems spanning a range of trophic states (Marshall and Alden 1990; Murrell and Lores 2004; Phlips et al. 1999). Because of their small size, picoplankton-dominated food webs tend to have much longer food chains and exhibit lower sinking fluxes than larger phytoplankton-dominated food webs (Hansen et al. 1994; Murrell and Lores 2004; Pedrós-Alió et al. 2000). Given predicted climatic changes, especially global warming, the strong positive relationship between picocyanobacteria biomass and temperature increases will be important to follow from ecosystem dynamics and management perspectives (Paerl and Huisman 2008).

Event Scale Variability

Episodic events, such as abnormal periods of high or low freshwater discharge as well as storms, can represent important drivers of phytoplankton dynamics in estuarine ecosystems. The wet spring of 2003 is an example of how short-term hydrologic forcing can profoundly affect phytoplankton biomass and community composition in the NRE. Photopigment concentrations began to increase soon after large freshwater pulses in the spring of 2003 and continued with a rainy summer (see Figs. 1, 6, and 7).

With respect to taxonomic composition of the Chl *a* response, fast-growing cryptophytes, characterized by alloxanthin, showed an immediate, rapid response to

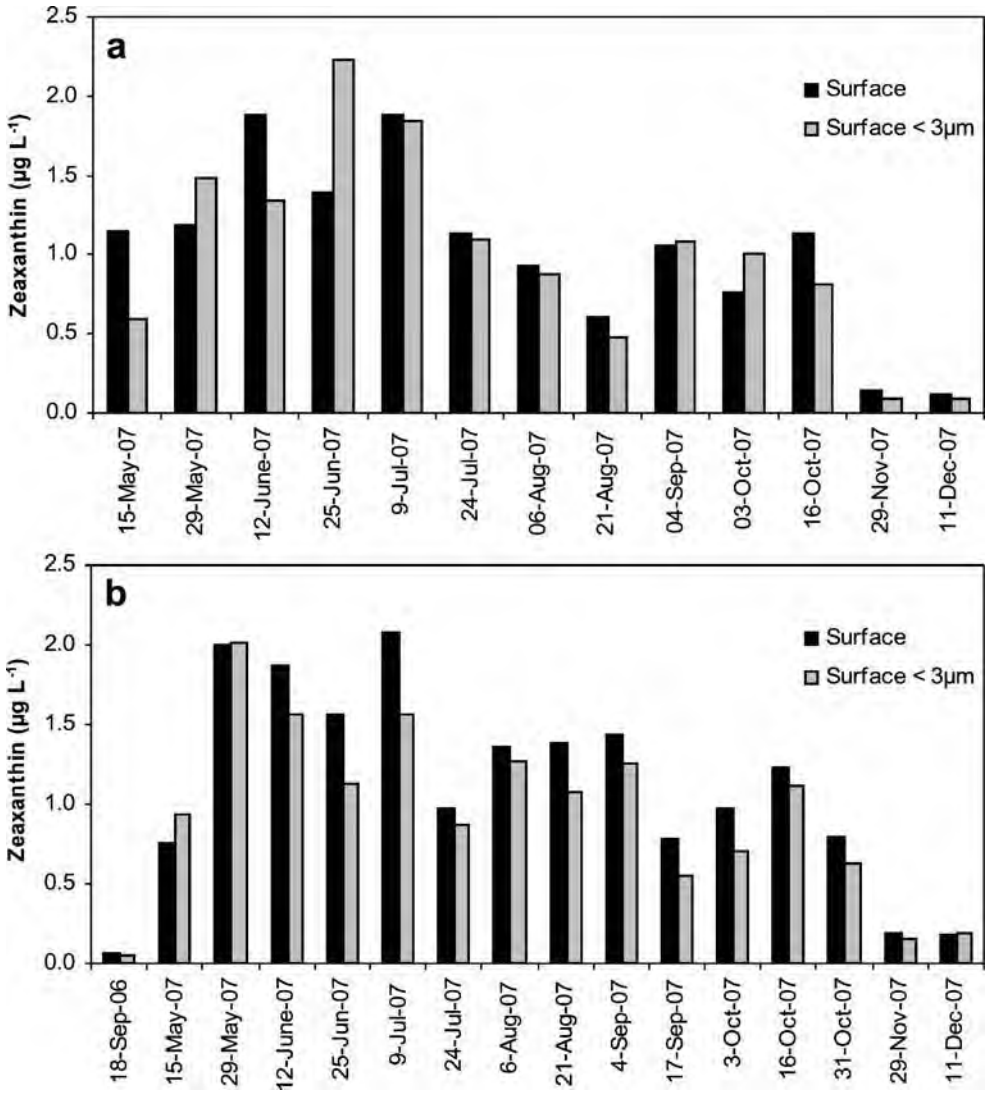

Fig. 8 Size-fractionated zeaxanthin concentration from surface waters of stations 180 (**a**) and 70 (**b**). The data illustrate that most of the zeaxanthin belongs in the picoplankton (<3 μm) size class

increased flow and nutrient enrichment associated with the commencement of the rainy period (Fig. 6). Then, as summer progressed and flow decreased, cyanobacteria, characterized by zeaxanthin, became a more dominant feature of the phytoplankton community (Fig. 7). These sequential differential taxonomic responses to the rainy spring–summer of 2003 demonstrate the roles flow, and hence water residence time, play in selecting for specific taxonomic responses. Fast-growing taxa like cryptomonads tend to respond to initial rapid increases in nutrient-laden flow, while slower-growing taxa that prefer warmer periods, typified by the cyanobacteria (Paerl and Huisman 2008), will tend to dominate during summer months.

Dinoflagellates, characterized by peridinin, also showed an increase in biomass following the elevated discharge in spring of 2003 (Fig. 6). However, like the cyanobacteria, dinoflagellates have relatively slow growth rates (Reynolds 2006), which may have kept their bloom response more modest than fast-growing cryptomonads during this high flow period. Unlike the cyanobacteria, dinoflagellates prefer cooler, relatively low light conditions in conjunction with periods of elevated hydrologic discharge to the NRE (Paerl et al. 1995). Indeed, they showed more profound bloom responses in fall and winter months following periods of freshwater discharge as experienced in 2005 and 2006 (Fig. 6).

Alterations in estuarine phytoplankton biomass and community structure have also been documented in response to the passage of other large, flood-inducing hurricanes, including Fran in 1996, Floyd, Dennis, and Irene 1999 (Paerl et al. 1998, 2001; Peierls et al. 2003), although it is not clear how representative the effects of those storms are given that storms span a continuum in terms of their characteristics (i.e., high rainfall vs. low rainfall; strong vs. light winds, etc.). Considerable effort has been put forth to understand the effects of different types of storms on phytoplankton dynamics in the NRE (Paerl et al. 2001, 2006a, b; Wetz and Paerl 2008). For instance, we found that relatively large storms such as Hurricane Isabel, which passed near the NRE as a category 2 hurricane in 2003, do not necessarily promote conditions favorable for phytoplankton growth. Isabel produced high winds (which would presumably mix nutrients from the sediments into the water column) and localized freshwater input, but nonetheless conditions became less favorable for phytoplankton growth after the storm. The potential stimulatory effects of Isabel's nutrient inputs were believed to be dampened in part by the fact that water column was already mixed and nutrient concentrations relatively high prior to the storm's passage (Paerl et al. 2006a, b; Wetz and Paerl 2008).

In contrast to Isabel which was a relative large and intense storm, but which did not elicit a phytoplankton response, several smaller storms have caused very large phytoplankton blooms. For instance, Tropical Storm Helene passed over the region in 2000 and led to a prolonged

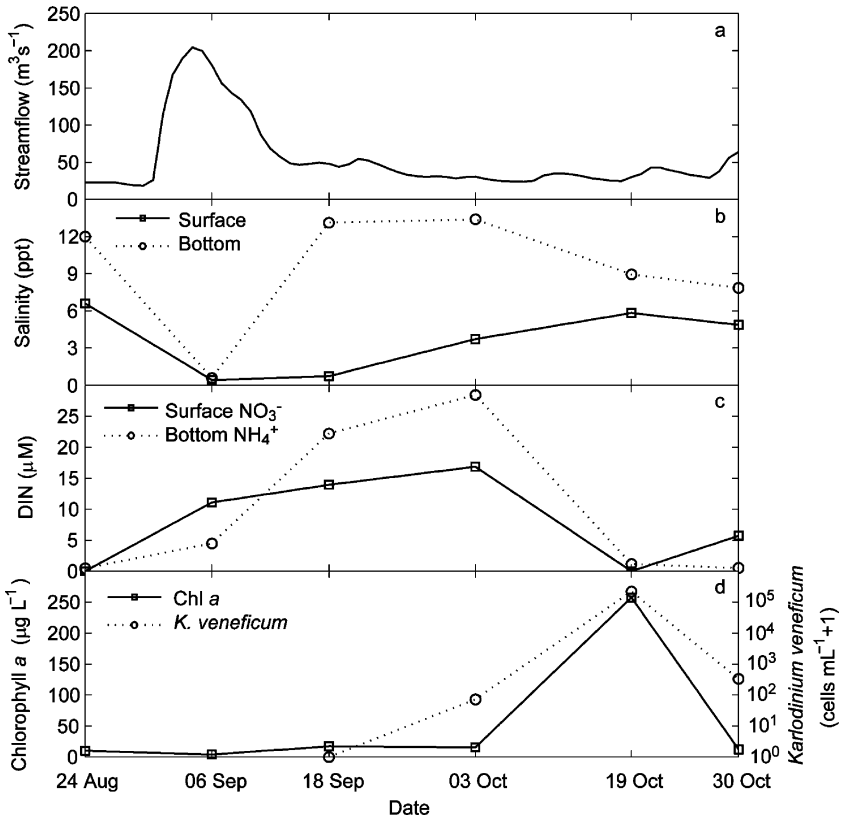

Fig. 9 Environmental conditions leading to the development of a toxic *Karlodinium veneficum* bloom. **a** Neuse River flow at Kinston, NC showing the runoff pulse from Tropical Storm Ernesto during the fall of 2006. **b** Time series of surface (0 m) and bottom (3 m) salinity at station 60 where the peak bloom biomass was observed on 19 October 2006. **c** Time series of the dominant sources of dissolved inorganic nitrogen to the bloom area; riverine NO_3^- input to the surface waters and accumulation of regenerated NH_4^+ in the bottom waters at station 60. **d** Time series of Chl *a* and *Karlodinium veneficum* cell abundance at station 60

(several weeks) increase in phytoplankton biomass in the mesohaline section of the NRE (Wetz and Paerl 2008). A more recent example was the impact of Tropical Storm Ernesto, which struck the North Carolina coast during September 2006 (Hall et al. 2008). This storm generated over 30 cm of rainfall within the NRE watershed and produced a flood pulse that temporarily increased flow by a factor of six over the previously dry conditions that dominated during the summer of 2006 (Fig. 9). The freshet delivered a large infusion of inorganic nitrogen primarily as nitrate (NO_3^-). Additionally, the strong salinity-based stratification and prevailing weak winds following the freshet prevented vertical mixing (Fig. 9) and allowed anomalously high concentrations of regenerated ammonium (NH_4^+) to accumulate in the saline bottom waters (Fig. 9). The unusual high nutrient availability fueled phytoplankton growth (Fig. 9), and a short-lived frontal circulation pattern additionally concentrated vertically migrating cells. The result was an intense (>200,000 cells ml^{-1}) but highly localized bloom of the toxic dinoflagellate *Karlodinium veneficum* in the mid-estuarine region (Fig. 2). The collapse of the bloom coincided spatially and temporally with several fish kills for which there was no other obvious cause of mortality (Hall et al. 2008).

Conclusions

More than a decade of intensive monitoring (ModMon, FerryMon) indicates that hydrological changes accompanying climatic events occurring on multiple timescales play a fundamentally important role in determining phytoplankton biomass and community composition in the NRE. Throughout this period, the system has been impacted by anthropogenic nutrient enrichment and subsequent management efforts. Complicating the nutrient management efforts are climatic changes, which are clearly playing an increasingly important role in mediating delivery of nutrients and freshwater to the estuary, both of which have been shown to strongly modulate phytoplankton community biomass and composition in the estuary.

The most recent decade of climatically influenced phytoplankton community data has shown highly significant shifts in composition and biomass, rivaling what was observed during the more anthropogenically influenced period of the 1970s–early 1990s, where increases in nutrient loading were the key drivers of ecological change in this community. While anthropogenic stressors (nutrient over-enrichment and sedimentation) still play a fundamentally important role in shaping phytoplankton production and compositional dynamics, the impacts of these stressors have been modified by climatic changes. To clarify and ultimately predict the trophic and biogeochemical cycling responses of estuaries to climate change, we must develop an understanding of the impacts of climate change on phytoplankton community structure and function.

Of additional concern is how potential shifts in phytoplankton community structure due to climatic changes alter habitat range. Using geographically diverse evidence, Paerl and Huisman (2008) proposed that cyanobacterial habitats have expanded northward in the Northern hemisphere due to global warming. In addition, it was shown that longer optimal growing seasons worldwide have enabled cyanobacterial species to be more competitive in a variety of habitats, including estuaries. This was attributed to the fact that, as a taxonomic group, cyanobacteria prefer higher temperatures for optimal growth than other taxa (i.e., diatoms, chlorophytes). Furthermore, changing hydrologic characteristics such as more extensive droughts would favor cyanobacterial bloom species, as they prefer long residence time conditions. Conversely, a more stormy time period, where frequent pulses of freshwater discharge dominate, would favor fast-growing taxa such as chlorophytes, cryptophytes, and diatoms. To some extent, this scenario may be unfolding in the NRE over the past decade.

Therefore, in managing (reducing) nutrient inputs to control eutrophication and minimize unwanted events, such as harmful algal blooms, parallel climatic changes need to be taken into consideration. Evidence presented here indicates that climatic changes and oscillations can at times overwhelm nutrient inputs in terms of controlling algal bloom thresholds, their duration, and spatial extent. This calls for water quality, eutrophication, and habitat models that incorporate hydrologic and other physical changes (i.e., water temperature, stratification, and circulation) in efforts to better predict phytoplankton community responses to nutrient inputs in estuarine and coastal ecosystems.

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Classifying aquatic macrophytes as indicators of eutrophication in European lakes

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Abstract Aquatic macrophytes are one of the biological quality elements in the Water Framework Directive (WFD) for which status assessments must be defined. We tested two methods to classify macrophyte species and their response to eutrophication pressure: one based on percentiles of occurrence along a phosphorous gradient and another based on trophic ranking of species using Canonical Correspondence Analyses in the ranking procedure. The methods were tested at Europe-wide, regional and national scale as well as by alkalinity category, using 1,147 lakes from 12 European states. The grouping of species as sensitive, tolerant or indifferent to eutrophication was evaluated for some taxa, such as the sensitive *Chara* spp. and the large isoetids, by analysing the (non-linear) response curve along a phosphorous gradient. These thresholds revealed in these response curves can be used to set boundaries among different ecological status classes. In total 48 taxa out of 114 taxa were classified identically regardless of dataset or classification method. These taxa can be considered the most consistent and reliable indicators of sensitivity or tolerance to eutrophication at European scale. Although the general response of well known indicator species seems to hold, there are many species that were evaluated differently according to the database

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selection and classification methods. This hampers a Europe-wide comparison of classified species lists as used for the status assessment within the WFD implementation process.

Keywords Aquatic vegetation · Indicators · Species classification · REBECCA · EU Water Framework Directive

Introduction

For implementing the Water Framework Directive (WFD) it is important to identify how aquatic macrophytes can be used to indicate pressures. The WFD uses aquatic macrophyte species composition and abundance as a biological quality element for ecological assessment. In order to ensure consistency in application of the directive, lakes with comparable macrophyte composition and abundance must be assigned a comparable ecological status. Many previous reports on the use of macrophytes as indicators of eutrophication in lakes are confined to single countries (e.g. Palmer et al. 1992), or to relatively small geographical regions within Europe (e.g. Rørslett 1991). Other approaches focus only on selected groups of macrophytes, such as isoetids (e.g. Murphy et al. 1990).

It is clear that macrophytes are confined to specific habitats (e.g. Schaminee et al. 1995; Murphy 2002) and that they react to many different changes in their optimal habitat (e.g. Barko et al. 1986; Van Geest 2005). It is therefore often suggested that macrophytes can be used as indicators for pressures such as eutrophication. In the most simplistic sense, certain macrophyte species are present in waters of good status and absent in bad status (e.g. Moss et al. 2003; Birk et al. 2006).

All EU member states are currently undertaking efforts to classify species and creating status assessment methods for lakes based on macrophytes, of which some are already available; Germany (Stelzer et al. 2005; Schaumburg et al. 2004), Denmark (Søndergaard et al. 2005), the Netherlands (Van den Berg 2004), Belgium (Leyssen et al. 2005), Sweden (Ecke 2006), Finland (Leka et al. 2007) and Ireland (Free et al. 2006), or in press; UK (Willby et al. 2006) and Norway (Mjelde 2007, unpublished).

In the Netherlands, classifying species is based on expert judgement by a group of ca. 30 experts, where not only species occurrence is classified according to a general 'ecosystem disturbance' for specific lake types but also the role that abundance of species plays in their classification. For example, low abundances of *Lemna* spp. are not assessed negatively, while high abundances of *Lemna* spp. are. Germany also bases its species classification system on expert judgement, while UK, Sweden and Norway base it on data analyses along a total phosphorous (TP) gradient.

Expert judgement and valuation of species sensitivity have proved to be highly country dependent, as was shown in the WFD intercalibration process (Lyche Solheim 2005). Therefore, we aim to describe the classification of sensitive and tolerant species on a Europe-wide scale.

Comparing classification or status assessment methods from individual member states has been completed for rivers (Birk et al. 2006), but it has not yet been carried out on a large scale for lakes, although an initial assessment of individual member state datasets show that there are large differences among resulting status descriptions (Tóth et al. 2008, this issue). One of the main problems in performing a Europe-wide assessment of macrophyte responses to pressures is the lack of a Europe-wide homogenous monitoring methodology, although attempts are being made to harmonise the methodology using a common CEN standard or lake habitat survey (CEN 2006).

In this article we use and discuss the results of two different methods to classify aquatic macrophytes according to their response to a eutrophication pressure represented by the summer average total phosphorous concentration. The results are part of a study aimed to determine the usability of macrophytes as indicators of eutrophication pressures throughout Europe, to examine the effects of scale and typology when classifying macrophyte species into eutrophication response classes, and to examine the effects of different methods on the final ecological quality assessment (see also Penning et al. 2008, this issue).

Methods

Data used

Data on macrophyte abundance and water quality were collected for as many European countries as possible. In total, data from 12 countries and 1,147 lakes are included in the REBECCA macrophyte

database. For some lakes samples of more years are available, resulting in an overall of 1,442 data points (Table 1; see Moe et al. 2008, this issue for further description on the database). Data providers assigned type and status (reference or non-reference lake) to most of the lakes provided to the database.

Only true aquatic macrophyte, of the growth forms isoetids, elodeids, nymphaeids, lemnids and charids were included (Mäkirinta 1978). Helophytes (emergent aquatic plants) were excluded from the analyses as their response to eutrophication is obscured by soil trophic characteristics, exposure, shoreline management and their ability to emerge from the water phase. Species for which it was unclear whether they represented the helophyte or aquatic form (e.g. *Juncus bulbosus*, *Sagittaria sagittifolia* and *Hippuris vulgaris*) were excluded from the analyses. Species occurring in less than 4 lakes were included, but were not given a response-class (i.e. sensitive, tolerant or indifferent to eutrophication). Macrophyte data were available at species level, with some exceptions of poorly identified groups, notably *Chara*, for which most surveys grouped species as *Chara* sp.

Table 1 Overview of content of the total REBECCA macrophyte database, showing the number of lakes from each country

Country	Total	Typed	Ref.	Chl. *a*	TotP	Secchi
Belgium	2	2	0	2	1	2
Estonia	4	4	0	3	2	4
Finland	527	345	154	179	396	459
Ireland	117	18	40	117	117	106
Latvia	144	144	15	144	137	144
Lithuania	5	5	2	5	4	5
Netherlands	46	46	34	46	46	46
Norway	269	60	168	74	269	74
Poland	5	5	2	5	5	5
Romania	19	17	8	18	17	17
Sweden	254	0	101	0	250	92
United Kingdom	50	33	13	50	50	0
Total	1442	679	537	643	1294	954

Total, total number of lakes; Typed, number of lakes for which there is an assigned intercalibration type; Reference, number of lakes defined as reference lakes for the intercalibration exercise. The number of lakes in which the environmental variables Chlorophyll *a* (μg l^{-1}), total P (μg l^{-1}) and Secchi depth (m) was available is also shown. Not all data has been used for the reported analyses

The main water quality parameters collected were concentrations of total phosphorus and chlorophyll *a* and Secchi depth. For most data points these are summer-average values, but in some cases the origin of the values is unknown, and these might be single sample values. In the Finnish lakes TP is given as a 10-year average. Information on lake typology, specifically size, mean depth, colour, alkalinity and altitude, was also collected for each lake. For some lakes, this information was only available on a nominal scale, conforming to the WFD intercalibration types.

The Geographic Intercalibration Group (GIG) typology proposed for the intercalibration exercise (Heiskanen et al. 2004) is based on lake size, alkalinity, depth and, depending on lake type, altitude and humic content. The typology was used in the structuring of the database and subsequent analyses.

The REBECCA macrophyte database contains partly incomplete data, as data were provided by various sources within individual member states. Not all data provided to the REBECCA database could therefore be used in the analyses that are here reported. Further discussion on the REBECCA macrophyte database, from a more technical standpoint can be found in Moe et al. (2008, this issue).

Classification of species

We tested species classifications based on the Percentile method (Mjelde 2007) and the LEAFPACS method (Willby et al. 2006), on Europe-wide, regional and national species lists. For identifying sensitive and tolerant species by Percentiles, we have used species presence or absence along a lake water total phosphorous gradient. This approach uses the 75th percentile of the phosphorous score to distinguish sensitive and tolerant species, and the 25th percentile to distinguish indifferent and tolerant species.

The following description of sensitive, tolerant and indifferent species was applied:

1. *Sensitive species*: species which are most apparent or only appear in reference lakes, often in high abundance. Frequency and abundance decreases (and often the species disappears entirely) with increased eutrophication pressure; the 75th percentile of observations is chosen as the phosphorous limit above which a species is no longer considered sensitive.

2. *Tolerant species*: species occurring at an increased frequency and abundance at higher eutrophication pressure. Often rare or with lower abundance in reference lakes; the 75th percentile of observations lies above the upper phosphorous limit and the 25th percentile lies above the lower phosphorous limit.
3. *Indifferent species*: species with wide preference, common not only in reference lakes but also in slightly eutrophic lakes; the 75th percentile of observations is more than the upper phosphorous limit and the 25th percentile is less than the lower phosphorous limit. These species disappear in hypertrophic lakes.

The LEAFPACS method is a more elaborate statistical approach for classifying species distributions. The method uses multivariate statistical methods to assign response-group classifications to aquatic macrophytes, and was designed especially to provide a relatively objective method for defining biological quality-element classifications for the EU Water Framework Directive. The method consists of two separate parts; the first part assigns a trophic rank to each macrophyte species, based on the global dataset, and the second part assigns a *lake-type specific* response group classification to each species. The first part of the analysis could easily be replaced by expert judgement based assessment of species trophic status, such as that produced for the UK by Hill and Ellenberg (1999). For the REBECCA project, it was decided that the dataset would best provide this trophic rank because the dataset had such a wide geographical distribution and there was no single expert-judgment system that covered the region known to the authors.

The second part of the method followed that of the LEAFPACS project (Willby et al. 2006). In summary, lake trophic rank scores were calculated for each lake as the average of the species trophic ranks of all species occurring in that lake. These lake trophic ranks were then used as the environmental variable in a further CCA analysis. The axis-1 and tolerance values produced by this analysis were used to define the response class of each species as follows:

– as a 'positive responder' if its axis-1 score was less than or equal to zero.
– as a 'negative responder' if its axis-1 score was greater than zero, and the result of subtracting its tolerance from its axis-1 score was less than or equal to zero.
– as a 'strongly negative responder' if the result of subtracting its tolerance from its axis-1 score was greater than zero.

The following deviations from the LEAFPACS method should be noted: site scores were calculated as the average of trophic rank (TR) scores of species occurring in each sample, only presence/absence data were used (no abundance data were used for weighting the site scores). We have not assigned any response groups to species occurring in <4 samples in a particular lake type.

The percentile method was calculated for the all data points in the whole REBECCA dataset, where TP values were available ($n = 1294$), for Northern and Central European Geographical Intercalibration Groups (GIG: as defined for the intercalibration exercise according to Heiskanen et al. 2004), low-moderate alkalinity (<1 meq l^{-1}) and high alkalinity (>1 meq l^{-1}) lakes within the GIGs, in addition to Norway, Finland and Latvia separately. The LEAFPACS classification was calculated separately for low-moderate and high alkalinity lakes within the two GIGs.

Results

In total the REBECCA database consists of 114 aquatic macrophyte taxa, comprising 105 species and 9 hybrids. The most common species in the database, found in more than 30% of the lakes, were *Nuphar lutea*, *Potamogeton natans*, *P. perfoliatus*, *Myriophyllum alterniflorum*, *Nymphaea alba*, *Isoetes lacustris*, *Ranunculus reptans*, *Utricularia vulgaris*, *Sparganium angustifolium*, *Isoetes echinospora* and *Eleocharis acicularis*. All these species are typical for low-moderate alkalinity, oligo-mesotrophic lakes (e.g. Murphy 2002), which reflect a dataset dominated by lakes from Northern Europe, although some of them are also found in more eutrophic lakes (e.g. Van den Berg 2004)

When comparing the whole dataset (percentile method) with the list for the Central GIG, 71% of the species were classified to the same group while 2% were diversely classified as tolerant or sensitive (see Appendix). Twenty-seven percent of the species showed small discrepancy (categorised as

indifferent-tolerant or sensitive-indifferent). These were mainly species with marked lower abundance in the Central GIG compared to the Northern GIG lakes. The same comparison with the Northern GIG list showed 77% species with a similar classification, while none had a different classification.

The percentage of species classified as sensitive differed among countries: 55%, 48% and 35%, respectively, for Norway, Finland and Latvia, which likely reflects the amount of impacted lakes compared to reference lakes in the datasets from these countries.

A comparison between the two different classification methods (percentile and LEAFPACS) were made for Central GIG, high and low-moderate alkalinity lakes, and for Northern GIG, high and low-moderate alkalinity lakes (Fig. 1). For the Central GIG low-moderate alkalinity lakes, the two tested methods gave similar results and 72% of the species had the same classification. Only *Chara globularis* and *Nitella flexilis* had opposing classifications, meaning that one method classified them as sensitive while the other as tolerant. They were both ranked as sensitive species in the percentiles method and tolerant in the LEAFPACS method. Six of the species classified as being indifferent in the percentile method were included as tolerant species in the LEAFPACS method. For the Central GIG high alkalinity lakes the two methods gave similar results, 62% were identically classified, while 2 species, *Najas marina* and *Nitella flexilis*, got opposing classifications. Twelve of the indifferent species were classified as sensitive in the LEAFPACS method, and five as tolerant. For the Northern GIG high alkalinity lakes, 70% of the species received the same classification while 12% had an opposing classification. The remaining 12% were variably classified as indifferent, tolerant or sensitive. For the low-moderate alkalinity lakes only 48% of the species had the same classification, while for 31% the classification was contradictory. Especially many elodeids like numerous *Potamogeton* species were not classified concordantly.

There is a clear positive relationship between total P and Secchi depth for the Northern GIG data ($r^2 = 0.63$), but not for Central GIG data ($r^2 = 0.24$). For the individual countries with sufficient data r^2 are

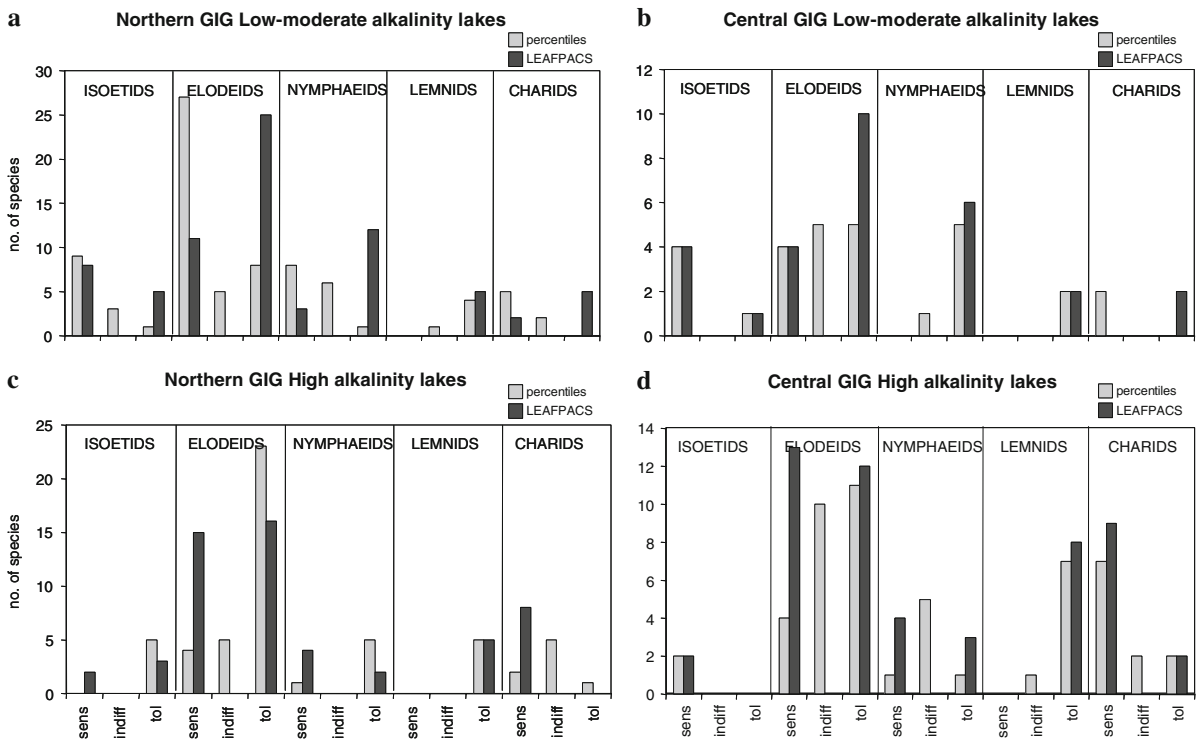

Fig. 1 Comparison between percentile method and LEAFPACS method for Northern GIG lakes (**a**, **c**) and Central GIG lakes (**b**, **d**). The negative and strongly negative responders (LEAFPACS method) are merged and treated as sensitive species, while the positive responders are treated as tolerant species

0.63, 0.79, and 0.41 for Finland, Norway and Latvia, respectively.

Based on all species classification analysis (Appendix) we obtained 48 species with identical classification, which can be regarded as the European list of aquatic macrophyte species that were more robustly defined as sensitive or tolerant against eutrophication (Table 2). The lists include all the large isoetids, and some *Chara* spp., among the sensitive species, while most lemnids are included among the tolerant species. The remaining species are partly indifferent to eutrophication pressure, but also include species missing or rare in some regions or countries.

Table 2 List of overall European sensitive and tolerant species based on the comparison of different methods and datasets to classify species as tolerant or sensitive

Overall European sensitive species	Overall European tolerant species
Eleocharis acicularis	*Callitriche cophocarpa*
Isoetes echinospora	*Ceratophyllum demersum*
I. lacustris	*Elodea nuttallii*
Littorella uniflora	*Myriophyllum verticillatum*
Lobelia dortmanna	*Potamogeton crispus*
Ranunculus reptans	*P. obtusifolius*
Subularia aquatica	*P. pectinatus*
Callitriche hamulata	*P. pusillus*
Myriophyllum alterniflorum	*P. trichoides*
M. sibiricum	*Ranunculus circinatus*
Potamogeton filiformis	*Zannichellia palustris*
P. polygonifolius	*Nymphoides peltata*
P. × *nitens*	*Sagittaria sagittifolia* × *natans*
P. × *zizii*	*Hydrocharis morsus-ranae*
Ranunculus confervoides	*Lemna minor*
R. peltatus	*Lemna trisulca*
Utricularia australis	*Salvinia natans*
U. intermedia	*Spirodela polyrrhiza*
U. minor	*Stratiotes aloides*
U. ochroleuca	*Trapha natans*
Nuphar lutea × *pumila*	*Nitellopsis obtusa*
Sparganium angustifolium	
S. hyperboreum	
Chara delicatula	
C. rudis	
C. strigosa	
Tolypella canadensis	

The species listed are those with the same classification regardless of classification method or dataset

The percentiles of observations used to define phosphorus levels separating sensitive, tolerant and indifferent species are based on expert judgement. However, we assume that the reference species *Isoetes lacustris* and *Lobelia dortmanna* in low-moderate alkalinity lakes, and *Chara* spp., in high alkalinity lakes, should have very low abundance or be completely absent in impacted lakes. For example, for Norway this gives a distinction between sensitive and tolerant species based on the 75th percentile at around 20 μg l^{-1} TP (Fig. 2), while for Latvia the 75th percentile boundary lines around 60 μg l^{-1} TP (Table 3). These thresholds can be evaluated by visual interpretation (Fig. 3a), or by use of more sophisticated statistical techniques such as quantile regressions (Fig. 3b). In a visual interpretation, the concentration is identified where a specific species or selected group of species, such as large isoetids, notably declines in total abundance along a total phosphorous gradient. Alternatively, this could also be an average value estimated to be the middle point of a gradient of decline. Using quantile regressions, a more objective line defining thresholds based on the quantiles below or above a certain percentile of the observations can be identified. For both these methods of threshold detection a clear drop in abundance of large isoetids in Northern lakes can be seen around 20 μg l^{-1} TP, and above 50 μg l^{-1} TP their abundance becomes very low.

Discussion

In essence, the LEAFPACS method and the percentile method are based on a similar idea of identifying groups of species as tolerant or sensitive to a eutrophication pressure. However, the results of the classification are not easy to compare as the percentile method distinguished a group of indifferent species that is lacking in the LEAFPACS method. Only species that are classified as tolerant and sensitive in both methods can therefore be used for the comparison of the results. Our suggested list of overall European sensitive and tolerant species consists of 48 taxa, which strongly agrees with earlier knowledge and expert judgement (e.g. Palmer et al. 1992; Ecke 2006; Rørslett 1991; Kurimo 1970; Pot 2003; Murphy 2002). The characterisation of the large and perennial isoetids as sensitive to eutrophication in low-moderate

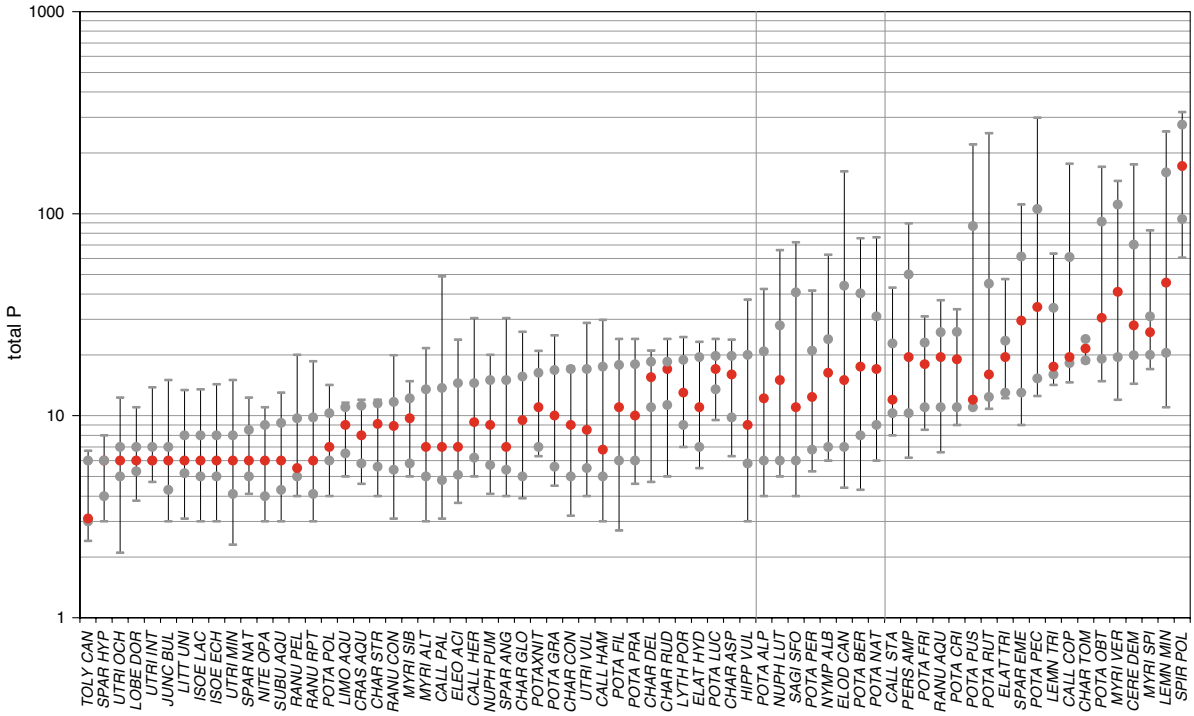

Fig. 2 The percentile method used for Norwegian lakes in which the grey points represent the 25th and 75th percentile and the red dots the 50th percentile. Sensitive species: 75th percentile less than 20 μg l^{-1} TP, Tolerant species: 75th percentile more than 20 and 25th percentile more than 10 μg l^{-1}, Indifferent species: 75th percentile more than 20 μg l^{-1} and 25th percentile less than 10 μg l^{-1}. Species recorded in less than four localities are excluded in the graph. Upper and lower phosphorus limits for the definition of sensitive and tolerant species are based on expert judgement (from Mjelde 2007). Vertical grey lines separate the sensitive, indifferent and tolerant species groups according to these limits

alkalinity lakes seems unambiguous. However, only three *Chara* species are included among the sensitive species. This may be because of the heterogeneous character of the high alkalinity lakes. In addition, many lake surveys included in the REBECCA database included several *Chara* spp. species merged, which make the discussion about the classification of the *Chara* species difficult.

Merging datasets from different countries results in classifications that differ with those based on country-wise judgement. For example, for Norway and Finland *Lobelia dortmanna* is characterised as a typical sensitive species, which does not appear in eutrophic waters. This species is missing from all Finnish and Norwegian high alkalinity lakes. In the Swedish dataset, this species exists in eight high alkalinity lakes, with total P varying

Table 3 Suggested limits of total P (μg l^{-1}) used for classifying aquatic macrophytes based on 25th and 75th percentiles for the overall REBECCA database, total Central and Nordic GIG; high and low alkalinity within the GIGs and for three individual countries

		All	Central GIG				Nordic GIG				
			Tot.	High	Low	Latvia	Tot.	High	Low	Norway	Finland
Sensitive species	75th less than	30	60	60	26	60	30	30	30	20	30
Tolerant species	75th more than	30	60	60	26	60	30	30	30	20	30
	25th more than	18	25	25	15	20	15	15	15	10	15
Indifferent species	75th more than	30	60	60	26	60	30	30	30	20	30
	25th less than	18	25	25	15	20	15	15	15	10	15
No of lakes		1294	231	151	40	137	932	106	751	269	369

High, High alkalinity; Low, Low-moderate alkalinity

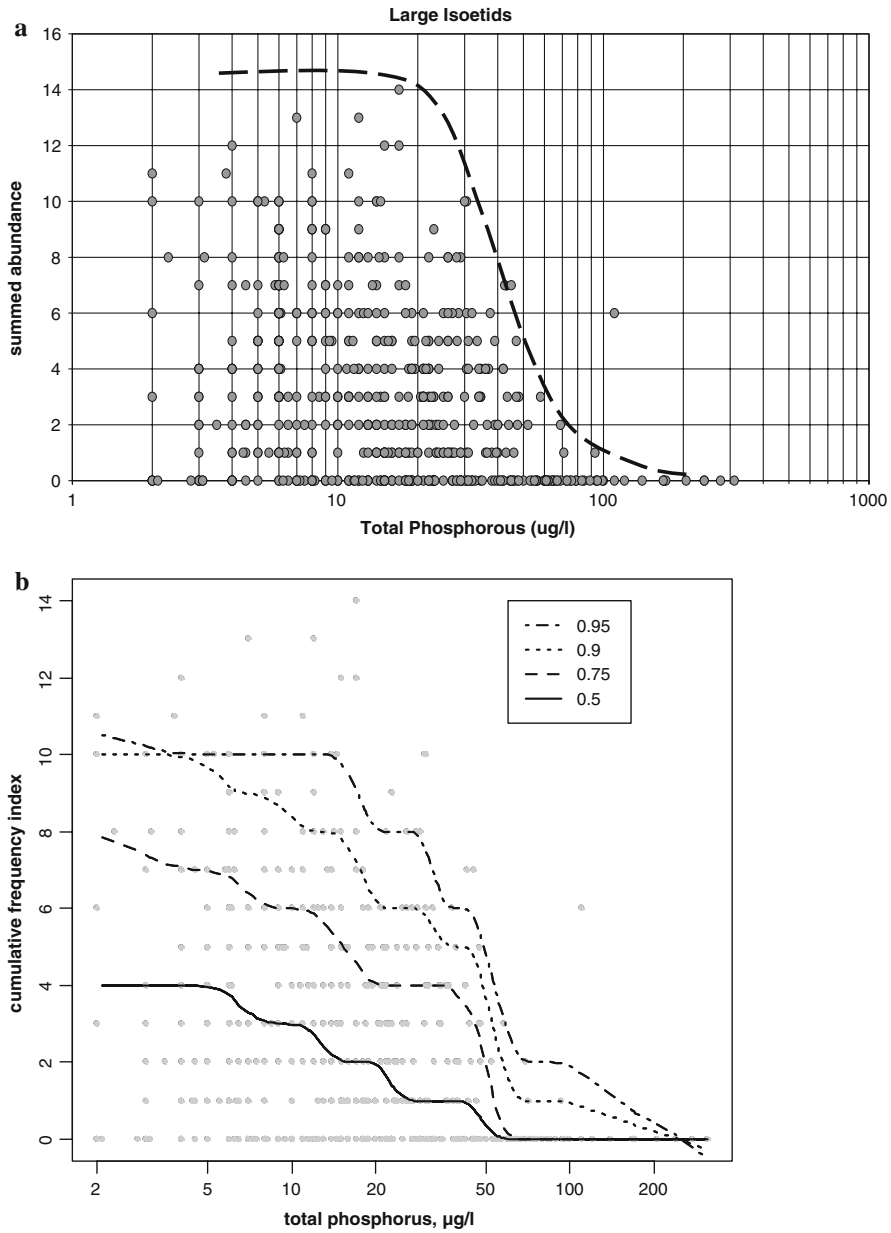

Fig. 3 (**a**) Sudden drop for large isoetids by visual interpretation. The y-axis gives the summed abundance of all large isoetid species occurring within a single lake recording. On the x-axis the summer average total P (µg l^{-1}) recorded for each lake sample. (**b**) Quantile regression for large isoetids in low-moderate alkalinity Nordic lakes. The y-axis shows a cumulative frequency index, which means sum of the semi-quantitative scores of all isoetid species at a given site

between 60 and 80 µg l^{-1}. These lakes may be shallow or very shallow allowing sensitive species to survive and grow despite low light conditions. Unfortunately, depth information at the sample locations was not available to test this hypothesis. However, such inequality can give 'incorrect' classifications and a list based on the whole GIG dataset will not be transferable to the individual country. Country-based lists provide the most consistent results, as they include data from more homogenous monitoring methods. Cluster analyses showed that potential other explanations for this country based homogeneity of data, such as spatial proximity, were not the main reason, as in that case countries such as Norway and Sweden should be closer together and Romania and the Netherlands further apart (see Lyche Solheim 2006). In some situations the full pressure gradient was not represented within the dataset, especially for specific lake types this was apparent. Extending the dataset with dedicated samples from the full gradient would help to reflect a truer optimum for many species. For this the cooperation between e.g. neighbouring countries might be necessary.

Some of the classified tolerant species (and positive responders) are not able to grow in hypertrophic conditions. However, some tolerant species are considered valuable indicators by experts in Central GIG for specific situations (e.g. *Nitellopsis obtusa*). Their absence can be indicative of hypertrophic situations (van den Berg 2004). This is the last step before complete disappearance of aquatic macrophytes and a transition to a cyanobacteria dominance state. The here described classification methods do not distinguish such species as sensitive to hypertrophic situation.

Ideally, a classification system including all aquatic macrophytes would probably be made separately for each individual lake type, or separated by alkalinity and humic content for each country. However, this could not be tested here since the subsets of data from the overall 1,147 lakes in the REBECCA database were not always sufficiently large (>20) to assess individual lake types.

In our dataset, combined data on light measurements and chlorophyll data was lacking for many lakes. Therefore, we used TP as an index for eutrophication (and indirectly for light conditions). Reduced light condition caused by increased phytoplankton biomass is probably the most important effect of eutrophication on aquatic macrophytes (Kirk 1994). Species sensitive to eutrophication are also often species that are less tolerant to poor light conditions which can be not only due to eutrophication but also due to increased water column suspended sediments (e.g. in association with wind induced sediment resuspension) (James et al. 2004). Unfortunately information on exposure, depth at sampling location and maximum depth of colonisation was not available to adequately assess the response of macrophytes to Secchi depth.

There is a large variation around the average response to eutrophication expressed as a change in total P concentration. If inorganic or humic substances are the main factors for poor light conditions, it is less obvious that total phosphorus can be used as an indicator for the effect of eutrophication. In humic lakes, total P is partly bound to organic particles unavailable for biomass production, resulting in a non-linear relationship between total P and light (Lamers et al. 2002). In lakes highly influenced by inorganic substances resuspended from the sediment the relationship between total P and light conditions will also be non-linear. In addition, many very shallow lakes, such as the Dutch border lakes and various lakes from the Danube delta (having large surface areas, and <3 m deep) can have acceptable light conditions despite relatively high phosphorus levels (Coops et al. 2007; Covaliov et al. 2003). Based on this, classification systems and ecological status assessments based on species occurrence along the phosphorus gradient alone will have limited value in the very shallow lakes such as many of the high alkaline lakes in Central GIG. However, the Northern GIG dataset is dominated by deeper lakes of low colour where variation in light conditions depends mainly on changes in phytoplankton biomass. Therefore, classification systems based on sensitive or tolerant species seem highly valuable for the lakes in the Northern GIG.

The data collected within the REBECCA project macrophyte database showed general trends in response to eutrophication pressure, but variation of responses of individual macrophyte species with respect to their classification in line with the pressure gradient throughout Europe and throughout lake types is high. This might be partly due to the fact that the total P data used now represent on average a summer-average value in most cases, but not in all (e.g. in Finnish lakes a 10-year summer average is used) and also seasonal changes in values are not available in this dataset. Gibson et al. (1996) indicate that these seasonal variations can be an important factor in the analyses of the interannual trends in trophic status in shallow lakes. Although the general response of well known indicator species seems to hold, there are many species that are classified differently according to the database selection and classification methods. How these different classifications affect the outcome of selected indices assessing ecological status is discussed in another article (see Penning et al. 2008, this issue).

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Appendix

List of species per species group included in REBECCA macrophyte study, with results of classifications. Column headings 'P' and 'L' represent methods based on percentiles or on LEAFPACS, respectively, and '*n*' is the number of lakes in which a species occurred

	Species	All		N-GIG		C-GIG		CH			CL			NH			NL			Norway		Finland		Latvia	
		P	*n*	P	*n*	P	*n*	P	L	*n*	P	L	*n*	P	L	*n*	P	L	*n*	P	*n*	P	*n*	P	*n*
I	*Baldellia ranunculoides*	o	8	+	5		1						1	+	+	4			1						
I	*Crassula aquatica*	o	24	o	24												o	+	24	–	8	o	8		
I	*Elatine hexandra*	–	20	o	8	–	5			1	+	+	4				o	+	8		3				
I	*Elatine hydropiper*	–	131	–	128		1			1							–	+	124	o	13	–	74		
I	*Elatine orthosperma*		3		3														3		3				
I	*Elatine triandra*	o	57	o	57												+	+	56	+	5	o	41		
I	*Eleocharis acicularis*	–	394	–	372	–	18	–	–	11	–	–	7	+	+	31	–	–	328	–	56	–	189	–	9
I	*Eriocaulon aquaticum*	–	20		1														1						
I	*Isoetes echinospora*	–	380	–	378											2	–	–	365	–	101	–	186		
I	*Isoetes lacustris*	–	487	–	424	–	13			2	–	–	11			3	–	–	405	–	117	–	197	–	7
I	*Limosella aquatica*	–	23	o	23											1	–	–	22	–	11	o	4		
I	*Littorella uniflora*	–	207	–	130	–	16	–	– –	7	–	–	9	+	+	12	–	–	118	–	55	–	16		
I	*Lobelia dortmanna*	–	292	–	260	–	4				–	–	4	+	–	6	–	–	251	–	81	–	102		
I	*Lythrum portula*	o	13	–	12		1						1			1	o	+	11	o	7				
I	*Ranunculus reptans*	–	462	–	462									+	–	21	–	–	419	–	88	–	256		
I	*Subularia aquatica*	–	323	–	319		3						3			2	–	–	302	–	75	–	157		
E	*Callitriche cophocarpa*	o	25	+	25											3	+	+	21	+	9	+	14		
E	*Callitriche hamulata*	–	93	–	78	o	5			1	–	–	4			2	–	–	76	–	63		1		
E	*Callitriche hermaphroditica*	o	101	–	79	o	13	o	–	8	+	+	5	+	–	19	–	+	58	–	27	–	24		
E	*Callitriche palustris*	o	224	–	223		1			1				o	–	4	–	–	210	–	46	o	112		
E	*Callitriche stagnalis*	o	15	o	8	o	5			3			2				o	+	8	o	6				
E	*Ceratophyllum demersum*	+	184	+	90	+	87	+	+	83	+	+	4	+	+	33	+	+	53	+	17	o	30	+	61
E	*Elodea canadensis*	o	237	o	107	o	78	o	–	60	+	+	18	+	+	30	–	+	75	o	23	o	39	o	49
E	*Elodea nuttallii*	+	20		2	+	15	+	+	14			1						2						
E	*Hydrilla verticillata*		2				2			2															2
E	*Myriophyllum alterniflorum*	–	482	–	419	–	24	–	– –	11	–	–	13	o	– –	22	–	–	387	–	115	–	185	–	12
E	*Myriophyllum sibiricum*	–	45	–	45									–	–	6	–	+	37	–	15	–	30		
E	*Myriophyllum spicatum*	+	184	+	68	o	90	o	+	79	o	+	11	+	+	44	+	+	24	+	9			o	56

Appendix continued

	Species	All		N-GIG		C-GIG		CH			CL			NH			NL			Norway		Finland		Latvia	
		P	*n*	P	*n*	P	*n*	P	L	*n*	P	L	*n*	P	L	*n*	P	L	*n*	P	*n*	P	*n*	P	*n*
E	*Myriophyllum verticillatum*	+	79	+	53	+	26	+	+	24			2	+	+	13	+	+	38	+	5	+	28	+	16
E	*Najas flexilis*	o	11	+	7											3	+	+	4		2		1		
E	*Najas marina*	+	24			+	24	+	–	24															
E	*Najas tenuissima*	–	3		3														3				3		
E	*Potamogeton acutifolius*		2				2			2															
E	*Potamogeton alpinus*	–	311	–	296	–	8			3	–	–	5	–	– –	29	–	+	255	o	90	o	144		
E	*Potamogeton berchtoldii*	–	322	o	284	–	8			2	o	+	6	+	–	27	–	+	245	o	83	–	138		
E	*Potamogeton compressus*	o	83	o	65	+	18	o	+	18				+	+	12	o	+	51		2	–	30	o	6
E	*Potamogeton crispus*	+	94	+	52	o	31	+	+	28			3	+	+	24	o	+	28	+	7		4	+	5
E	*Potamogeton filiformis*	–	82	–	63	–	11	–	– –	9			2	–	–	30	–	–	33	–	38	–	11		
E	*Potamogeton friesii*	o	61	o	45	o	14	o	–	14				+	+	36	–	+	9	+	21				2
E	*Potamogeton gramineus*	–	317	–	285	–	20	o	–	11	–	–	9	o	–	33	–	+	245	–	66	–	128	+	8
E	*Potamogeton lucens*	o	181	+	64	o	93	o	–	84	+	+	9	+	+	37	–	+	26	+	11	–	5	o	74
E	*Potamogeton obtusifolius*	o	177	+	151	+	11	+	+	7	+	+	4	+	+	28	+	+	120	+	30	+	55		1
E	*Potamogeton pectinatus*	+	128	+	45	+	71	+	+	68			3	+	+	30	o	+	15	+	10		1	o	9
E	*Potamogeton perfoliatus*	o	627	–	470	o	115	o	–	99	o	+	16	o	–	56	–	+	394	o	73	–	239	–	75
E	*Potamogeton polygonifolius*	–	23	–	18		1			1							–	– –	18	–	16				
E	*Potamogeton praelongus*	–	224	–	199	–	22	–	–	16	o	+	6	o	–	43	–	+	153	–	47	–	62	–	18
E	*Potamogeton pusillus*	+	56	o	19	+	30	+	+	29			1	+	+	7	–	+	12	+	4	+	6		
E	*Potamogeton rutilus*	o	23	o	17	o	5			3			2	+	+	5	o	+	12	+	9	+	4		1
E	*Potamogeton trichoides*	+	10			+	10	+	+	10															
E	*Potamogeton vaginatus*		2		2											1			1		1		1		
E	*Potamogeton gramineus × perfoliatus*	o	39	o	31	o	6	o	–	4			2	+	–	14	–	+	17	–	12				1
E	*Potamogeton gramineus × natans*	–	4	–	4												–	–	4		3				
E	*Potamogeton filiformis × pectinatus*		3		3											2			1		3				
E	*Potamogeton gramineus × lucens*	–	25	–	24									–	–	10	–	+	14		3				
E	*Ranunculus aquatilis*	o	58	o	25	–	31	–	–	29			2	+	+	10	–	+	15	o	12			–	24
E	*Ranunculus baudoti*		3				2			2															

Appendix continued

	Species	All		N-GIG		C-GIG		CH			CL			NH			NL			Norway		Finland		Latvia	
		P	*n*	P	*n*	P	*n*	P	L	*n*	P	L	*n*	P	L	*n*	P	L	*n*	P	*n*	P	*n*	P	*n*
E	*Ranunculus circinatus*	+	36	+	25	+	10	+	+	9			1	+	+	20	+	+	5						
E	*Ranunculus confervoides*	–	43	–	43									–	– –	9	–	–	34	–	32	–	4		
E	*Ranunculus peltatus*	–	249	–	249									+	–	18	–	–	222	–	29	–	133		
E	*Ranunculus penicillatus*		1																						
E	*Utricularia australis*	–	7	–	7												–	+	7			–	7		
E	*Utricularia intermedia*	–	130	–	127									–	–	7	–	–	114	–	38	o	57		
E	*Utricularia minor*	–	85	–	83		1						1	–	–	4	–	–	77	–	50	o	24		1
E	*Utricularia ochroleuca*	–	30	–	30												–	– –	30	–	27		3		
E	*Utricularia vulgaris*	o	418	–	364	o	38	o	–	34	o	+	4	–	+	41	–	+	308	–	53	o	173	–	25
E	*Zannichellia palustris*	+	36	+	15	+	20	+	+	17			3	+	+	8	+	+	7		2				
N	*Nuphar lutea*	o	766	o	572	o	154	o	–	122	+	+	32	+	+	73	–	+	473	o	111	o	248	+	118
N	*Nuphar pumila*	o	206	–	178	o	25	o	–	14	+	+	11			3	–	+	165	–	19	o	117	+	24
N	*Nuphar lutea × pumila*	–	174	–	172		2			1			1				–	–	154			–	134		
N	*Nymphaea alba*	o	454	o	416	o	26	o	+	22	o	+	4	–	–	56	–	+	350	o	109	o	163	–	11
N	*Nymphaea candida × tetragona*		2		2														2				2		
N	*Nymphaea candida*	o	86	o	30	o	56	o	+	46	+	+	10			3	–	+	27					–	50
N	*Nymphaea tetragona*	o	24	o	24												o	+	23			o	24		
N	*Nymphaea alba × candida*		3				3			3															
N	*Nymphoides peltata*	+	4			+	4	+	+	4															
N	*Persicaria amphibia*	o	208	o	184	–	13	–	–	8	+	+	5	+	+	43	o	+	140	o	35	o	63		
N	*Potamogeton natans*	o	669	o	513	–	129	o	–	102	+	+	27	–	–	83	o	+	422	o	133	o	189	o	107
N	*Sagittaria natans*	o	68	o	68												o	+	66			o	53		
N	*Sagittaria sagittifolia × natans*	+	8	+	8												+	+	7			+	8		
N	*Sparganium angustifolium*	–	396	–	388		2						2	–	– –	15	–	–	348	–	147	–	196		
N	*Sparganium gramineum*	o	205	o	205											2	o	+	201		1	o	138		
N	*Sparganium hyperboreum*	–	27	–	27											1	–	– –	26	–	20	–	6		
N	*Sparganium natans*	o	99	o	97									–	–	5	o	+	88	–	12	o	54		
N	*Sparganium angustifolium × gramineum*	–	21	–	21												–	+	21				2		
L	*Hydrocharis morsus-ranae*	+	120	+	76	+	44	+	+	37	+	+	7	+	+	25	+	+	48			+	30	+	25
L	*Lemna gibba*	+	7		2	o	5	o	+	5						1			1						1

Appendix continued

	Species	All		N-GIG		C-GIG		CH			CL			NH			NL			Norway		Finland		Latvia	
		P	*n*	P	*n*	P	*n*	P	L	*n*	P	L	*n*	P	L	*n*	P	L	*n*	P	*n*	P	*n*	P	*n*
L	*Lemna minor*	+	264	+	215	+	47	+	+	42	+	+	5	+	+	53	+	+	158	+	56	+	81	+	18
L	*Lemna trisulca*	+	146	+	78	+	36	+	+	33			3	+	+	26	+	+	51	+	4	+	23	o	14
L	*Salvinia natans*	+	7			+	7	+	+	7															
L	*Spirodela polyrhiza*	+	84	+	58	+	26	+	+	24			2	+	+	22	+	+	34	+	13	+	19	+	9
L	*Stratiotes aloides*	+	60	+	56	+	4	+	+	4				+	+	26	o	+	28			o	13		
L	*Trapa natans*	+	5			+	5	+	+	4			1												2
C	*Chara aspera*	o	76	o	49	–	23	–	– –	21			2	o	–	28	–	+	20	o	12	–	11		3
C	*Chara connivens*	+	15			–	15	–	– –	15															
C	*Chara contraria*	o	47	o	22	–	25	–	– –	24			1	o	–	16	o	+	6	–	5			–	5
C	*Chara delicatula*	–	15	–	12		3			3				–	–	9			3	–	10				
C	*Chara filiformis*		2		1		1			1						1									
C	*Chara fragilis*	o	10	o	10											1	o	+	9			o	6		
C	*Chara globularis*	o	135	–	97	–	33	–	–	26	–	+	7	o	–	30	–	+	63	–	40	–	25		
C	*Chara hispida*	o	22	o	7	–	14	–	– –	14				o	–	4			3					–	4
C	*Chara intermedia*		2		1		1			1						1					1				1
C	*Chara rudis*	o	20	–	15	–	5	–	– –	5				–	–	14			1	–	11				2
C	*Chara strigosa*	–	7	–	6		1			1						3			3	–	6				
C	*Chara tomentosa*	o	24	+	10	o	14	o	– –	14				–	–	9			1	+	4			o	9
C	*Chara vulgaris*	o	12			–	10	–	–	10															2
C	*Nitella confervacea*		2		2														2		1		1		
C	*Nitella flexilis*	o	48	–	29	o	10	+	–	6	–	+	4				–	+	27			–	25		
C	*Nitella mucronata*	o	8		2	+	6	o	+	6									2		2				
C	*Nitella opaca*	–	142	–	132	o	6			3			3	o	–	6	–	–	121	–	67	–	51		
C	*Nitella translucens*		3		2		1						1						2						
C	*Nitella wahlbergiana*		3		3														3				2		
C	*Nitellopsis obtuse*	+	41		1	+	40	+	+	40						1								o	10
C	*Tolypella canadensis*	–	5	–	5												–	– –	5	–	5				
C	*Tolypella glomerata*		3				2			2															

Classifications in the percentiles method are Sensitive (–), Tolerant (+) and Indifferent (o). LEAFPACS classifications are positive responder (+), negative responder (–), or strongly negative responder (– –).C = Central GIG, N = Northern GIG, H = High alkalinity, L = Low-moderate alkalinity. Species groups: I—isoetids, E—elodeids, N—nymphaeids, L—lemnids, C—charids

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The Natural Flow Regime

A paradigm for river conservation and restoration

N. LeRoy Poff, J. David Allan, Mark B. Bain, James R. Karr, Karen L. Prestegaard, Brian D. Richter, Richard E. Sparks, and Julie C. Stromberg

Humans have long been fascinated by the dynamism of free-flowing waters. Yet we have expended great effort to tame rivers for transportation, water supply, flood control, agriculture, and power generation. It is now recognized that harnessing of streams and rivers comes at great cost: Many rivers no longer support socially valued native species or sustain healthy ecosystems that provide important goods and services (Naiman et al. 1995, NRC 1992).

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The ecological integrity of river ecosystems depends on their natural dynamic character

The extensive ecological degradation and loss of biological diversity resulting from river exploitation is eliciting widespread concern for conservation and restoration of healthy river ecosystems among scientists and the lay public alike (Allan and Flecker 1993, Hughes and Noss 1992, Karr et al. 1985, TNC 1996, Williams et al. 1996). Extirpation of species, closures of fisheries, groundwater depletion, declines in water quality and availability, and more frequent and intense flooding are increasingly recognized as consequences of current river management and development policies (Abramovitz 1996, Collier et al. 1996, Naiman et al. 1995). The broad social support in the United States for the Endangered Species Act, the recognition of the intrinsic value of noncommercial native species, and the proliferation of watershed councils and riverwatch teams are evidence of society's interest in maintaining the ecological integrity and self-sustaining productivity of free-flowing river systems.

Society's ability to maintain and restore the integrity of river ecosystems requires that conservation and management actions be firmly grounded in scientific understanding. However, current management approaches often fail to recognize the fundamental scientific principle that the integrity of flowing water systems depends largely on their natural dynamic character; as a result, these methods frequently prevent successful river conservation or restoration. Streamflow quantity and timing are critical components of water supply, water quality, and the ecological integrity of river systems. Indeed, streamflow, which is strongly correlated with many critical physicochemical characteristics of rivers, such as water temperature, channel geomorphology, and habitat diversity, can be considered a "master variable" that limits the distribution and abundance of riverine species (Power et al. 1995, Resh et al. 1988) and regulates the ecological integrity of flowing water systems (Figure 1). Until recently, however, the importance of natural streamflow variability in maintaining healthy aquatic ecosystems has been virtually ignored in a management context.

Historically, the "protection" of river ecosystems has been limited in scope, emphasizing water quality and only one aspect of water quantity: minimum flow. Water resources management has also suffered from the often incongruent perspectives and fragmented responsibility of agencies (for example, the US Army Corps of Engineers and Bureau of Reclamation are responsible for water supply and flood control, the US Environmental Protection Agency and state environmental agencies for water quality, and the US Fish &

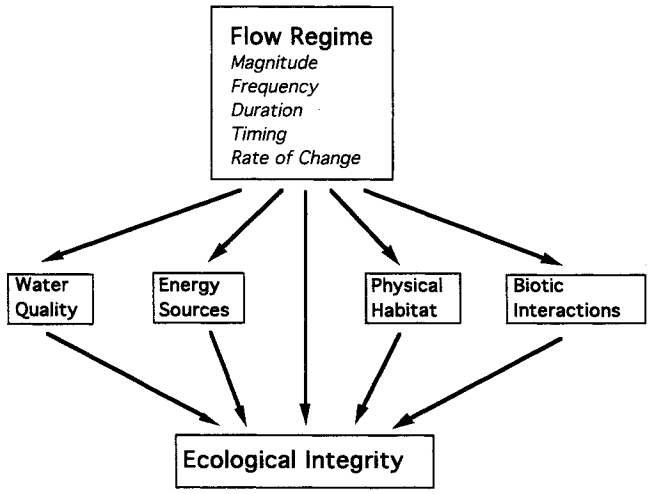

Figure 1. Flow regime is of central importance in sustaining the ecological integrity of flowing water systems. The five components of the flow regime—magnitude, frequency, duration, timing, and rate of change—influence integrity both directly and indirectly, through their effects on other primary regulators of integrity. Modification of flow thus has cascading effects on the ecological integrity of rivers. After Karr 1991.

Wildlife Service for water-dependent species of sporting, commercial, or conservation value), making it difficult, if not impossible, to manage the entire river ecosystem (Karr 1991). However, environmental dynamism is now recognized as central to sustaining and conserving native species diversity and ecological integrity in rivers and other ecosystems (Holling and Meffe 1996, Hughes 1994, Pickett et al. 1992, Stanford et al. 1996), and coordinated actions are therefore necessary to protect and restore a river's natural flow variability.

In this article, we synthesize existing scientific knowledge to argue that the natural flow regime plays a critical role in sustaining native biodiversity and ecosystem integrity in rivers. Decades of observation of the effects of human alteration of natural flow regimes have resulted in a well-grounded scientific perspective on why altering hydrologic variability in rivers is ecologically harmful (e.g., Arthington et al. 1991, Castleberry et al. 1996, Hill et al. 1991, Johnson et al. 1976, Richter et al. 1997, Sparks 1995, Stanford et al. 1996, Toth 1995, Tyus 1990). Current pressing demands on water use and the continuing alteration of watersheds require scientists to help develop management protocols that can accommodate economic uses while protecting ecosystem functions. For humans to continue to rely on river ecosystems for sustainable food production, power production, waste assimilation, and flood control, a new, holistic, ecological perspective on water management is needed to guide society's interactions with rivers.

The natural flow regime

The natural flow of a river varies on time scales of hours, days, seasons, years, and longer. Many years of observation from a streamflow gauge are generally needed to describe the characteristic pattern of a river's flow quantity, timing, and variability—that is, its natural flow regime. Components of a natural flow regime can be characterized using various time series (e.g., Fourier and wavelet) and probability analyses of, for example, extremely high or low flows, or of the entire range of flows expressed as average daily discharge (Dunne and Leopold 1978). In watersheds lacking long-term streamflow data, analyses can be extended statistically from gauged streams in the same geographic area. The frequency of large-magnitude floods can be estimated by paleohydrologic studies of debris left by floods and by studies of historical damage to living trees (Hupp and Osterkamp 1985, Knox 1972). These historical techniques can be used to extend existing hydrologic records or to provide estimates of flood flows for ungauged sites.

River flow regimes show regional patterns that are determined largely by river size and by geographic variation in climate, geology, topography, and vegetative cover. For example, some streams in regions with little seasonality in precipitation exhibit relatively stable hydrographs due to high groundwater inputs (Figure 2a), whereas other streams can fluctuate greatly at virtually any time of year (Figure 2b). In regions with seasonal precipitation, some streams are dominated by snowmelt, resulting in pronounced, predictable runoff patterns (Figure 2c), and others lack snow accumulation and exhibit more variable runoff patterns during the rainy season, with peaks occurring after each substantial storm event (Figure 2d).

Five critical components of the flow regime regulate ecological processes in river ecosystems: the magnitude, frequency, duration, timing, and rate of change of hydrologic conditions (Poff and Ward 1989, Richter et al. 1996, Walker et al. 1995). These components can be used to characterize the entire range of flows and specific hydrologic phenomena, such as floods or low flows, that are critical to the integrity of river ecosystems. Furthermore, by defining flow regimes in these terms, the ecological consequences of particular human activities that modify one or more components of the flow regime can be considered explicitly.

- The *magnitude* of discharge[1] at any given time interval is simply the amount of water moving past a fixed location per unit time. Magnitude can refer either to absolute or to relative discharge (e.g., the amount of water that inundates a floodplain). Maximum and minimum magnitudes of flow vary with climate and watershed size both within and among river systems.
- The *frequency* of occurrence refers to how often a flow above a given magnitude recurs over some specified time interval. Frequency of occurrence is inversely related to flow magnitude. For example, a 100-year flood is equaled or exceeded on average once every 100 years (i.e., a chance of 0.01 of occurring in any given year). The average (median)

[1]Discharge (also known as streamflow, flow, or flow rate) is always expressed in dimensions of volume per time. However, a great variety of units are used to describe flow, depending on custom and purpose of characterization: Flows can be expressed in near-instantaneous terms (e.g., ft^3/s and m^3/s) or over long time intervals (e.g., acre-ft/yr).

flow is determined from a data series of discharges defined over a specific time interval, and it has a frequency of occurrence of 0.5 (a 50% probability).

•The *duration* is the period of time associated with a specific flow condition. Duration can be defined relative to a particular flow event (e.g., a floodplain may be inundated for a specific number of days by a ten-year flood), or it can be a defined as a composite expressed over a specified time period (e.g., the number of days in a year when flow exceeds some value).

•The *timing*, or *predictability*, of flows of defined magnitude refers to the regularity with which they occur. This regularity can be defined formally or informally and with reference to different time scales (Poff 1996). For example, annual peak flows may occur with low seasonal predictability (Figure 2b) or with high seasonal predictability (Figure 2c).

•The *rate of change*, or *flashiness*, refers to how quickly flow changes from one magnitude to another. At the extremes, "flashy" streams have rapid rates of change (Figure 2b), whereas "stable" streams have slow rates of change (Figure 2a).

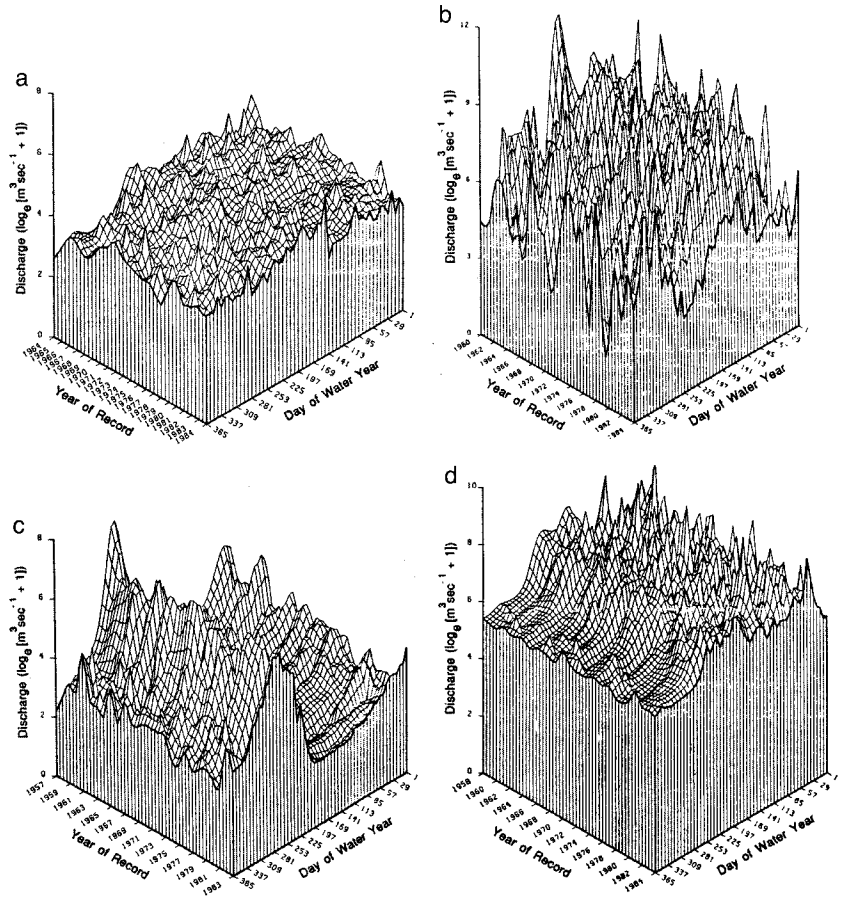

Figure 2. Flow histories based on long-term, daily mean discharge records. These histories show within- and among-year variation for (**a**) Augusta Creek, MI, (**b**) Satilla River, GA, (**c**) upper Colorado River, CO, and (**d**) South Fork of the McKenzie River, OR. Each water year begins on October 1 and ends on September 30. Adapted from Poff and Ward 1990.

Hydrologic processes and the flow regime. All river flow derives ultimately from precipitation, but in any given time and place a river's flow is derived from some combination of surface water, soil water, and groundwater. Climate, geology, topography, soils, and vegetation help to determine both the supply of water and the pathways by which precipitation reaches the channel. The water movement pathways depicted in Figure 3a illustrate why rivers in different settings have different flow regimes and why flow is variable in virtually all rivers. Collectively, overland and shallow subsurface flow pathways create hydrograph peaks, which are the river's response to storm events. By contrast, deeper groundwater pathways are responsible for baseflow, the form of delivery during periods of little rainfall.

Variability in intensity, timing, and duration of precipitation (as rain or as snow) and in the effects of terrain, soil texture, and plant evapotranspiration on the hydrologic cycle combine to create local and regional flow patterns. For example, high flows due to rainstorms may occur over periods of hours (for permeable soils) or even minutes (for impermeable soils), whereas snow will melt over a period of days or weeks, which slowly builds the peak snowmelt flood. As one proceeds downstream within a watershed, river flow reflects the sum of flow generation and routing processes operating in multiple small tributary watersheds. The travel time of flow down the river system, combined with nonsynchronous tributary inputs and larger downstream channel and floodplain storage capacities, act to attenuate and to dampen flow peaks. Consequently, annual hydrographs in large streams typically show peaks created by widespread storms or snowmelt events and broad seasonal influences that affect many tributaries together (Dunne and Leopold 1978).

The natural flow regime organizes and defines river ecosystems. In rivers, the physical structure of the environment and, thus, of the habitat, is defined largely by physical processes, especially the movement of water and sediment within the channel and between the channel and floodplain. To understand the biodiversity, production, and sustainability of river ecosystems, it is necessary to appreciate the central organizing role played by a dynamically varying physical environment.

The physical habitat of a river includes sediment size and heterogeneity, channel and floodplain morphology, and other geomorphic features. These features form as the available sediment, woody debris, and other transportable materials are moved and deposited by flow. Thus, habitat conditions associated with channels and floodplains vary among

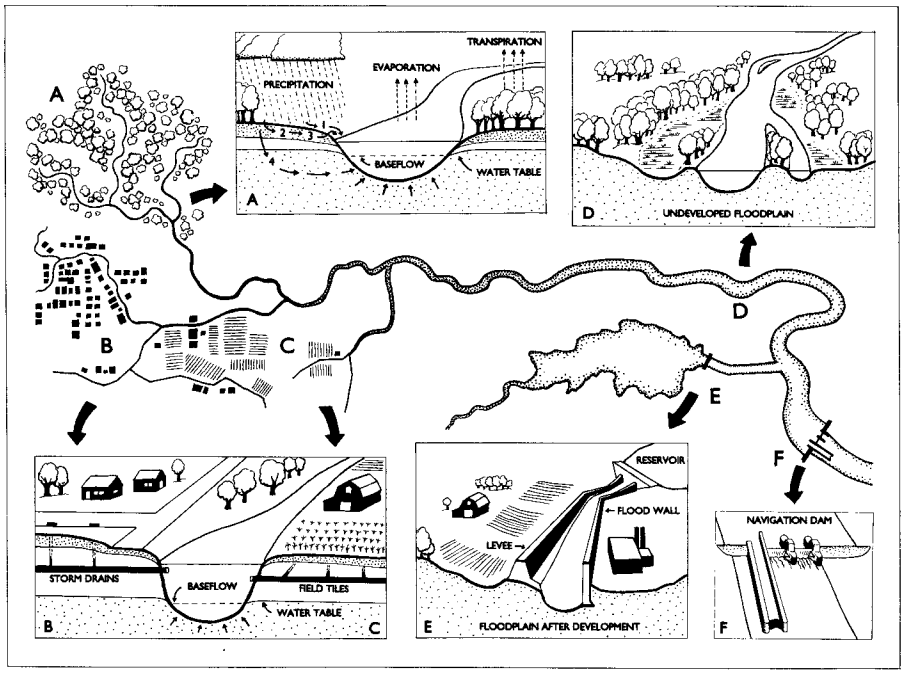

Figure 3. Stream valley cross-sections at various locations in a watershed illustrate basic principles about natural pathways of water moving downhill and human influences on hydrology. Runoff, which occurs when precipitation exceeds losses due to evaporation and plant transpiration, can be divided into four components (**a**): overland flow (1) occurs when precipitation exceeds the infiltration capacity of the soil; shallow subsurface stormflow (2) represents water that infiltrates the soil but is routed relatively quickly to the stream channel; saturated overland flow (3) occurs where the water table is close to the surface, such as adjacent to the stream channel, upstream of first-order tributaries, and in soils saturated by prior precipitation; and groundwater flow (4) represents relatively deep and slow pathways of water movement and provides water to the stream channel even during periods of little or no precipitation. Collectively, overland and shallow subsurface flow pathways create the peaks in the hydrograph that are a river's response to storm events, whereas deeper groundwater pathways are responsible for baseflow. Urbanized (**b**) and agricultural (**c**) land uses increase surface flow by increasing the extent of impermeable surfaces, reducing vegetation cover, and installing drainage systems. Relative to the unaltered state, channels often are scoured to greater depth by unnaturally high flood crests and water tables are lowered, causing baseflow to drop. Side-channels, wetlands, and episodically flooded lowlands comprise the diverse floodplain habitats of unmodified river ecosystems (**d**). Levees or flood walls (**e**) constructed along the banks retain flood waters in the main channel and lead to a loss of floodplain habitat diversity and function. Dams impede the downstream movement of water and can greatly modify a river's flow regime, depending on whether they are operated for storage (**e**) or as "run-of-river," such as for navigation (**f**).

rivers in accordance with both flow characteristics and the type and the availability of transportable materials.

Within a river, different habitat features are created and maintained by a wide range of flows. For example, many channel and floodplain features, such as river bars and riffle–pool sequences, are formed and maintained by dominant, or bankfull, discharges. These discharges are flows that can move significant quantities of bed or bank sediment and that occur frequently enough (e.g., every several years) to continually modify the channel (Wolman and Miller 1960). In many streams and rivers with a small range of flood flows, bankfull flow can build and maintain the active floodplain through stream migration (Leopold et al. 1964). However, the concept of a dominant discharge may not be applicable in all flow regimes (Wolman and Gerson 1978). Furthermore, in some flow regimes, the flows that build the channel may differ from those that build the floodplain. For example, in rivers with a wide range of flood flows, floodplains may exhibit major bar deposits, such as berms of boulders along the channel, or other features that are left by infrequent high-magnitude floods (e.g., Miller 1990).

Over periods of years to decades, a single river can consistently provide ephemeral, seasonal, and persistent types of habitat that range from free-flowing, to standing, to no water. This predictable diversity of in-channel and floodplain habitat types has promoted the evolution of species that exploit the habitat mosaic created and maintained by hydrologic variability. For many riverine species, completion of the life cycle requires an array of different habitat types, whose availability over time is regulated by the flow regime (e.g., Greenberg et al. 1996, Reeves et al. 1996, Sparks 1995). Indeed, adaptation to this environmental dynamism allows aquatic and floodplain species to persist in the face of seemingly harsh conditions, such as floods and droughts, that regularly destroy and re-create habitat elements.

From an evolutionary perspective, the pattern of spatial and temporal habitat dynamics influences the relative success of a species in a particular environmental setting. This habitat template (Southwood 1977), which is dictated largely by flow regime, creates both subtle and profound differences in the natural histories of species in different segments of their ranges. It also influences species distribution and abundance, as well as ecosystem function (Poff and Allan 1995, Schlosser 1990, Sparks 1992, Stanford et al. 1996). Human alteration of flow regime changes the established pattern of natural hydrologic variation and disturbance, thereby altering habitat dynamics and creating new conditions to which the native biota may be poorly adapted.

Human alteration of flow regimes

Human modification of natural hydrologic processes disrupts the dynamic equilibrium between the movement of water and the movement of sediment that exists in free-flowing rivers (Dunne and Leopold 1978). This disruption alters both gross- and fine-scale geomorphic features that constitute habitat for aquatic and riparian species (Table 1). After

Table 1. Physical responses to altered flow regimes.

Source(s) of alteration	Hydrologic change(s)	Geomorphic response(s)	Reference(s)
Dam	Capture sediment moving downstream	Downstream channel erosion and tributary headcutting	Chien 1985, Petts 1984, 1985, Williams and Wolman 1984
		Bed armoring (coarsening)	Chien 1985
Dam, diversion	Reduce magnitude and frequency of high flows	Deposition of fines in gravel	Sear 1995, Stevens et al. 1995
		Channel stabilization and narrowing	Johnson 1994, Williams and Wolman 1984
		Reduced formation of point bars, secondary channels, oxbows, and changes in channel planform	Chien 1985, Copp 1989, Fenner et al. 1985
Urbanization, tiling, drainage	Increase magnitude and frequency of high flows	Bank erosion and channel widening	Hammer 1972
		Downward incision and floodplain disconnection	Prestegaard 1988
	Reduced infiltration into soil	Reduced baseflows	Leopold 1968
Levees and channelization	Reduce overbank flows	Channel restriction causing downcutting	Daniels 1960, Prestegaard et al. 1994
		Floodplain deposition and erosion prevented	Sparks 1992
		Reduced channel migration and formation of secondary channels	Shankman and Drake 1990
Groundwater pumping	Lowered water table levels	Streambank erosion and channel downcutting after loss of vegetation stability	Kondolf and Curry 1986

such a disruption, it may take centuries for a new dynamic equilibrium to be attained by channel and floodplain adjustments to the new flow regime (Petts 1985); in some cases, a new equilibrium is never attained, and the channel remains in a state of continuous recovery from the most recent flood event (Wolman and Gerson 1978). These channel and floodplain adjustments are sometimes overlooked because they can be confounded with long-term responses of the channel to changing climates (e.g., Knox 1972). Recognition of human-caused physical changes and associated biological consequences may require many years, and physical restoration of the river ecosystem may call for dramatic action (see box on the Grand Canyon flood, page 774).

Dams, which are the most obvious direct modifiers of river flow, capture both low and high flows for flood control, electrical power generation, irrigation and municipal water needs, maintenance of recreational reservoir levels, and navigation. More than 85% of the inland waterways within the continental United States are now artificially controlled (NRC 1992), including nearly 1 million km of rivers that are affected by dams (Echeverria et al. 1989). Dams capture all but the finest sediments moving down a river, with many severe downstream consequences. For example, sediment-depleted water released from dams can erode finer sediments from the receiving channel. The coarsening of the streambed can, in turn, reduce habitat availability for the many aquatic species living in or using interstitial spaces. In addition, channels may erode, or downcut, triggering rejuvenation of tributaries, which themselves begin eroding and migrating headward (Chien 1985, Petts 1984). Fine sediments that are contributed by tributaries downstream of a dam may be deposited between the coarse particles of the streambed (e.g., Sear 1995). In the absence of high flushing flows, species with life stages that are sensitive to sedimentation, such as the eggs and larvae of many invertebrates and fish, can suffer high mortality rates.

For many rivers, it is land-use activities, including timber harvest, livestock grazing, agriculture, and urbanization, rather than dams, that are the primary causes of altered flow regimes. For example, logging and the associated building of roads have contributed greatly to degradation of salmon streams in the Pacific Northwest, mainly through effects on runoff and sediment delivery (NRC 1996). Converting forest or prairie lands to agricultural lands generally decreases soil infiltration and results in increased overland flow, channel incision, floodplain isolation, and headward erosion of stream channels (Prestegaard 1988). Many agricultural areas were drained by the construction of ditches or tile-and-drain systems, with the result that many channels have become entrenched (Brookes 1988).

These land-use practices, combined with extensive draining of wetlands or overgrazing, reduce retention of water in watersheds and,

A controlled flood in the Grand Canyon

Since the Glen Canyon dam first began to store water in 1963, creating Lake Powell, some 430 km (270 miles) of the Colorado River, including Grand Canyon National Park, have been virtually bereft of seasonal floods. Before 1963, melting snow in the upper basin produced an average peak discharge exceeding 2400 m^3/s; after the dam was constructed, releases were generally maintained at less than 500 m^3/s. The building of the dam also trapped more than 95% of the sediment moving down the Colorado River in Lake Powell (Collier et al. 1996).

This dramatic change in flow regime produced drastic alterations in the dynamic nature of the historically sediment-laden Colorado River. The annual cycle of scour and fill had maintained large sandbars along the river banks, prevented encroachment of vegetation onto these bars, and limited bouldery debris deposits from constricting the river at the mouths of tributaries (Collier et al. 1997). When flows were reduced, the limited amount of sand accumulated in the channel rather than in bars farther up the river banks, and shallow low-velocity habitat in eddies used by juvenile fishes declined. Flow regulation allowed for increased cover of wetland and riparian vegetation, which expanded into sites that were regularly scoured by floods in the constrained fluvial canyon of the Colorado River; however, much of the woody vegetation that established after the dam's construction is composed of an exotic tree, salt cedar (*Tamarix* sp.; Stevens et al. 1995). Restoration of flood flows clearly would help to steer the aquatic and riparian ecosystem toward its former state and decrease the area of wetland and riparian vegetation, but precisely how the system would respond to an artificial flood could not be predicted.

In an example of adaptive management (i.e., a planned experiment to guide further actions), a controlled, seven-day flood of 1274 m^3/s was released through the Glen Canyon dam in late March 1996. This flow, roughly 35% of the pre-dam average for a spring flood (and far less than some large historical floods), was the maximum flow that could pass through the power plant turbines plus four steel drainpipes, and it cost approximately $2 million in lost hydropower revenues (Collier et al. 1997). The immediate result was significant beach building: Over 53% of the beaches increased in size, and just 10% decreased in size. Full documentation of the effects will continue to be monitored by measuring channel cross-sections and studying riparian vegetation and fish populations.

instead, route it quickly downstream, increasing the size and frequency of floods and reducing baseflow levels during dry periods (Figure 3b; Leopold 1968). Over time, these practices degrade in-channel habitat for aquatic species. They may also isolate the floodplain from overbank flows, thereby degrading habitat for riparian species. Similarly, urbanization and suburbanization associated with human population expansion across the landscape create impermeable surfaces that direct water away from subsurface pathways to overland flow (and often into storm drains). Consequently, floods increase in frequency and intensity (Beven 1986), banks erode, and channels widen (Hammer 1972), and baseflow declines during dry periods (Figure 3c).

Whereas dams and diversions affect rivers of virtually all sizes, and land-use impacts are particularly evident in headwaters, lowland rivers are greatly influenced by efforts to sever channel–floodplain linkages. Flood control projects have shortened, narrowed, straightened, and leveed many river systems and cut the main channels off from their floodplains (NRC 1992). For example, channelization of the Kissimmee River above Lake Okeechobee, Florida, by the US Army Corps of Engineers transformed a historical 166 km meandering river with a 1.5 to 3 km wide floodplain into a 90 km long canal flowing through a series of five impoundments, resulting in great loss of river channel habitat and adjacent floodplain wetlands (Toth 1995). Because levees are designed to prevent increases in the width of flow, rivers respond by cutting deeper channels, reaching higher velocities, or both.

Channelization and wetland drainage can actually increase the magnitude of extreme floods, because reduction in upstream storage capacity results in accelerated water delivery downstream. Much of the damage caused by the extensive flooding along the Mississippi River in 1993 resulted from levee failure as the river reestablished historic connections to the floodplain. Thus, although elaborate storage dam and levee systems can "reclaim" the floodplain for agriculture and human settlement in most years, the occasional but inevitable large floods will impose increasingly high disaster costs to society (Faber 1996). The severing of floodplains from rivers also stops the processes of sediment erosion and deposition that regulate the topographic diversity of floodplains. This diversity is essential for maintaining species diversity on floodplains, where relatively small differences in land elevation result in large differences in annual inundation and soil moisture regimes, which regulate plant distribution and abundance (Sparks 1992).

Ecological functions of the natural flow regime

Naturally variable flows create and maintain the dynamics of in-channel and floodplain conditions and habitats that are essential to aquatic and riparian species, as shown schematically in Figure 4. For purposes of illustration, we treat the components of a flow regime individually, although in reality they interact in complex ways to regulate geomorphic and ecological processes. In describing the ecological functions associated with the components of a flow regime, we pay particular attention to high- and low-flow events, because they often serve as ecological "bottlenecks" that present critical stresses and opportunities for a wide array of riverine species (Poff and Ward 1989).

The magnitude and frequency of high and low flows regulate numerous ecological processes. Frequent, moderately high flows effectively transport sediment through the channel (Leopold et al. 1964). This sediment movement, combined with the force of moving water, exports organic resources, such as detritus and attached algae, rejuvenating the biological community and allowing many species with fast life cycles and good colonizing ability to reestablish (Fisher 1983). Consequently, the composition and relative abundance of species that are present in a stream or river often reflect the frequency and intensity of high flows (Meffe and Minckley 1987, Schlosser 1985).

High flows provide further ecological benefits by maintaining ecosystem productivity and diversity. For example, high flows remove and transport fine sediments that would otherwise fill the interstitial spaces in productive gravel habitats (Beschta and Jackson 1979). Floods import woody debris into the channel (Keller and Swanson 1979), where it creates new, high-quality habitat (Figure 4; Moore and Gregory 1988, Wallace and Benke 1984). By connecting the channel to the floodplain, high overbank flows also maintain broader productivity and diversity. Floodplain wetlands provide important nursery grounds for fish and export organic matter and organisms back into the main channel (Junk et al. 1989, Sparks 1995, Welcomme 1992). The scouring of floodplain soils rejuvenates habitat for plant species that germinate only on barren, wetted surfaces that are free of competition (Scott et al. 1996) or that require access to shallow water tables (Stromberg et al. 1997). Flood-resistant, disturbance-adapted riparian communities are maintained by flooding along river corridors, even in river sections that have steep banks and lack floodplains (Hupp and Osterkamp 1985).

Flows of low magnitude also provide ecological benefits. Periods of low flow may present recruitment opportunities for riparian plant species in regions where floodplains are frequently inundated (Wharton et al. 1981). Streams that dry temporarily, generally in arid regions, have aquatic (Williams and Hynes 1977) and riparian (Nilsen et al. 1984) species with special behavioral or physiological adaptations that suit them to these harsh conditions.

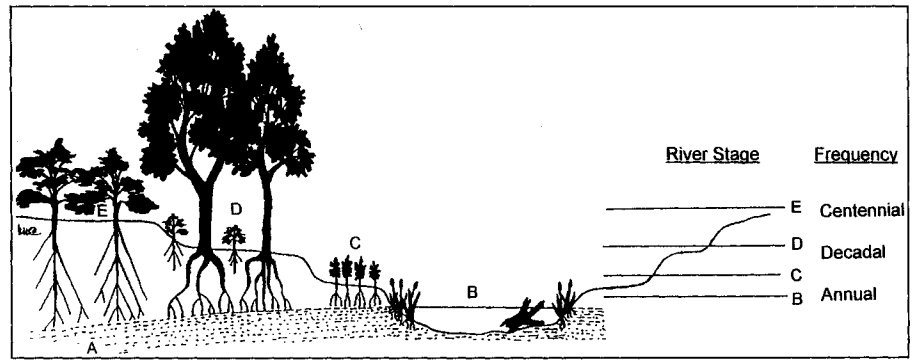

Figure 4. Geomorphic and ecological functions provided by different levels of flow. Water tables that sustain riparian vegetation and that delineate in-channel baseflow habitat are maintained by groundwater inflow and flood recharge (A). Floods of varying size and timing are needed to maintain a diversity of riparian plant species and aquatic habitat. Small floods occur frequently and transport fine sediments, maintaining high benthic productivity and creating spawning habitat for fishes (B). Intermediate-size floods inundate low-lying floodplains and deposit entrained sediment, allowing for the establishment of pioneer species (C). These floods also import accumulated organic material into the channel and help to maintain the characteristic form of the active stream channel. Larger floods that recur on the order of decades inundate the aggraded floodplain terraces, where later successional species establish (D). Rare, large floods can uproot mature riparian trees and deposit them in the channel, creating high-quality habitat for many aquatic species (E).

The duration of a specific flow condition often determines its ecological significance. For example, differences in tolerance to prolonged flooding in riparian plants (Chapman et al. 1982) and to prolonged low flow in aquatic invertebrates (Williams and Hynes 1977) and fishes (Closs and Lake 1996) allow these species to persist in locations from which they might otherwise be displaced by dominant, but less tolerant, species.

The timing, or predictability, of flow events is critical ecologically because the life cycles of many aquatic and riparian species are timed to either avoid or exploit flows of variable magnitudes. For example, the natural timing of high or low streamflows provides environmental cues for initiating life cycle transitions in fish, such as spawning (Montgomery et al. 1983, Nesler et al. 1988), egg hatching (Næsje et al. 1995), rearing (Seegrist and Gard 1978), movement onto the floodplain for feeding or reproduction (Junk et al. 1989, Sparks 1995, Welcomme 1992), or migration upstream or downstream (Trépanier et al. 1996). Natural seasonal variation in flow conditions can prevent the successful establishment of nonnative species with flow-dependent spawning and egg incubation requirements, such as striped bass (*Morone saxatilis*; Turner and Chadwick 1972) and brown trout (*Salmo trutta*; Moyle and Light 1996, Strange et al. 1992).

Seasonal access to floodplain wetlands is essential for the survival of certain river fishes, and such access can directly link high wetland productivity with fish production in the stream channel (Copp 1989, Welcomme 1979). Studies of the effects on stream fishes of both extensive and limited floodplain inundation (Finger and Stewart 1987, Ross and Baker 1983) indicate that some fishes are adapted to exploiting floodplain habitats, and these species decline in abundance when floodplain use is restricted. Models indicate that catch rates and biomass of fish are influenced by both maximum and minimum wetland area (Power et al. 1995, Welcomme and Hagborg 1977), and empirical work shows that the area of floodplain water bodies during nonflood periods influences the species richness of those wetland habitats (Halyk and Balon 1983). The timing of floodplain inundation is important for some fish because migratory and reproductive behaviors must coincide with access to and avail-

Table 2. Ecological responses to alterations in components of natural flow regime.[a]

Flow component	Specific alteration	Ecological response	Reference(s)
Magnitude and frequency	Increased variation	Wash-out and/or stranding	Cushman 1985, Petts 1984
		Loss of sensitive species	Gehrke et al. 1995, Kingsolving and Bain 1993, Travnichek et al. 1995
		Increased algal scour and wash-out of organic matter	Petts 1984
		Life cycle disruption	Scheidegger and Bain 1995
		Altered energy flow	Valentin et al. 1995
	Flow stabilization	Invasion or establishment of exotic species, leading to:	
		Local extinction	Kupferberg 1996, Meffe 1984
		Threat to native commercial species	Stanford et al. 1996
		Altered communities	Busch and Smith 1995, Moyle 1986, Ward and Stanford 1979
		Reduced water and nutrients to floodplain plant species, causing:	
		Seedling desiccation	Duncan 1993
		Ineffective seed dispersal	Nilsson 1982
		Loss of scoured habitat patches and secondary channels needed for plant establishment	Fenner et al. 1985, Rood et al. 1995, Scott et al. 1997, Shankman and Drake 1990
		Encroachment of vegetation into channels	Johnson 1994, Nilsson 1982
Timing	Loss of seasonal flow peaks	Disrupt cues for fish:	
		Spawning	Fausch and Bestgen 1997, Montgomery et al. 1993, Nesler et al. 1988
		Egg hatching	Næsje et al. 1995
		Migration	Williams 1996
		Loss of fish access to wetlands or backwaters	Junk et al. 1989, Sparks 1995
		Modification of aquatic food web structure	Power 1992, Wootton et al. 1996
		Reduction or elimination of riparian plant recruitment	Fenner et al. 1985
		Invasion of exotic riparian species	Horton 1977
		Reduced plant growth rates	Reily and Johnson 1982
Duration	Prolonged low flows	Concentration of aquatic organisms	Cushman 1985, Petts 1984
		Reduction or elimination of plant cover	Taylor 1982
		Diminished plant species diversity	Taylor 1982
		Desertification of riparian species composition	Busch and Smith 1995, Stromberg et al. 1996
		Physiological stress leading to reduced plant growth rate, morphological change, or mortality	Kondolf and Curry 1986, Perkins et al. 1984, Reily and Johnson 1982, Rood et al. 1995, Stromberg et al. 1992
	Prolonged baseflow "spikes"	Downstream loss of floating eggs	Robertson 1997
	Altered inundation duration	Altered plant cover types	Auble et al. 1994
	Prolonged inundation	Change in vegetation functional type	Bren 1992, Connor et al. 1981
		Tree mortality	Harms et al. 1980
		Loss of riffle habitat for aquatic species	Bogan 1993
Rate of change	Rapid changes in river stage	Wash-out and stranding of aquatic species	Cushman 1985, Petts 1984
	Accelerated flood recession	Failure of seedling establishment	Rood et al. 1995

[a]Only representative studies are listed here. Additional references are located on the Web at http://lamar.colostate.edu/~poff/natflow.html.

ability of floodplain habitats (Welcomme 1979). The match of reproductive period and wetland access also explains some of the yearly variation in stream fish community composition (Finger and Stewart 1987).

Many riparian plants also have life cycles that are adapted to the seasonal timing components of natural flow regimes through their "emergence phenologies"—the seasonal sequence of flowering, seed dispersal, germination, and seedling growth. The interaction of emergence phenologies with temporally varying environmental stress from flooding or drought helps to maintain high species diversity in, for example, southern floodplain forests (Streng et al. 1989). Productivity of riparian forests is also influenced by flow timing and can increase when short-duration flooding occurs in the growing season (Mitsch and Rust 1984, Molles et al. 1995).

The rate of change, or flashiness, in flow conditions can influence spe-

cies persistence and coexistence. In many streams and rivers, particularly in arid areas, flow can change dramatically over a period of hours due to heavy storms. Non-native fishes generally lack the behavioral adaptations to avoid being displaced downstream by sudden floods (Minckley and Deacon 1991). In a dramatic example of how floods can benefit native species, Meffe (1984) documented that a native fish, the Gila topminnow (*Poeciliopsis occidentalis*), was locally extirpated by the introduced predatory mosquitofish (*Gambusia affinis*) in locations where natural flash floods were regulated by upstream dams, but the native species persisted in naturally flashy streams.

Rapid flow increases in streams of the central and southwestern United States often serve as spawning cues for native minnow species, whose rapidly developing eggs are either broadcast into the water column or attached to submerged structures as floodwaters recede (Fausch and Bestgen 1997, Robertson in press). More gradual, seasonal rates of change in flow conditions also regulate the persistence of many aquatic and riparian species. Cottonwoods (*Populus* spp.), for example, are disturbance species that establish after winter–spring flood flows, during a narrow "window of opportunity" when competition-free alluvial substrates and wet soils are available for germination. A certain rate of floodwater recession is critical to seedling germination because seedling roots must remain connected to a receding water table as they grow downward (Rood and Mahoney 1990).

Ecological responses to altered flow regimes

Modification of the natural flow regime dramatically affects both aquatic and riparian species in streams and rivers worldwide. Ecological responses to altered flow regimes in a specific stream or river depend on how the components of flow have changed relative to the natural flow regime for that particular stream or river (Poff and Ward 1990) and how specific geomorphic and ecological processes will respond to this relative change. As a result of variation in flow regime within and among rivers (Figure 2), the same human activity in different locations may cause different degrees of change relative to unaltered conditions and, therefore, have different ecological consequences.

Flow alteration commonly changes the magnitude and frequency of high and low flows, often reducing variability but sometimes enhancing the range. For example, the extreme daily variations below peaking power hydroelectric dams have no natural analogue in freshwater systems and represent, in an evolutionary sense, an extremely harsh environment of frequent, unpredictable flow disturbance. Many aquatic populations living in these environments suffer high mortality from physiological stress, from wash-out during high flows, and from stranding during rapid dewatering (Cushman 1985, Petts 1984). Especially in shallow shoreline habitats, frequent atmospheric exposure for even brief periods can result in massive mortality of bottom-dwelling organisms and subsequent severe reductions in biological productivity (Weisberg et al. 1990). Moreover, the rearing and refuge functions of shallow shoreline or backwater areas, where many small fish species and the young of large species are found (Greenberg et al. 1996, Moore and Gregory 1988), are severely impaired by frequent flow fluctuations (Bain et al. 1988, Stanford 1994). In these artificially fluctuating environments, specialized stream or river species are typically replaced by generalist species that tolerate frequent and large variations in flow. Furthermore, life cycles of many species are often disrupted and energy flow through the ecosystem is greatly modified (Table 2). Short-term flow modifications clearly lead to a reduction in both the natural diversity and abundance of many native fish and invertebrates.

At the opposite hydrologic extreme, flow stabilization below certain types of dams, such as water supply reservoirs, results in artificially constant environments that lack natural extremes. Although production of a few species may increase greatly, it is usually at the expense of other native species and of systemwide species diversity (Ward and Stanford 1979). Many lake fish species have successfully invaded (or been intentionally established in) flow-stabilized river environments (Moyle 1986, Moyle and Light 1996). Often top predators, these introduced fish can devastate native river fish and threaten commercially valuable stocks (Stanford et al. 1996). In the southwestern United States, virtually the entire native river fish fauna is listed as threatened under the Endangered Species Act, largely as a consequence of water withdrawal, flow stabilization, and exotic species proliferation. The last remaining strongholds of native river fishes are all in dynamic, free-flowing rivers, where exotic fishes are periodically reduced by natural flash floods (Minckley and Deacon 1991, Minckley and Meffe 1987).

Flow stabilization also reduces the magnitude and frequency of overbank flows, affecting riparian plant species and communities. In rivers with constrained canyon reaches or multiple shallow channels, loss of high flows results in increased cover of plant species that would otherwise be removed by flood scour (Ligon et al. 1995, Williams and Wolman 1984). Moreover, due to other related effects of flow regulation, including increased water salinity, non-native vegetation often dominates, such as the salt cedar (*Tamarix* sp.) in the semiarid western United States (Busch and Smith 1995). In alluvial valleys, the loss of overbank flows can greatly modify riparian communities by causing plant desiccation, reduced growth, competitive exclusion, ineffective seed dispersal, or failure of seedling establishment (Table 2).

The elimination of flooding may also affect animal species that depend on terrestrial habitats. For example, in the flow-stabilized Platte River of the United States Great Plains, the channel has narrowed dramatically (up to 85%) over a period of decades (Johnson 1994). This narrowing has been facilitated by vegetative colonization of sandbars that formerly provided nesting habitat for the threatened piping plover (*Charadius melodius*) and endangered least tern (*Sterna antillarum*; Sidle et al. 1992). Sand-

hill cranes (*Grus canadensis*), which made the Platte River famous, have abandoned river segments that have narrowed the most (Krapu et al. 1984).

Changes in the duration of flow conditions also have significant biological consequences. Riparian plant species respond dramatically to channel dewatering, which occurs frequently in arid regions due to surface water diversion and groundwater pumping. These biological and ecological responses range from altered leaf morphology to total loss of riparian vegetation cover (Table 2). Changes in duration of inundation, independent of changes in annual volume of flow, can alter the abundance of plant cover types (Auble et al. 1994). For example, increased duration of inundation has contributed to the conversion of grassland to forest along a regulated Australian river (Bren 1992). For aquatic species, prolonged flows of particular levels can also be damaging. In the regulated Pecos River of New Mexico, artificially prolonged high summer flows for irrigation displace the floating eggs of the threatened Pecos bluntnose shiner (*Notropis sinius pecosensis*) into unfavorable habitat, where none survive (Robertson in press).

Modification of natural flow timing, or predictability, can affect aquatic organisms both directly and indirectly. For example, some native fishes in Norway use seasonal flow peaks as a cue for egg hatching, and river regulation that eliminates these peaks can directly reduce local population sizes of these species (Næsje et al. 1995). Furthermore, entire food webs, not just single species, may be modified by altered flow timing. In regulated rivers of northern California, the seasonal shifting of scouring flows from winter to summer indirectly reduces the growth rate of juvenile steelhead trout (*Oncorhyncus mykiss*) by increasing the relative abundance of predator-resistant invertebrates that divert energy away from the food chain leading to trout (Wootton et al. 1996). In unregulated rivers, high winter flows reduce these predator-resistant insects and favor species that are more palatable to fish.

Riparian plant species are also strongly affected by altered flow timing (Table 2). A shift in timing of peak flows from spring to summer, as often occurs when reservoirs are managed to supply irrigation water, has prevented reestablishment of the Fremont cottonwood (*Populus fremontii*), the dominant plant species in Arizona, because flow peaks now occur after, rather than before, its germination period (Fenner et al. 1985). Non-native plant species with less specific germination requirements may benefit from changes in flood timing. For example, salt cedar's (*Tamarix* sp.) long seed dispersal period allows it to establish after floods occurring any time during the growing season, contributing to its abundance on floodplains of the western United States (Horton 1977).

Altering the rate of change in flow can negatively affect both aquatic and riparian species. As mentioned above, loss of natural flashiness threatens most of the native fish fauna of the American Southwest (Minckley and Deacon 1991), and artificially increased rates of change caused by peaking power hydroelectric dams on historically less flashy rivers creates numerous ecological problems (Table 2; Petts 1984). A modified rate of change can devastate riparian species, such as cottonwoods, whose successful seedling growth depends on the rate of groundwater recession following floodplain inundation. In the St. Mary River in Alberta, Canada, for example, rapid drawdowns of river stage during spring have prevented the recruitment of young trees (Rood and Mahoney 1990). Such effects can be reversed, however. Restoration of the spring flood and its natural, slow recession in the Truckee River in California has allowed the successful establishment of a new generation of cotton-

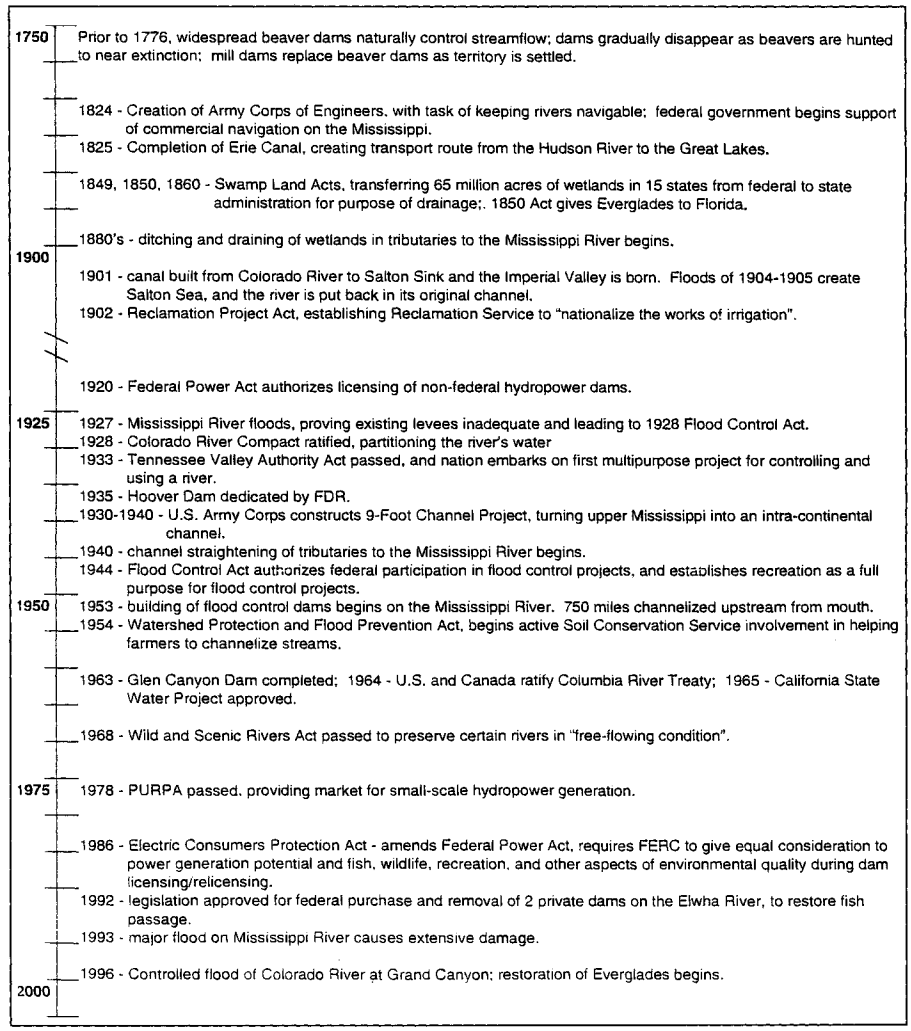

Figure 5. A brief history of flow alteration in the United States.

Table 3. Recent projects in which restoration of some component(s) of natural flow regimes has occurred or been proposed for specific ecological benefits.

Location	Flow component(s)	Ecological purpose(s)	Reference
Trinity River, CA	Mimic timing and magnitude of peak flow	Rejuvenate in-channel gravel habitats; restore early riparian succession; provide migration flows for juvenile salmon	Barinaga 1996[a]
Truckee River, CA	Mimic timing, magnitude, and duration of peak flow, and its rate of change during recession	Restore riparian trees, especially cottonwoods	Klotz and Swanson 1997
Owens River, CA	Increase base flows; partially restore overbank flows	Restore riparian vegetation and habitat for native fishes and non-native brown trout	Hill and Platts in press
Rush Creek, CA (and other tributaries to Mono Lake)	Increase minimum flows	Restore riparian vegetation and habitat for waterfowl and non-native fishes	LADWP 1995
Oldman River and tributaries, southern Alberta, Canada	Increase summer flows; reduce rates of postflood stage decline; mimic natural flows in wet years	Restore riparian vegetation (cottonwoods) and cold-water (trout) fisheries	Rood et al. 1995
Green River, UT	Mimic timing and duration of peak flow and duration and timing of nonpeak flows; reduce rapid baseflow fluctuations from hydropower generation	Recovery of endangered fish species; enhance other native fishes	Stanford 1994
San Juan River, UT/NM	Mimic magnitude, timing, and duration of peak flow; restore low winter baseflows	Recovery of endangered fish species	—[b]
Gunnison River, CO	Mimic magnitude, timing, and duration of peak flow; mimic duration and timing of nonpeak flows	Recovery of endangered fish species	—[b]
Rio Grande River, NM	Mimic timing and duration of floodplain inundation	Ecosystem processes (e.g., nitrogen flux, microbial activity, litter decomposition)	Molles et al. 1995
Pecos River, NM	Regulate duration and magnitude of summer irrigation releases to mimic spawning flow "spikes"; maintain minimum flows	Determine spawning and habitat needs for threatened fish species	Robertson 1997
Colorado River, AZ	Mimic magnitude and timing	Restore habitat for endangered fish species and scour riparian zone	Collier et al. 1997
Bill Williams River, AZ (proposed)	Mimic natural flood peak timing and duration	Promote establishment of native trees	USCOE 1996
Pemigewasset River, NH	Reduce frequency (i.e., to no more than natural frequency) of high flows during summer low-flow season; reduce rate of change between low and high flows during hydropower cycles	Enhance native Atlantic salmon recovery	FERC 1995
Roanoke River, VA	Restore more natural patterning of monthly flows in spring; reduce rate of change between low and high flows during hydropower cycles	Increased reproduction of striped bass	Rulifson and Manooch 1993
Kissimmee River, FL	Mimic magnitude, duration, rate of change, and timing of high- and low-flow periods	Restore floodplain inundation to recover wetland functions; reestablish in-channel habitats for fish and other aquatic species	Toth 1995

[a]J. Polos, 1997, personal communication. US Fish & Wildlife Service, Arcata, CA.
[b]F. Pfeifer, 1997, personal communication. US Fish & Wildlife Service, Grand Junction, CO.

wood trees (Klotz and Swanson 1997).

Recent approaches to streamflow management

Methods to estimate environmental flow requirements for rivers focus primarily on one or a few species that live in the wetted river channel. Most of these methods have the narrow intent of establishing minimum allowable flows. The simplest make use of easily analyzed flow data, of assumptions about the regional similarity of rivers, and of professional opinions of the minimal flow needs for certain fish species (e.g., Larson 1981).

A more sophisticated assessment of how changes in river flow affect aquatic habitat is provided by the Instream Flow Incremental Methodology (IFIM; Bovee and Milhous

1978). IFIM combines two models, a biological one that describes the physical habitat preferences of fishes (and occasionally macroinvertebrates) in terms of depth, velocity, and substrate, and a hydraulic one that estimates how the availability of habitat for fish varies with discharge. IFIM has been widely used as an organizational framework for formulating and evaluating alternative water management options related to production of one or a few fish species (Stalnaker et al. 1995).

As a predictive tool for ecological management, the IFIM modeling approach has been criticized both in terms of the statistical validity of its physical habitat characterizations (Williams 1996) and the limited realism of its biological assumptions (Castleberry et al. 1996). Field tests of its predictions have yielded mixed results (Morehardt 1986). Although this approach continues to evolve, both by adding biological realism (Van Winkle et al. 1993) and by expanding the range of habitats modeled (Stalnaker et al. 1995), in practice it is often used only to establish minimum flows for "important" (i.e., game or imperiled) fish species. But current understanding of river ecology clearly indicates that fish and other aquatic organisms require habitat features that cannot be maintained by minimum flows alone (see Stalnaker 1990). A range of flows is necessary to scour and revitalize gravel beds, to import wood and organic matter from the floodplain, and to provide access to productive riparian wetlands (Figure 4). Interannual variation in these flow peaks is also critical for maintaining channel and riparian dynamics. For example, imposition of only a fixed high-flow level each year would simply result in the equilibration of in-channel and floodplain habitats to these constant peak flows.

Moreover, a focus on one or a few species and on minimum flows fails to recognize that what is "good" for the ecosystem may not consistently benefit individual species, and that what is good for individual species may not be of benefit to the ecosystem. Long-term studies of naturally variable systems show that some species do best in wet years, that other species do best in dry years, and that overall biological diversity and ecosystem function benefit from these variations in species success (Tilman et al. 1994). Indeed, experience in river restoration clearly shows the impossibility of simultaneously engineering optimal conditions for all species (Sparks 1992, 1995, Toth 1995). A holistic view that attempts to restore natural variability in ecological processes and species success (and that acknowledges the tremendous uncertainty that is inherent in attempting to mechanistically model all species in the ecosystem) is necessary for ecosystem management and restoration (Franklin 1993).

Managing toward a natural flow regime

The first step toward better incorporating flow regime into the management of river ecosystems is to recognize that extensive human alteration of river flow has resulted in widespread geomorphic and ecological changes in these ecosystems. The history of river use is also a history of flow alteration (Figure 5). The early establishment of the US Army Corps of Engineers is testimony to the importance that the nation gave to developing navigable water routes and to controlling recurrent large floods. However, growing understanding of the ecological impacts of flow alteration has led to a shift toward an appreciation of the merits of free-flowing rivers. For example, the Wild and Scenic Rivers Act of 1968 recognized that the flow of certain rivers should be protected as a national resource, and the recent blossoming of natural flow restoration projects (Table 3) may herald the beginning of efforts to undo some of the damage of past flow alterations. The next century holds promise as an era for renegotiating human relationships with rivers, in which lessons from past experience are used to direct wise and informed action in the future.

A large body of evidence has shown that the natural flow regime of virtually all rivers is inherently variable, and that this variability is critical to ecosystem function and native biodiversity. As we have already discussed, rivers with highly altered and regulated flows lose their ability to support natural processes and native species. Thus, to protect pristine or nearly pristine systems, it is necessary to preserve the natural hydrologic cycle by safeguarding against upstream river development and damaging land uses that modify runoff and sediment supply in the watershed.

Most rivers are highly modified, of course, and so the greatest challenges lie in managing and restoring rivers that are also used to satisfy human needs. Can reestablishing the natural flow regime serve as a useful management and restoration goal? We believe that it can, although to varying degrees, depending on the present extent of human intervention and flow alteration affecting a particular river. Recognizing the natural variability of river flow and explicitly incorporating the five components of the natural flow regime (i.e., magnitude, frequency, duration, timing, and rate of change) into a broader framework for ecosystem management would constitute a major advance over most present management, which focuses on minimum flows and on just a few species. Such recognition would also contribute to the developing science of stream restoration in heavily altered watersheds, where, all too often, physical channel features (e.g., bars and woody debris) are re-created without regard to restoring the flow regime that will help to maintain these re-created features.

Just as rivers have been incrementally modified, they can be incrementally restored, with resulting improvements to many physical and biological processes. A list of recent efforts to restore various components of a natural flow regime (that is, to "naturalize" river flow) demonstrates the scope for success (Table 3). Many of the projects summarized in Table 3 represent only partial steps toward full flow restoration, but they have had demonstrable ecological benefits. For example, high flood flows followed by mimicked natural rates of flow decline in the Oldman River of Alberta, Canada, resulted in a massive cottonwood recruitment that extended for more than 500 km downstream from the Oldman Dam. Dampening of the unnatural flow fluctuations caused by hydroelectric generation on the Roanoke River in

Virginia has increased juvenile abundances of native striped bass. Mimicking short-duration flow spikes that are historically caused by summer thunderstorms in the regulated Pecos River of New Mexico has benefited the reproductive success of the Pecos bluntnose shiner.

We also recognize that there are scientific limits to how precisely the natural flow regime for a particular river can be defined. It is possible to have only an approximate knowledge of the historic condition of a river, both because some human activities may have preceded the installation of flow gauges, and because climate conditions may have changed over the past century or more. Furthermore, in many rivers, year-to-year differences in the timing and quantity of flow result in substantial variability around any average flow condition. Accordingly, managing for the "average" condition can be misguided. For example, in human-altered rivers that are managed for incremental improvements, restoring a flow pattern that is simply proportional to the natural hydrograph in years with little runoff may provide few if any ecological benefits, because many geomorphic and ecological processes show nonlinear responses to flow. Clearly, half of the peak discharge will not move half of the sediment, half of a migration-motivational flow will not motivate half of the fish, and half of an overbank flow will not inundate half of the floodplain. In such rivers, more ecological benefits would accrue from capitalizing on the natural between-year variability in flow. For example, in years with above-average flow, "surplus" water could be used to exceed flow thresholds that drive critical geomorphic and ecological processes.

If full flow restoration is impossible, mimicking certain geomorphic processes may provide some ecological benefits. Well-timed irrigation could stimulate recruitment of valued riparian trees such as cottonwoods (Friedman et al. 1995). Strategically clearing vegetation from river banks could provide new sources of gravel for sediment-starved regulated rivers with reduced peak flows (e.g., Ligon et al. 1995). In all situations, managers will be required to make judgments about specific restoration goals and to work with appropriate components of the natural flow regime to achieve those goals. Recognition of the natural flow variability and careful identification of key processes that are linked to various components of the flow regime are critical to making these judgments.

Setting specific goals to restore a more natural regime in rivers with altered flows (or, equally important, to preserve unaltered flows in pristine rivers) should ideally be a cooperative process involving river scientists, resource managers, and appropriate stakeholders. The details of this process will vary depending on the specific objectives for the river in question, the degree to which its flow regime and other environmental variables (e.g., thermal regime, sediment supply) have been altered, and the social and economic constraints that are in play. Establishing specific criteria for flow restoration will be challenging because our understanding of the interactions of individual flow components with geomorphic and ecological processes is incomplete. However, quantitative, river-specific standards can, in principle, be developed based on the reconstruction of the natural flow regime (e.g., Richter et al. 1997). Restoration actions based on such guidelines should be viewed as experiments to be monitored and evaluated—that is, adaptive management—to provide critical new knowledge for creative management of natural ecosystem variability (Table 3).

To manage rivers from this new perspective, some policy changes are needed. The narrow regulatory focus on minimum flows and single species impedes enlightened river management and restoration, as do the often conflicting mandates of the many agencies and organizations that are involved in the process. Revisions of laws and regulations, and redefinition of societal goals and policies, are essential to enable managers to use the best science to develop appropriate management programs.

Using science to guide ecosystem management requires that basic and applied research address difficult questions in complex, real-world settings, in which experimental controls and statistical replication are often impossible. Too little attention and too few resources have been devoted to clarifying how restoring specific components of the flow regime will benefit the entire ecosystem. Nevertheless, it is clear that, whenever possible, the natural river system should be allowed to repair and maintain itself. This approach is likely to be the most successful and the least expensive way to restore and maintain the ecological integrity of flow-altered rivers (Stanford et al. 1996). Although the most effective mix of human-aided and natural recovery methods will vary with the river, we believe that existing knowledge makes a strong case that restoring natural flows should be a cornerstone of our management approach to river ecosystems.

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Benthic cyanobacteria and filamentous chlorophytes affect macroinvertebrate assemblages in a large fluvial lake

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Abstract. Proliferations of filamentous chlorophytes and mats of cyanobacteria (hereafter termed metaphyton) are increasingly observed in rivers, lakes, wetlands, and estuaries undergoing eutrophication, but their contribution to invertebrate production and overall ecological significance remains poorly understood. In Lake Saint-Pierre, a shallow widening of the St. Lawrence River (Québec), vascular macrophytes (mainly *Vallisneria americana*) grow in combination with filamentous chlorophytes (*Hydrodictyon*, *Oedogonium*) in the upstream reach, which is fed by nutrient-rich waters from the tributaries, and in association with filamentous cyanobacteria (*Lyngbya wollei*) in the chronically NO_3^--depleted downstream reach. We hypothesized that different vegetation types (macrophytes, filamentous chlorophytes, and cyanobacteria) would support macroinvertebrate communities with different biomasses and taxonomic compositions. We expected a higher invertebrate biomass in the upstream reach and, within the reach, a higher biomass on metaphyton than on macrophytes. Total macroinvertebrate biomass was significantly higher at the enriched stations in the upstream reach (75–100 mg/g vegetation, dry mass) than farther downstream (8–38 mg/g). In addition, macrophytes and metaphyton in the upstream reach sustained taxonomically different invertebrate assemblages. Gastropods dominated the fauna associated with macrophytes throughout the lake (43–73%) and probably benefitted from a structurally simple and solid substratum on which to crawl and feed. Small mobile taxa, such as cladocerans, copepods, chironomids, and ostracods, were more abundant on filamentous metaphyton, both up- and downstream. Amphipods were dominant (59%) in metaphytic mats of *L. wollei*. At the scale of the river reach, macrophytes supported most of the invertebrate biomass. Chlorophytes in the upstream reach contributed <5% of the total biomass, representing an alternative, albeit temporary, habitat. In contrast, in the downstream reach where macrophytes were scarce, cyanobacterial mats hosted a significant fraction of macroinvertebrates (36%). Shifts in vegetation between the 2 reaches affected the quantity and availability of prey items for fish predators.

Key words: vascular macrophytes, macroinvertebrates, *Vallisneria americana*, filamentous algae, *Lyngbya wollei*, St. Lawrence River, Lake Saint-Pierre, *Hydrodictyon*, *Oedogonium*.

Proliferation of metaphytic filamentous chlorophytes and cyanobacteria is a recurring problem in rivers, lakes, wetlands, and estuaries (Wu and Mitsch 1998, Havens et al. 2003, Elmetri and Bell 2004, Hagerthey and Kerfoot 2005). Excessive algal growth is a well-documented consequence of eutrophication of aquatic ecosystems caused by nutrient-rich agricultural or urban waste waters (Leland and Porter 2000, Dodds et al. 2002, Sabater et al. 2003).

Metaphyton is a major primary producer (Stevenson et al. 1996) that exerts both positive and negative effects on the aquatic environment. Metaphyton can reduce aqueous nutrient concentrations by uptake (Wu and Mitsch 1998), or it can release dissolved nutrients during decomposition (Keiper and Foote 1999, Kraufvelin et al. 2006). In addition, metaphyton hosts a diversified invertebrate fauna (Liston and

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Trexler 2005, Tessier et al. 2007), functions as a food source for invertebrate grazers (Cattaneo 1983, Valiela et al. 1997), and provides a refuge from fish predation (Mbahinzireki et al. 1991, Pihl et al. 1995, Kraufvelin et al. 2006). Conversely, metaphytic blooms can have a negative impact on macroinvertebrate communities by amplifying daily dissolved O_2 variations and inducing episodic hypoxic or anoxic events (Valiela et al. 1997). In addition, some species, mostly cyanobacteria, produce toxins (Onodera et al. 1997, Chorus and Bartram 1999, Camacho 2008, Gérard et al. 2008) or have morphological structures (e.g., thick sheaths) (Camacho and Thacker 2006) that deter grazers. Metaphytic blooms usually are dominated by chlorophytes (Valiela et al. 1997), although massive developments of benthic cyanobacteria have been documented in many systems with low N:P ratios (Whitton and Potts 2000, Sabater et al. 2003, Albert et al. 2005, Vis et al. 2008). Under high P concentration, some benthic cyanobacteria (e.g., *Lyngbya*) are able to fix atmospheric N (Elmetri and Bell 2004).

Macroinvertebrates are a crucial trophic link between primary producers and vertebrate consumers. Because of their high abundance and their diversified response to environmental changes, they are a useful tool for evaluating the effects of anthropogenic perturbations, particularly eutrophication (Rosenberg and Resh 1993, McCormick et al. 2004). However, few studies have compared assemblages in different vegetative habitats within the same aquatic system (McCreary Waters and San Giovanni 2002, White and Irvine 2003, Liston and Trexler 2005, Tessier et al. 2007).

Metaphyton and vascular macrophytes differ greatly in their architecture and in their surrounding environment, which can influence the associated macroinvertebrate assemblage (Rosenberg and Resh 1993, Norkko et al. 2000, Salovius and Kraufvelin 2004). One gram of metaphyton supports a considerably higher biomass of macroinvertebrates than do submerged and emergent macrophytes, but their effect might be negligible at the whole-lake scale because blooms of filamentous algae are short-lived and spatially dispersed (Tessier et al. 2007). Changes in the dominant vegetation type (between macrophytes and metaphyton) could lead to a shift in the dominant invertebrate species (Liston and Trexler 2005).

Vis et al. (2008) showed that, as nutrient-rich water flowed slowly through submerged plant beds along the south shore of Lake Saint-Pierre, a 30-km-long widening of the St. Lawrence River, metaphyton communities initially dominated by filamentous chlorophytes were progressively replaced by benthic cyanobacterial mats (*Lyngbya wollei*). Vascular macrophytes (mainly *Vallisneria americana*) grew in combination with either metaphytic chlorophytes (upstream reach) or metaphytic cyanobacteria (downstream reach). Such a shift in metaphyton composition might affect the macroinvertebrates and, ultimately, the entire trophic network (Kornijów et al. 2005). The consequences of such a shift could be significant because Lake Saint-Pierre (LSP) is the largest (>300 km^2) of the fluvial lakes of the St. Lawrence River (Jean et al. 2002) and is a key habitat for 13 amphibian and 79 fish species, many of which are exploited by sports and commercial fisheries (SLC 1996). The ecological value of Lake Saint-Pierre has been recognized by its status as a Ramsar Wetland (in 1998; www.ramsar.org) and a United Nations Educational, Scientific, and Cultural Organization (UNESCO) Biosphere Reserve (in 2000; www.unesco.org/mab/).

We hypothesized that: 1) stations in the upstream reach of LSP, under the direct influence of enriched tributaries, would support a higher biomass of invertebrates than the stations in the downstream reach, and that 2) within a reach, structurally complex metaphyton would support significantly more invertebrate biomass/g vegetation dry mass (DM) than would macrophytes.

We characterized macroinvertebrate biomass (/g vegetation) and taxonomic composition on macrophytes and metaphyton (filamentous chlorophytes or cyanobacteria) at 5 stations located at increasing distances from the nutrient-rich tributaries along the south shore of LSP. We then assessed the relative importance of metaphyton as a support for invertebrates at the scale of the river reach, taking into account the areal biomass of each vegetation type over a 15-km-long section of LSP.

Methods

Study area

LSP (lat 46°12′N, long 72°49′W) is a large fluvial lake (~300 km^2) of the St. Lawrence River, located ~75 km downstream of Montreal (Fig. 1). The river broadens to 10–12 km in LSP proper; this reach of the river is relatively shallow (mean depth ~3 m) and slow-flowing (<0.5 m/s), with the exception of a 250-m-wide, man-made (1854–2001) central navigation channel (>11-m deep). This fluvial lake is characterized by large, sheltered bays and a shallow sloping shoreline that supports large emergent marshes and extensive beds of submerged aquatic vegetation to a depth of ~3 m (Hudon 1997). During the summer, submerged aquatic plants cover 260 km^2 (85%) of the lake surface

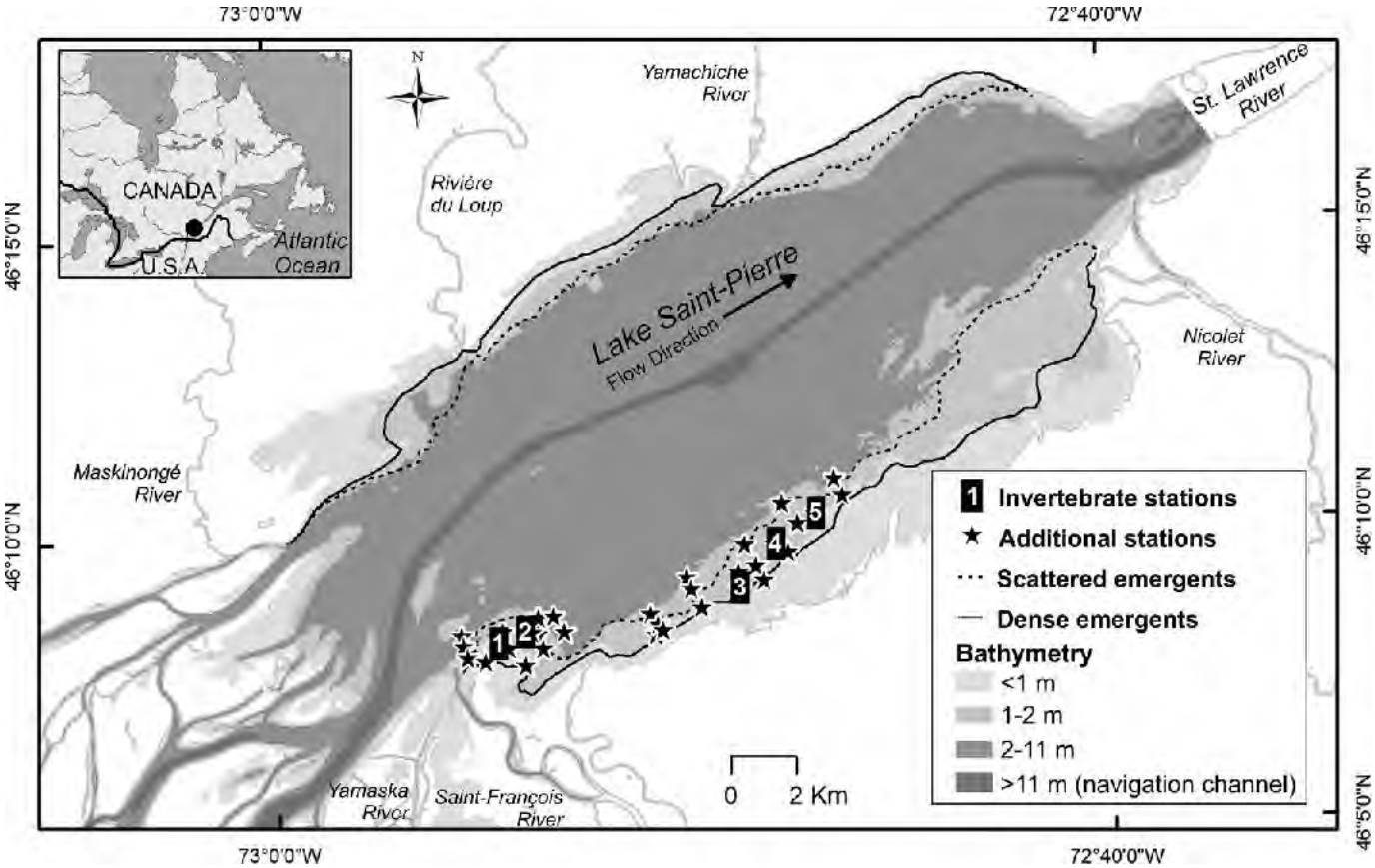

FIG. 1. Map of the study sites in Lake Saint-Pierre (St. Lawrence River, Québec, Canada, inset). Stations 1 and 2 were in the upstream reach of Lake Saint-Pierre, near the mouth of the Yamaska and Saint-François Rivers. Stations 3, 4, and 5 were downstream. Additional stations (stars) sampled in August and September 2006 were used to extrapolate results to the scale of each river reach, characterized by shallow (1–2 m) littoral areas where a mixture of submerged (dotted line) and scattered emergent (full line) wetland vegetation was found.

area (mean biomass 54 g DM/m^2) and represent an annual production of 8700 tonnes (t) of C (Vis et al. 2007). Lake Saint-Pierre is eutrophying because of excessive inputs of nutrients entering from tributaries draining agricultural lands (Hudon and Carignan 2008).

Sampling was done at 5 stations situated on the south shore of LSP (Fig. 1). The first 2 stations were in the upstream area close to the inflow of 2 tributaries draining farmlands (i.e., the Saint-François and Yamaska rivers). In this reach, macrophyte biomass was high and proliferations of metaphytic chlorophytes were commonly observed as a response to high concentrations of N and P. As water slowly percolated through the shallow, dense macrophyte bed, intense biological activity induced a sharp drop in dissolved inorganic N (DIN) concentration, probably from bacterial denitrification (Hudon and Carignan 2008, Hudon et al. 2009). The other 3 stations were in the downstream part of this gradient, where total P (TP) remained high but DIN dropped to concentrations near the detection limit, coinciding with the area of massive developments of the metaphytic cyanobacterium *L. wollei* (Vis et al. 2008).

Characterization of physical and chemical variables

Sampling was done on 19–21 September 2006, at the time of highest vegetation biomass in this system (Vis et al. 2006). At each station, water temperature (°C), conductivity (μS/cm), pH, and dissolved O_2 (mg/L and % of saturation) were measured 30 cm below the surface with a YSI 600 XLM multiprobe (Yellow Springs Instruments, Inc., Yellow Springs, Ohio). Current velocity was measured at a depth of 30 cm (FlowTracker acoustic Doppler velocimeter; SonTek/YSI, San Diego, California). Water samples were collected below the surface and brought back to the laboratory for analyses of suspended particulate matter (SPM) (APHA 1998), total P (TP) (Environne-

ment Canada 2005) and total N (TN) (LACHAT Continuous Flow Quick-Chem 8000, Method No 10-107-06-2E). Samples were filtered (Whatman GF/C) for analysis of total dissolved P (TDP), NH_4, NO_2-NO_3, dissolved organic C (DOC), and color (Platinum-Cobalt units [Pt-Co]) (Environnement Canada 2005).

Estimation of macrophyte and metaphyton biomass

Submerged vegetation was collected over a 1-m bottom strip with a 35-cm-wide (0.35 m^2), double-headed rake (Yin et al. 2000). Comparison between the wet plant areal biomass collected with rakes (semiquantitative sampling from the surface) and quadrats (in situ quantitative sampling by a diver) yielded significant linear relationships (Pearson r parametric correlations, $\log_{10}(x)$-transformed biomass values) for macrophytes ($r = 0.70$, $p < 0.001$, $n = 114$ pairs of samples), filamentous chlorophytes ($r = 0.63$, $p < 0.05$, $n = 11$), and filamentous cyanobacteria ($r = 0.91$, $p < 0.001$, $n = 53$) (CH, unpublished data). Mean plant biomass and dominant vascular species composition were estimated from 8–10 rake samples collected systematically around the boat at each site after sampling the macroinvertebrates. Masses of filamentous metaphyton were separated by hand from the macrophytes in a bucket of water. Metaphyton and macrophytes were weighed on the boat after washing off sediment and wringing out excess water. Wet mass of each vegetation type was converted to DM with previously published equations (Hudon and Lalonde 1998). Metaphyton was subsampled and preserved in Lugol's solution to characterize proportional representation of the different algal genera occurring on a total of 100 microscopic fields (10 slides × 10 fields) at a magnification of 100× (Vis et al. 2008).

Macroinvertebrate sampling and biomass estimation

Macroinvertebrates associated with macrophytes were sampled at all 5 stations, whereas those associated with metaphyton were sampled at 1 station upstream (2) and 1 station downstream (4), which were dominated by filamentous chlorophytes and cyanobacteria, respectively. Macrophytes and metaphyton growing side by side were collected separately by a diver, who gently closed a rectangular Plexiglas box (5.7 L) around the vegetation and cut around the edges of the box before retrieval (Downing and Cyr 1985). This device allowed quantitative sampling of all invertebrates, including those loosely associated with the vegetation. Four replicates of both vegetation types (macrophytes and metaphyton) were collected at stations 2 and 4. Three replicates of macrophytes alone were collected at stations 1, 3, and 5. The water in the Plexiglas box was washed through a 500-μm sieve to retain floating invertebrates. All organisms were preserved in ethanol (70%) until they could be analyzed. Invertebrates were sorted under a dissecting microscope and generally identified to the order level. Gastropods, the dominant group, were identified to the family level. Cladocerans and heteropterans also were identified to the family level to improve estimates of their biomass by length–mass relationships, but statistical analyses were done at the order level, as for the other groups.

After sorting the invertebrates and rinsing off the debris, macrophytes and metaphyton retained by the Plexiglas box were dried (48 h at 60°C) and weighed (±0.05 mg) to estimate macroinvertebrate density/g vegetation DM. Invertebrate length was measured with an image-analyzer system connected to a dissecting microscope (Image Pro-Plus; Media Cybernetics Inc., Bethesda, Maryland). At each station, gastropod length was measured in each replicate sample of all individuals, or of a subsample when >50 organisms were present for a given family. For the other invertebrate groups, length of all individuals was measured in the replicate with the greatest number of organisms. Body-length measurements were converted to DM with published length–biomass equations (Tourville Poirier 2009). Biomass of invertebrate taxa was expressed as mg/g vegetation DM.

Extrapolation of invertebrate biomass to the reach scale

Areal invertebrate biomass (mg invertebrates/m^2 river bottom) at the reach scale was calculated as the product of the invertebrate biomass/g vegetation DM measured at stations 2 and 4 and vegetation biomass/m^2 at each reach. For this calculation, we used vegetation biomass data obtained from additional stations surveyed in August and September 2006 in the upstream ($n = 17$ stations) and downstream ($n = 24$ stations) reaches (Fig. 1). These additional stations allowed us to ascertain the stability of the physical and chemical gradients and to refine our biomass estimates of the different types of vegetation in each reach. This refinement was particularly important for chlorophytes, whose biomass was highly variable in time and space. Physical and chemical characteristics of the water and biomasses of macrophytes and metaphyton were measured at each of these additional sites with the methods described above. Furthermore, epiphytic biomass (μg Chl *a*/g vegetation DM) on *V. americana* was measured at a subset of these stations ($n = 10$ upstream, $n = 8$ downstream) to

TABLE 1. Environmental characteristics and vegetation biomass at 5 stations in Lake Saint-Pierre in September 2006. TDP = total dissolved P, TP = total P, TN = total N, DOC = dissolved organic C, DM = dry mass.

Variables	1	2	3	4	5
Reach	Upstream	Upstream	Downstream	Downstream	Downstream
Distance from effluent mouth (km)	2.35	3.78	9.74	11.67	13.26
Depth (m)	0.65	0.65	0.84	0.67	0.92
Temperature (°C)	15.10	14.75	18.58	18.42	18.32
Conductivity (μS/cm)	219	226	192	191	185
Current speed (cm/s)	4.0	0.5	2.3	3.7	4.5
pH	8.59	7.96	8.9	9.09	8.86
O_2 (% saturation)	50.0	30.6	46.6	43.5	32.2
O_2 (mg/L)	5.03	3.10	4.36	4.07	3.05
Light extinction coefficient (K_{water}, m^{-1})	2.13	2.24	0.59	2.37	1.12
TDP (μg P/L)	13	9	7	8	8
TP (μg P/L)	29	17	9	14	9
NH_4 (μg N/L)	<10	<10	13	<10	<10
NO_2-NO_3 (μg N/L)	320	330	<30	<30	<30
TN (μg N/L)	691	709	339	362	354
DOC (mg C/L)	6.80	6.64	6.74	6.79	7.04
Color (Pt-Co)	32.96	31.60	26.78	28.5	26.43
Suspended matter (mg/L)	4.7	5.8	<3	6.7	<3
Macrophytes DM (g/m^2)	220.3	670.1	140.3	39.1	107.8
Cyanobacteria DM (g/m^2)	0.0	0.0	9.67	4.3	12.2
Chlorophytes DM (g/m^2)	0.0	0.8	0.0	0.0	0.0

determine the food resources available to invertebrate grazers. Epiphytes were removed manually from 10 *V. americana* leaves, which were subsequently dried (60°C) and weighed (0.1 mg). Chl *a* content of the epiphytes was determined using spectrophotometry after cold ethanol extraction (Nusch 1980). Areal values of invertebrate biomass were then used to compare the relative contribution of macrophytes and metaphyton to the total biomass of phytophilous macroinvertebrates in the 2 reaches of the fluvial lake.

Statistical analyses

Differences in total macroinvertebrate biomass associated with macrophytes along the upstream and downstream gradient were assessed by contrasting values recorded at the 5 stations (n = 3, 4, 3, 4, 3 at stations 1–5, respectively), with a 1-way analysis of variance (ANOVA) between stations ($\log_{10}[x]$-transformed data) with permutation tests (R-package; Ihaka and Gentleman 1996, Legendre 2009), followed by Tukey's comparison of means between stations (Statistix for Windows, version 8; Analytical Software, Tallahassee, Florida). Testing the significance of ANOVA F-values with a permutation test improves the robustness of the test to unbalanced sampling design because the probability distribution is generated from the random permutation of values rather than from a standard table.

Differences in total invertebrate biomass/g DM of macrophytes (n = 4) and metaphyton (n = 4) were tested between upstream station 2 and downstream station 4 (2-tailed t-test for samples of unequal variance; Microsoft Office Excel 2003; Microsoft Corporation, Redmond, Washington). We also compared total invertebrate biomass/g DM of chlorophytes (n = 4 replicate samples at upstream station 2) and cyanobacteria (n = 4 replicate samples at downstream station 4) with the same statistical method. Epiphyte biomass on *V. americana* (μg Chl *a*/g vegetation DM) at the upstream (n = 10) and downstream (n = 8) reaches was compared with a 1-tailed t-test for samples of unequal variance (Microsoft Office Excel).

The association between major macroinvertebrate taxa and vegetation types at stations 2 and 4 was assessed with a redundancy analysis (RDA; rdaTest function, R-package; Legendre and Durand 2009). A matrix of binary codes for vegetation types and stations was used in this analysis. To minimize the impact of taxon absences, biomass data (y_{ij}) were transformed prior to analysis using the equation $y_{ij}' = \sqrt{(y_{ij}/y_{i+})}$ (Hellinger transformation; Legendre and Gallagher 2001).

Results

Characteristics of the sampling stations

All stations were in shallow (<1 m), slow-flowing (<5 cm/s) beds of submerged aquatic vegetation (Table 1) dominated by *V. americana*, interspersed

Table 2. Mean (±SD) total biomass (mg/g vegetation dry mass) of the major taxa of macroinvertebrates found in macrophytes and metaphyton (filamentous chlorophytes and *Lyngbya wollei*) at 5 stations in Lake Saint-Pierre in September 2006. – indicates taxon not present.

		Upstream			Downstream			
		Station 1	Station 2		Station 3	Station 4		Station 5
Taxa	Code	Macrophytes $n = 3$	Macrophytes $n = 4$	Chlorophytes $n = 4$	Macrophytes $n = 3$	Macrophytes $n = 4$	*L. wollei* $n = 4$	Macrophytes $n = 3$
Turbellaria	PLA	0.1 (0.05)	1.9 (0.7)	2.0 (0.6)	0.3 (0.1)	0.2 (0.1)	1.8 (1.30)	0.024 (0.04)
Mollusca								
Gastropoda	GAS	88.5 (16.5)	49.2 (18.2)	26.7 (16.5)	3.8 (1.7)	9.1 (8.5)	4.9 (2.6)	8.4 (8.3)
Ancylidae	ANC	3.3 (3.1)	0.8 (0.5)	0.5 (0.3)	0.4 (0.4)	0.4 (0.7)	0.01 (0.02)	0.2 (0.07)
Bithyniidae	BIT	37.1 (4.1)	22.0 (10.4)	9.6 (8.3)	2.6 (1.7)	6.9 (6.0)	4.0 (3.1)	8.3 (6.5)
Physidae	PHY	6.1 (7.9)	21.2 (18.8)	12.5 (7.0)	0.8 (0.8)	0.05 (0.07)	0.2 (0.3)	–
Planorbidae	PBD	31.8 (10.0)	1.3 (1.5)	0.9 (0.5)	–	–	0.02 (0.04)	–
Valvatidae	VAL	4.9 (1.1)	0.6 (1.1)	0.05 (0.06)	–	–	0.3 (0.6)	–
Viviparidae	VIV	5.4 (1.0)	3.4 (4.1)	4.0 (7.3)	–	1.8 (3.2)	0.3 (0.4)	0.3 (0.5)
Annelida								
Hirudinea	HIR	–	–	0.03 (0.06)	–	–	–	–
Oligochaeta	OLI	0.9 (0.03)	9.3 (6.7)	12.7 (5.8)	0.2 (0.03)	0.03 (0.04)	0.4 (0.2)	0.4 (0.2)
Hydrachnidia	HCA	<0.01	0.01 (0.005)	0.03 (0.01)	<0.01	<0.01	0.01 (0.01)	<0.01
Insecta								
Coleoptera	COL	–	–	–	–	–	0.2 (0.4)	–
Diptera								
Chironomidae								
Larvae	CHI	5.1 (2.5)	0.6 (0.4)	2.2 (0.9)	2.1 (0.5)	0.3 (0.2)	3.8 (2.0)	0.6 (0.8)
Pupae	NYM	0.5 (0.3)	0.06 (0.04)	0.2 (0.3)	0.05 (0.02)	–	0.08 (0.1)	0.4 (0.7)
Tipulidae	TIP	–	–	–	–	–	<0.01	–
Ephemeroptera	EPH	<0.01	–	–	–	–	<0.01	0.03 (0.05)
Hemiptera								
Corixidae	HEC	–	0.08 (0.15)	0.07 (0.003)	–	–	–	–
Hebridae	HEH	–	0.08 (0.04)	0.13 (0.02)	–	<0.01	0.06 (0.1)	–
Lepidoptera	LEP	0.09 (0.08)	0.2 (0.1)	0.08 (0.1)	0.3 (0.3)	0.1 (0.1)	<0.01	–
Odonata	ODO	–	–	<0.01	0.06 (0.08)	–	–	0.01 (0.02)
Trichoptera	TRI	0.06 (0.03)	1.0 (0.6)	6.4 (2.4)	0.1 (0.1)	0.07 (0.05)	0.1 (0.1)	0.1 (0.2)
Crustacea								
Amphipoda	AMP	0.1 (0.07)	2.8 (0.7)	8.2 (2.7)	0.3 (0.1)	2.5 (2.0)	25.4 (7.8)	1.3 (1.7)
Cladocera	CLA	0.2 (0.1)	1.0 (0.6)	3.5 (1.6)	0.01 (0.005)	<0.01	0.08 (0.03)	0.04 (0.01)
Copepoda	COP	0.04 (0.03)	0.2 (0.1)	1.5 (1.2)	<0.01	<0.01	0.4 (0.2)	<0.01
Ostracoda	OST	<0.01	0.04 (0.01)	0.02 (0.01)	0.02 (0.007)	<0.01	0.7 (0.4)	<0.01

TABLE 2. Continued.

Taxa	Code	Upstream			Downstream			
		Station 1	Station 2		Station 3	Station 4		Station 5
		Macrophytes $n = 3$	Macrophytes $n = 4$	Chlorophytes $n = 4$	Macrophytes $n = 3$	Macrophytes $n = 4$	*L. wollei* $n = 4$	Macrophytes $n = 3$
Hydra	HYD	0.2 (0.1)	2.8 (0.9)	3.8 (1.9)	0.9 (0.1)	0.2 (0.3)	0.4 (0.3)	1.3 (0.7)
Nematoda	NEM	–	–	–	0.05 (0.03)	–	0.03 (0.03)	0.3 (0.4)
Total biomass		95.8 (14.1)	69.3 (24.7)	68.1 (10.6)	8.1 (1.4)	12.4 (10.5)	38.3 (7.7)	12.9 (8.5)

with *Potamogeton richardsonii* and *Heteranthera dubia* (upstream only). Several environmental characteristics at the 5 stations showed a strong spatial variation (Table 1). Upstream stations 1 and 2 had high nutrient and suspended solid concentrations and elevated macrophyte biomass (Table 1). Conspicuous patches of filamentous chlorophytes, forming cloudy masses loosely attached to macrophytes or floating mats at the surface, were visible throughout the upstream reach but present in our samples at station 2 only. These sparse mats were formed by *Hydrodictyon* (72%), *Oedogonium* (27%), and *Spirogyra* (1%) (Table 1).

As water percolated slowly through the aquatic vegetation, NO_3^- concentrations dropped below the detection level concomitantly with a decrease in water conductivity. This decline was associated at downstream stations 3, 4, and 5 with reduced macrophyte biomass and the presence on the bottom of thick mats of the cyanobacterium *L. wollei* (99%), with only traces of chlorophytes (*Oedogonium* and *Rhizoclonium*, 1%). At these stations, metaphytic biomass was found mostly near the water bottom where it grew close to the sediment or tangled within the lower segment of vascular macrophytes.

Differences in macroinvertebrate biomass between stations and vegetation types

The total macroinvertebrate biomass associated with macrophytes was significantly higher at the upstream stations (1 and 2) than at the downstream stations (3–5) (1-way ANOVA, $F = 24.37$, $p = 0.001$, followed by multiple comparisons, $p < 0.05$; Table 2, Fig. 2A).

At station 2, macrophytes and metaphytic chlorophytes supported similarly high (69.3 and 68.1 mg invertebrates/g vegetation DM, respectively) biomasses of macroinvertebrates ($t = 0.087$, $p = 0.935$) (Table 2, Fig. 2B). In contrast, at station 4, macrophytes harbored a significantly smaller macroinvertebrate biomass (12.4 mg invertebrates/g vegetation DM) than metaphytic cyanobacteria (38.3 mg invertebrates/g vegetation DM) ($t = -3.993$, $p = 0.010$; Table 2, Fig. 2). As a result, metaphytic chlorophytes growing at the upstream station supported nearly 2× the invertebrate biomass hosted by cyanobacteria downstream ($t = 4.562$, $p = 0.006$; Fig. 2B).

Macroinvertebrate taxonomic composition

A total of 27 macroinvertebrate taxa were identified. Gastropods were composed of 6 families. Two taxa were found exclusively upstream (Heteroptera: Corixidae and Hirudinea), and 3 were found exclu-

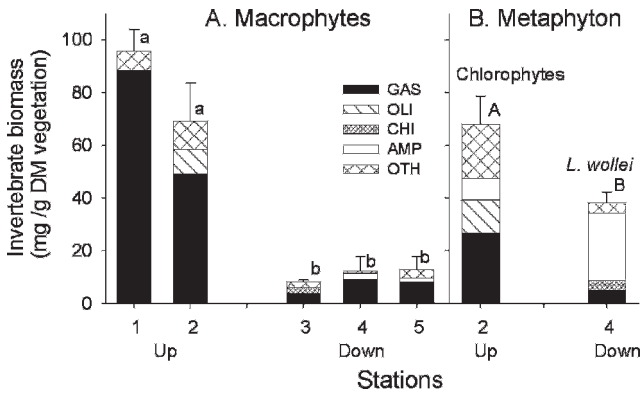

FIG. 2. Mean (±1 SE) total biomass (mg/g vegetation dry mass [DM]) of macroinvertebrates collected on macrophytes at 5 stations in the upstream (up) and downstream (down) reaches of Lake Saint-Pierre (A) and on metaphytic chlorophytes and cyanobacteria at stations 2 (upstream) and 4 (downstream) (B). Shading in bars shows the contributions of gastropods (GAS), oligochaetes (OLI), chironomids (CHI), amphipods (AMP), and other less abundant taxa (OTH; <10% of total biomass) to the total biomass. Bars with the same lowercase letters (stations) or uppercase letters (reaches) are not significantly different (macrophytes at all stations: 1-way analysis of variance and Tukey comparison of means between stations, $p < 0.001$; chlorophytes and *Lyngbya wollei* at stations 2 and 4: 2-tailed *t*-test, $p < 0.006$). $n = 4$ at stations 2 and 4, $n = 3$ at stations 1, 3, 5.

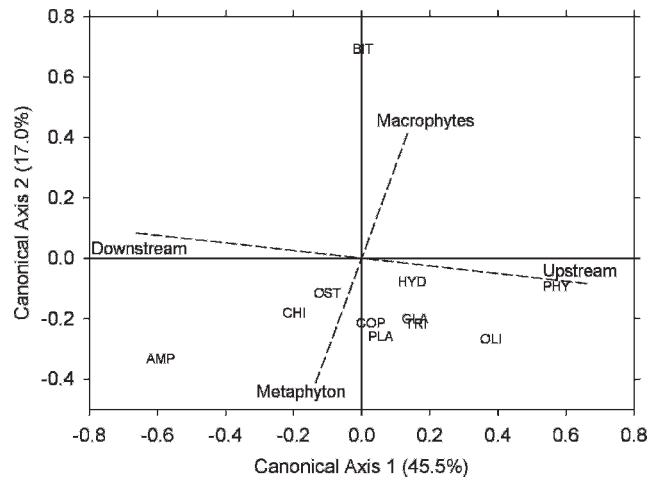

FIG. 3. Redundancy analysis (RDA) of macroinvertebrate taxa biomass (mg/g vegetation dry mass) in chlorophytes and *Lyngbya wollei* (metaphyton) and vascular macrophytes (macrophytes) sampled at stations 2 (upstream) and 4 (downstream) in Lake Saint-Pierre. Only taxa with >50% of their variance explained by RDA are represented. See Table 2 for abbreviations for macroinvertebrate taxa. Percent variance explained by each axis is shown in parentheses.

sively downstream (Coleoptera:Elmidae, Nematoda, and Diptera:Tipulidae; Table 2). Most nongastropod taxa were rare. Other than the 6 families of gastropods, only 9 taxa (amphipods, chironomid larvae, cladocerans, copepods, *Hydra*, oligochaetes, ostracods, planarians, and trichopterans) had biomass >1 mg g vegetation DM in ≥1 vegetation type. Gastropods dominated macroinvertebrate biomass (43 to 73% of total biomass) in the chlorophytes and macrophytes (Table 2, Fig. 2B). Amphipods were the dominant group (59%) associated with the benthic cyanobacterium *L. wollei* (Fig. 2B).

Among gastropods, Bithyniidae were the most important family and made up ~40% of the gastropod biomass at stations 1 and 2 and 69 to 94% at stations 3, 4, and 5. Stations 1 and 2 harbored all 6 gastropod families, whereas only Bithyniidae and rare Ancylidae and Viviparidae (3% each) were found at station 5 (Table 2).

The composition of the entire invertebrate assemblage was contrasted between vegetation types and stations with RDA (Fig. 3). The 1st axis (45.5% of variance explained) distinguished between assemblages found at upstream and downstream stations, whereas the 2nd axis (17.6% of variance) distinguished between macrophytes and metaphyton (filamentous chlorophytes and cyanobacteria). The analysis explained 57% of the total variance of the invertebrate assemblage (adjusted R^2). Stations were differentiated on the basis of the biomass of oligochaetes (82% of variance explained), Physidae (79%), *Hydra* (58%), and Trichoptera (54%), which were most abundant upstream, and amphipods (88%) and chironomids (60%), which dominated downstream. Gastropods (especially Bithyniidae [62%]) were associated with macrophytes, whereas most other invertebrates, including Turbellaria (50%) and several zooplanktonic taxa (copepods [74%], cladocerans [86%], ostracods [55%]), were strongly associated with filamentous algae (chlorophytes and cyanobacteria).

Contribution of different vegetation types at the reach scale

The ecological significance of the differences in invertebrate biomass in different types of vegetation was assessed by generalizing the measurements described above to a broader area of LSP with vegetation estimates from additional stations (Fig. 1) sampled in late summer 2006 in the upstream (mean ± SE, macrophytes: 107.3 ± 24.2 g DM/m^2, metaphytic chlorophytes: 5.7 ± 4.3 g DM/m^2, $n = 17$) and downstream (macrophytes: 28.5 ± 4.6 g DM/m^2, metaphytic *L. wollei*: 9.4 ± 1.6 g DM/m^2, $n = 24$) reaches (AC and CH, unpublished data). These

estimates were used to calculate metaphyton contribution to the total macroinvertebrate areal biomass. Metaphytic chlorophytes supported only 4.6% of the upstream macroinvertebrate biomass, whereas the metaphytic cyanobacterium *L. wollei* supported 36.4% of the downstream macroinvertebrate biomass (Fig. 4A–E). *Lyngbya wollei* was a particularly important habitat for chironomids and amphipods (Fig. 4D, E).

Reaches also differed markedly in terms of the biomass of epiphytes growing on *V. americana*, which was 2× higher upstream (17.5 ± 14.1 mg Chl a/m^2, n = 10 stations) than downstream (8.5 ± 10.0 mg Chl a/m^2, n = 8 stations), but this difference was only marginally significant (t = 1.59, 1-tailed p = 0.07) because of high variability.

Discussion

Comparison between upstream and downstream stations

The markedly higher invertebrate biomass found upstream in the system strongly supported our first hypothesis. Such a difference probably was the result of the influence of the enriched tributaries, which altered the carrying capacity of upstream and downstream stations through various mechanisms. First, macrophytes, which provide physical support for invertebrate colonization and shelter from predators within the water column, supported a considerably higher invertebrate biomass upstream than downstream. Second, epiphyte biomass serving as a food resource was twice as high upstream than downstream. Third, metaphyton present at each site also differed in nutritional value: chlorophytes (upstream) are considered a better food resource than cyanobacteria (downstream), which hinder grazing by producing toxins and presenting thick sheaths (Carmichael 1981, Chorus and Bartram 1999, Gérard et al. 2008, Camacho 2008).

Effect of vegetation types: metaphyton and macrophytes

Our 2nd hypothesis that, within a reach, metaphyton would support significantly more invertebrate biomass/g vegetation than would macrophytes was invalidated in the upstream reach but supported in the downstream reach. At station 2, total macroinvertebrate biomass did not differ significantly between metaphytic chlorophytes and macrophytes, and both types of vegetation supported high macroinvertebrate biomass/g DM. In contrast, at station 4, metaphytic cyanobacteria supported significantly higher invertebrate biomass, particularly of amphipods, than did adjacent macrophytes.

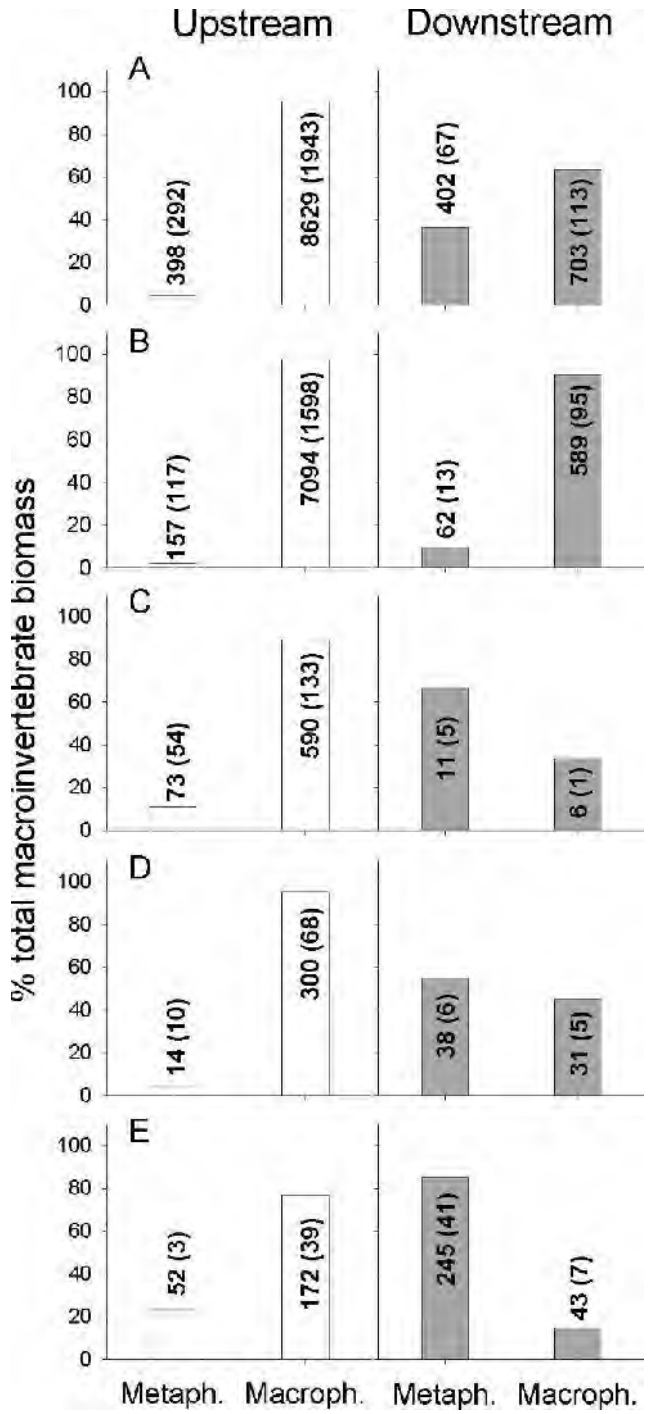

FIG. 4. Percentage of total macroinvertebrate biomass on metaphyton (Metaph.) and macrophytes (Macroph.) in upstream and downstream reaches for total macroinvertebrates (A), gastropods (B), oligochaetes (C), chironomids (D), and amphipods (E). Numbers above bars show mean (1 SE) biomass (mg/m^2 DM) of organisms on metaphyton and macrophytes.

Within each station, macrophytes and metaphyton sustained taxonomically different invertebrate assemblages. These differences might be explained in part by differences in vegetative architecture. Macrophytes were dominated by *V. americana*, which formed rosettes of linear, ribbon-like leaves above the river bottom. In both reaches, the fauna associated with macrophytes was dominated by gastropods, which might have benefited from the availability of a more stable and robust substratum upon which to crawl and feed (Salovius and Kraufvelin 2004) than that provided by the metaphyton found upstream (chlorophytes) or downstream (cyanobacteria). Both types of metaphyton offered a dissected architecture in the form of a loose mat of tangled filaments, which differed markedly from the structurally simple and free-floating leaves of *V. americana*. The occurrence and abundance of different macroinvertebrate groups on metaphyton can be explained by a combination of their behavior, mobility, feeding habits, tolerance to stress, and vulnerability to predators (Österling and Pihl 2001). Mobile taxa, such as cladocerans, copepods, chironomids, and ostracods, can benefit from the presence of metaphytic filamentous algae. Proliferations of metaphytic chlorophytes are typically temporary, so invertebrate taxa that can colonize new habitats rapidly and resist eventual drifting are favored by this alternative habitat (Norkko et al. 2000, Salovius and Kraufvelin 2004). Efficient swimmers like amphipods hide from predators in benthic mats of *L. wollei* during the day and move away to feed at night (Kraufvelin et al. 2006).

Differences in macroinvertebrate assemblages between the 2 types of metaphyton could stem partly from their distribution in the water column. Chlorophytes were found throughout the water column and near the surface, whereas cyanobacteria grew as mats (1–10 cm thick) close to anoxic sediments. Taxa usually associated with the sediments, such as amphipods, ostracods, and chironomid larvae, had higher absolute biomass (by factors of 3, 35 and 2, respectively) in the cyanobacteria than in the chlorophytes, despite the generally scarce resources found in the downstream reach. The association of amphipods with *Lyngbya* already has been reported (Camacho and Thacker 2006), but to our knowledge, the preference of ostracods and chironomids for cyanobacterial mats has not been reported.

Generalization of the results to the river reach

When our results were expressed as areal invertebrate biomass, the upstream reach supported an order of magnitude more invertebrate biomass/area river bottom than did the downstream reach. This pattern stems from the strong differences in total macroinvertebrate biomass/g vegetation combined with variations in macrophytic areal biomass between reaches. Macroinvertebrate densities are often positively related to an increase in biomass and primary production of aquatic vegetation (Cyr and Downing 1988, Strayer et al. 2003, Giorgi et al. 2005). Dense plant beds also offer protection from fish predation (Crowder and Cooper 1982, Rennie and Jackson 2005). Thus, high resource availability and protection from predators in the upstream reach might explain the large differences in invertebrate biomass between reaches.

Macrophytes support most of the invertebrate biomass in LSP. Metaphytic chlorophytes provide high-quality, albeit very localized and transient, shelter and food resources. The absolute contribution of chlorophytes to total invertebrate biomass is generally marginal at the river scale because they are sparsely distributed and occur sporadically, as has been documented in other areas of LSP (Tessier et al. 2007). Nevertheless, metaphytic chlorophytes occasionally become relevant during transient episodes of low water levels, when their biomass can equal that of macrophytes (AC, unpublished data). In contrast, the metaphytic cyanobacterium *L. wollei* hosted a significant proportion (~⅓) of the total invertebrate biomass in the downstream reach. Here, where submerged macrophytes are scarce and do not form dense patches, the thick mats of *L. wollei* are a suitable alternative habitat for invertebrates and offer a refuge against predation. *Lyngbya wollei* produces saxitoxin (Carmichael et al. 1997, Onodera et al. 1997, CH, unpublished data), but laboratory studies suggest that amphipods are not affected by these toxins (Camacho and Thacker 2006).

Macroinvertebrates are the primary resource for most littoral fish species inhabiting the St. Lawrence River (Scott and Crossman 1974), and their impoverished biomass and reduced availability among the dense filaments of *L. wollei* could lead to a critical decline of the carrying capacity of the downstream reach. Our results indicate that the replacement of dense macrophyte beds by cyanobacterial mats over large areas of Lake Saint-Pierre might seriously compromise fish resources in that area and should be the focus of future studies.

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Responsive Management™

WEST VIRGINIA RESIDENTS' OPINIONS ON AND TOLERANCE LEVELS OF ALGAE IN WEST VIRGINIA WATERS

Conducted for the West Virginia Department of Environmental Protection

by Responsive Management

2012

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2012

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EXECUTIVE SUMMARY

INTRODUCTION AND METHODOLOGY

The West Virginia Department of Environmental Protection's (DEP) Water Quality Standards Program is designed to monitor and control water quality in accordance with the Clean Water Act. Water Quality Standards (hereinafter referred to as "Standards") are legal criteria established to control the amount of pollution entering West Virginia waters from multiple sources, including industry, wastewater treatment facilities, and runoff.

As part of the agency's efforts to develop Standards regarding algae in streams and waterways in West Virginia, this study was conducted for the DEP to identify the public's threshold for algae and to assess the impact of algae levels on pubic recreational water use, such as swimming, fishing, and boating. The study entailed a statewide telephone survey of West Virginia residents.

A portion of this study entailed assessing respondents' reactions to images of seven levels of algae cover on West Virginia waters to determine the public's threshold for algae (see Appendix A for the images used in this study). Each aquatic image depicts a different level of algae cover on a different West Virginia body of water. The level of algae cover for each image used in the study was assessed and determined by the DEP.

The survey design required the respondents to have the images in front of them for reference on specific questions while they were being interviewed. Respondents were first called to secure their participation; if they agreed to participate, they were supplied with the images via postal mail or via the Internet, according to their preference. Each respondent could view the images online or receive a printed packet of the images. Those who could access the images online while on the telephone were directed to a website address for the images and were asked to complete the survey during the initial call. Those who could not access the images online while on the telephone (or who preferred to look at paper images) were either e-mailed the website address to access the images online at another time or were mailed a printed packet, and then they were called to complete the survey at a subsequent date. Those who received the printed packet in the mail but who did not have it readily available when they were called to complete the survey were again encouraged to view the images online, if possible, and were provided the

website address for the images. Alternately, a call-back was scheduled for a time when they would have the images available.

In the image portion of the survey, respondents were assigned a random aquatic image as a starting point with the quantity and order of subsequent images based on their responses to each question throughout the image portion of the survey. Specifically, respondents were presented an image of algae cover and asked if it was acceptable or unacceptable. If it was deemed acceptable, respondents were directed to the next highest level of algae cover and asked about its acceptability, moving on up until they found the level that was unacceptable (or until the highest level was reached). Conversely, if they found the first photograph to which they were directed unacceptable, they moved down in algae coverage levels until they found the level that was acceptable (or until the lowest level was reached). In this way, the survey recorded the highest level of algae cover that respondents felt was acceptable.

The telephone survey questionnaire was developed cooperatively by Responsive Management and the DEP, based on the research team's familiarity with natural resource management, water quality, and outdoor recreation. The sample was representative of all West Virginia residents. A central polling site at the Responsive Management office allowed for rigorous quality control over the interviews and data collection. Telephone surveying times are Monday through Friday from 9:00 a.m. to 9:00 p.m., Saturday from noon to 5:00 p.m., and Sunday from 5:00 p.m. to 9:00 p.m., local time. The survey was conducted in February and March 2012.

The software used for data collection was Questionnaire Programming Language. Responsive Management obtained a total of 1,001 completed interviews. The analysis of data was performed using Statistical Package for the Social Sciences as well as proprietary software developed by Responsive Management. Throughout this report, findings of the telephone survey are reported at a 95% confidence interval (or higher). For the entire sample of West Virginia residents, the sampling error is at most plus or minus 3.10 percentage points.

PARTICIPATION IN WATER ACTIVITIES

Many West Virginia residents participate in water-based recreation, which means that there is likely to be wide exposure to algae issues. In particular, rivers and streams are commonly used, with somewhat less use being made of lakes/reservoirs, and much less use being made of ponds. The three rivers of interest (the Greenbrier, the South Branch of the Potomac, and the Cacapon, chosen for focus in the study because of the prevalence of algae issues on them) are widely used: about a third of water-based recreationists (32%) used one or more of these rivers for recreation.

KNOWLEDGE OF AND RATINGS OF THE WEST VIRGINIA DEPARTMENT OF ENVIRONMENTAL PROTECTION

Perceptions of credibility and trustworthiness of the DEP are positive, with only 4% saying that the DEP is *not at all* credible and 4% saying that the DEP is *not at all* trustworthy; however, the ratings are not uniformly positive, with substantial percentages saying that the DEP is *somewhat* credible (36%) or *somewhat* trustworthy (40%). Ratings of the DEP's efforts at protecting and restoring the environment are positive: 70% rate the efforts as excellent or good. Finally, in general, residents want the DEP to expend more effort on protecting and restoring the environment. In general, this suggests that the DEP will have credibility among residents regarding any communications relating to algae in West Virginia waters.

KNOWLEDGE AND AWARENESS OF ALGAE

In general, while West Virginia resident do not profess much knowledge of algae, they are aware of algae in West Virginia waters. About a third of residents (32%) think that algae has been increasing in West Virginia waters over the past 2 years. This perception of increasing algae levels is particularly strong among those who participate in water-based recreation on one of the three rivers of interest in this study (42% of those recreationists say that algae has been increasing over the past 2 years).

OPINIONS ON ALGAE IN GENERAL

General opinions of algae run from some mild concern ("Algae doesn't bother me, but I worry about problems it may cause") to more robust concern ("I generally regard algae as a nuisance"). When asked directly about whether algae is a problem in West Virginia, more than a third of

residents indicate that algae is a problem. Furthermore, about a quarter indicate being concerned about algae in West Virginia waters.

A series of questions elicited opinions on various aspects of algae. Aesthetics plays a major role in feelings about algae, with 79% of West Virginia residents agreeing with the statement, "Algae is generally unattractive and unappealing." Safety also plays a role in opinions about algae, with well more than half of West Virginia residents (57%) agreeing that "algae causes safety hazards for people participating in activities on or in West Virginia waters." Those who participate in water-based recreation on the three rivers of interest have more negative opinions regarding algae, compared to those who do not engage in recreation on these rivers. The above should not be taken to mean that West Virginia residents misunderstand that some amount of algae in natural waters is part of a "normal" ecosystem. A large majority of residents (79%) agree that "algae is beneficial for the ecosystem in some situations."

LEVELS OF ACCEPTANCE OF ALGAE

The primary focus of the survey was to help determine the level of tolerance West Virginia residents have regarding algae in the state's natural waters. As discussed in the methodology, respondents examined photographs that showed varying levels of algae cover to determine at which point the level becomes unacceptable to them. The photograph that shows 20 percent coverage has only about a quarter of respondents (27%) thinking that this amount is unacceptable. However, the next photograph, showing 26 percent coverage, has nearly half of respondents (49%) thinking that the level is unacceptable. This suggests that waters with any more than a quarter coverage will be unacceptable to a majority of residents.

Crosstabulations of tolerance levels by participation in various activities and by demographic factors found that certain groups are less tolerant of algae than are other groups. In particular, people who go rafting or inner tubing, power boaters and users of personal watercraft, and those who engage in walking along the banks and shores of natural waters are less tolerant of algae than are their counterparts. Demographically, those who are the median age or older have less tolerance of algae than do younger residents. Additionally, females have less tolerance of algae than do males.

CONTACTING AGENCIES AND ORGANIZATIONS REGARDING ALGAE

The substantial concern expressed about algae is not reflected in contacts with the DEP or other organizations about algae. The survey found that less than 1% of West Virginia residents contacted the DEP about algae in West Virginia waters in the past 2 years, and a little less than 3% contacted any other agency or organization about algae.

ALGAE-RELATED CONSTRAINTS TO PARTICIPATION IN WATER-BASED ACTIVITIES IN WEST VIRGINIA

At present, algae is not a major constraint to water-based recreation in the state. A general open-ended question asked respondents if there were things that took away from their participation in or their enjoyment of water-based recreation in West Virginia, and water quality was named by only 4%, and less than 1% named algae specifically. Nonetheless, if algae levels increase, the data suggest that water-based recreation could be affected. This would be particularly true if algae levels routinely reach more than about a quarter coverage in rivers and streams.

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INTRODUCTION AND METHODOLOGY

The West Virginia Department of Environmental Protection's (DEP) Water Quality Standards Program is designed to monitor and control water quality in accordance with the Clean Water Act. Water Quality Standards (hereinafter referred to as "Standards") are legal criteria established to control the amount of pollution entering West Virginia waters from multiple sources, including industry, wastewater treatment facilities, and runoff. Standards have both numeric and narrative limits and are developed to protect and maintain water quality for the designated or assigned uses, such as aquatic life, water contact recreation, water supply, power generation, agriculture, navigation, and more.

As part of the agency's efforts to develop Standards regarding algae in streams and waterways in West Virginia, this study was conducted for the DEP to identify the public's threshold for algae and to assess the impact of algae levels on pubic recreational water use, such as swimming, fishing, and boating. The study entailed a statewide telephone survey of West Virginia residents. Specific aspects of the research methodology are discussed below.

OVERALL STUDY DESIGN

A portion of this study entailed assessing respondents' reactions to images of seven levels of algae cover on West Virginia waters to determine the public's threshold for algae (see Appendix A for the images used in this study). The survey design required the respondents to have the images in front of them for reference on specific questions. Respondents were first called to secure their participation; if they agreed to participate, they were supplied with the images. Each respondent could view the images online or receive a printed packet of the images. Those who could access the images online while on the telephone were directed to a website address for the images and were asked to complete the survey during the initial call. Those who could not access the images online while on the telephone (or who preferred to look at paper images) were either e-mailed the website address to access the images online at another time or were mailed a printed packet, and then they were called to complete the survey at a subsequent date. Those who received the printed packet in the mail but who did not have it readily available when they were called to complete the survey were again encouraged to view the images online, if possible,

and were provided the website address for the images. Alternately, a call-back was scheduled for a time when they would have the images available.

In addition to the seven images depicting different levels of algae cover on West Virginia waters, five non-aquatic images of wildlife species and various habitats were also included to reduce any potential bias that predisposing respondents to images only of water and algae cover prior to participating in the survey may have produced. The same seven aquatic algae cover images and five non-aquatic images were used online and in each printed packet.

Each aquatic image depicts a different level of algae cover on a different West Virginia body of water. The level of algae cover for each image used in the study was assessed and determined by the DEP. The seven levels of algae cover depicted in the images are 4 percent, 15 percent, 20 percent, 26 percent, 39 percent, 47 percent, and 65 percent cover. Respondents were not informed of the percentage of algae cover depicted in each aquatic image. Although each printed packet and the website featured the aquatic images in the same order, the images were not featured in a logical order; that is, the order did not represent a linear increase in algae cover or a logical progression based on any other factor within the images, and the order was intended to appear random to the respondent.

Each image (aquatic and non-aquatic) was assigned an alphanumeric code by the researchers to enable the interviewer to direct the respondent to a specific image during survey administration and to ensure respondents were answering each question about the correct image. Images were displayed one per page both online and in the printed packet. The tabulation on the following page shows each image code, description, and algae level (if applicable) in the order in which the images appeared in the printed packet as well as the order in which the links to each image were displayed on the website menu (see Appendix B for an image of the primary website menu for this study). A second tabulation on the next page shows only the aquatic images asked about in the survey in order of increasing algae cover.

All Images (Aquatic and Non-Aquatic) in Order of Display

Image Code	Image Description	Algae Cover Level (aquatic images only)
A 12	Wildflowers in West Virginia	
B 83	West Virginia water body with algae cover	20%
D 96	West Virginia water body with algae cover	47%
E 45	Black bear in West Virginia forest fire area	
H 85	West Virginia water body with algae cover	39%
I 74	Fawn (white-tailed deer) in West Virginia	
K 97	West Virginia water body with algae cover	65%
M 51	West Virginia water body with algae cover	4%
O 36	Eastern cottontail rabbit in West Virginia	
Q 52	West Virginia water body with algae cover	15%
U 27	Hardwood trees in West Virginia	
Z 34	West Virginia water body with algae cover	26%

Aquatic Images in Order of Increasing Algae Cover Level

Image Code	Image Description
M 51	West Virginia water body with 4% algae cover
Q 52	West Virginia water body with 15% algae cover
B 83	West Virginia water body with 20% algae cover
Z 34	West Virginia water body with 26% algae cover
H 85	West Virginia water body with 39% algae cover
D 96	West Virginia water body with 47% algae cover
K 97	West Virginia water body with 65% algae cover

Note that respondents were not directed to the images in the order in which they were featured in the online menu or the printed packet. Respondents were assigned a random aquatic image as a starting point with the quantity and order of subsequent images based on their responses to each question throughout the image portion of the survey. Respondents were presented an image of algae cover and asked if it was acceptable or unacceptable. If it was deemed acceptable, respondents were directed to the next highest level of algae cover and asked about its acceptability, moving on up until they found the level that was unacceptable (or until the highest level was reached). Conversely, if they found the first photograph to which they were directed unacceptable, they moved down in algae coverage levels until they found the level that was acceptable (or until the lowest level was reached). In this way, the survey recorded the highest level of algae cover that respondents felt was acceptable.

QUESTIONNAIRE DESIGN

The telephone survey questionnaire was developed cooperatively by Responsive Management and the DEP, based on the research team's familiarity with natural resource management, water quality, and outdoor recreation. Responsive Management conducted pre-tests of the questionnaire to ensure proper wording, flow, and logic in the survey.

SURVEY SAMPLE

The sample of West Virginia residents was obtained from Survey Sampling International and Database 101, providers of scientific samples of populations. The sample was representative of all West Virginia residents 18 years old and older.

TELEPHONE INTERVIEWING FACILITIES

A central polling site at the Responsive Management office allowed for rigorous quality control over the interviews and data collection. Responsive Management maintains its own in-house telephone interviewing facilities. These facilities are staffed by interviewers with experience conducting computer-assisted telephone interviews on the subjects of outdoor recreation and natural resources.

To ensure the integrity of the telephone survey data, Responsive Management has interviewers who have been trained according to the standards established by the Council of American Survey Research Organizations. Methods of instruction included lecture and role-playing. The Survey Center Managers and other professional staff conducted a project briefing with the interviewers prior to the administration of this survey. Interviewers were instructed on type of study, study goals and objectives, handling of survey questions, interview length, termination points and qualifiers for participation, interviewer instructions within the survey questionnaire, reading of the survey questions, skip patterns, and probing and clarifying techniques necessary for specific questions on the survey questionnaire.

INTERVIEWING DATES AND TIMES

Telephone surveying times are Monday through Friday from 9:00 a.m. to 9:00 p.m., Saturday from noon to 5:00 p.m., and Sunday from 5:00 p.m. to 9:00 p.m., local time. A five-callback

design was used to maintain the representativeness of the sample, to avoid bias toward people easy to reach by telephone, and to provide an equal opportunity for all to participate. When a respondent could not be reached on the first call, subsequent calls were placed on different days of the week and at different times of the day. The survey was conducted in February and March 2012.

TELEPHONE SURVEY DATA COLLECTION AND QUALITY CONTROL

The software used for data collection was Questionnaire Programming Language (QPL). The survey data were entered into the computer as each interview was being conducted, eliminating manual data entry after the completion of the survey and the concomitant data entry errors that may occur with manual data entry. The survey questionnaire was programmed so that QPL branched, coded, and substituted phrases in the survey based on previous responses to ensure the integrity and consistency of the data collection.

The Survey Center Managers and statisticians monitored the data collection, including monitoring of the actual telephone interviews without the interviewers' knowledge, to evaluate the performance of each interviewer and ensure the integrity of the data. The survey questionnaire itself contains error checkers and computation statements to ensure quality and consistent data. After the surveys were obtained by the interviewers, the Survey Center Managers and/or statisticians checked each completed survey to ensure clarity and completeness.

Responsive Management obtained a total of 1,001 completed interviews. The total sample size on some questions is less than 1,001 because the survey asked some questions only of specific respondents in the survey. In particular, this was done when a follow-up question did not apply to some respondents. For instance, only those who participated in activities on or in West Virginia waters were asked follow-up questions about the specific bodies of water in which they participated in recreation.

DATA ANALYSIS

The analysis of data was performed using Statistical Package for the Social Sciences as well as proprietary software developed by Responsive Management. The results were weighted by demographic characteristics so that the sample was representative of residents in West Virginia as a whole.

On questions that asked respondents to provide a number (e.g., age), the graph shows ranges of numbers rather than the precise numbers. Nonetheless, in the survey each respondent provided a precise number, and the dataset includes this precise number, even if the graph only shows ranges of numbers. Note that the calculation of means and medians used the precise numbers that the respondents provided.

Crosstabulations were run on many questions, including crosstabulations by participation in water-based recreation on three rivers of interest, chosen because of the prevalence of algae issues in the past on those rivers: the Greenbrier, the South Branch of the Potomac, and the Cacapon. Other crosstabulations were run, as appropriate, as part of the analysis. These crosstabulations are indicated on the graphs.

SAMPLING ERROR

Throughout this report, findings of the telephone survey are reported at a 95% confidence interval (or higher). For the entire sample of West Virginia residents, the sampling error is at most plus or minus 3.10 percentage points. This means that if the survey were conducted 100 times on different samples that were selected in the same way, the findings of 95 out of the 100 surveys would fall within plus or minus 3.10 percentage points of each other. Sampling error was calculated using the formula described on the following page, with a sample size of 1,001 and a population size of 1,465,576 residents 18 years old and older.

Sampling Error Equation

$$B = \left(\sqrt{\frac{\frac{N_p(.25)}{N_s} - .25}{N_p - 1}} \right)(1.96)$$

Where: B = maximum sampling error (as decimal)
N_P = population size (i.e., total number who could be surveyed)
N_S = sample size (i.e., total number of respondents surveyed)

Derived from formula: p. 206 in Dillman, D. A. 2000. *Mail and Internet Surveys*. John Wiley & Sons, NY.

Note: This is a simplified version of the formula that calculates the maximum sampling error using a 50:50 split (the most conservative calculation because a 50:50 split would give maximum variation).

ADDITIONAL INFORMATION ABOUT THE PRESENTATION OF RESULTS IN THE REPORT

In examining the results, it is important to be aware that the questionnaire included several types of questions:

- Open-ended questions are those in which no answer set is read to the respondents; rather, they can respond with anything that comes to mind from the question.
- Closed-ended questions have an answer set from which to choose.
- Single or multiple response questions: Some questions allow only a single response, while other questions allow respondents to give more than one response or choose all that apply. Those that allow more than a single response are indicated on the graphs with the label, "Multiple Responses Allowed."
- Scaled questions: Many closed-ended questions (but not all) are in a scale, such as excellent-good-fair-poor.
- Series questions: Many questions are part of a series, and the results are primarily intended to be examined relative to the other questions in that series (although results of the questions individually can also be valuable). Typically, results of all questions in a series are shown together.

Some graphs show an average, either the mean or median (or both). The mean is simply the sum of all numbers divided by the number of respondents. Because outliers (extremely high or low numbers relative to most of the other responses) may skew the mean, the median may be shown. The median is the number at which half the sample is above and the other half is below. In other words, a median of 150 means that half the sample gave an answer of more than 150 and the other half gave an answer of less than 150.

Most graphs show results rounded to the nearest integer; however, all data are stored in decimal format, and all calculations are performed on unrounded numbers. For this reason, some results may not sum to exactly 100% because of this rounding on the graphs. Additionally, rounding may cause apparent discrepancies of 1 percentage point between the graphs and the reported results of combined responses (e.g., when “strongly agree” and “moderately agree” are summed to determine the total percentage in agreement).

PARTICIPATION IN WATER ACTIVITIES

- A large majority of West Virginia residents participated, in the past 2 years, in walking along water banks or shores (79%), a majority went swimming in West Virginia waters (55%), and a majority engaged in fishing activities (52%). Considering all boating activities together, 44% did a boating activity. Note that this question's response set was read to respondents, and they were asked if they did or did not do each one; 14% had done none of the activities.
  - The survey followed up the above question by asking if there were other water-based activities that respondents did that were not covered in the first question. A low percentage (less than 3%) indicated that they participated in other water-based activities, including other miscellaneous waterside activities, other in-water activities, camping by the water, conservation activities, and picnicking by the water.
  - Another follow-up question asked respondents to name the single activity in which they participated most often in the past 2 years. The leading activities are fishing (32%) and walking along a water bank or shore (29%), distantly followed by swimming or wading (12%), and various boating activities (all at 6% or less).

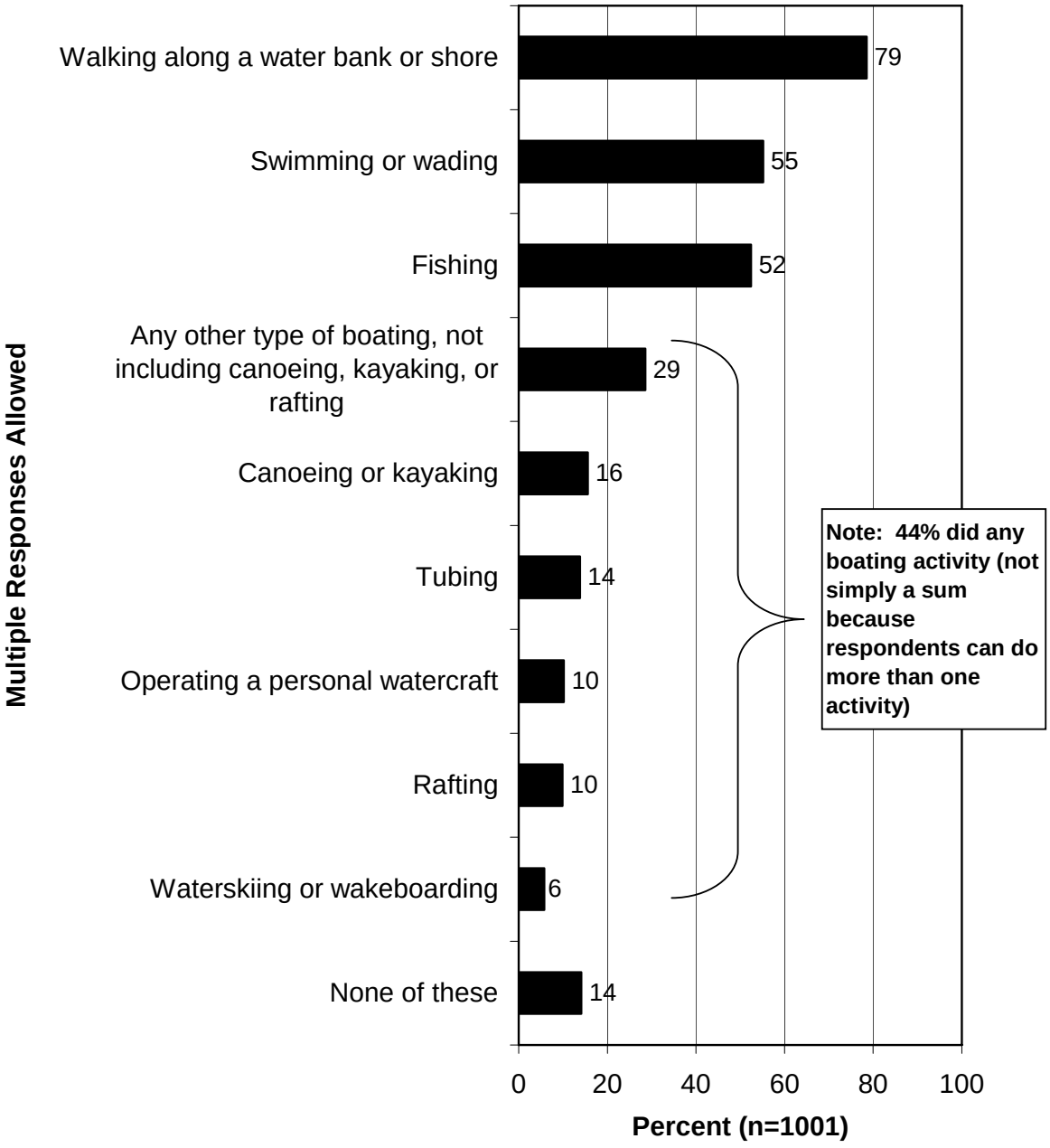
- Two questions asked about employment related to West Virginia waters: a little more than 1% of respondents said that they or members of their household work as outfitters or guides on or associated with West Virginia waters, and 3% said that they or household members work in any other position associated with West Virginia waters.

- Those who had participated in at least one activity on or in West Virginia waters in the past 2 years were asked to name the types of water bodies on or in which they had participated. Rivers and streams were most popular (80% of those who participated in an activity), followed by lakes and reservoirs (48%), and ponds (22%).
  - An additional locational question asked respondents (those who had participated in water-based recreation) if they had participated in any of the water-based activities on any of the three rivers that were chosen for this study: 16% of these respondents had participated on or in the Greenbrier River, 14% on or in the South Branch of the Potomac, and 6% on or in the Cacapon River. In total, 32% of these respondents had

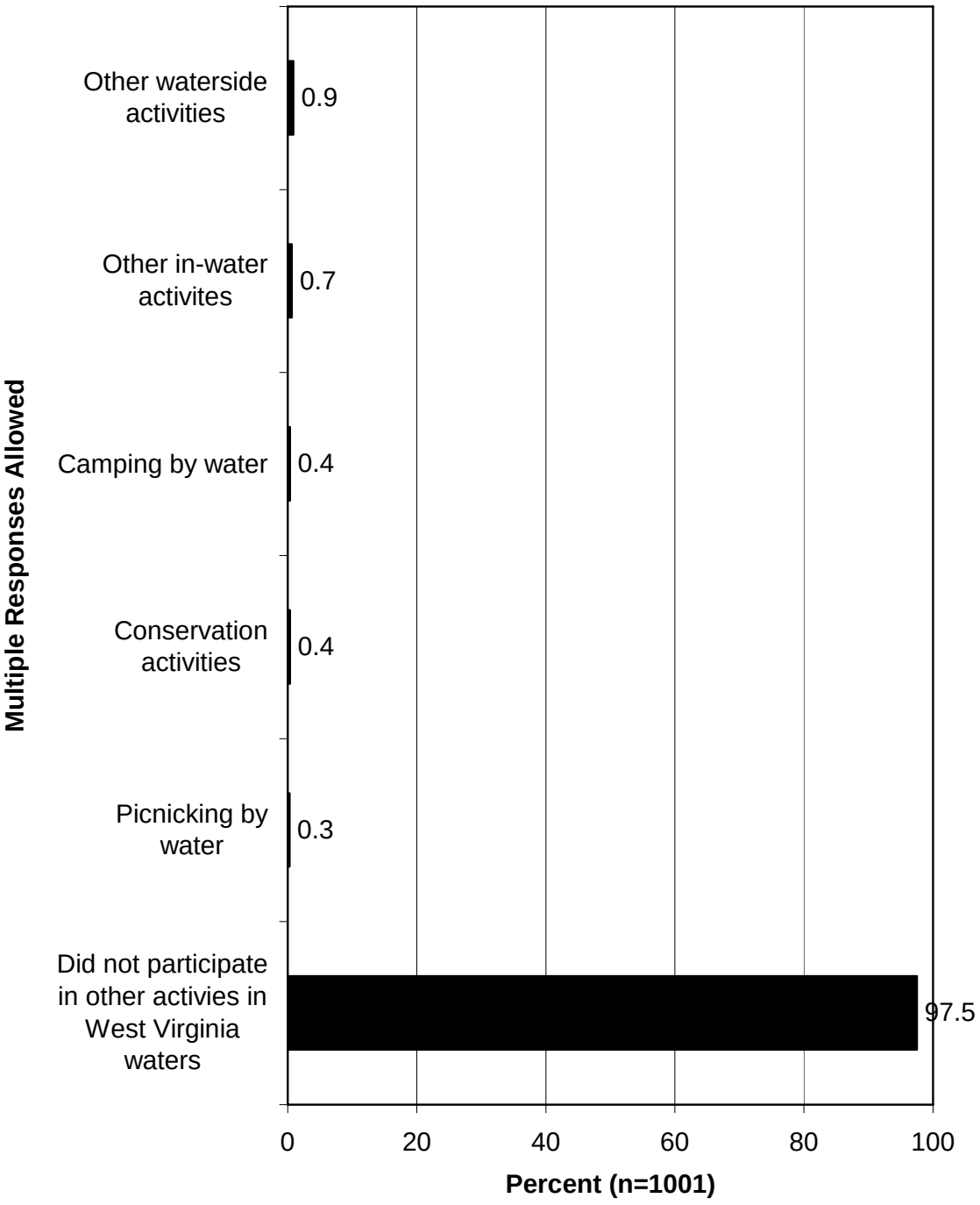
participated in at least one of these three; conversely, 68% had participated in water-based recreation but not on any of these three rivers.

- Another question in this section asked respondents about their likelihood to participate in water-based recreation on or in West Virginia waters in the next 2 years: 77% indicated being likely to do so, mostly *very* likely (60%), while 22% indicated being unlikely.

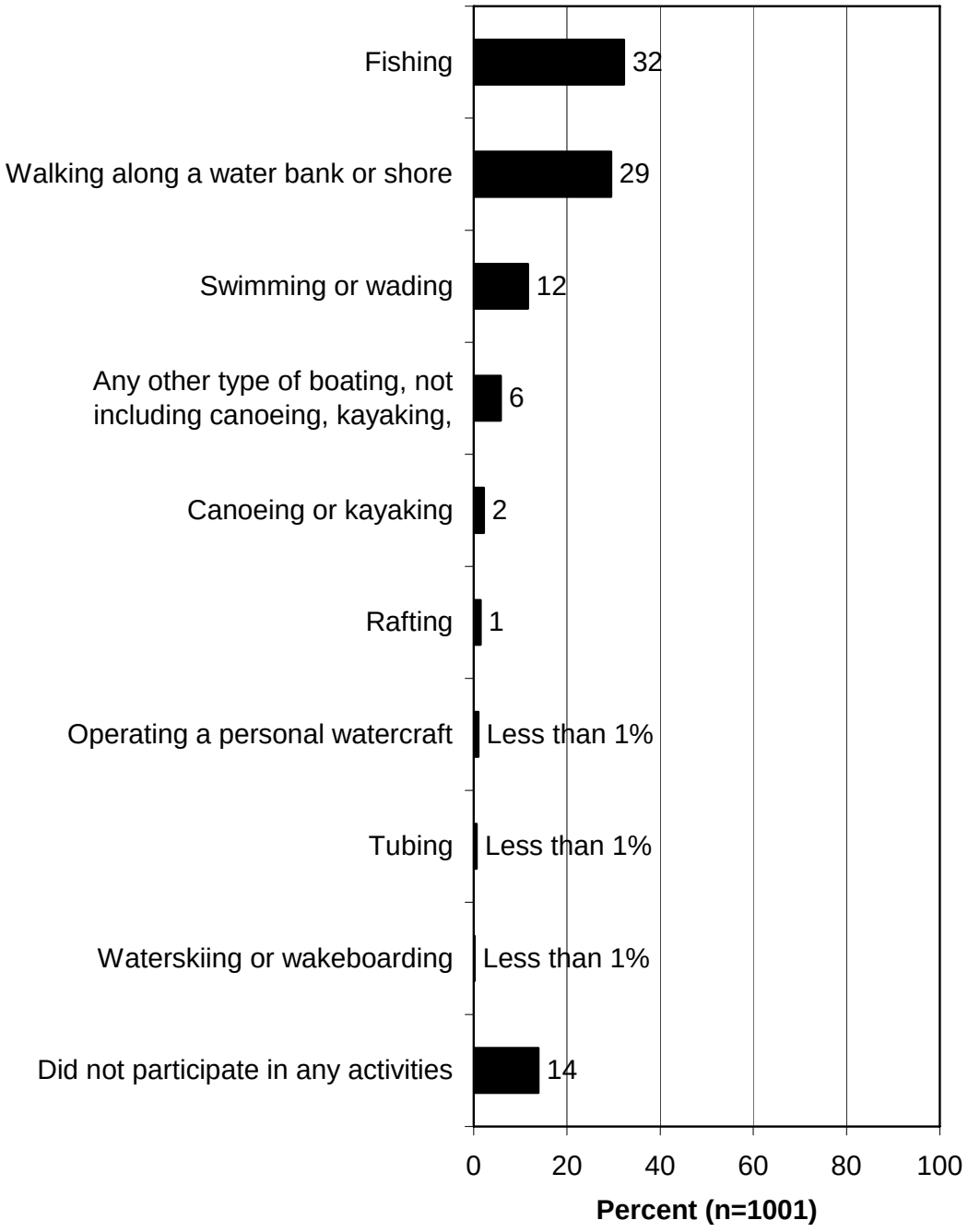
Q22. I would like to know if you personally participated in any of the following activities on or in West Virginia waters, including rivers and streams, lakes and reservoirs, and ponds, in the past 2 years.



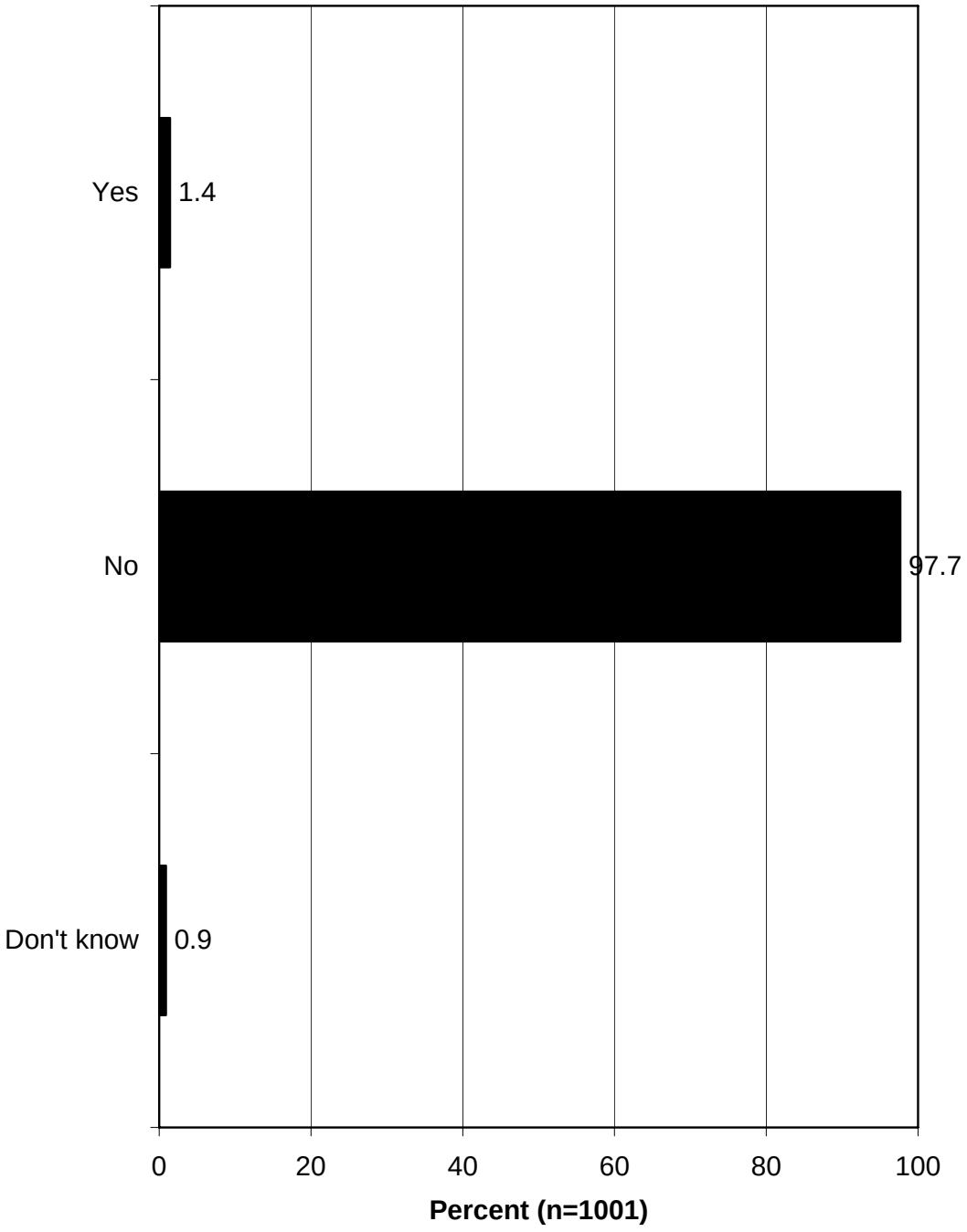
Q24. What other activities did you participate in?



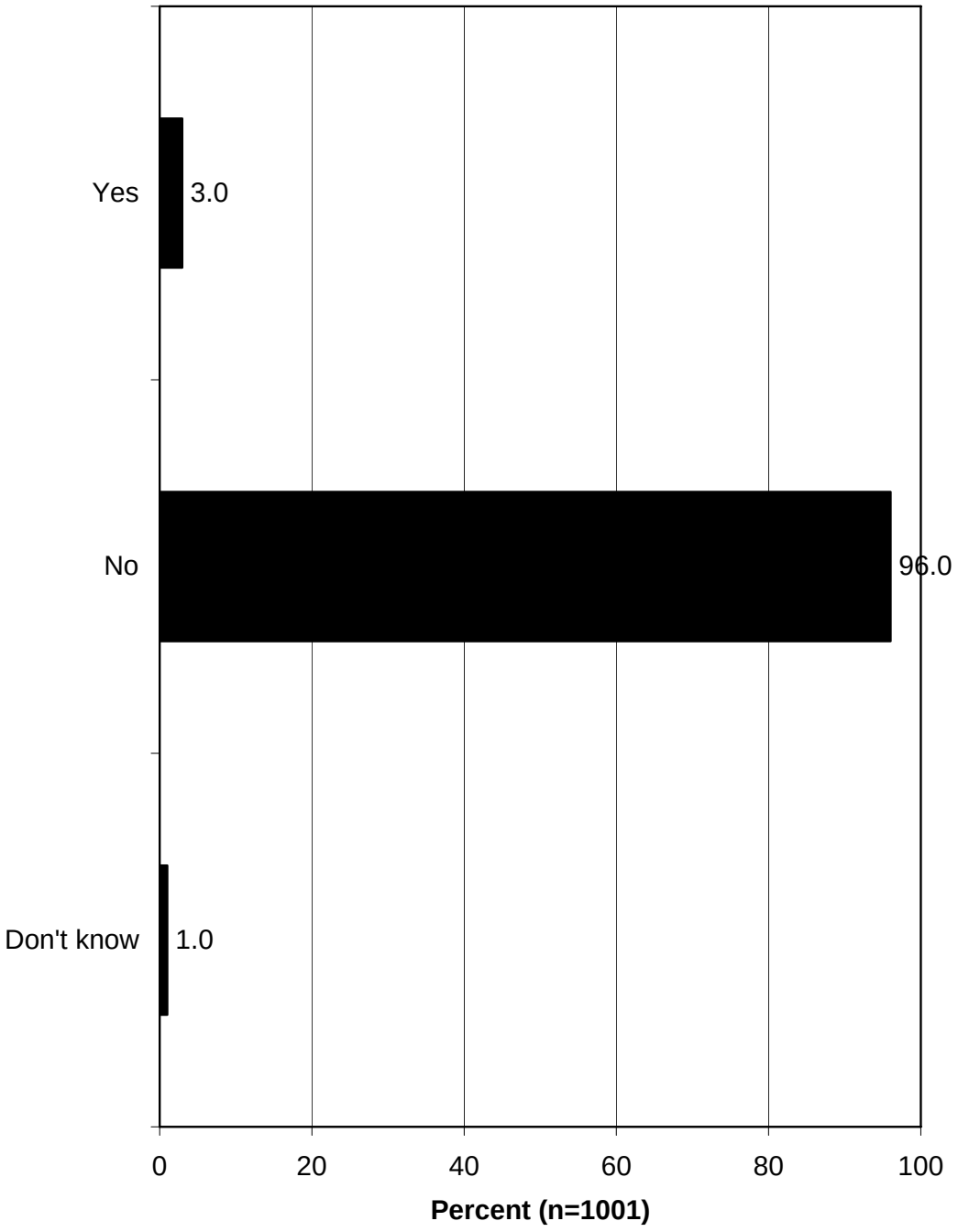
Q26. Which one of these activities did you participate in most often on or in West Virginia waters in the past 2 years?



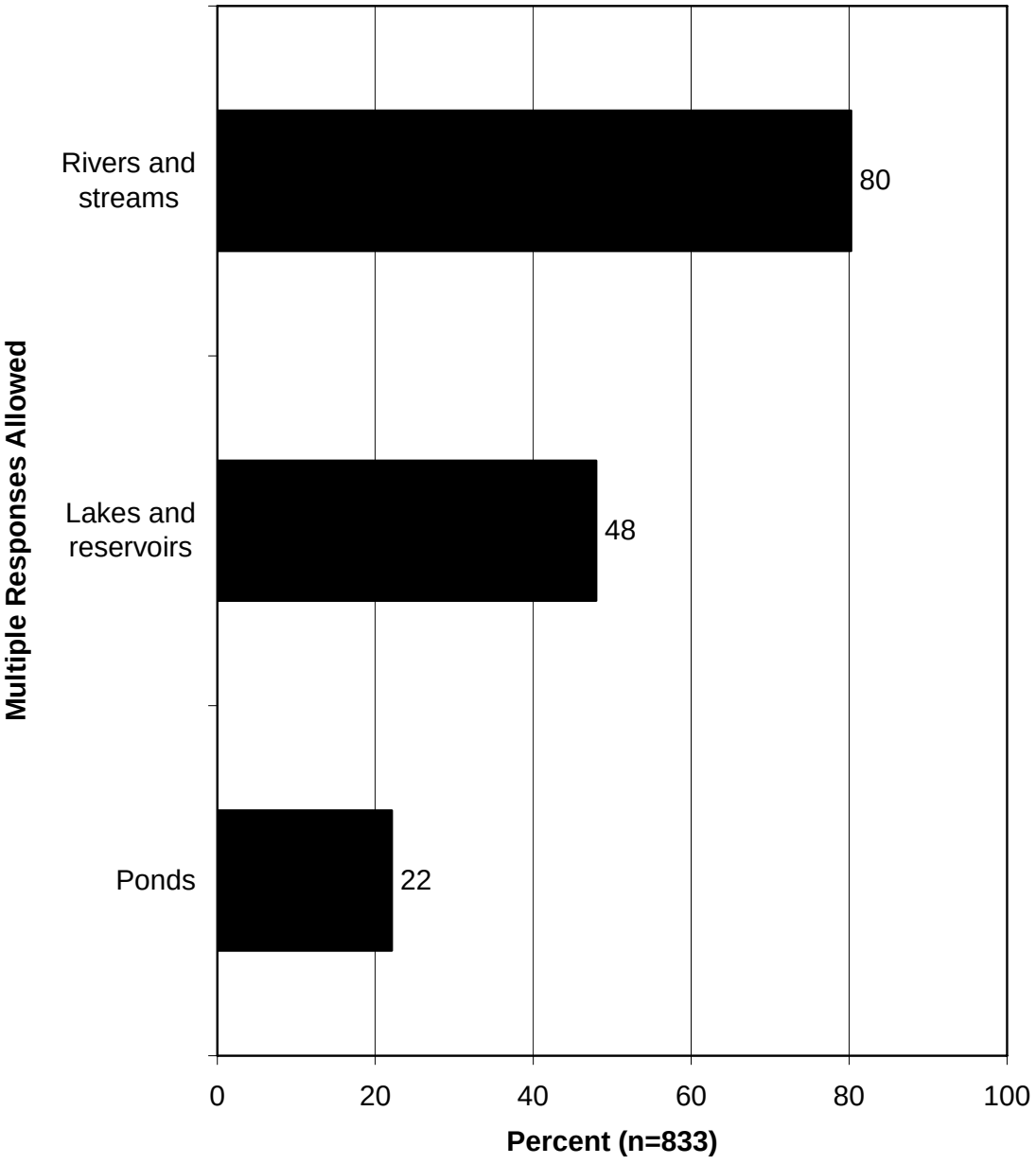
Q100. Do you or does anyone in your household currently work as an outfitter or guide on or associated with West Virginia waters?



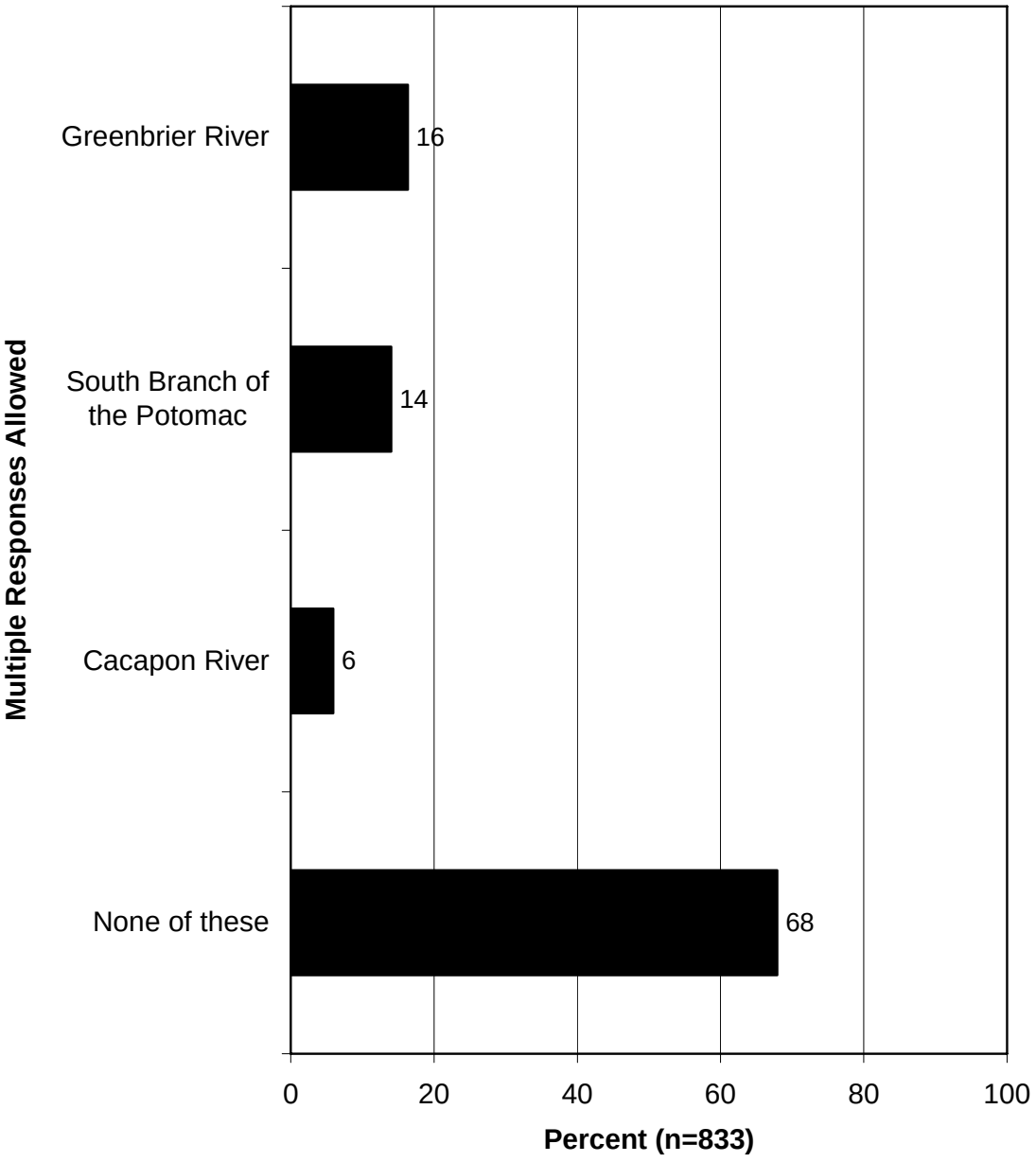
Q101. Do you or does anyone else in your household currently work in any other position on or associated with West Virginia waters?



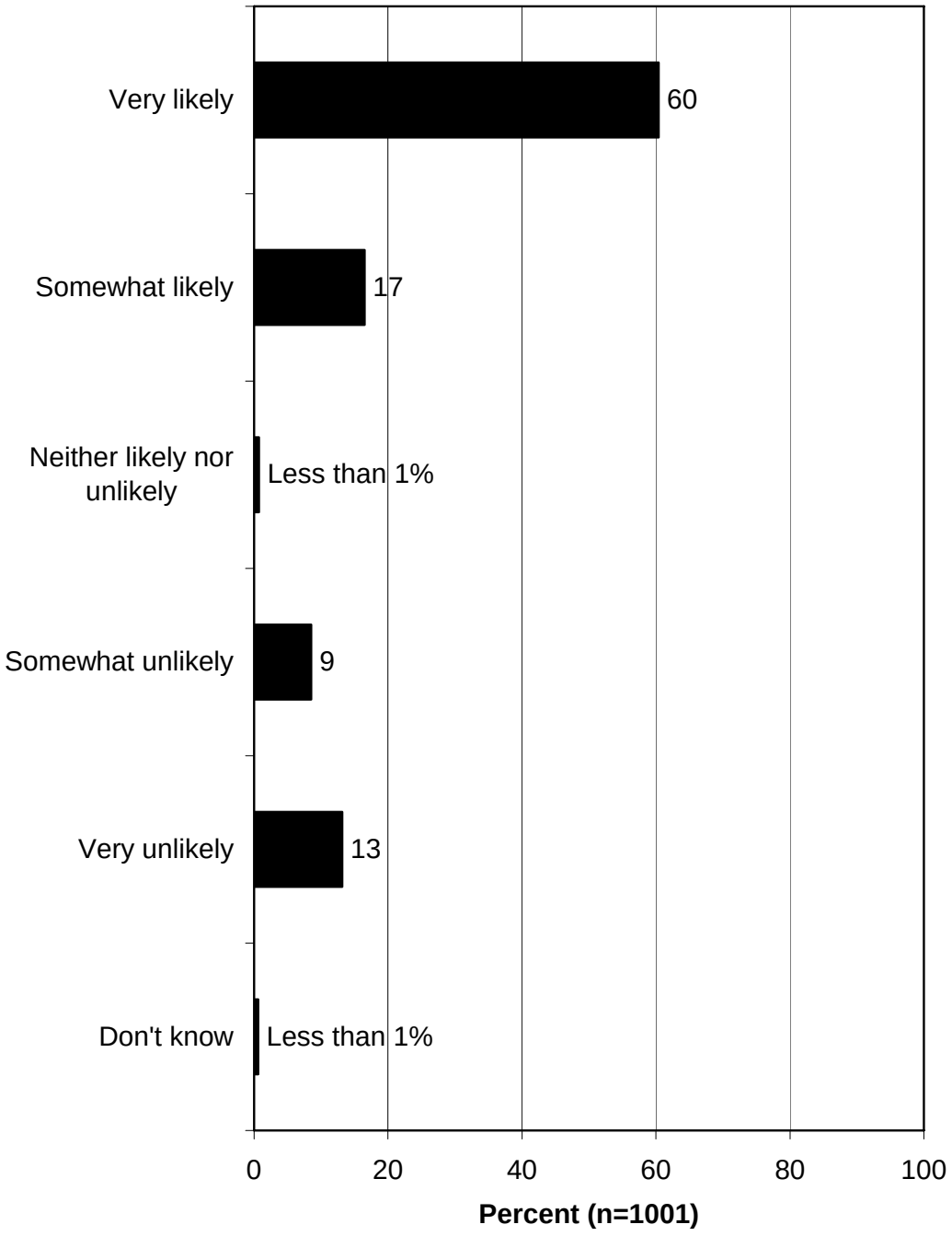
Q30. In which of the following types of water bodies did you participate in these activities in West Virginia? (Asked of those who participated in at least one activity on or in West Virginia waters in the past 2 years.)



Q33. In the past 2 years, did you participate in any activities during the Summer on or in the following water bodies? (Asked of those who participated in at least one activity on or in West Virginia waters in the past 2 years.)



Q42. How likely or unlikely are you to participate in activities on or in West Virginia waters in the next 2 years?



KNOWLEDGE OF AND RATINGS OF THE WEST VIRGINIA DEPARTMENT OF ENVIRONMENTAL PROTECTION

- Self-professed knowledge levels of the DEP are relatively low: the majority of respondents are in the bottom half of the knowledge scale, with 58% saying that they know only *a little* and 15% saying that they know *nothing* (a sum of 73% with no more than a little knowledge).

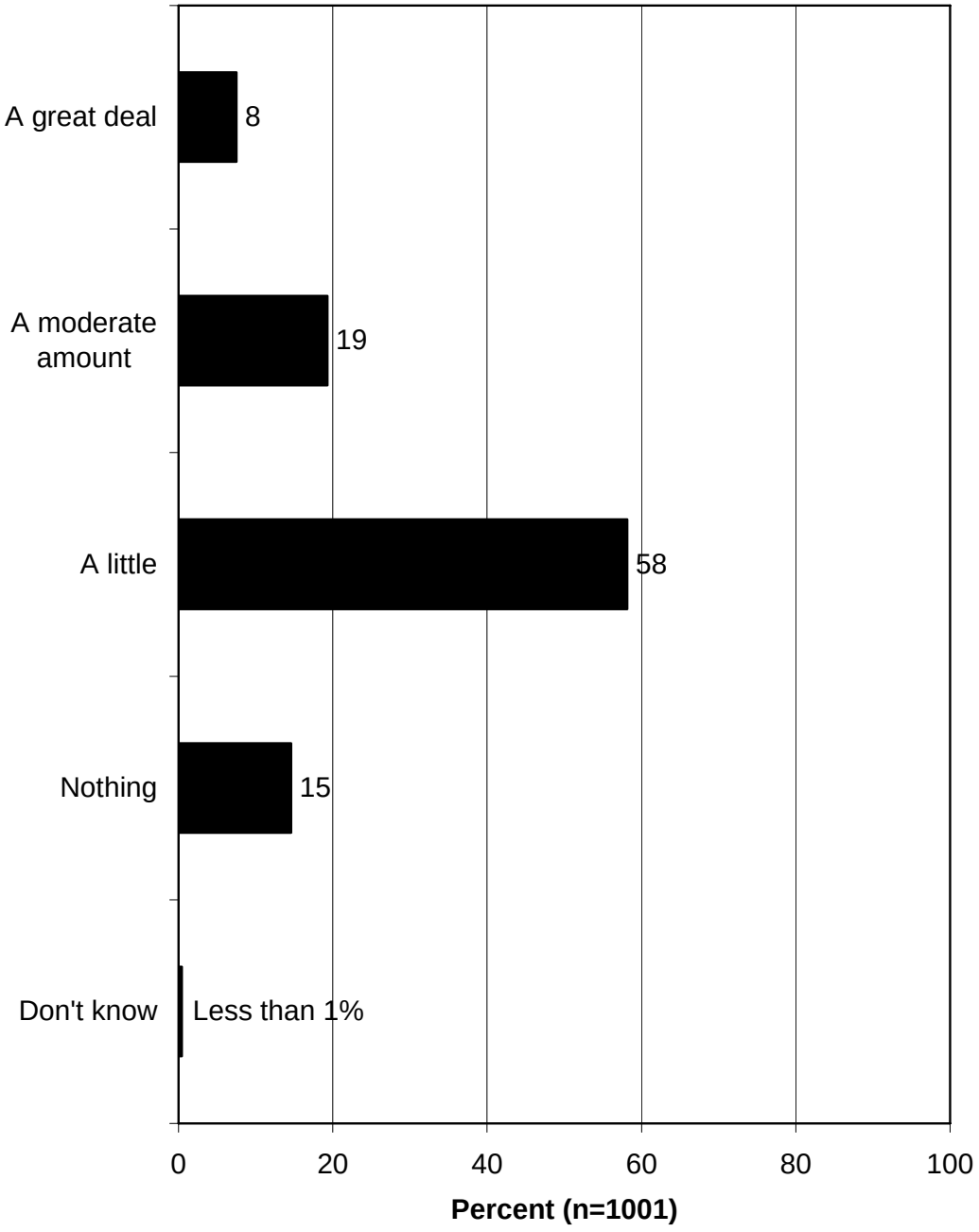
- The survey asked about the credibility of the DEP. Just under half rate the agency as *very* credible (49%), and another 36% rate it as *somewhat* credible (a sum of 85%); at the other end, 4% rate it as *not at all* credible. The remainder say that they do not know.
  - A similar question asked respondents to rate how trustworthy the agency is: 43% say *very* trustworthy, 40% say *somewhat* trustworthy (a sum of 83%), and 4% rate it *not at all* trustworthy.

- The questions about credibility and trustworthiness were crosstabulated by self-professed knowledge of the agency. In general, those at higher self-professed knowledge levels are less likely to answer don't know to either of these questions, compared to those with lower self-professed knowledge levels. Because of this, respondents at higher knowledge levels are more likely to say that the agency is *very* credible or *very* trustworthy, but also more likely to say that the agency is *not at all* credible or trustworthy.

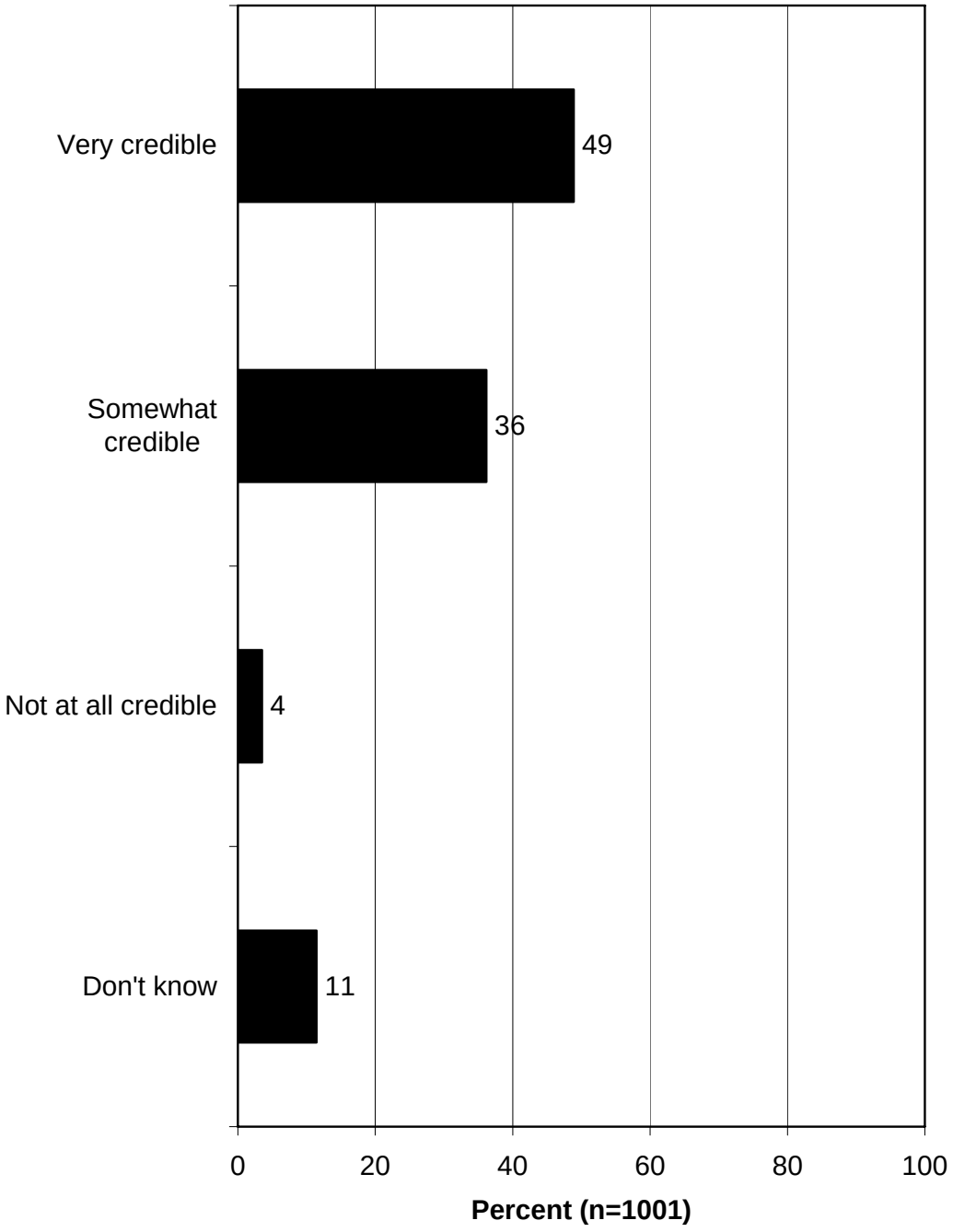
- Respondents rated the efforts of the DEP at protecting and restoring the environment in West Virginia. The ratings were generally positive, with a majority in the top half of the scale: 19% rated the efforts as excellent, and 51% rated the efforts as good (a sum of 70%). Meanwhile, 17% gave a rating of fair, and 4% rated them as poor.
  - The crosstabulation by self-professed knowledge level found that those with higher self-professed knowledge of the agency were more likely than those at lower knowledge levels to rate the effort as excellent, but also more likely to rate it poor. Again, the lower "don't know" percentage among those with higher knowledge levels allows both the excellent and poor percentages to increase.

- The majority of respondents want the DEP to expend more effort protecting and restoring the environment (58% want *much* or *slightly* more effort expended), and about a third are satisfied with the current amount (32% said the *same*). Only 3% indicated wanting *less* effort.
  - Those who profess higher knowledge levels, compared to those at lower self-professed knowledge levels, have a greater tendency to want *much* more effort expended by the DEP at protecting and restoring the environment (44% to 32%). Overall, the two groups are the same regarding wanting *much* or *slightly* more effort: 57% to 58%. However, those at higher self-professed knowledge levels also have a greater tendency to say that they want *less* effort.

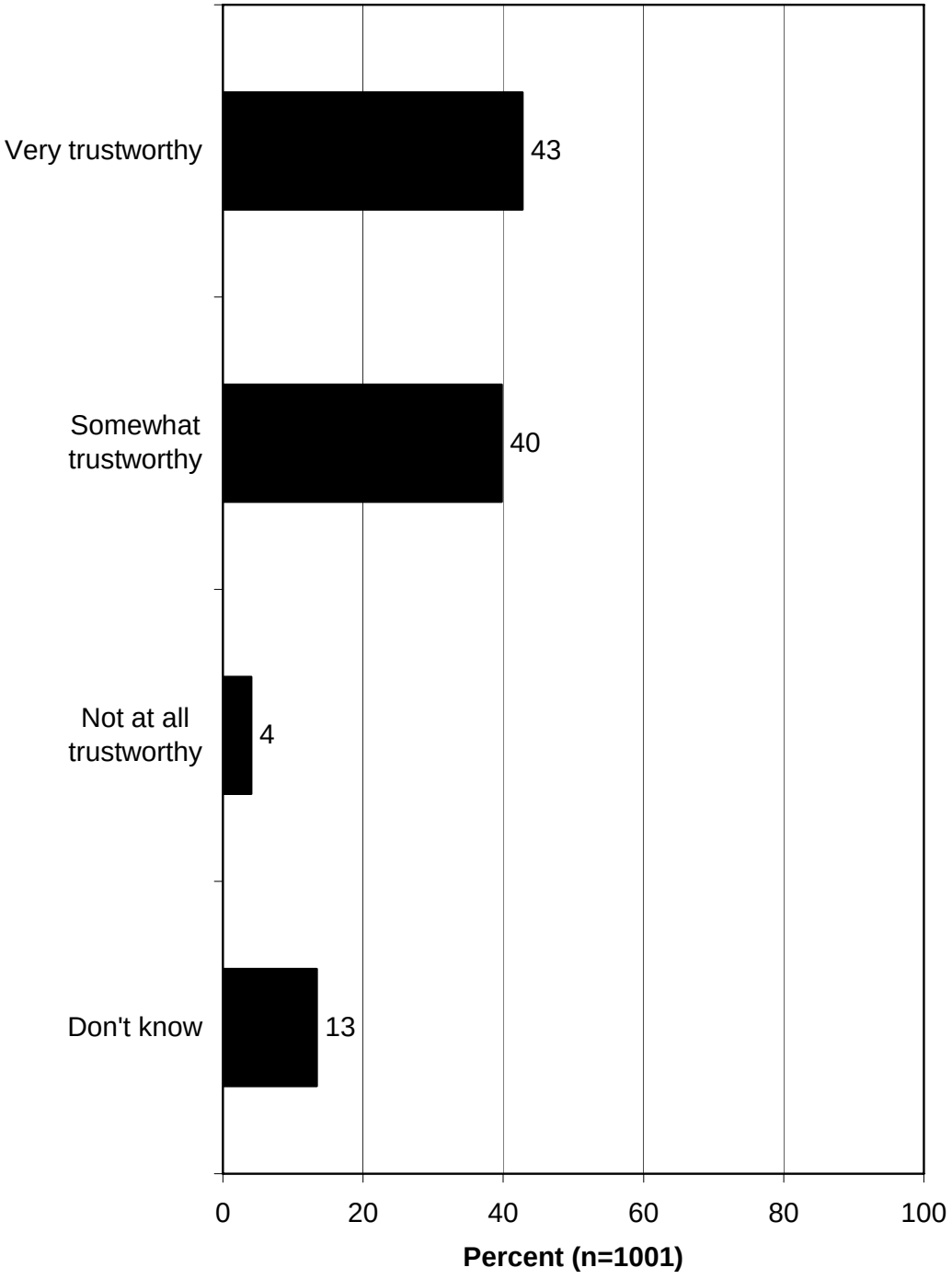
Q43. Would you say you know a great deal, a moderate amount, a little, or nothing about the West Virginia Department of Environmental Protection, also called the DEP?



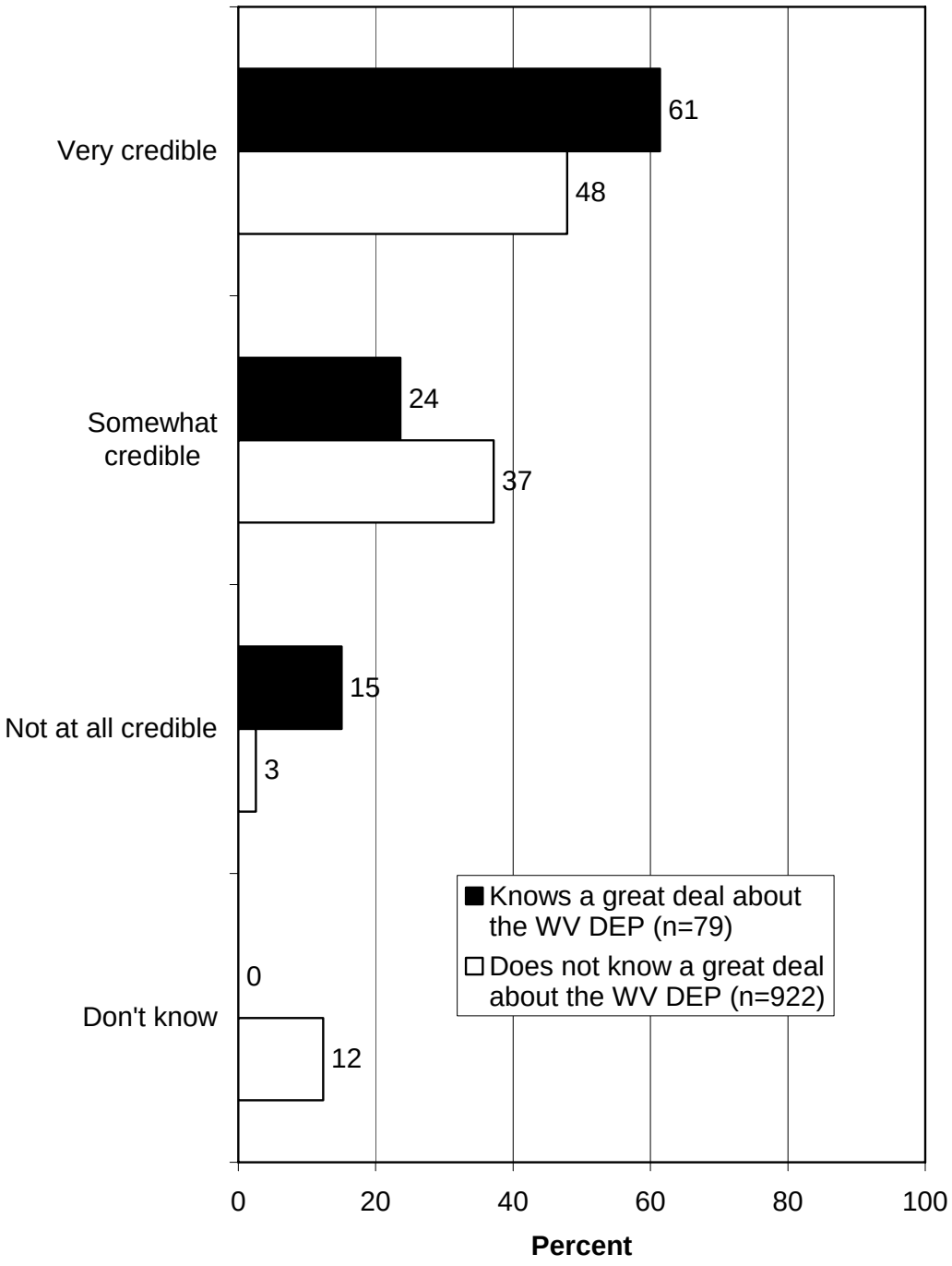
Q46. Do you think the DEP is very credible, somewhat credible, or not at all credible as a source of information on the environment?



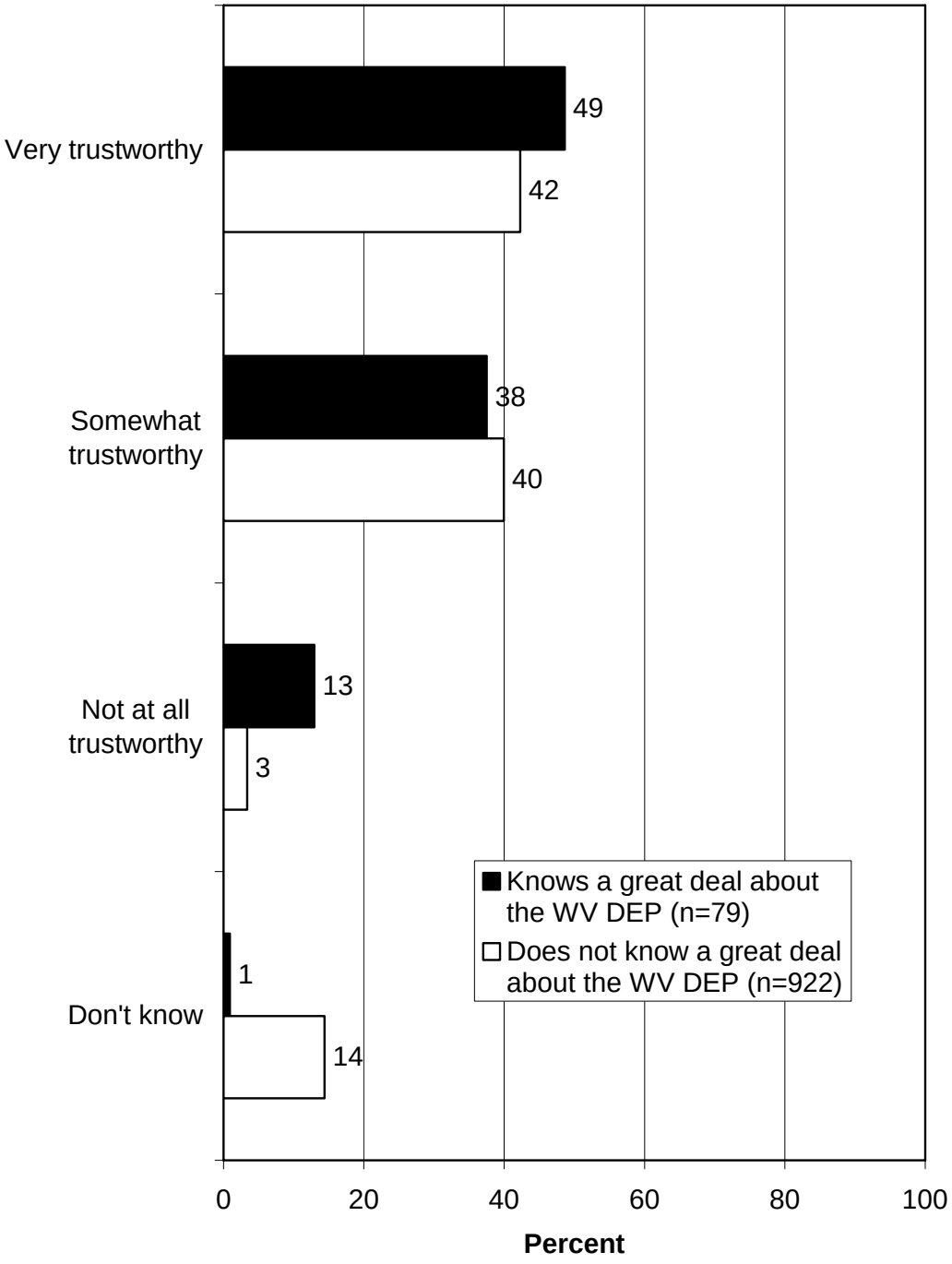
Q47. In your opinion, is the DEP very trustworthy, somewhat trustworthy, or not at all trustworthy?



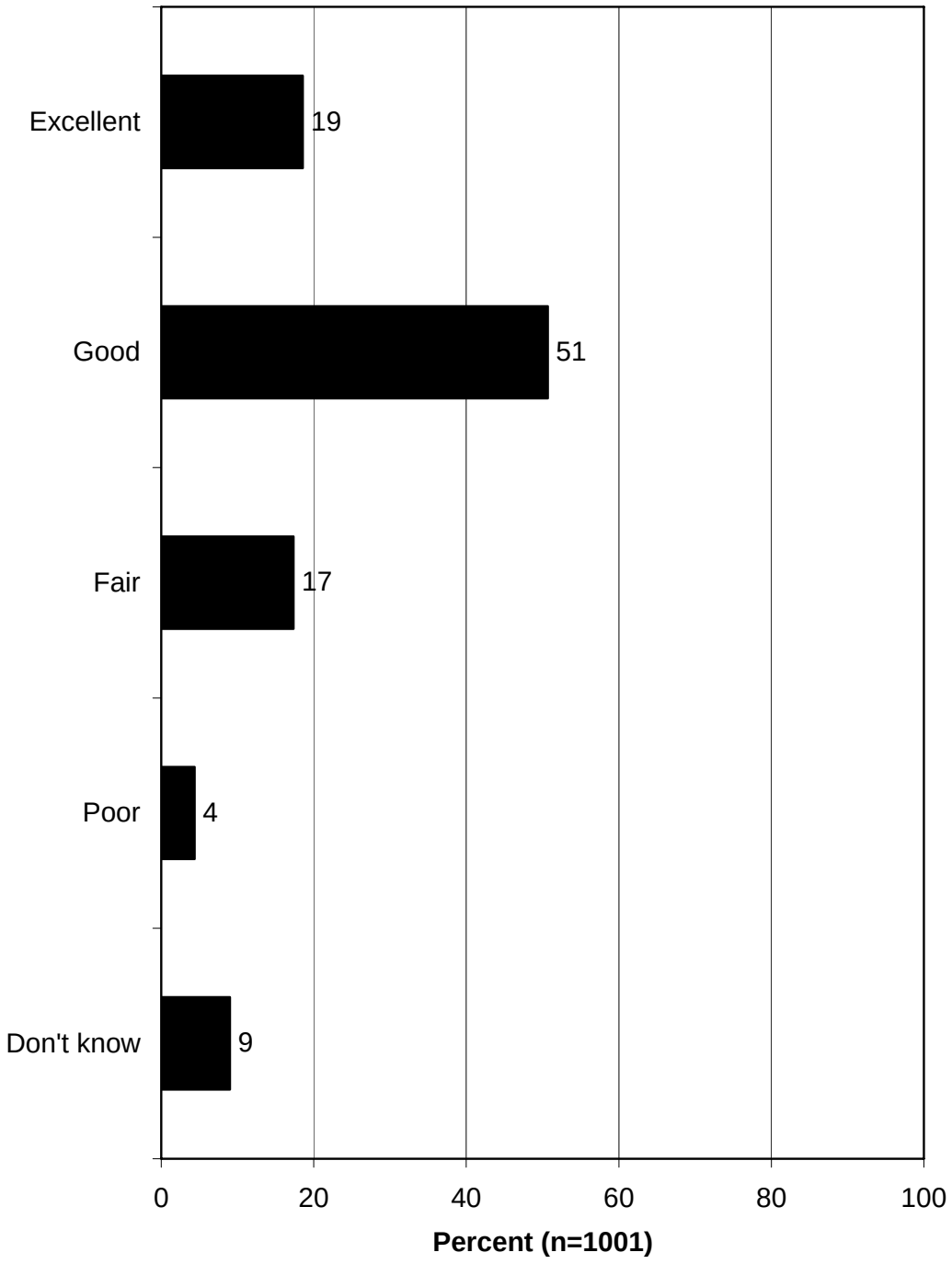
Q46. Do you think the DEP is very credible, somewhat credible, or not at all credible as a source of information on the environment?



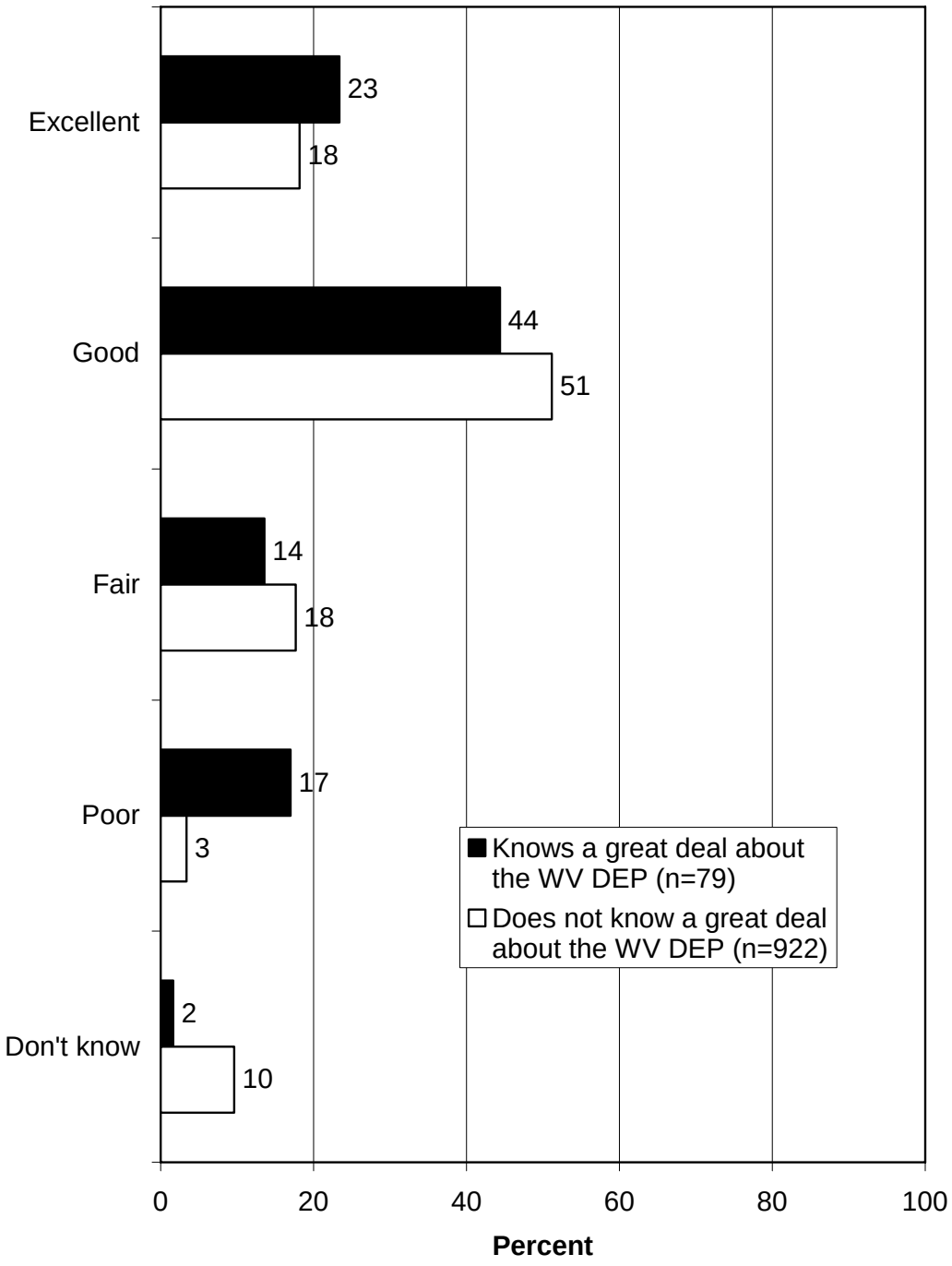
Q47. In your opinion, is the DEP very trustworthy, somewhat trustworthy, or not at all trustworthy?



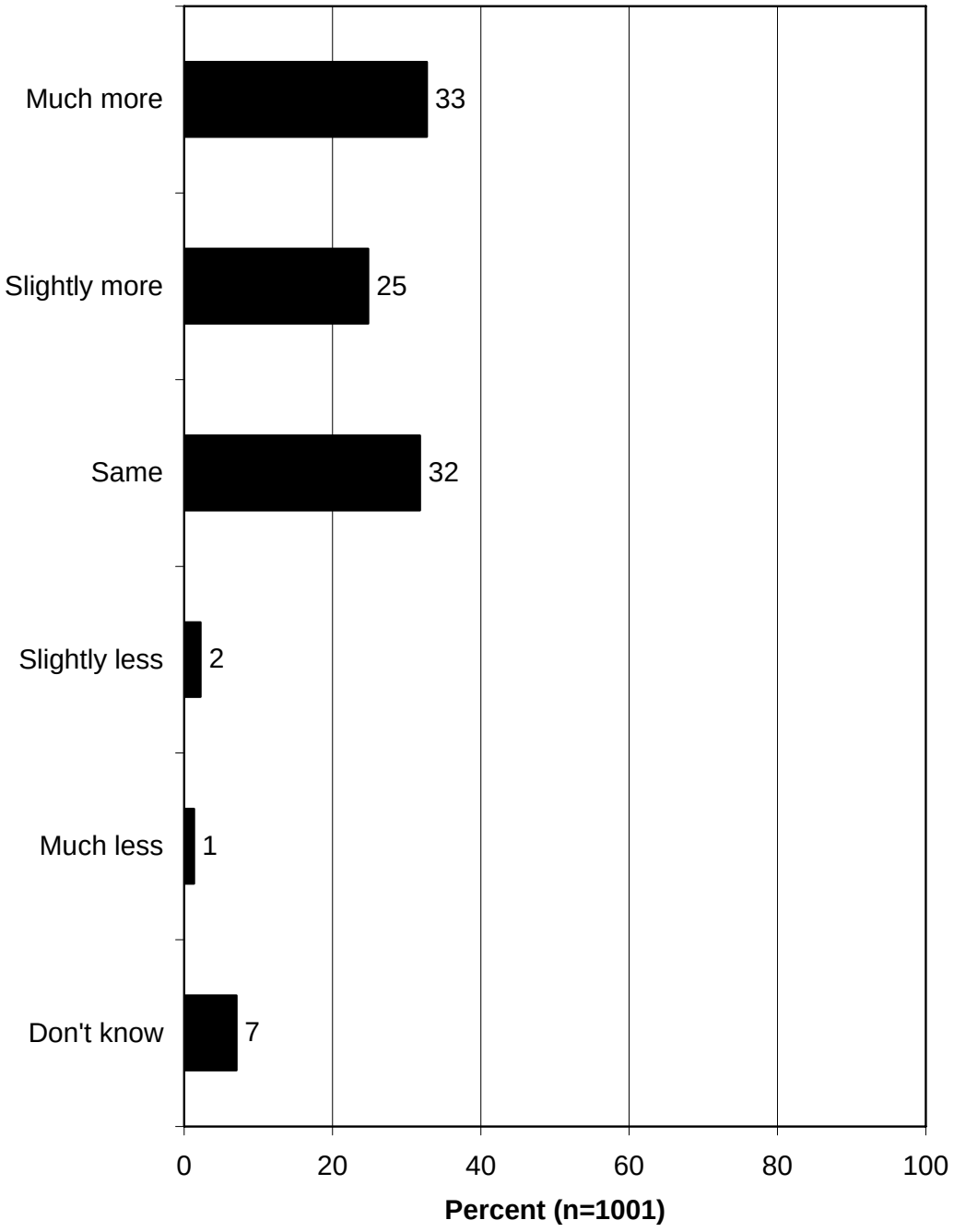
Q48. Overall, how would you rate the DEP's efforts to protect and restore the environment in West Virginia?



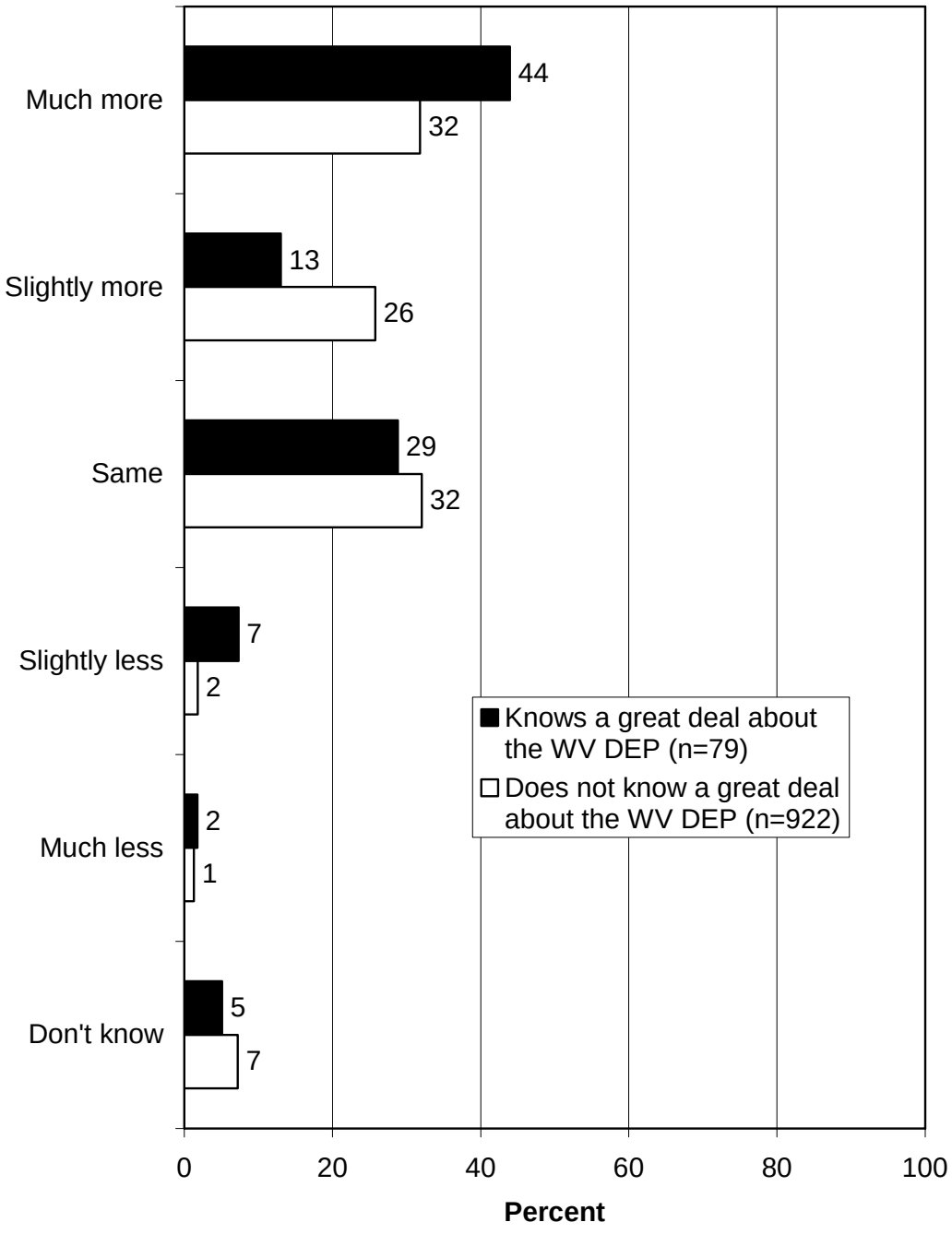
Q48. Overall, how would you rate the DEP's efforts to protect and restore the environment in West Virginia?



Q49. Do you think the DEP should spend more, the same, or less effort to protect and restore the environment?



Q49. Do you think the DEP should spend more, the same, or less effort to protect and restore the environment?



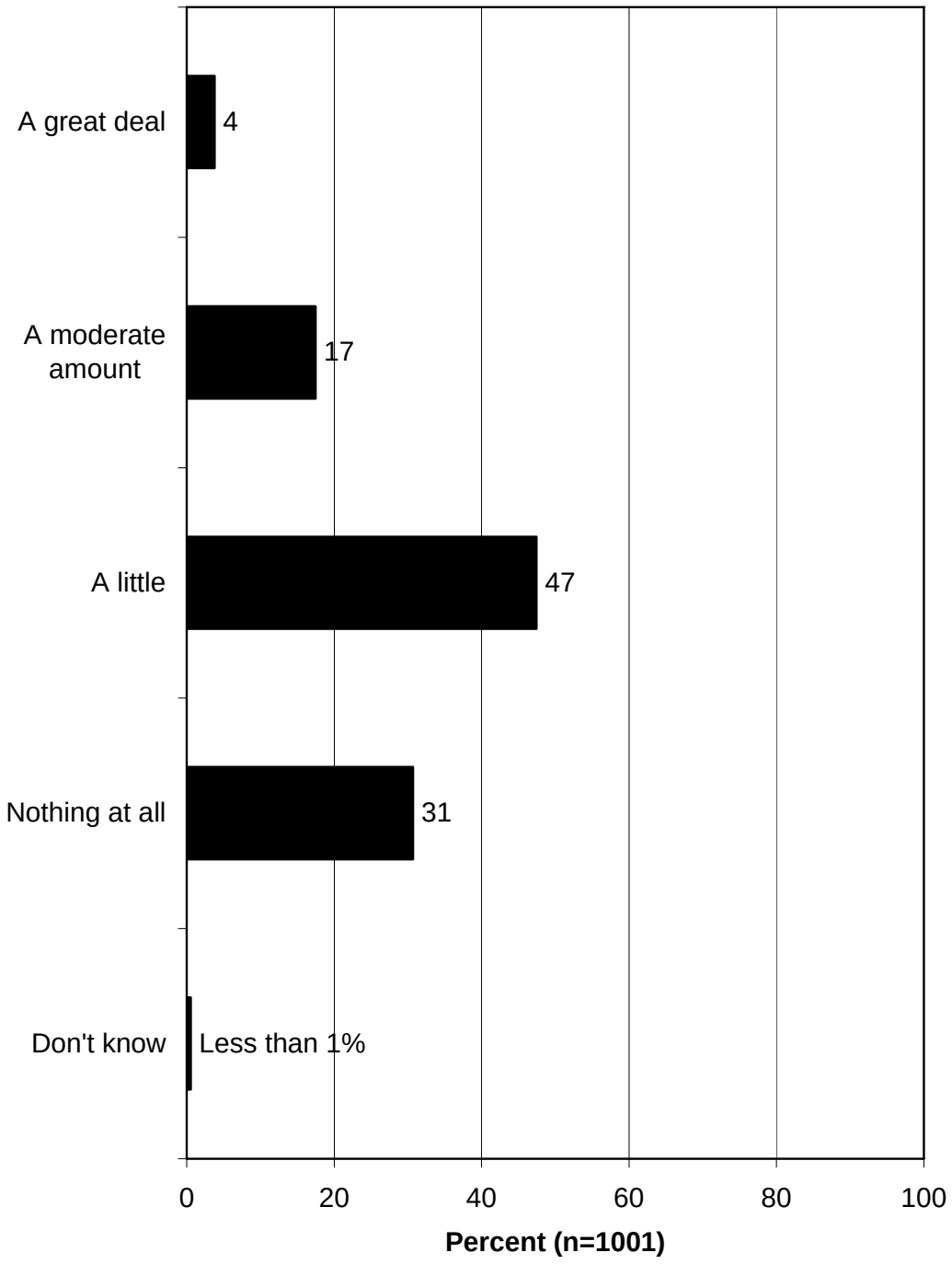
OPINIONS ON ALGAE IN WEST VIRGINIA WATERS

KNOWLEDGE AND AWARENESS OF ALGAE

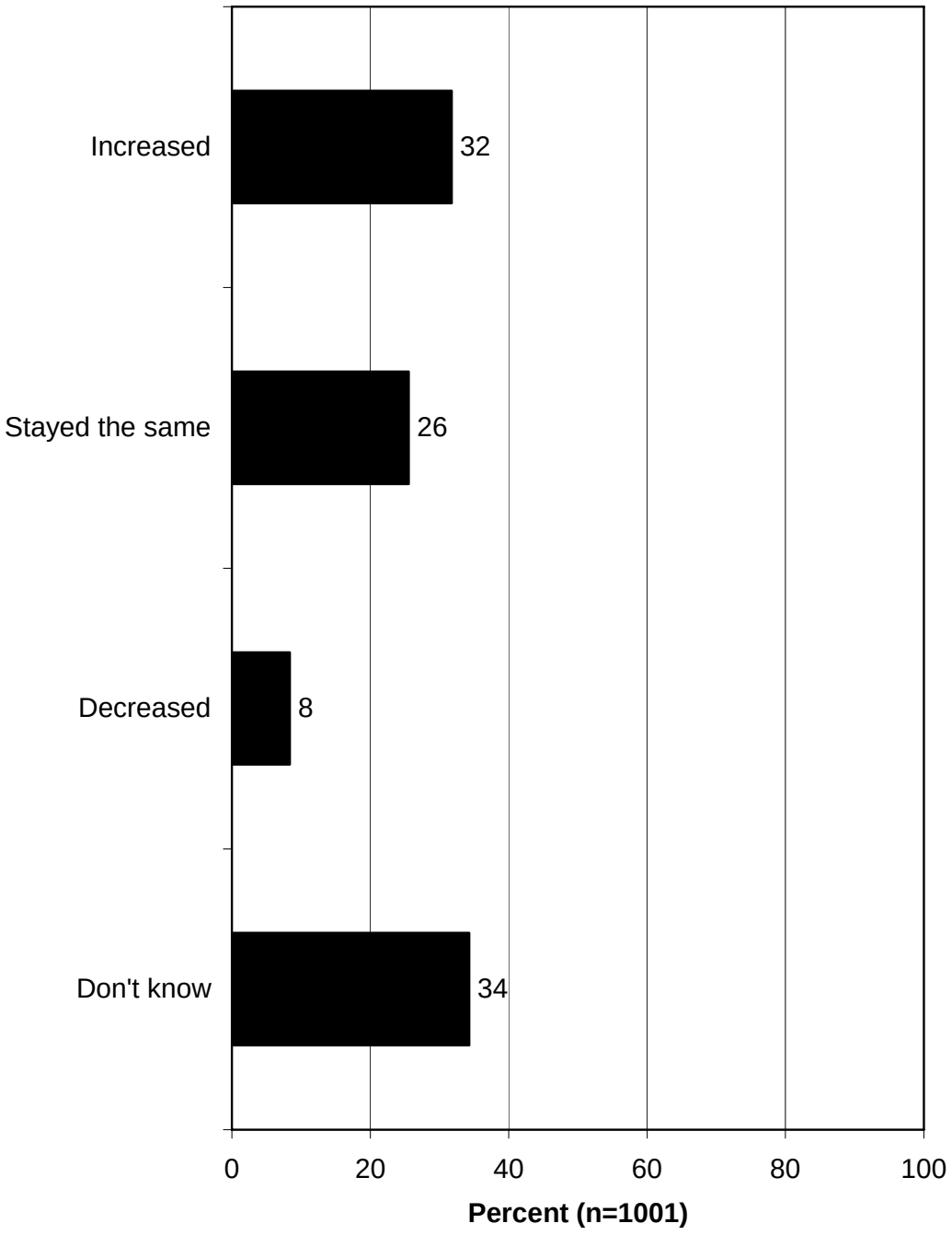
- Self-professed knowledge levels of algae in West Virginia waters is relatively low: 78% indicate knowing *a little* or *nothing at all* about it. At the other end, 21% say that they know *a great deal* or *moderate amount* (with only 4% at the highest level of *a great deal*).

- Respondents were asked if algae in West Virginia waters over the past 2 years has increased, stayed the same, or decreased. Most commonly, respondents with an opinion (i.e., those who did not say "don't know") say the levels have increased (32%), closely followed by the percentage saying that the levels have stayed the same (26%). Only 8% think levels have decreased. More than a third (34%) answered that they do not know.
  - This question was crosstabulated by participation in water-based activities on one of the three rivers of interest in this study (the three rivers of interest, chosen because of past algae issues on them, are the Greenbrier River, the South Branch of the Potomac, and the Cacapon River). This crosstabulation found that those who recreated on one of those rivers are more likely to think that algae has increased, compared to those who participated in water-based recreation but not on one of those three rivers.

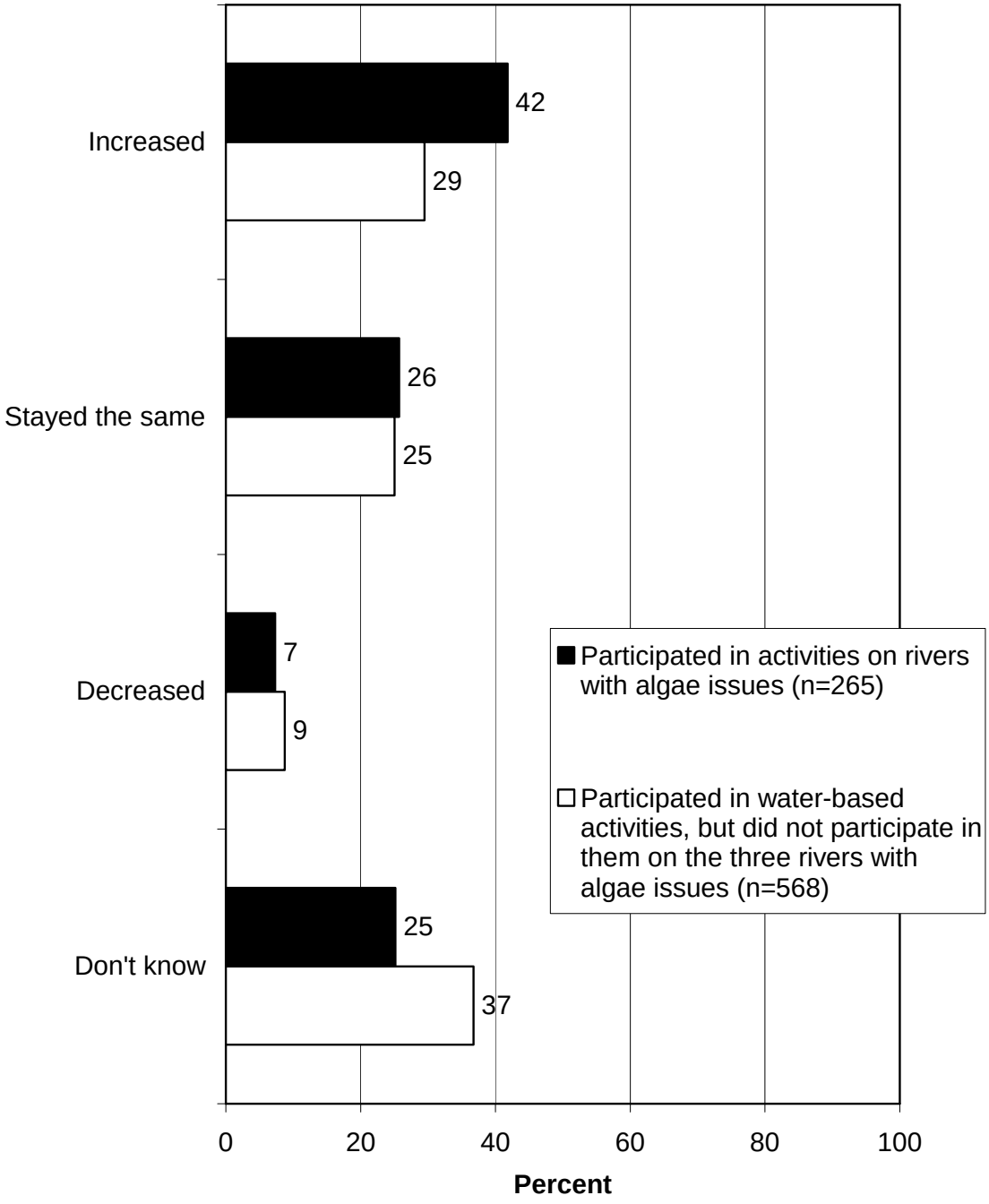
Q53. How much would you say you know about algae in West Virginia waters?



Q54. Would you say algae in West Virginia waters has increased, stayed about the same, or decreased in the past 2 years?



Q54. Would you say algae in West Virginia waters has increased, stayed about the same, or decreased in the past 2 years?



OPINIONS ON ALGAE IN GENERAL

- The survey presented respondents with a scale regarding whether algae bothers them or not, and they are more bothered than not. The scale ran from "algae doesn't bother me at all" through "algae doesn't bother me, but I worry about problems it may cause" to "I generally regard algae as a nuisance." (To allow all answers, there is a neutral answer of "I have no particular feelings about algae.") Respondents are predominantly in the middle to the least tolerant of algae on the scale: 32% are in the middle ("algae doesn't bother me, but I worry about problems it may cause") and 26% are at the least tolerant ("I generally regard algae as a nuisance"); meanwhile, only 10% are not bothered at all about algae.
  - This question was crosstabulated by participation in water-based activities on one of the three rivers of interest (the Greenbrier River, the South Branch of the Potomac, and the Cacapon River). These participants are more likely to express concern about algae and are less likely to have no opinion about it, compared to their counterparts.

- Another line of questioning asked respondents directly if they agreed or disagreed that "algae is a problem in West Virginia." They are split in their opinions. Note that the sample was split in half to eliminate bias, and the question was asked in two ways. Respondents in the first half of the sample were asked if they agreed or disagreed with this statement: "Algae is a problem in West Virginia." Respondents in the second half of the sample were asked if they agreed or disagreed with this statement: "Algae is not a problem in West Virginia."
  - In coding the two questions together (reverse coding the "not" question), the analysis found that from 32% to 44% think that algae is a problem, and from 29% to 34% think that algae is not a problem. A relatively large percentage gave a neutral answer (neither agreed nor disagreed or answered that they did not know) (from 27% to 34%).

- The survey asked respondents whether they had been concerned about algae in West Virginia waters over the past 2 years: a quarter (25%) answered that they had been.

- A series of 10 questions gauged residents' opinions on algae. For each question, a statement about algae (some negative, some positive) was read to respondents, who indicated if they agreed or disagreed with the statement. This allows for a comparison of various themes as they relate to algae. The tabulation below shows the themes, the statements read to respondents, and an indication of whether the statement is negative or positive.
  - Because some statements reflected a positive opinion and others a negative opinion, for comparison, the negative statements were reverse coded. In other words, strong agreement to a negative statement was compared to strong *dis*agreement to a positive statement, and so forth. In this way, the themes could be compared.

Theme	**Statement Read to Respondents**	**Agreeing Is a Negative or Positive Opinion About Algae**
Aesthetics	Algae is generally unattractive and unappealing.	Negative
Ecological	Algae is beneficial for the ecosystem in some situations.	Positive
	Algae makes natural public waters unhealthy for fish and plant life.	Negative
Recreational	The current algae levels in West Virginia waters do not bother me when participating in water activities.	Positive
	Algae causes safety hazards for people participating in activities on or in West Virginia waters.	Negative
Health	Algae in natural public waters is not a health hazard for people.	Positive
Overall (in General)	Algae is a problem in West Virginia.	Negative
	Algae is not a problem in West Virginia.	Positive

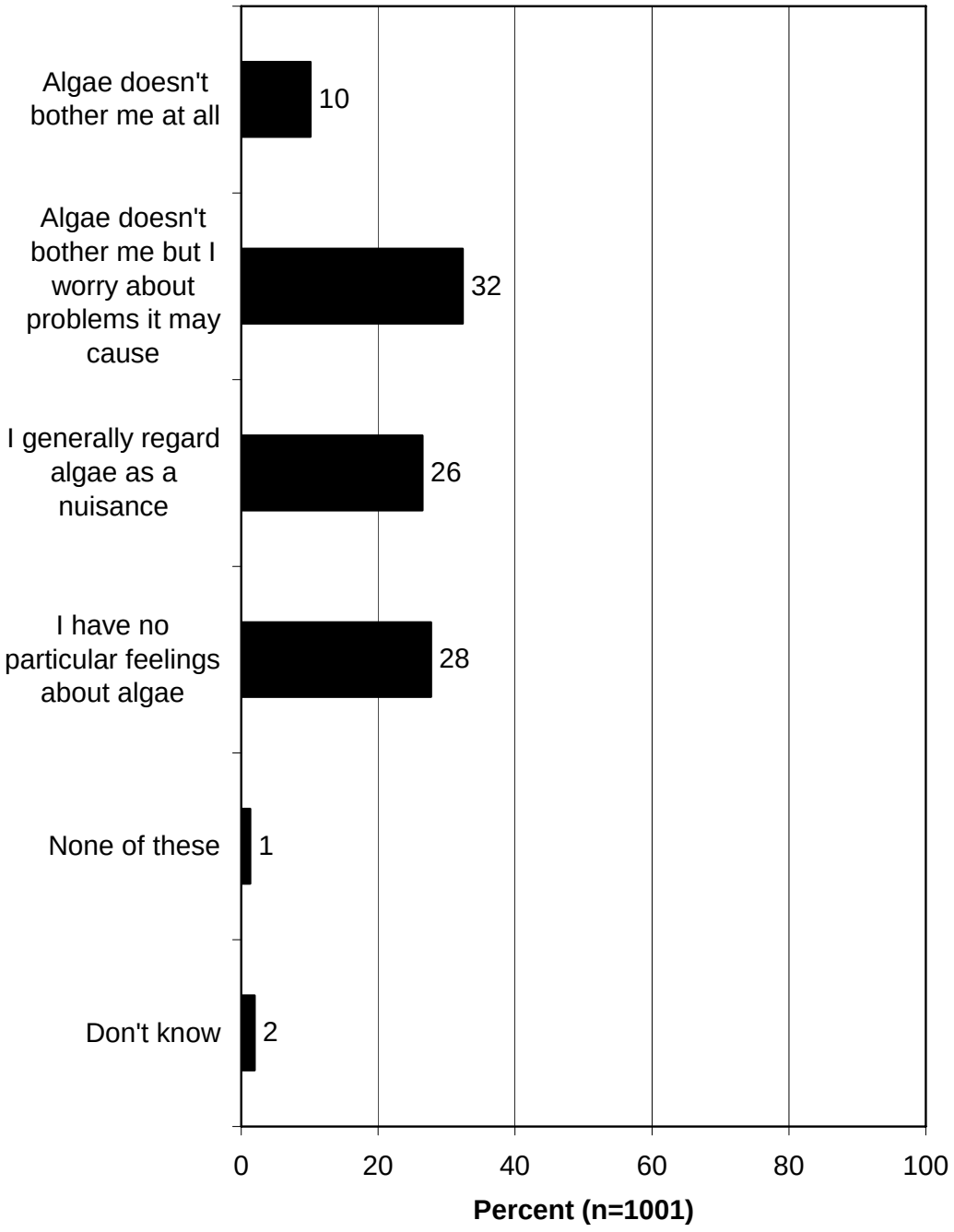
  - In looking at holding a strong negative opinion, the aesthetic theme resonated with respondents: the top statement by far is "Algae is generally unattractive and unappealing" (45% strongly agree).
  - In examining a strong or moderate negative opinion, the aforementioned aesthetic theme was the top ranked item by far (79% agreed), followed by the recreational theme: "Algae causes safety hazards for people participating in activities on or in West Virginia waters" (57% agreed).
  - In looking at any positive opinions about algae, it appears that West Virginia residents understand that some amount of algae is part of a natural ecosystem: the top positive

opinion about algae is that "Algae is beneficial for the ecosystem in some situations" (79% of residents agree with this statement, including 36% who strongly agree). The second spot in the positive realm is recreational: "The current algae levels in West Virginia waters do not bother me when participating in water activities" (55% agree, with 29% strongly agreeing).

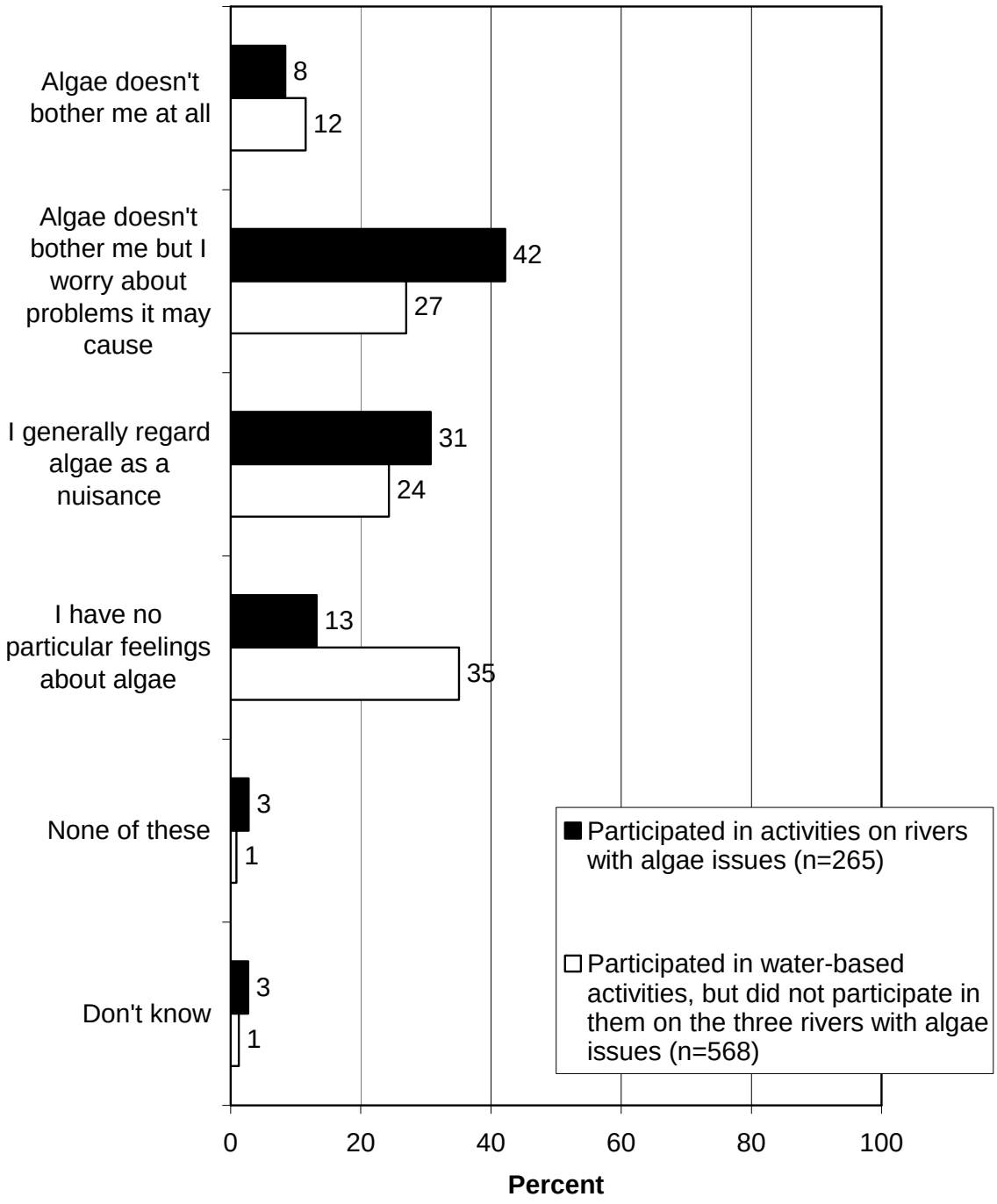
- The fact that a recreational theme resonates both negatively and positively suggest a dichotomy among recreationists: some recreationists are not bothered by algae when they participate in water-based recreational activities, while other recreationists consider algae a safety hazard, but there is no consensus among recreationists.
- Crosstabulations were run of two of the statements to further explore this dichotomy: "The current algae levels in West Virginia waters do not bother me when participating in water activities" and "Algae causes safety hazards for people participating in activities on or in West Virginia waters." The crosstabulations found that boaters using human power only were more likely than power boaters to disagree that "current algae levels in West Virginia waters do not bother me when participating in water activities" (in other words, more likely to be bothered by algae). Also, human powered boaters were more likely to agree that "algae causes safety hazards for people participating in activities on or in West Virginia waters." In short, boaters using human power show a more negative opinion of algae, compared to power boaters.

- Crosstabulations of this series of questions found that those who participated in activities on or in West Virginia waters of the three rivers of interest (the Greenbrier, the South Branch of the Potomac, or the Cacapon) have more negative opinions of algae, compared to those who participated in water-based activities but not on any of the three rivers of interest.

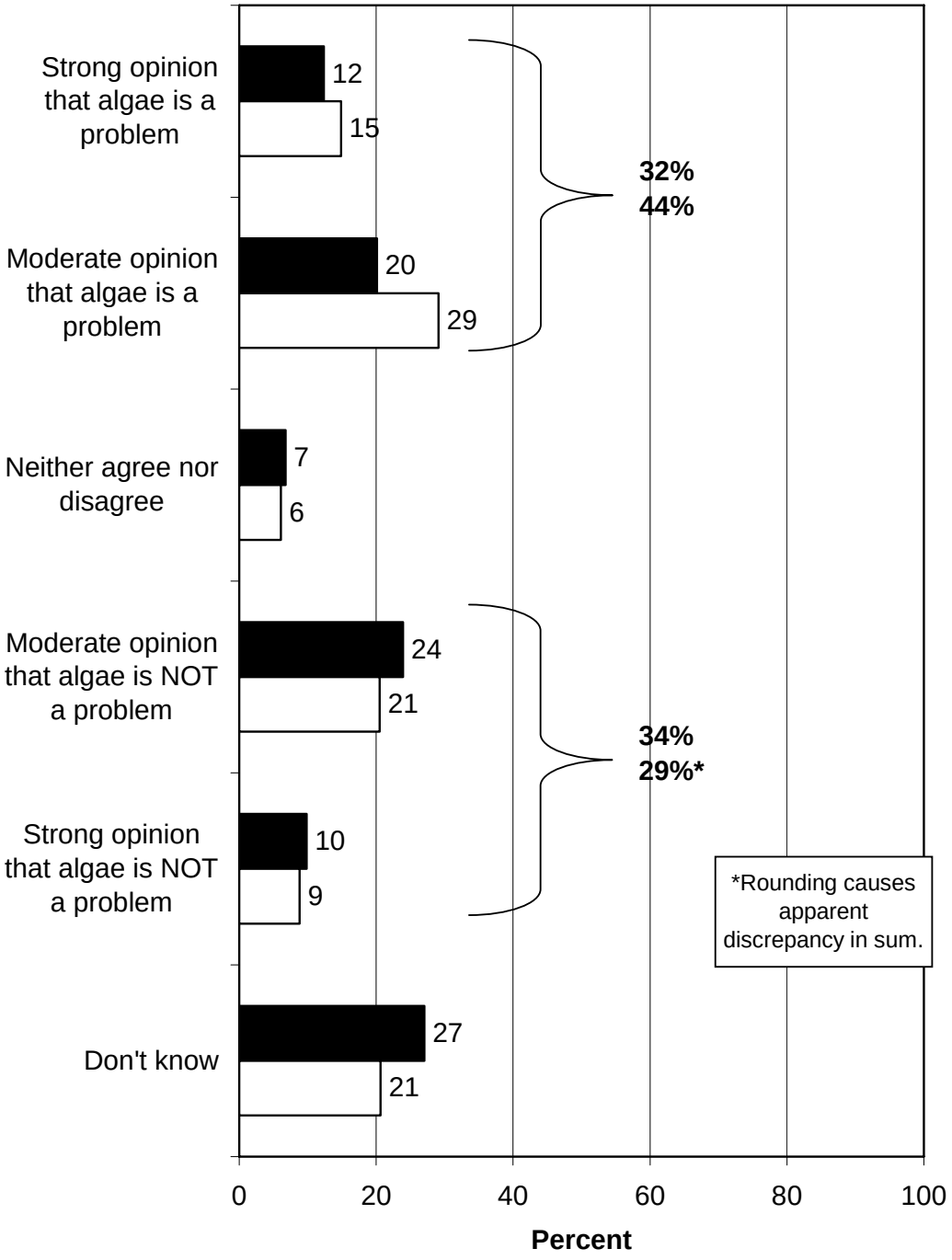
Q55. Generally, which of the following best describes your feelings about algae in West Virginia waters in the past 2 years?



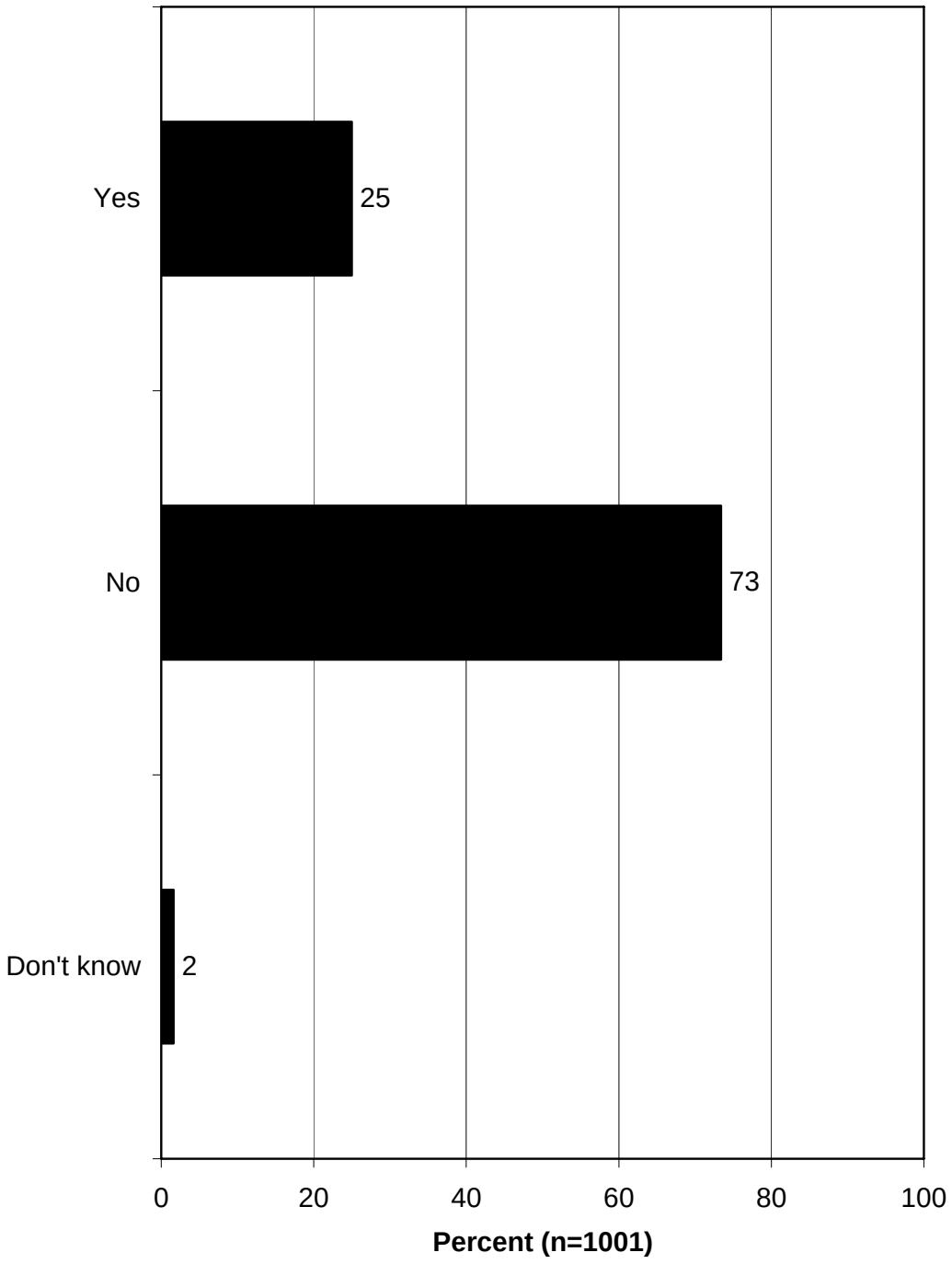
Q55. Generally, which of the following best describes your feelings about algae in West Virginia waters in the past 2 years?



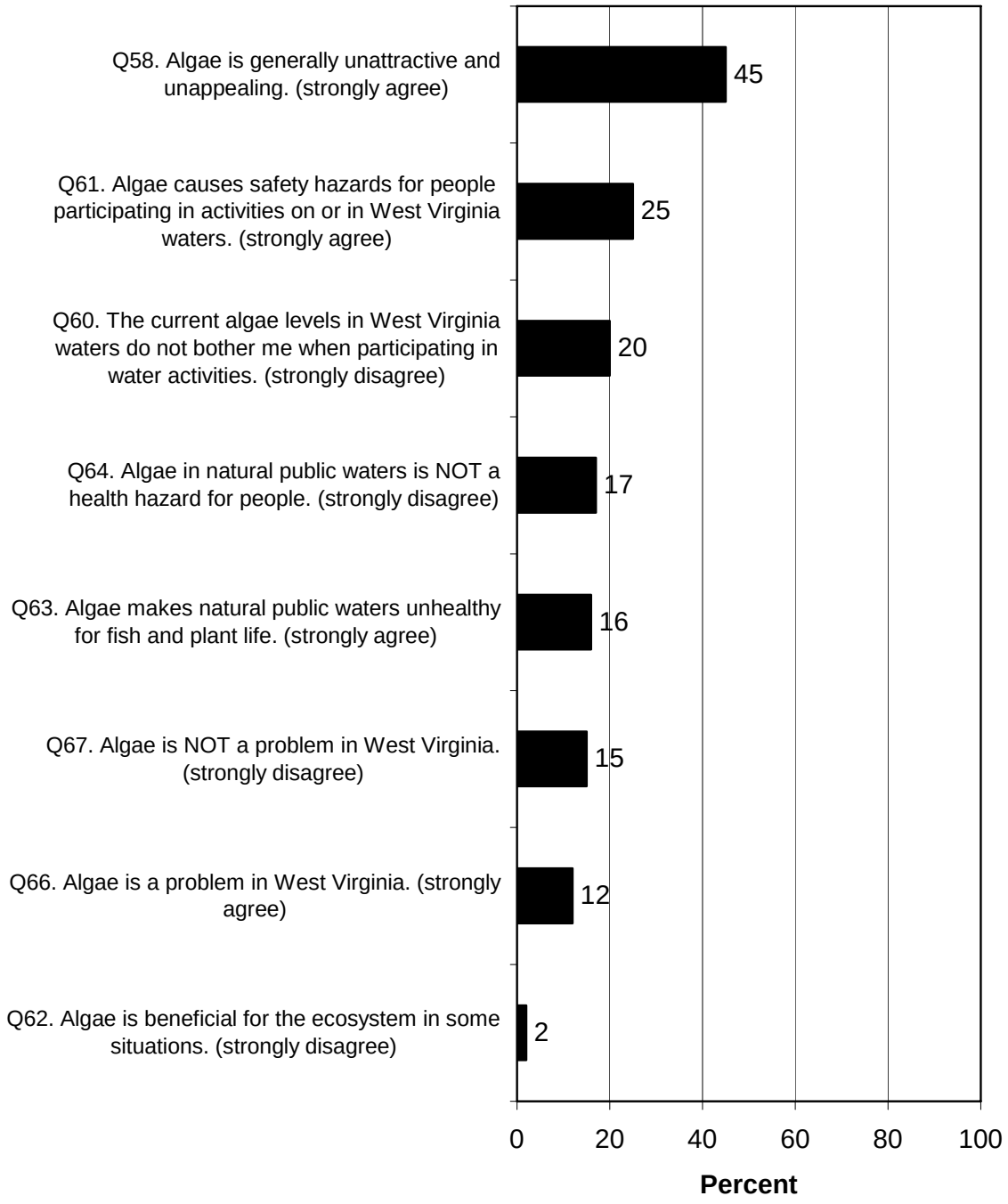
Q66. Algae is a problem in West Virginia.
Q67. Algae is NOT a problem in West Virginia.
(Q67 has been reverse coded for comparison.)



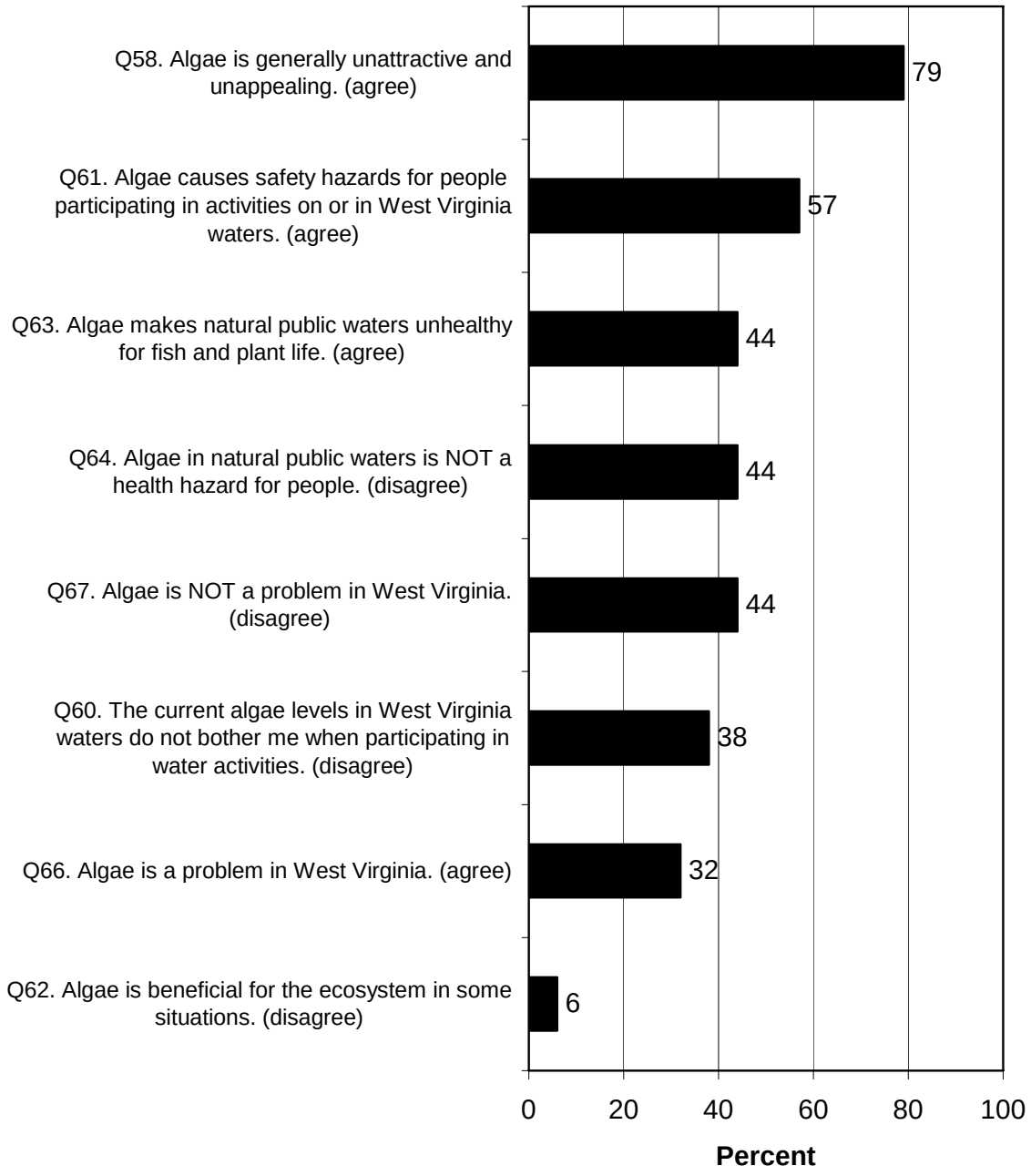
Q86. Over the past 2 years, have you been concerned at all about algae in West Virginia waters?



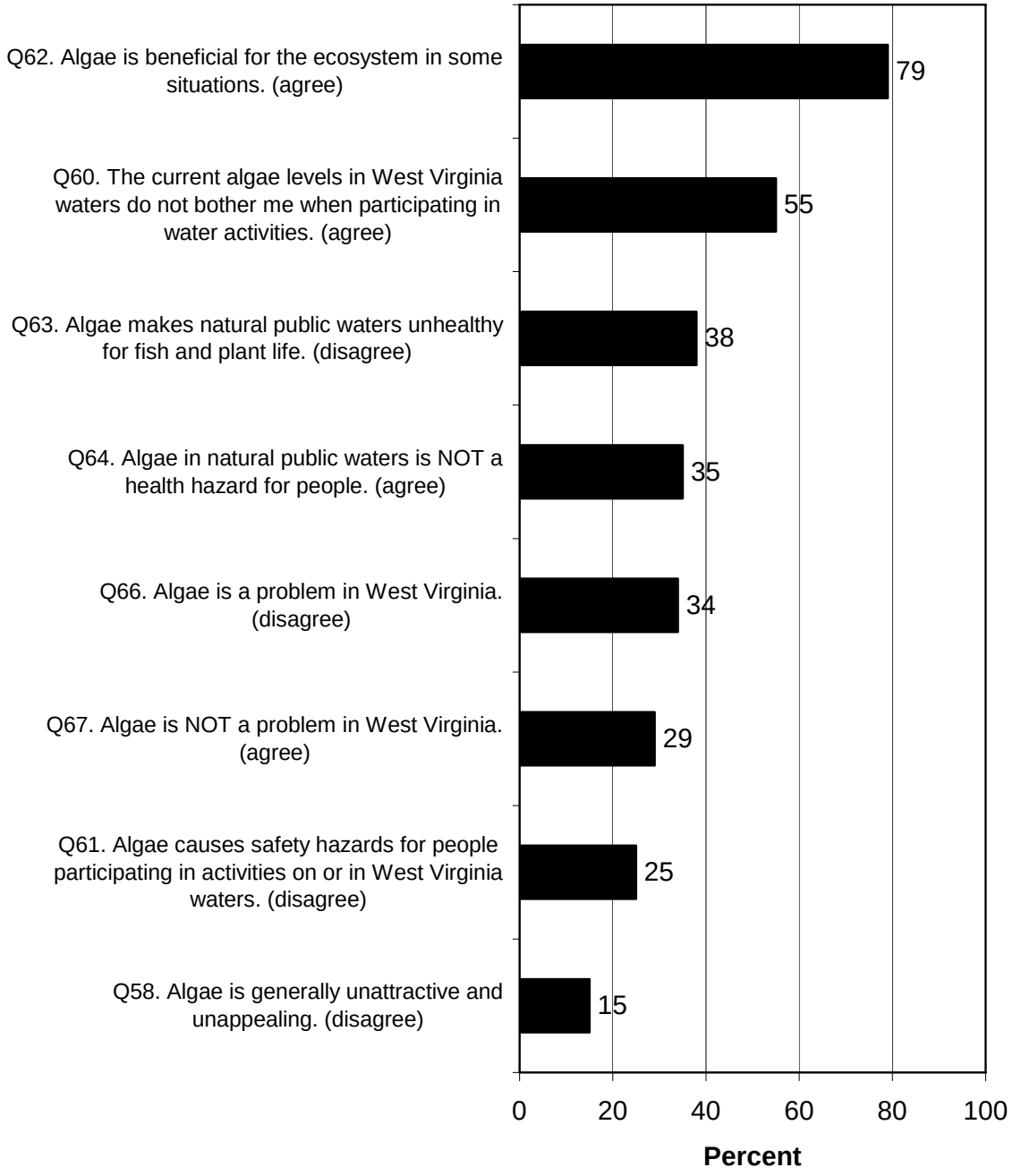
Q58-67. Percent of respondents who have a strong negative opinion about algae:



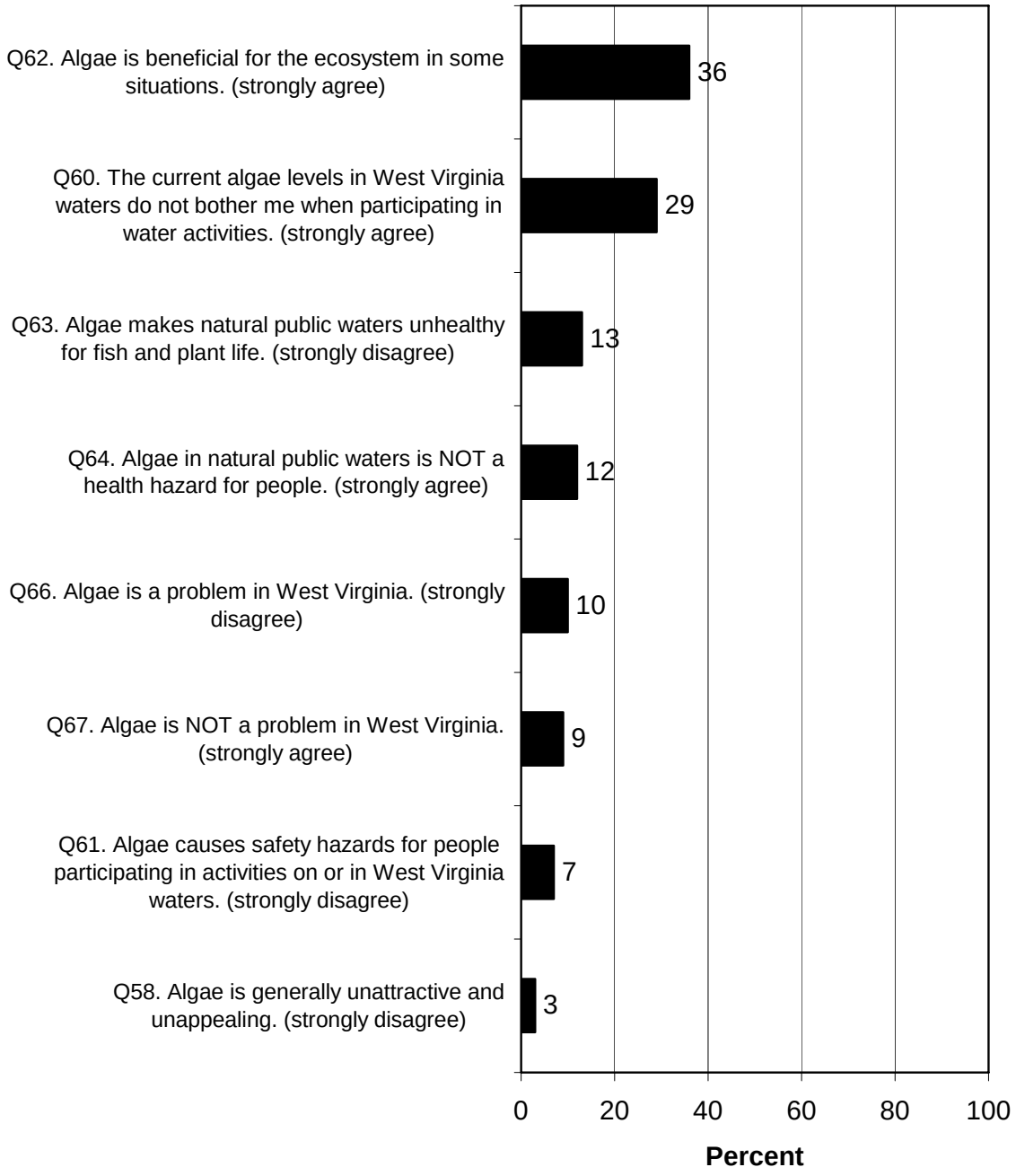
Q58-67. Percent of respondents who have a strong or moderate negative opinion about algae:



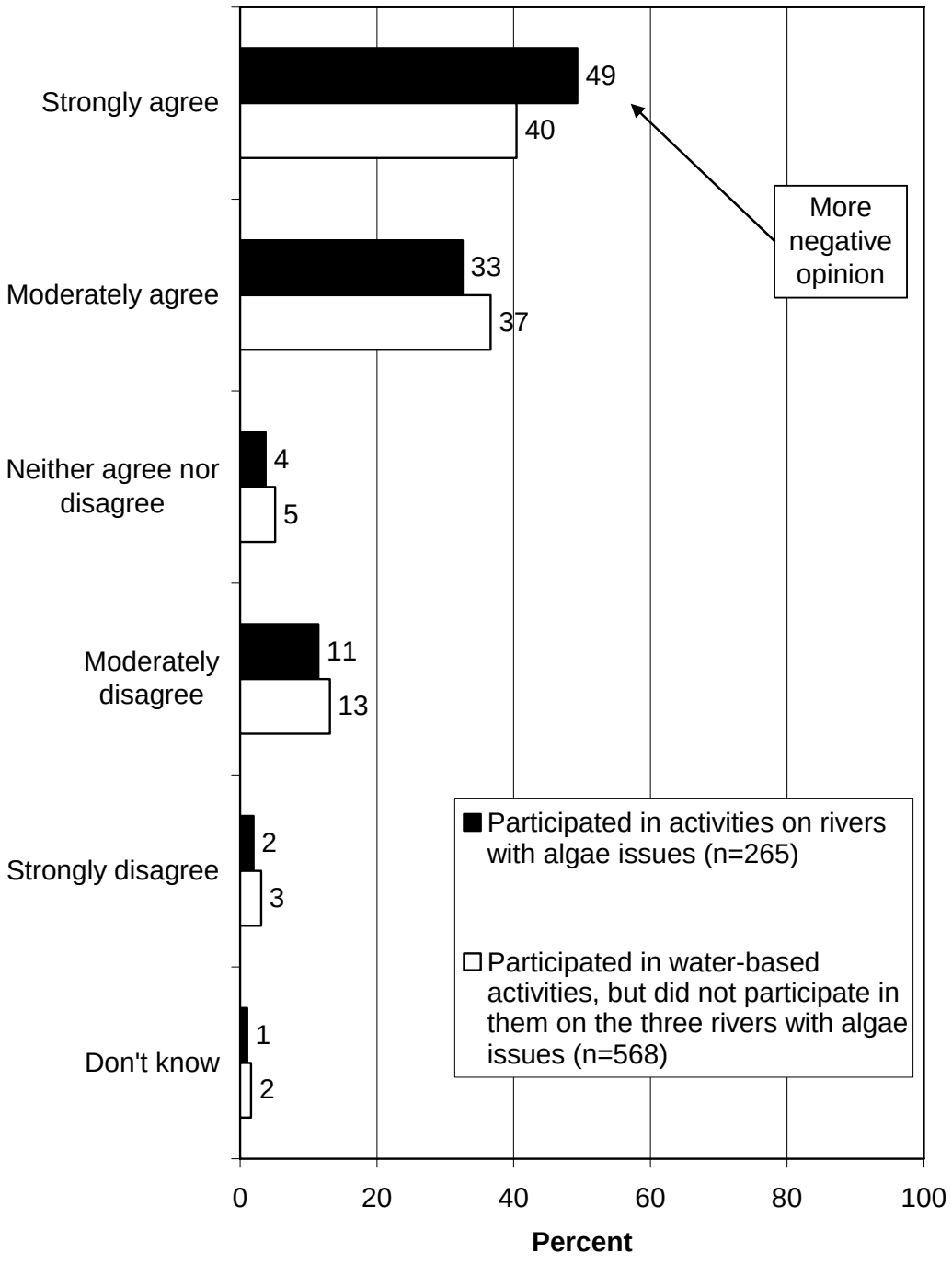
Q58-67. Percent of respondents who have a strong or moderate positive opinion about algae:



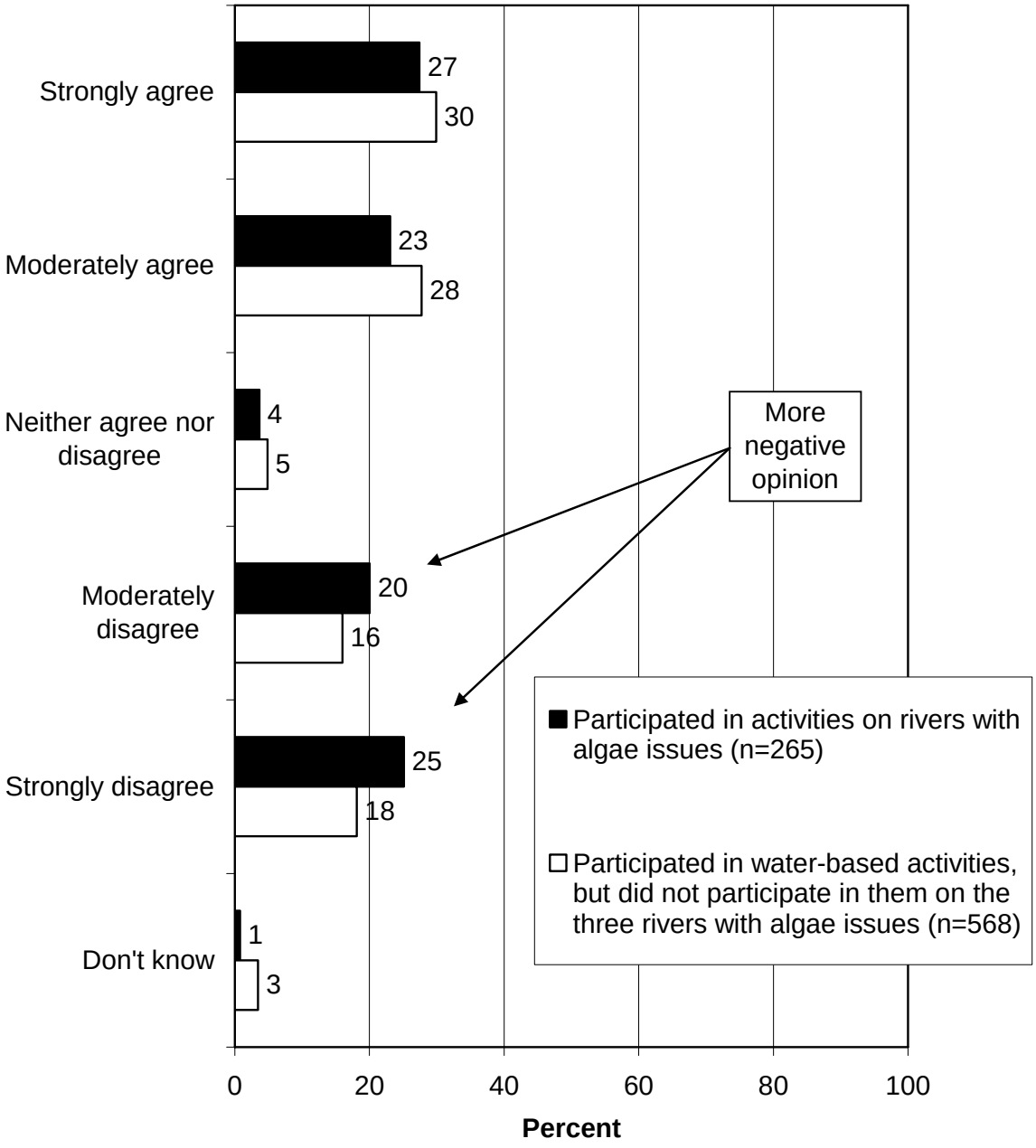
Q58-67. Percent of respondents who have a strong positive opinion about algae:



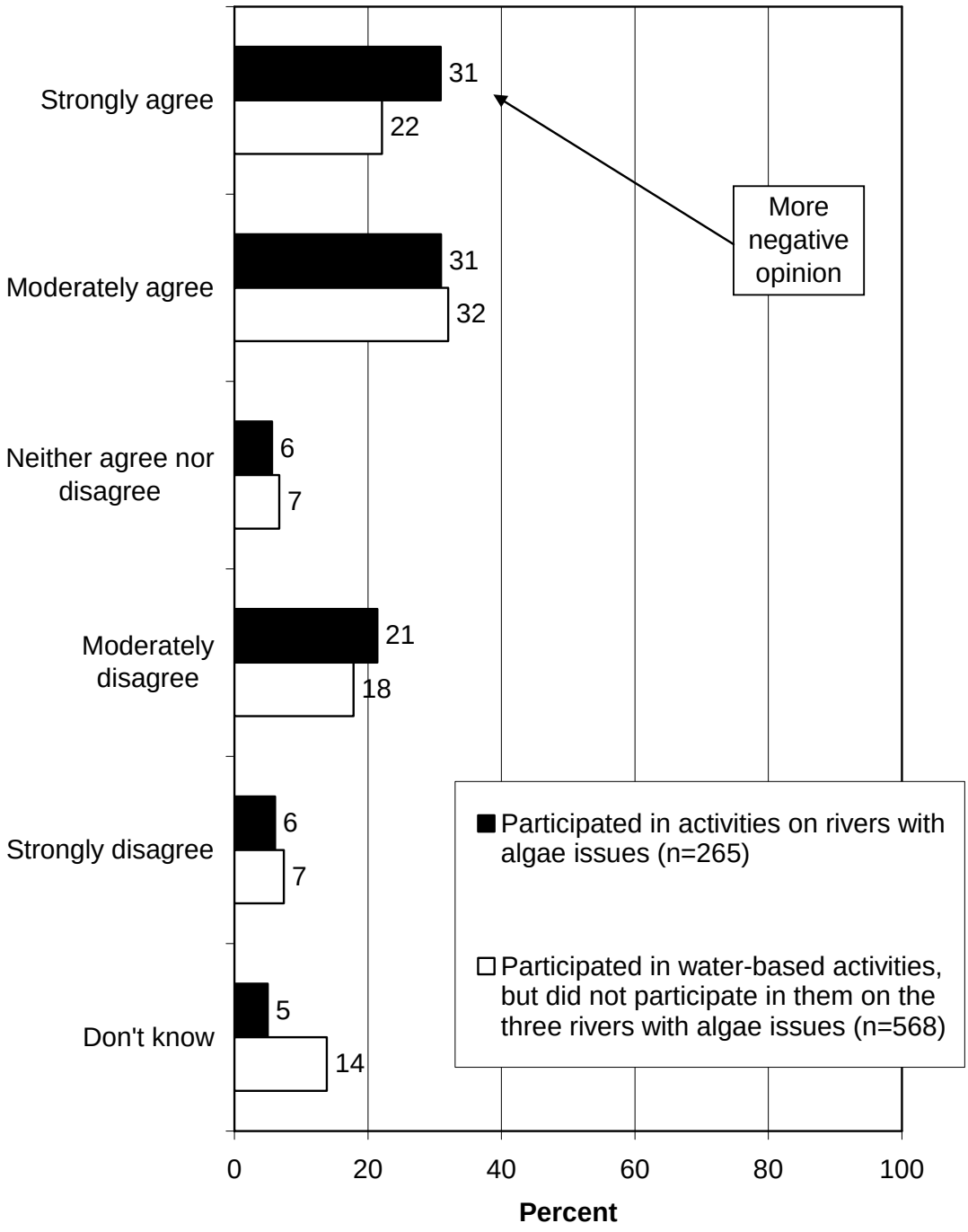
Q58. Algae is generally unattractive and unappealing.



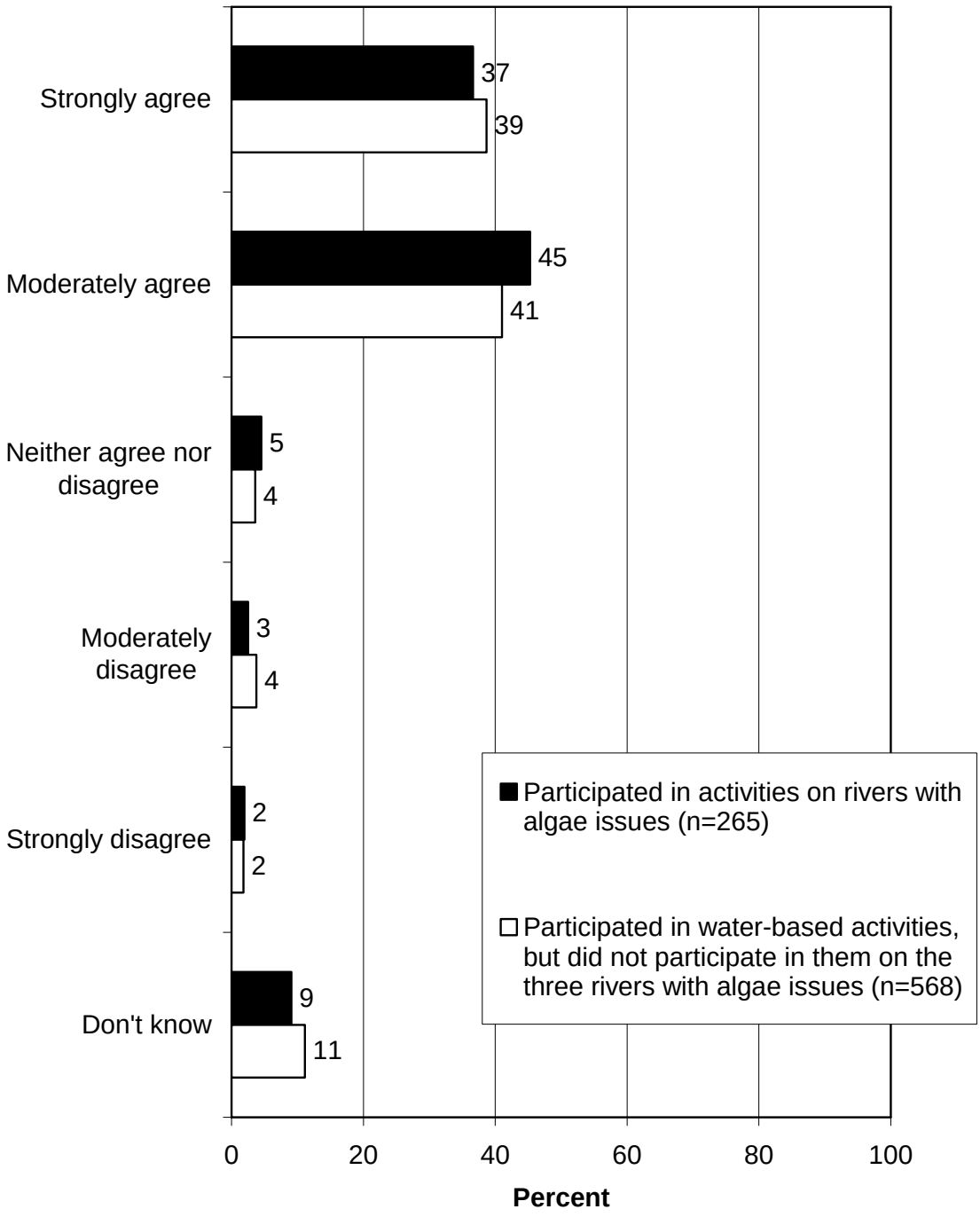
Q60. The current algae levels in West Virginia waters do not bother me when participating in water activities. (Asked of those who participated in at least one activity on or in West Virginia waters in the past 2 years.)



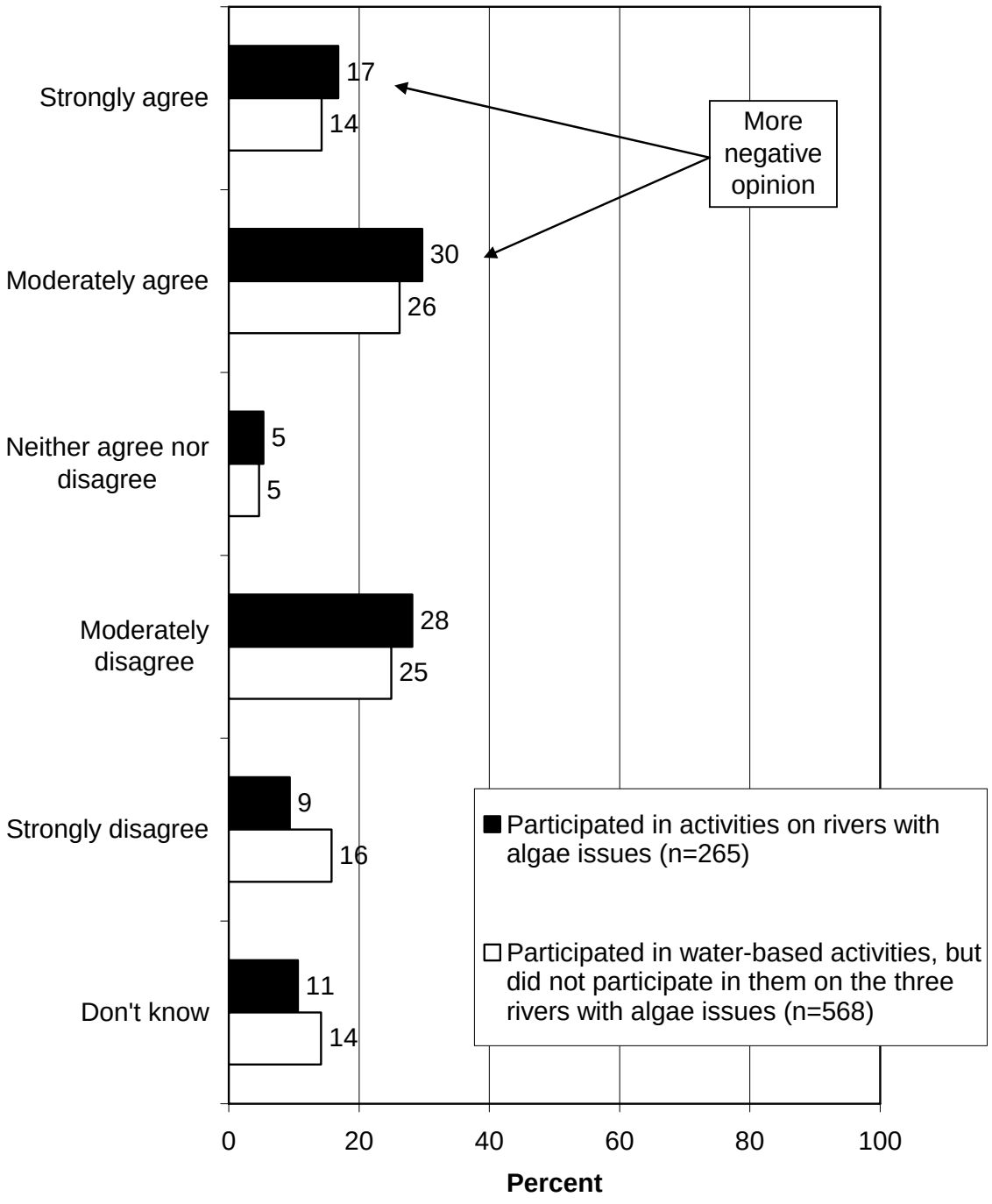
Q61. Algae causes safety hazards for people participating in activities on or in West Virginia waters.



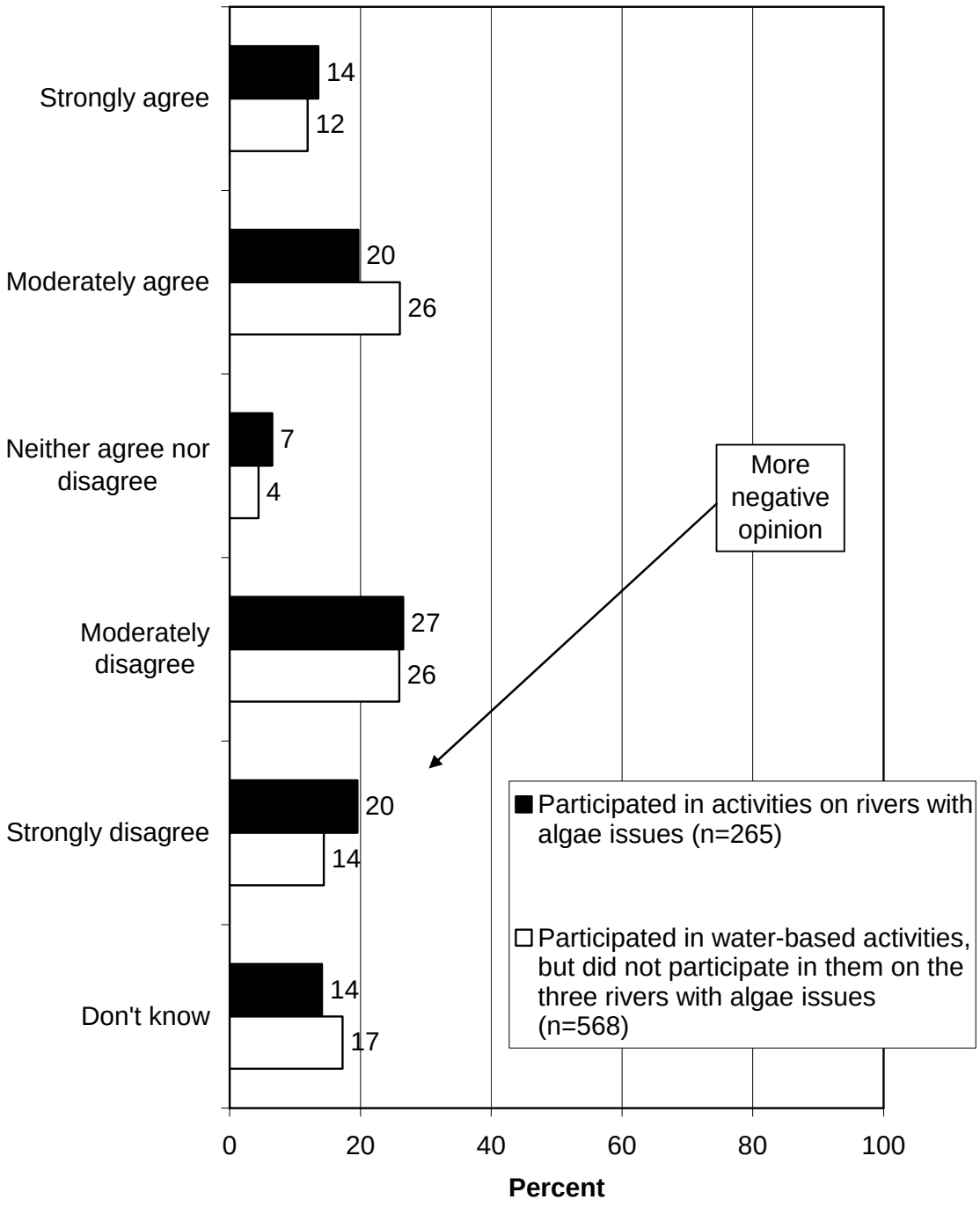
Q62. Algae is beneficial for the ecosystem in some situations.



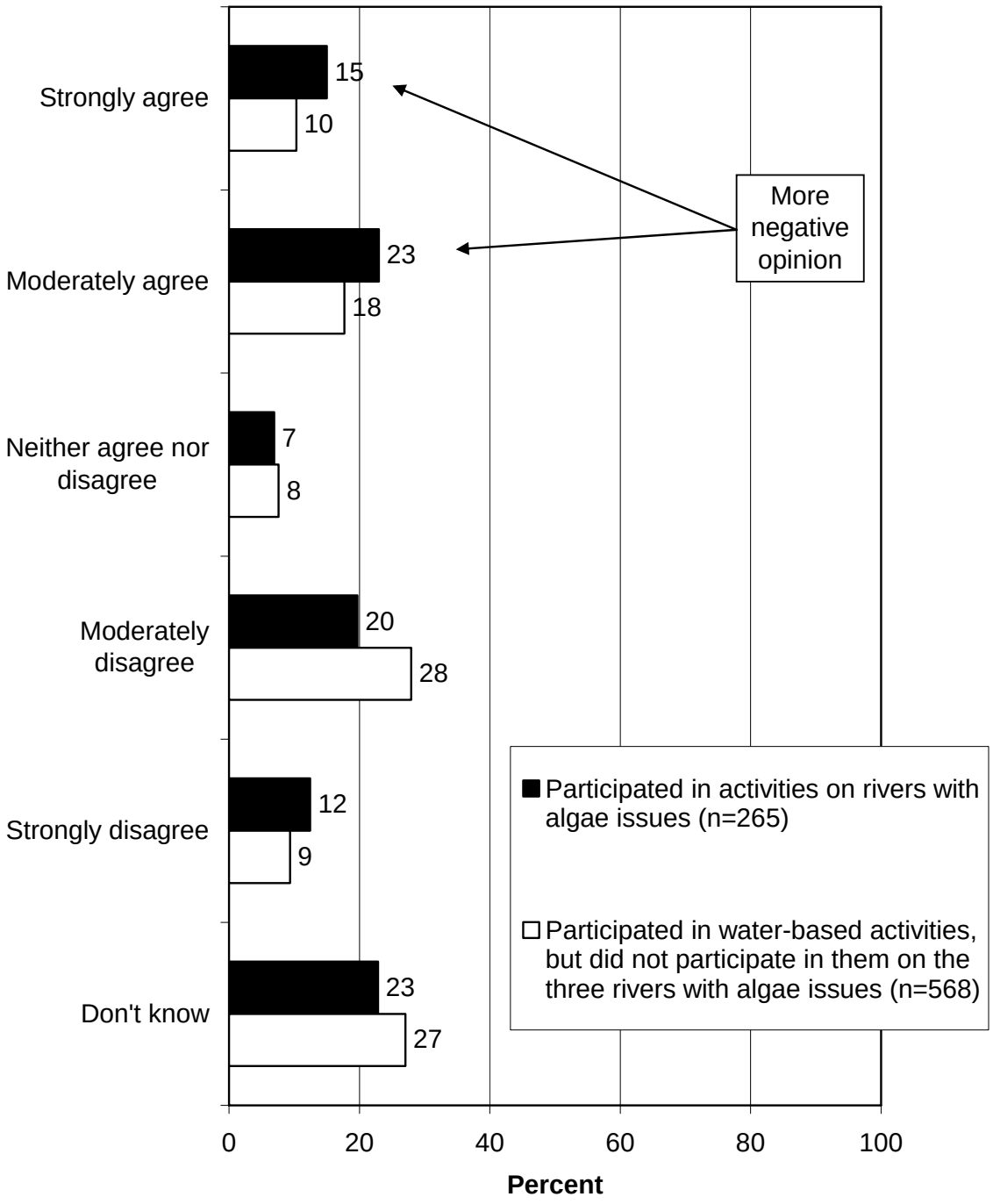
Q63. Algae makes natural public waters unhealthy for fish and plant life.



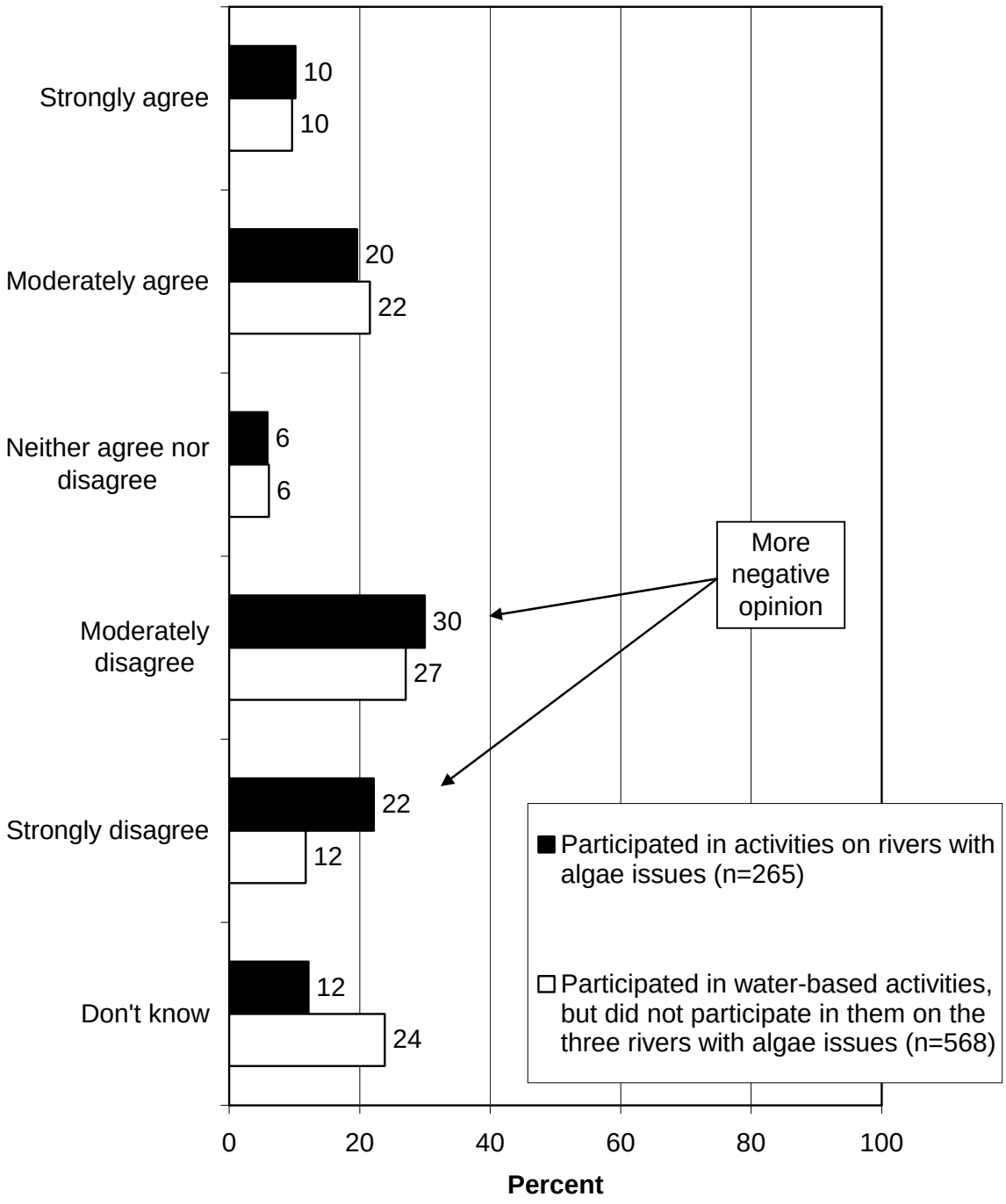
Q64. Algae in natural public waters is NOT a health hazard for people.



Q66. Algae is a problem in West Virginia. (Asked of randomly selected half of sample.)



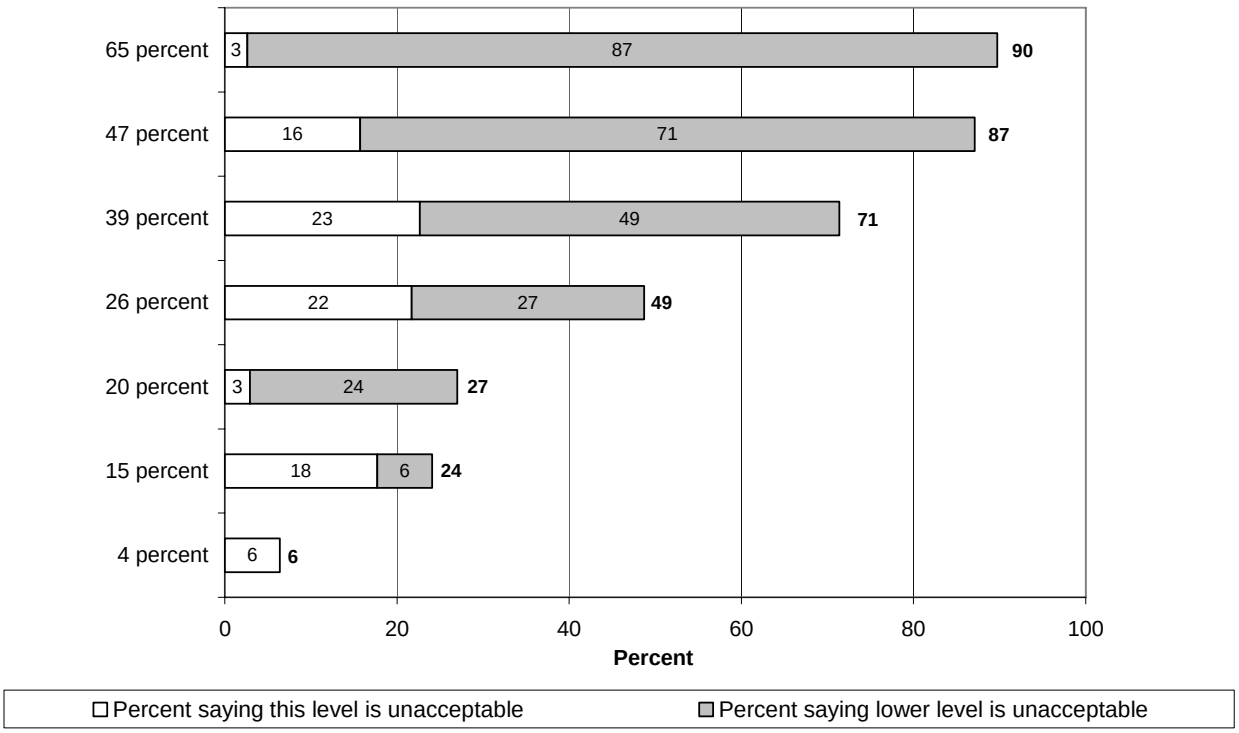
Q67. Algae is NOT a problem in West Virginia.
(Asked of randomly selected half of sample.)

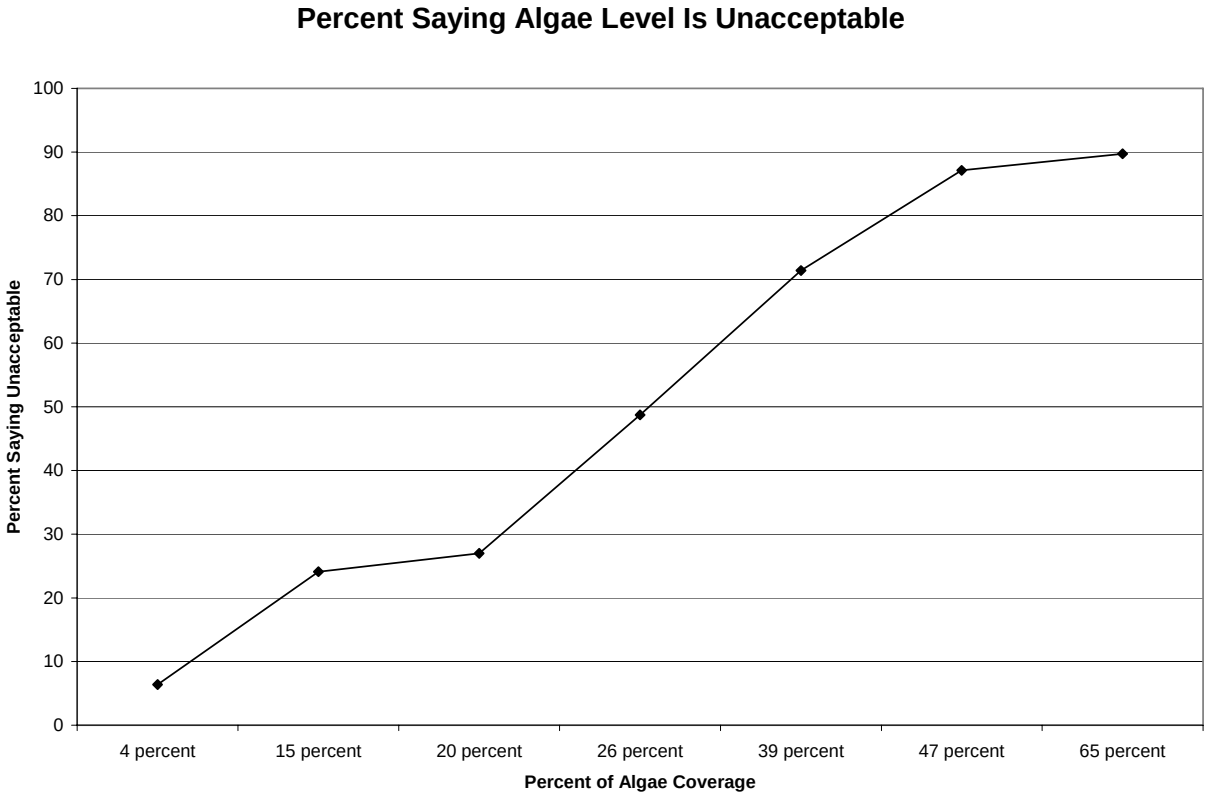


LEVELS OF ACCEPTANCE OF ALGAE

- The primary focus of the survey was to help determine the level of tolerance West Virginia residents have regarding algae in the state's natural waters. As discussed in the methodology, respondents examined photographs that showed varying levels of algae cover to determine at which point the level becomes unacceptable to them. The photograph that shows 20 percent coverage has only about a quarter of respondents (27%) thinking that this amount is unacceptable. However, the next photograph, showing 26 percent coverage, has nearly half of respondents (49%) thinking that the level is unacceptable. This suggests that waters with any more than a quarter coverage will be unacceptable to a majority of residents. A line graph on the following page illustrates this in addition to the stacked bar graph below.

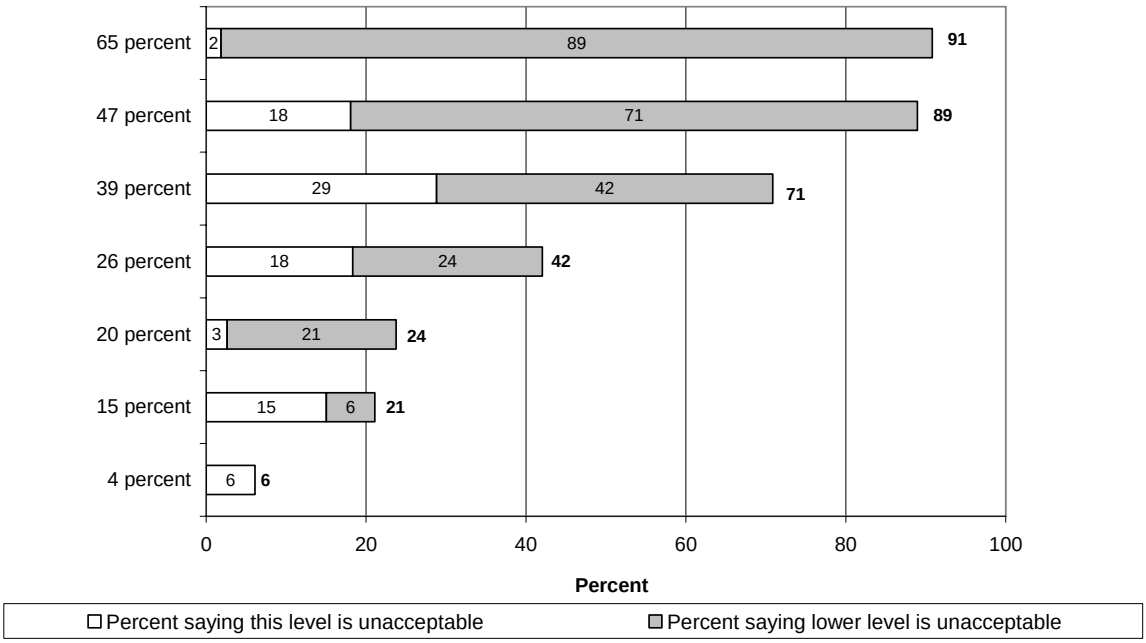
Percent Saying Algae Level Is Unacceptable



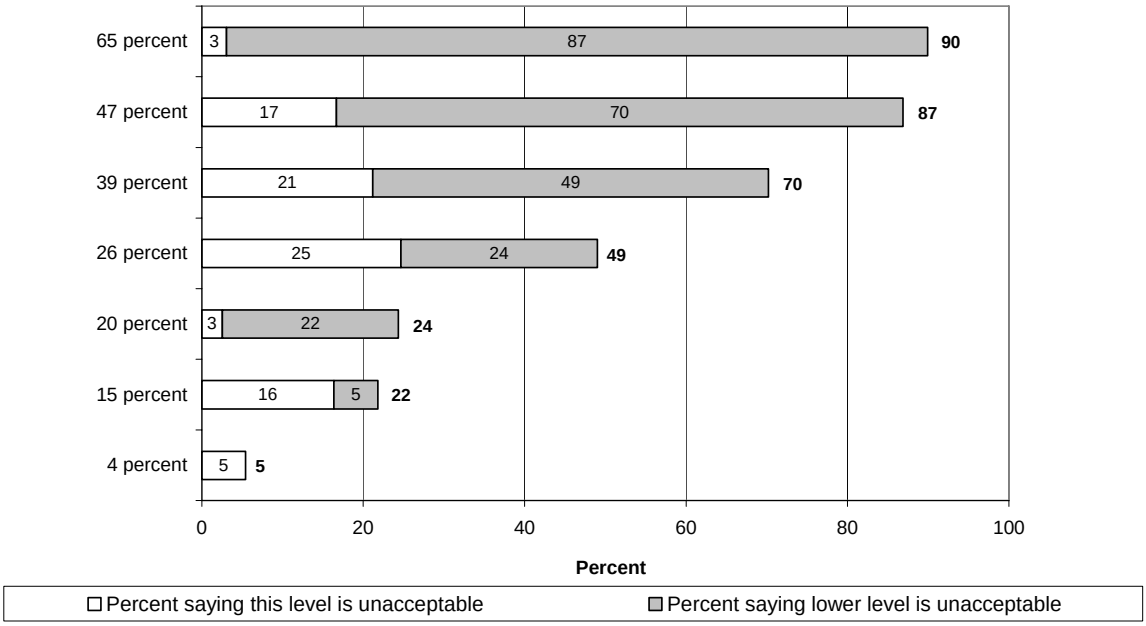

Percent Saying Algae Level Is Unacceptable
Percent Saying Unacceptable
100
90
80
70
60
50
40
30
20
10
0
4 percent
15 percent
20 percent
26 percent
39 percent
47 percent
65 percent
Percent of Algae Coverage

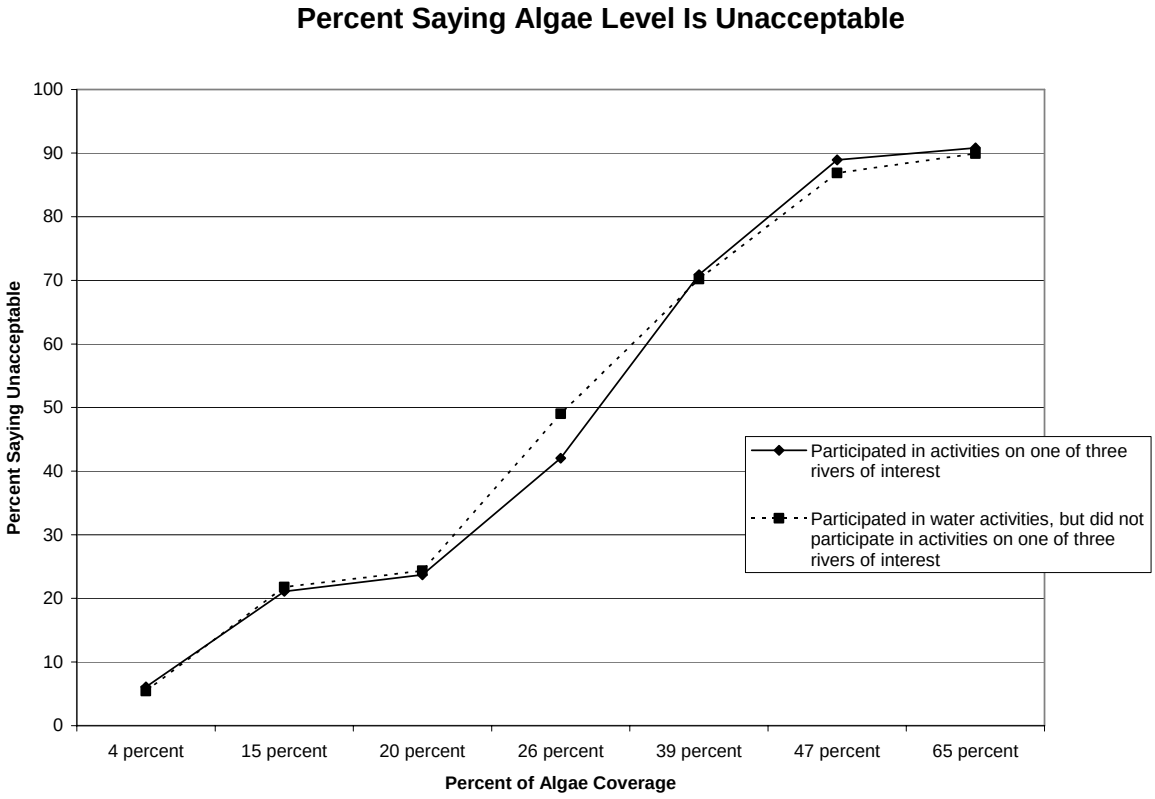
- The above graphs, which show all respondents, were also run among specific activity groups and specific demographic groups, as shown in the graphs that follow (note that groups might overlap in that, for instance, a person who fished may also have swam or waded):
  - Among those who participated in water-based activities on one of the three rivers of interest.
  - Among those who participated in water-based activities, but not on one of the three rivers of interest.
  - Among those who fished.
  - Among those who swam or waded.
  - Among those who went tubing or rafting: This group had a relatively low tolerance of algae, particularly those whose greatest participation was in tubing or rafting, compared to other groups.
  - Among those who boated (other than tubing or rafting) or used a personal watercraft: This group had a relatively low tolerance of algae, particularly those whose greatest participation was in this type of boating, compared to other groups.
  - Among those who walked along the bank or shore: This group had a relatively low tolerance of algae, particularly those whose greatest participation was in walking along a bank or shore, compared to other groups.
  - Among those who waterskied or wakeboarded.
  - Among those who did not participate in any of the listed water-based recreation: This group had a relatively low tolerance of algae, compared to the other groups.
  - Among those under the median age of respondents.
  - Among those who are the median age or older: This group had a lower tolerance of algae than did younger residents.
  - Among males.
  - Among females: This group had a lower tolerance of algae than did males.

Percent Saying Algae Level Is Unacceptable
(Among those who participated in any activities in or on the three rivers of interest in the past 12 months.)

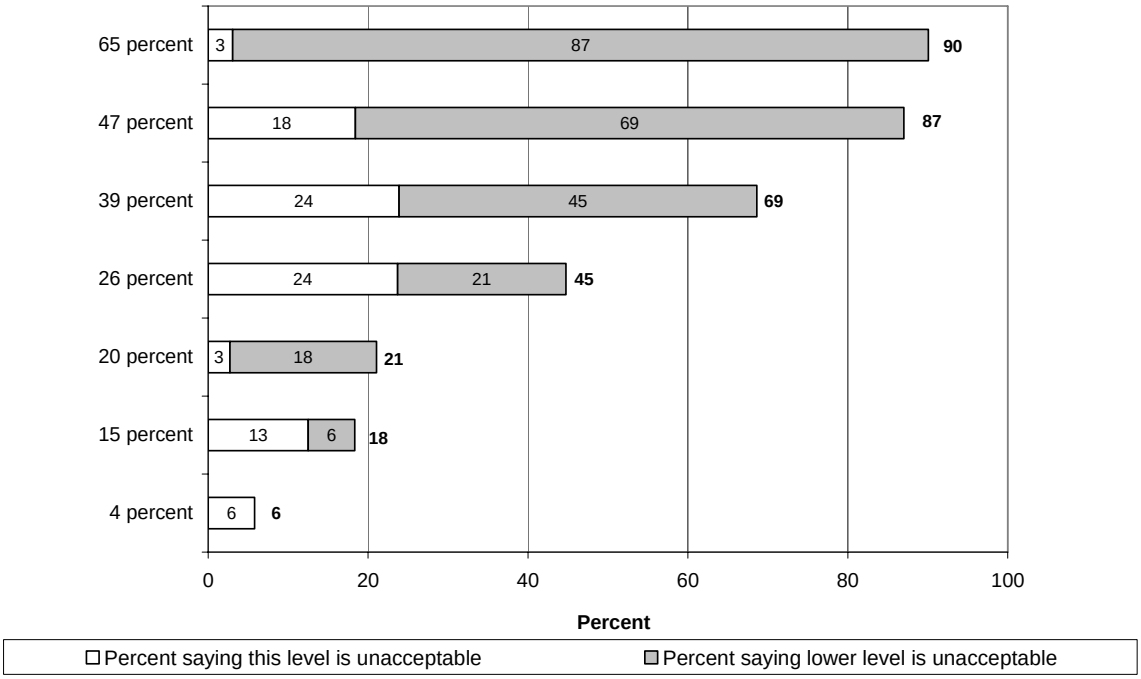


Percent Saying Algae Level Is Unacceptable
(Among those who participated in activities in or on West Virginia waters in the past 12 months, but did not participate on or in the three rivers of interest.)

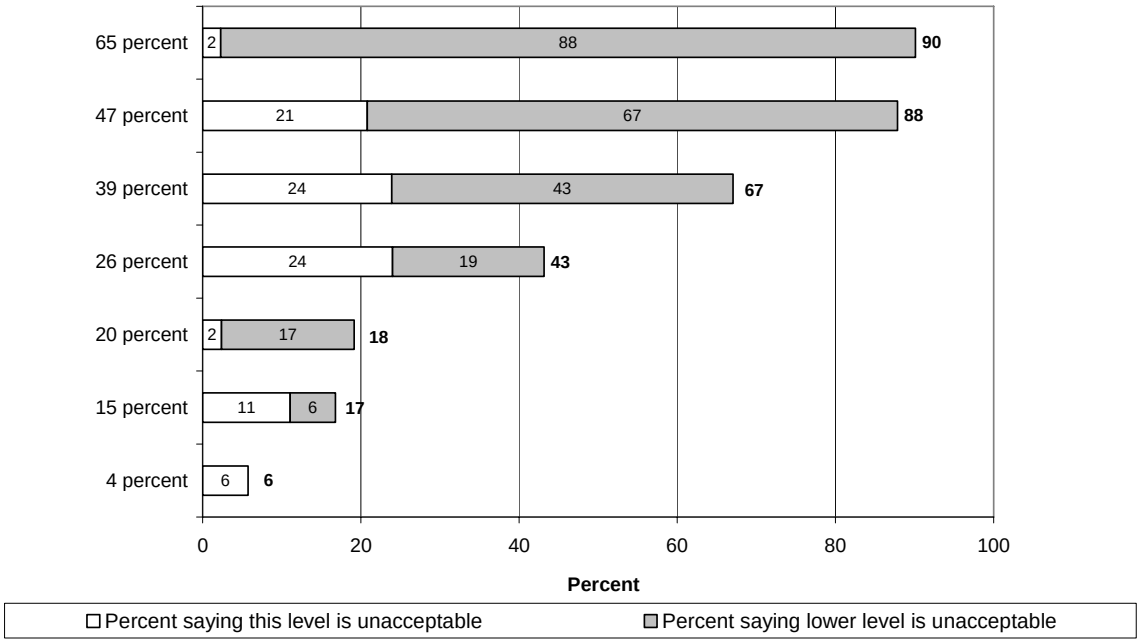



Percent Saying Algae Level Is Unacceptable
Percent Saying Unacceptable
100
90
80
70
60
50
40
30
20
10
0
4 percent
15 percent
20 percent
26 percent
39 percent
47 percent
65 percent
Percent of Algae Coverage
Participated in activities on one of three rivers of interest
Participated in water activities, but did not participate in activities on one of three rivers of interest

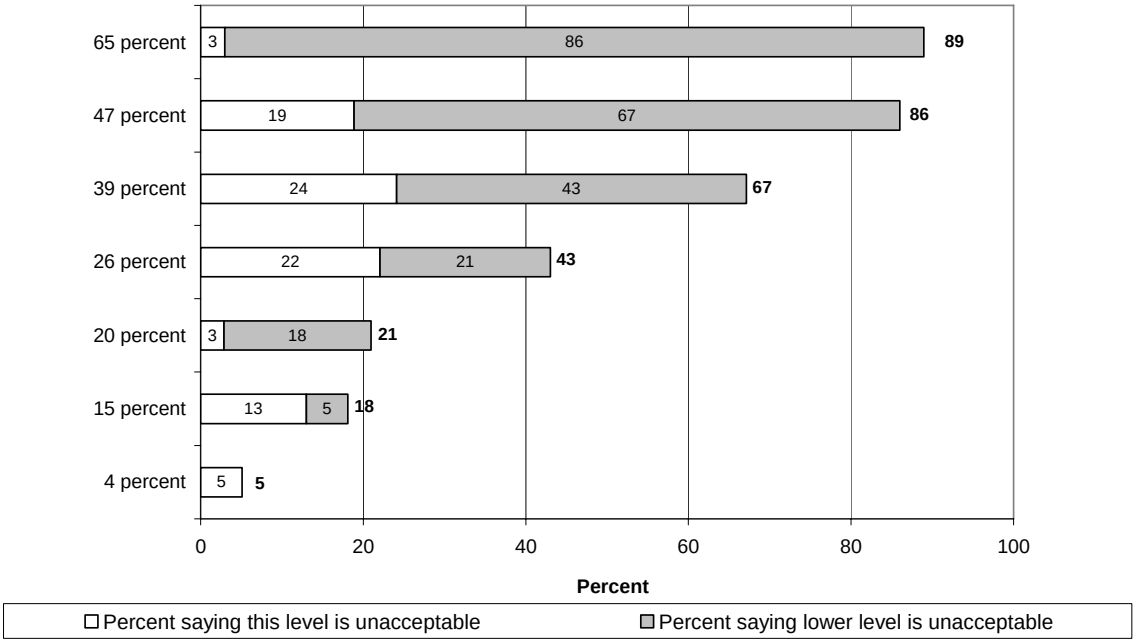
Percent Saying Algae Level Is Unacceptable
(Among those who fished in West Virginia in the past 2 years.)



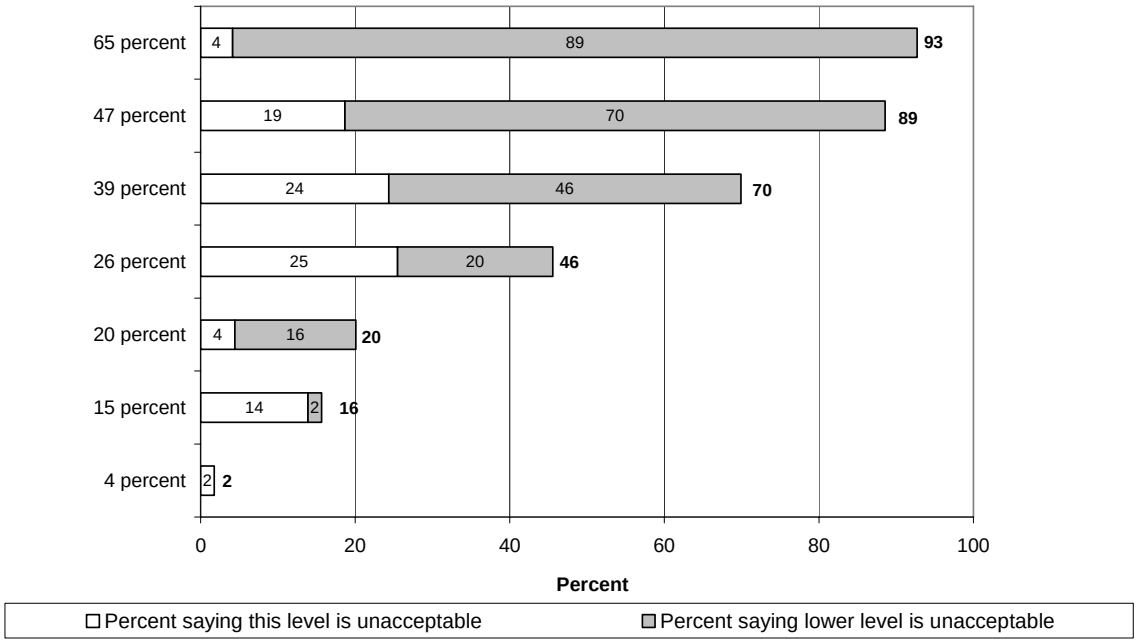
Percent Saying Algae Level Is Unacceptable
(Among those whose greatest participation is in fishing in or on West Virginia waters in the past 12 months.)



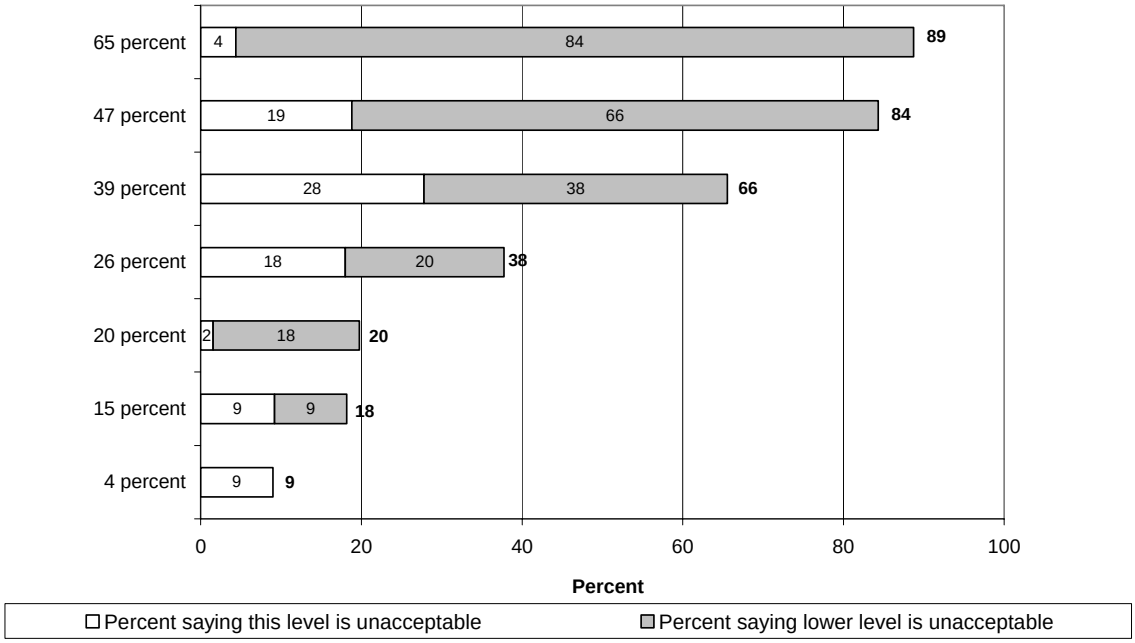
Percent Saying Algae Level Is Unacceptable
(Among those who swam or waded in West Virginia waters in the past 2 years.)



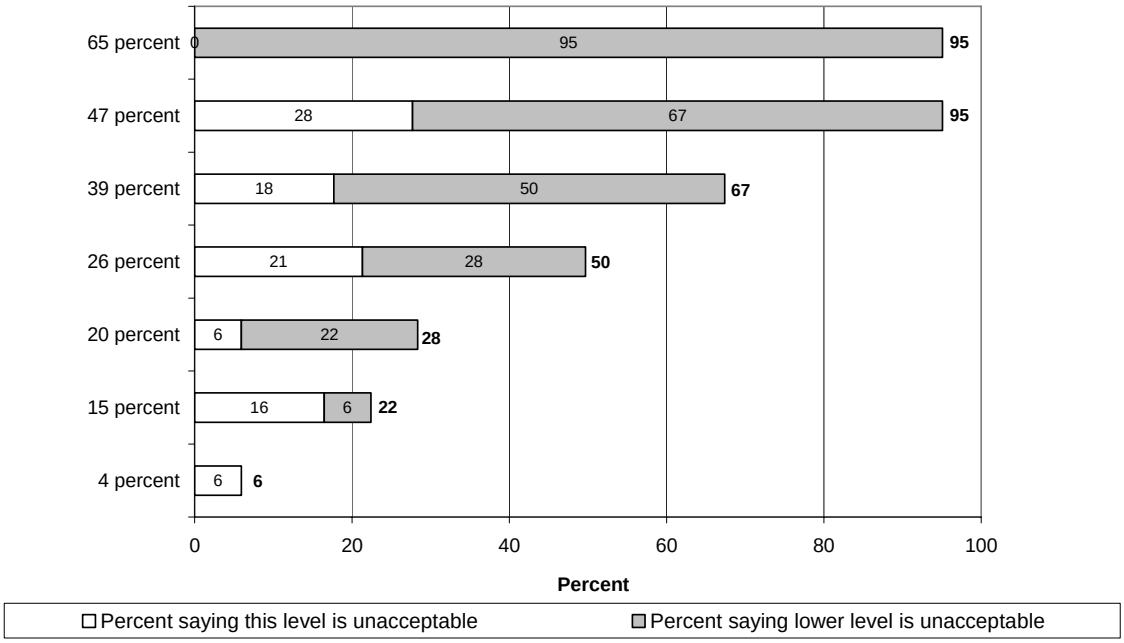
Percent Saying Algae Level Is Unacceptable
(Among those whose greatest participation is in swimming or wading in or on West Virginia waters in the past 2 years.)



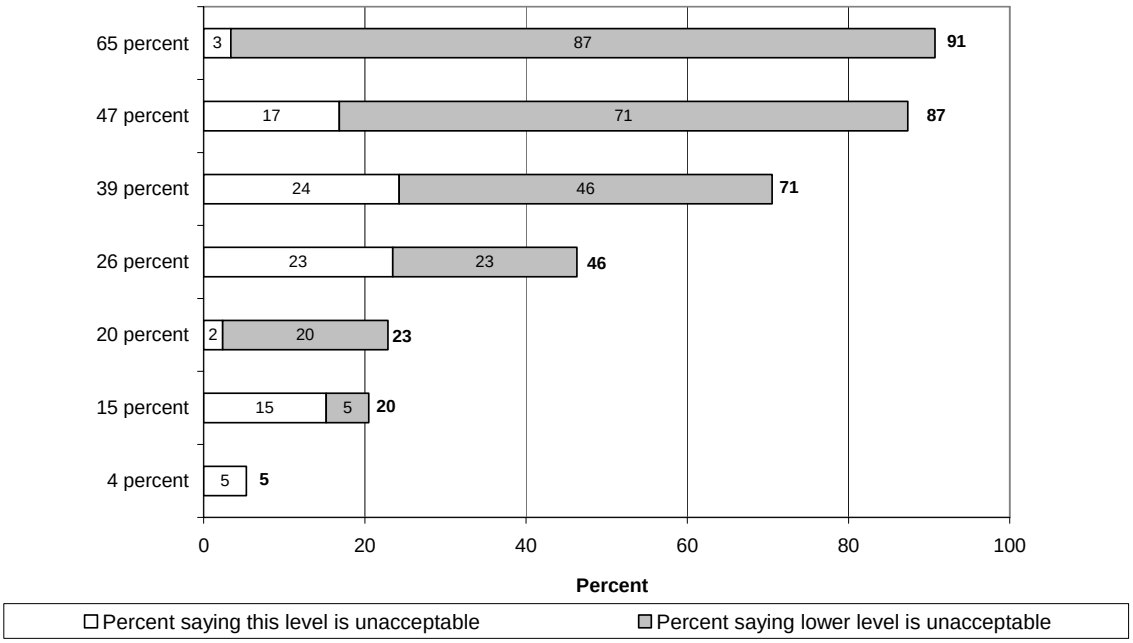
Percent Saying Algae Level Is Unacceptable
(Among those who tubed or rafted in West Virginia waters in the past 2 years.)



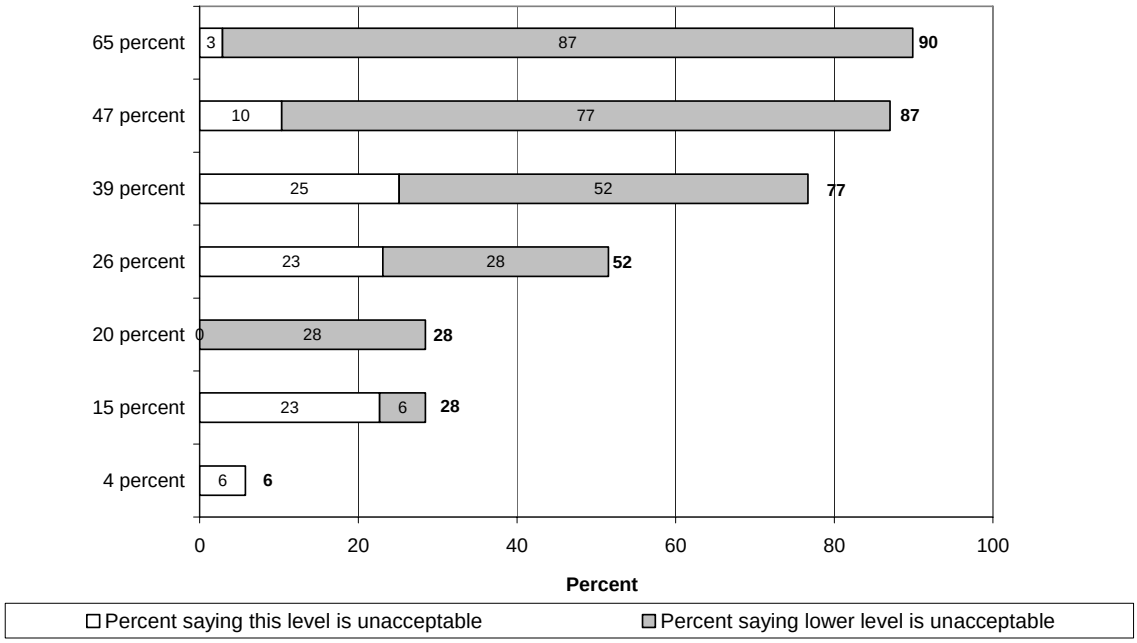
Percent Saying Algae Level Is Unacceptable
(Among those whose greatest participation is in tubing or rafting in West Virginia waters in the past 2 years.)



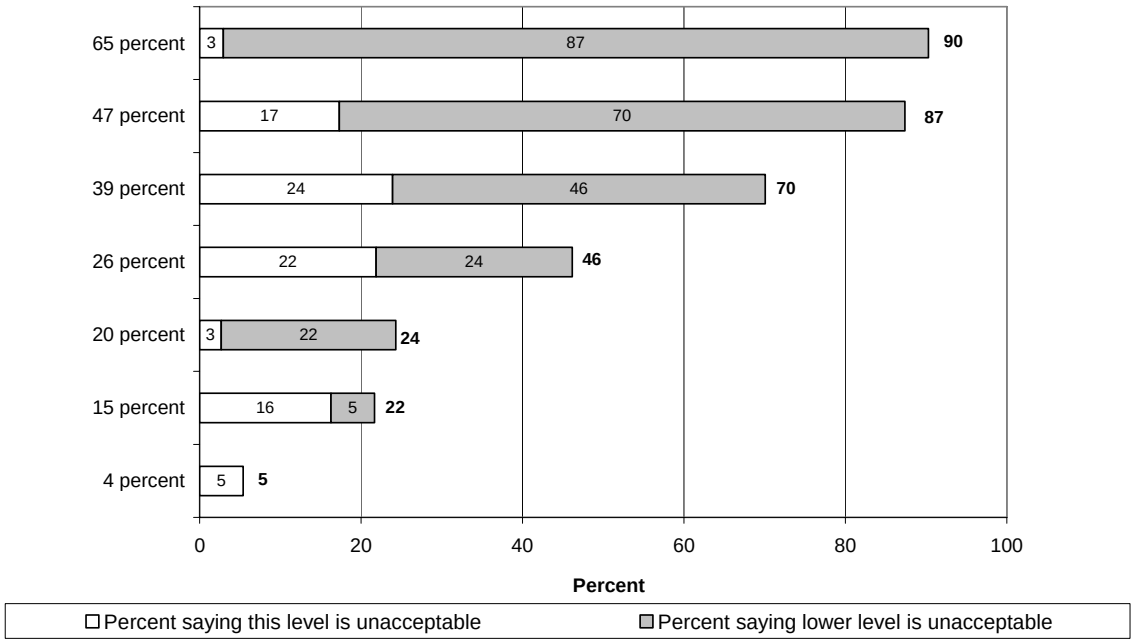
Percent Saying Algae Level Is Unacceptable
(Among those who boated (other than tubing or rafting) or used a personal watercraft in West Virginia waters in the past 2 years.)



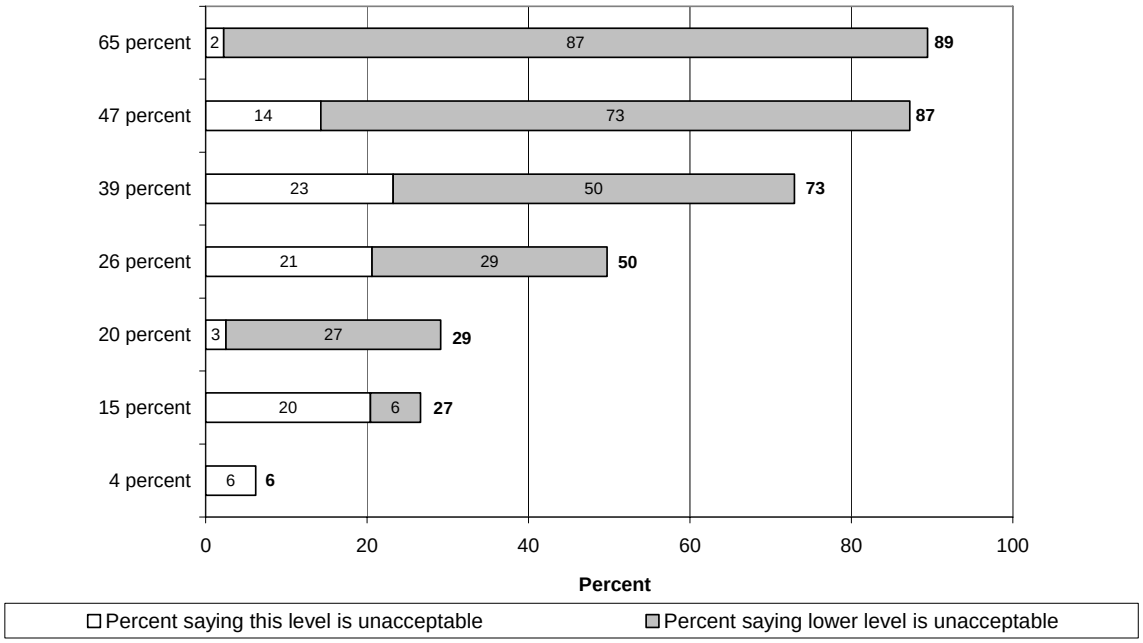
Percent Saying Algae Level Is Unacceptable
(Among those whose greatest participation is in boating (other than tubing or rafting) or personal watercraft use in West Virginia waters in the past 2 years.)



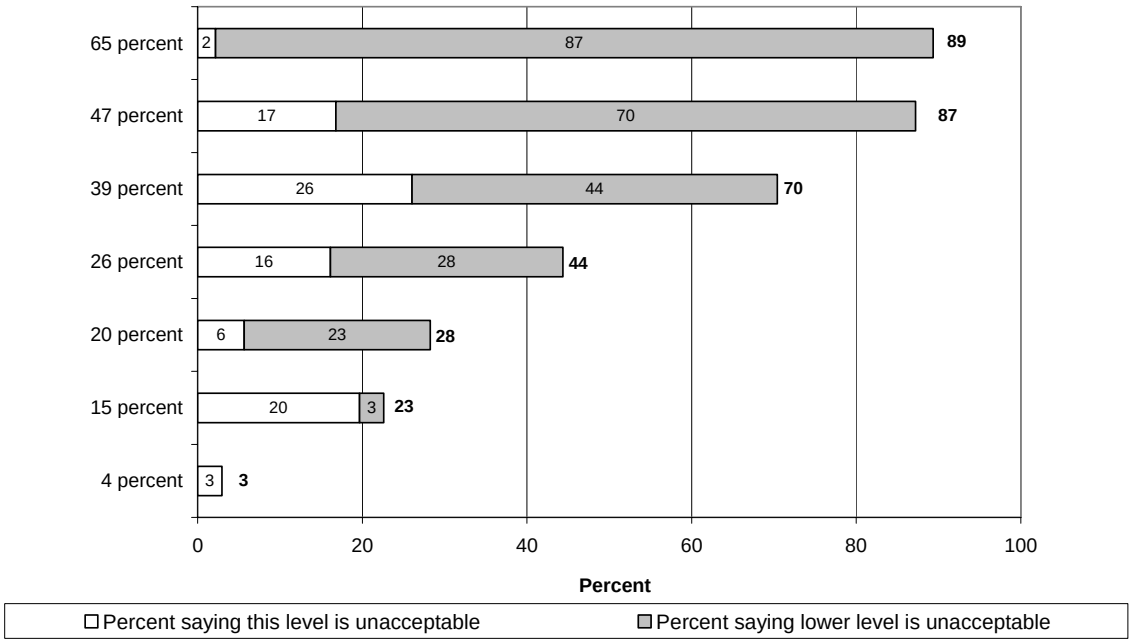
Percent Saying Algae Level Is Unacceptable
(Among those who walked along a bank or shore in West Virginia in the past 2 years.)



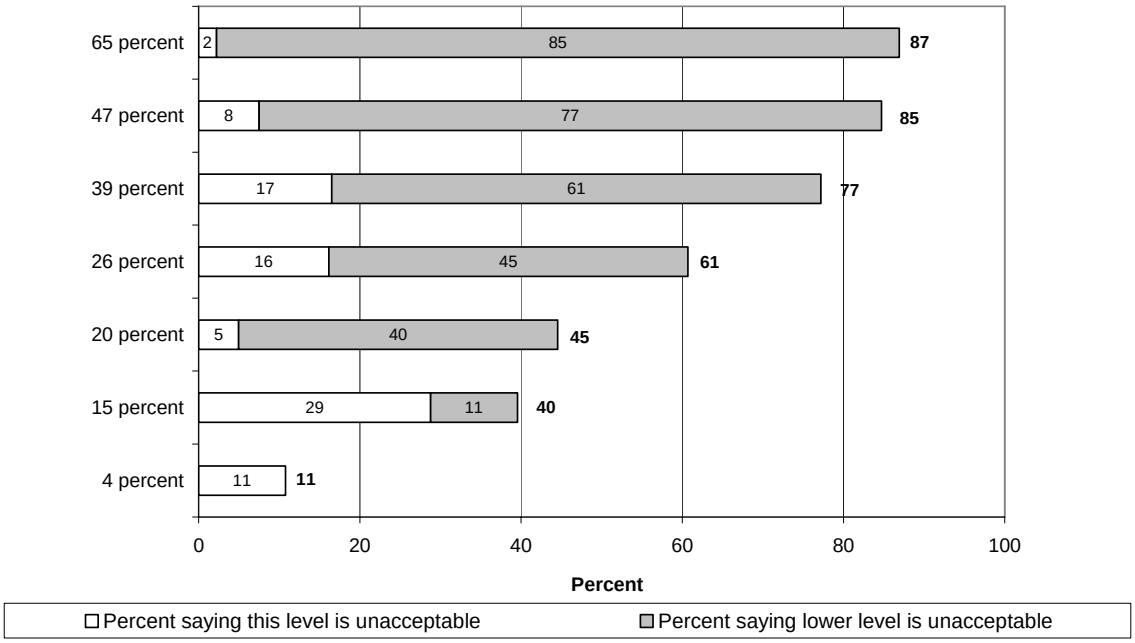
Percent Saying Algae Level Is Unacceptable
(Among those whose greatest participation is in walking along a bank or shore near West Virginia waters in the past 2 years.)

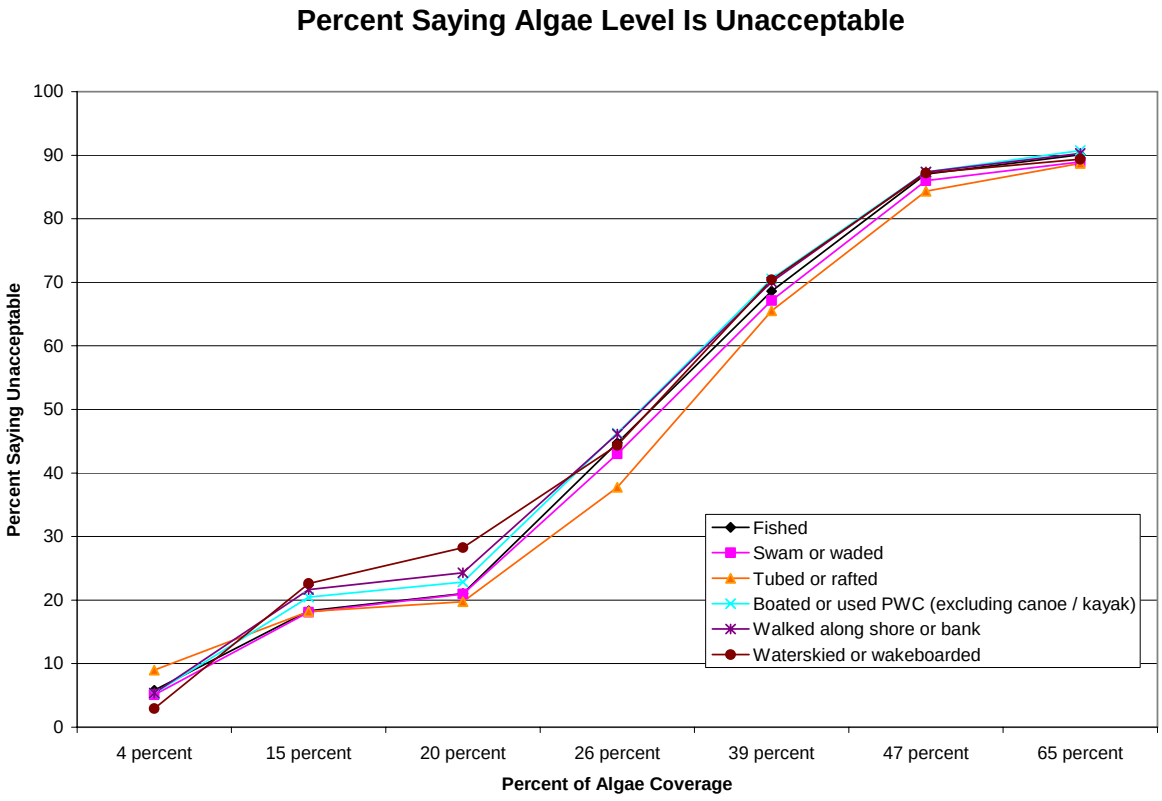


Percent Saying Algae Level Is Unacceptable
(Among those who waterskied or wakeboarded in West Virginia in the past 2 years.)

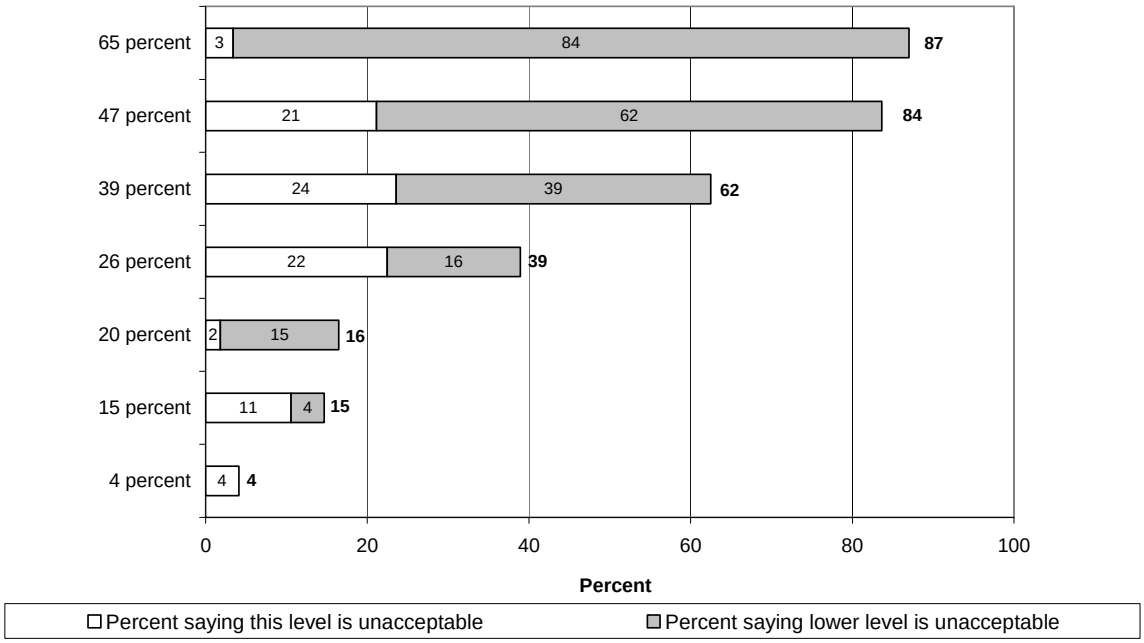


Percent Saying Algae Level Is Unacceptable
(Among those who did not participate in any activities in or on West Virginia waters in the past 2 years.)

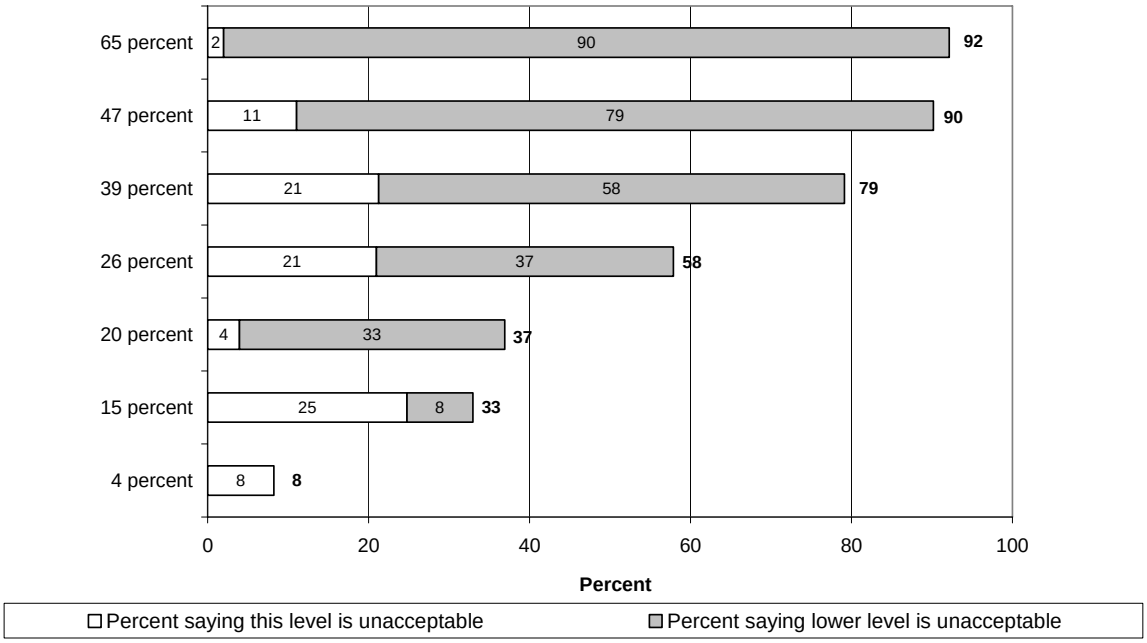



Percent Saying Algae Level Is Unacceptable
Percent Saying Unacceptable
100
90
80
70
60
50
40
30
20
10
0
4 percent
15 percent
20 percent
26 percent
39 percent
47 percent
65 percent
Percent of Algae Coverage
Fished
Swam or waded
Tubed or rafted
Boated or used PWC (excluding canoe / kayak)
Walked along shore or bank
Waterskied or wakeboarded

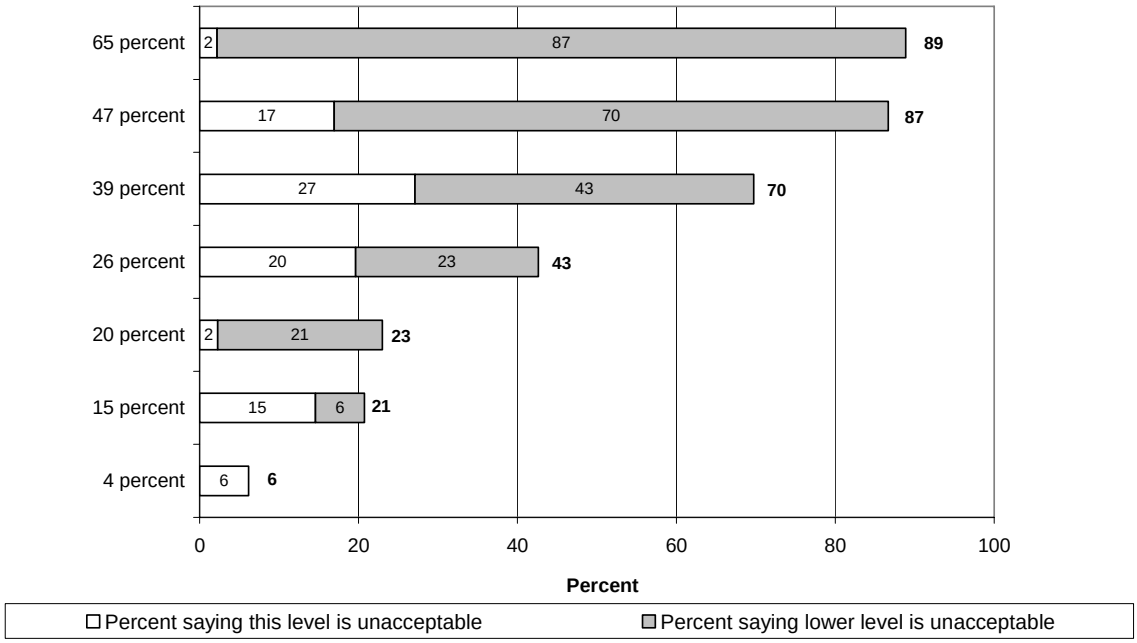
Percent Saying Algae Level Is Unacceptable
(Among those under the median age of 49.)



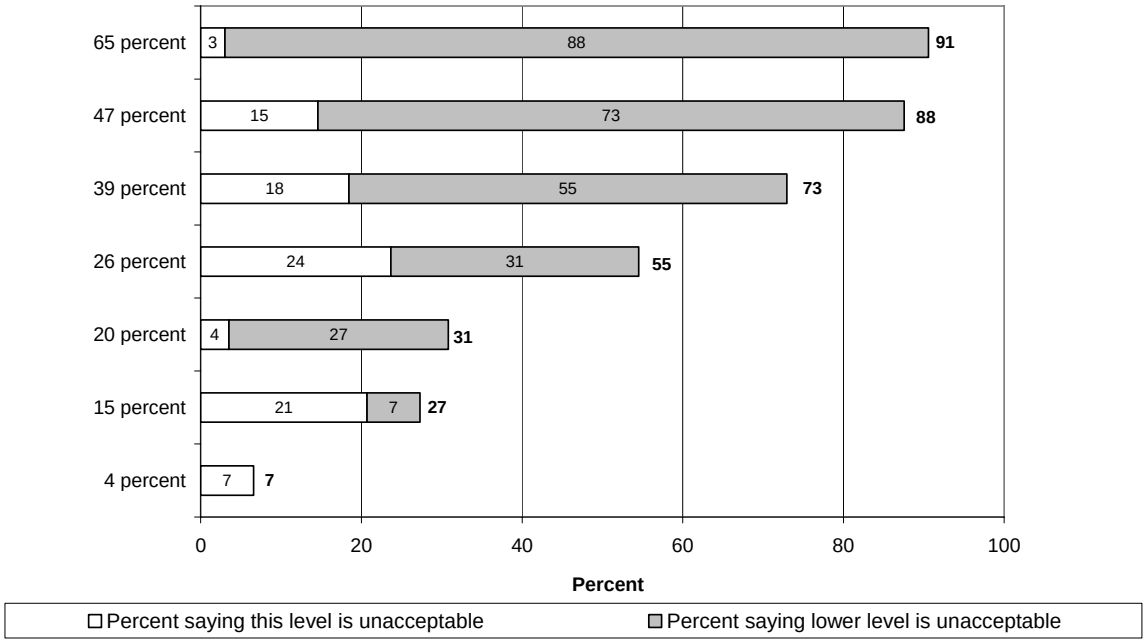
Percent Saying Algae Level Is Unacceptable
(Among those who are 49 years old or older.)

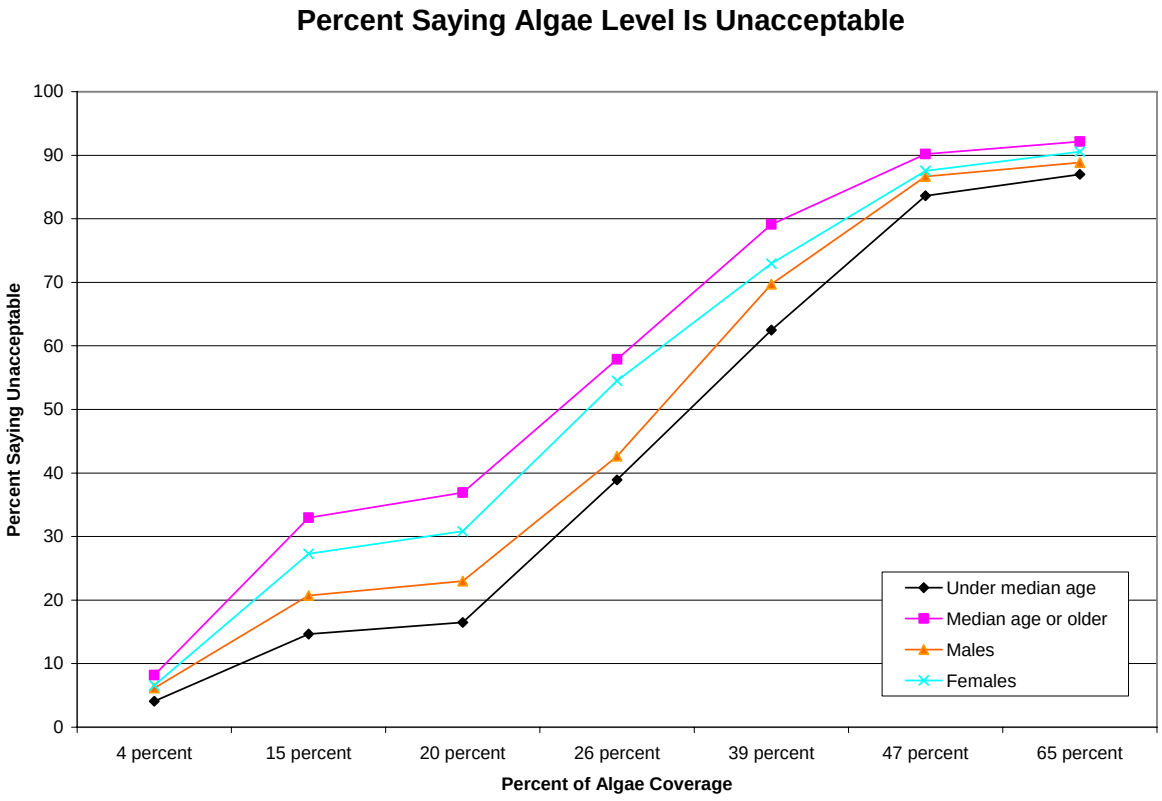


Percent Saying Algae Level Is Unacceptable
(Among males.)



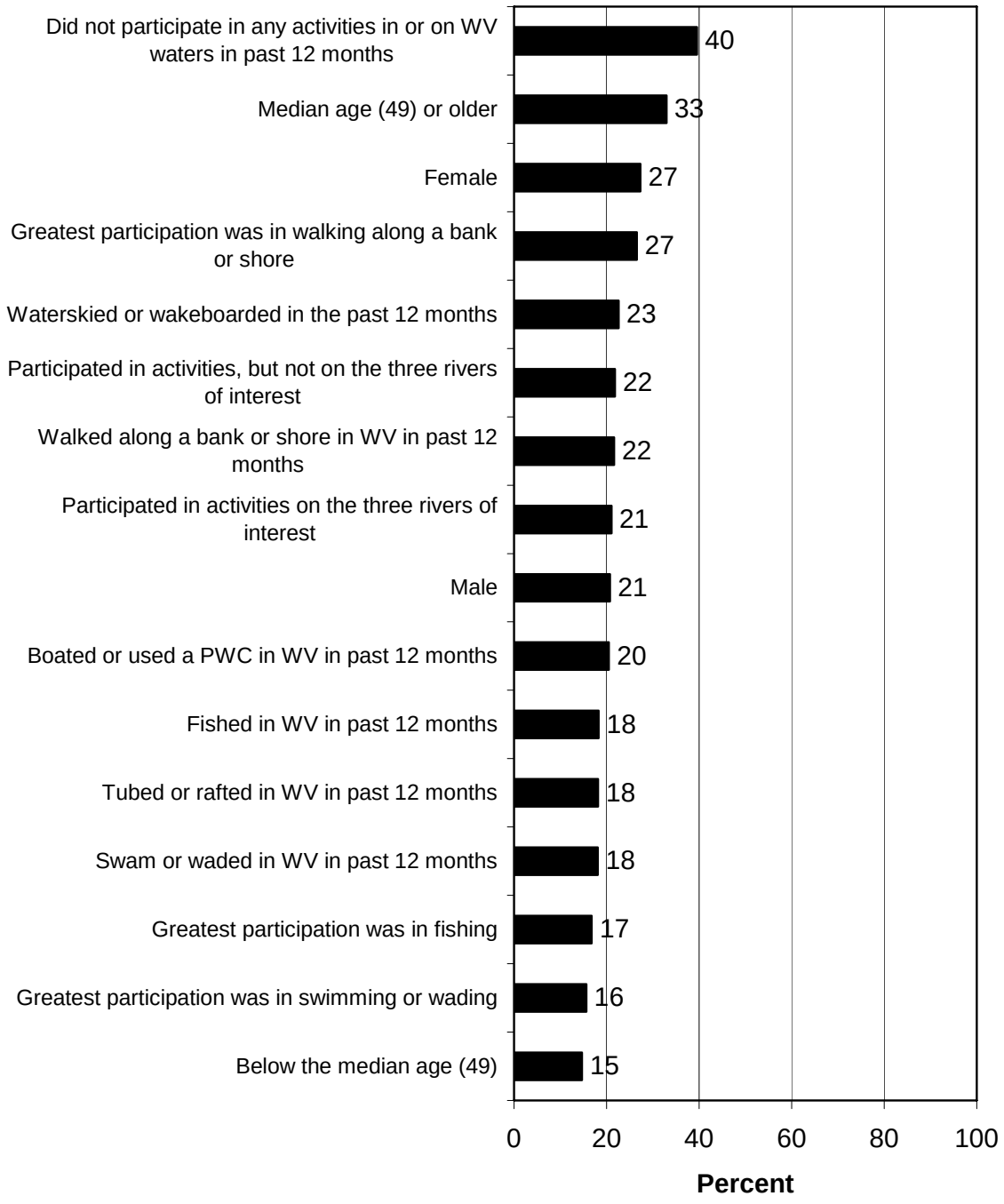
Percent Saying Algae Level Is Unacceptable
(Among females.)



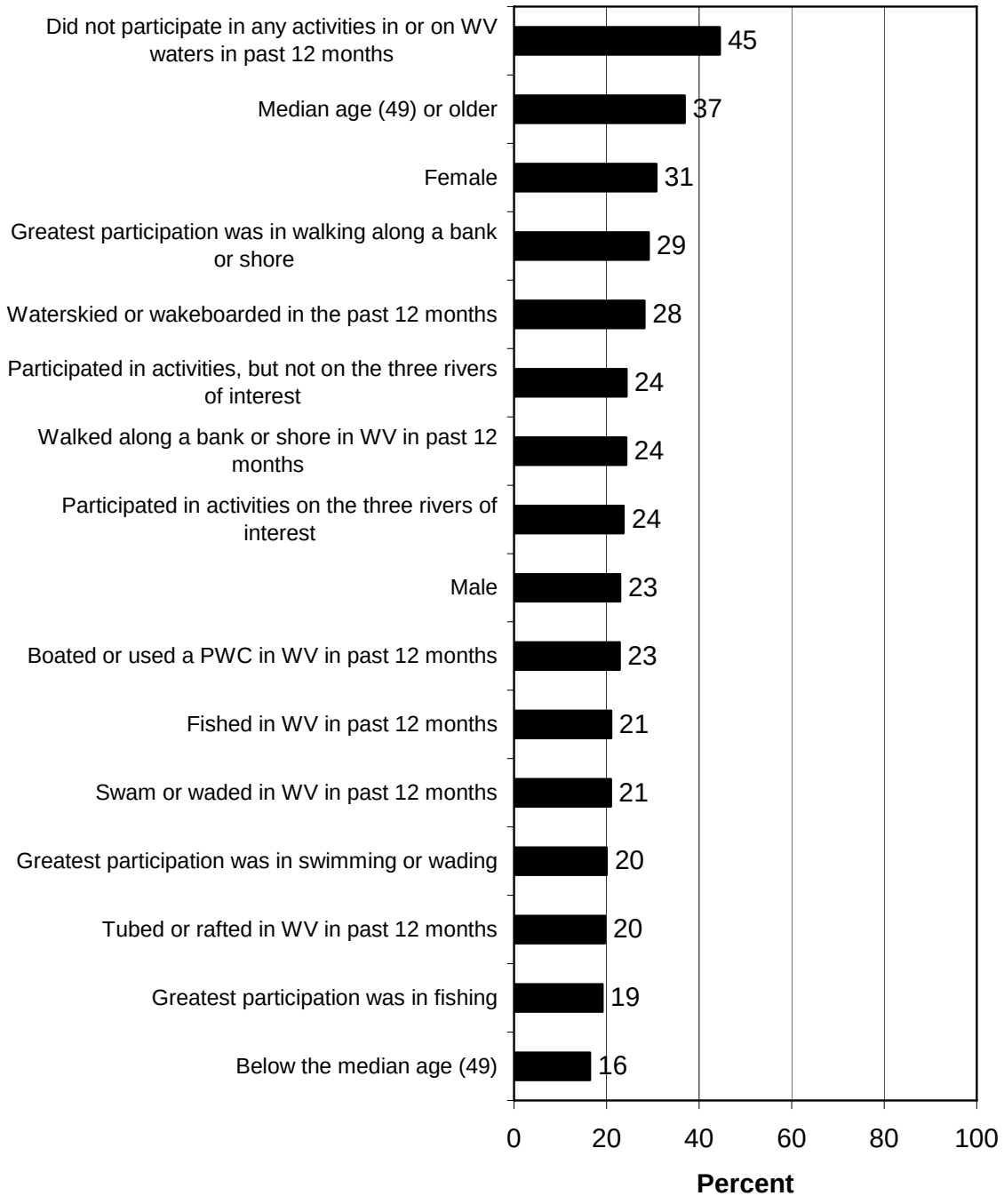

Percent Saying Algae Level Is Unacceptable
Percent Saying Unacceptable
100
90
80
70
60
50
40
30
20
10
0
4 percent
15 percent
20 percent
26 percent
39 percent
47 percent
65 percent
Percent of Algae Coverage
Under median age
Median age or older
Males
Females

- A final way to examine the data is to see the percentage of various groups at specific thresholds for considering algae coverage to be unacceptable. Four graphs are shown: the percent of various groups thinking that 15 percent algae cover is unacceptable, and the same for 20 percent, 26 percent, and 39 percent. These graphs also demonstrate that certain groups appear to have relatively less tolerance of algae: those who participated in no water-based activities, older respondents, and females.

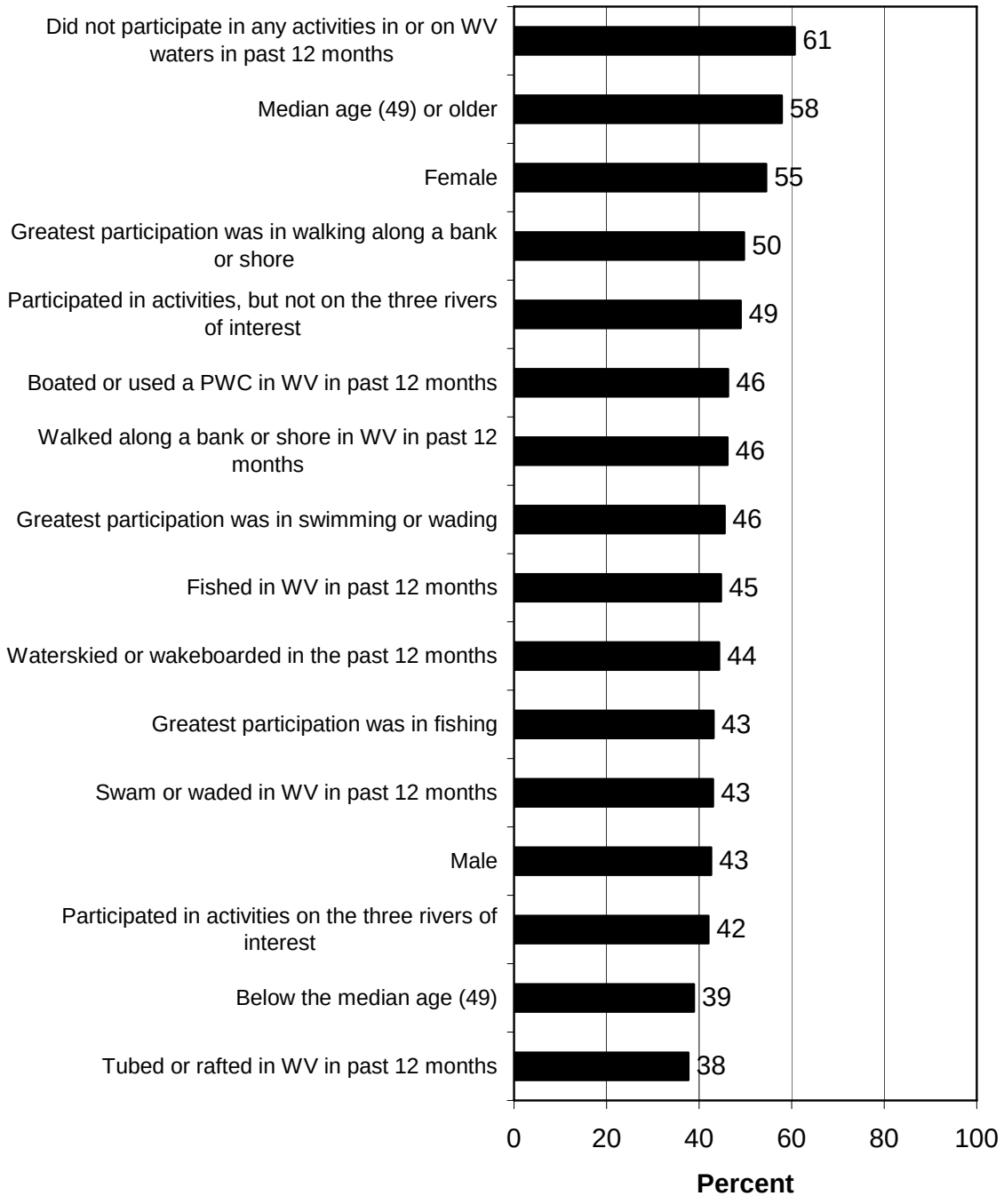
Percent of each of the following groups that considers 15 percent algae cover unacceptable:



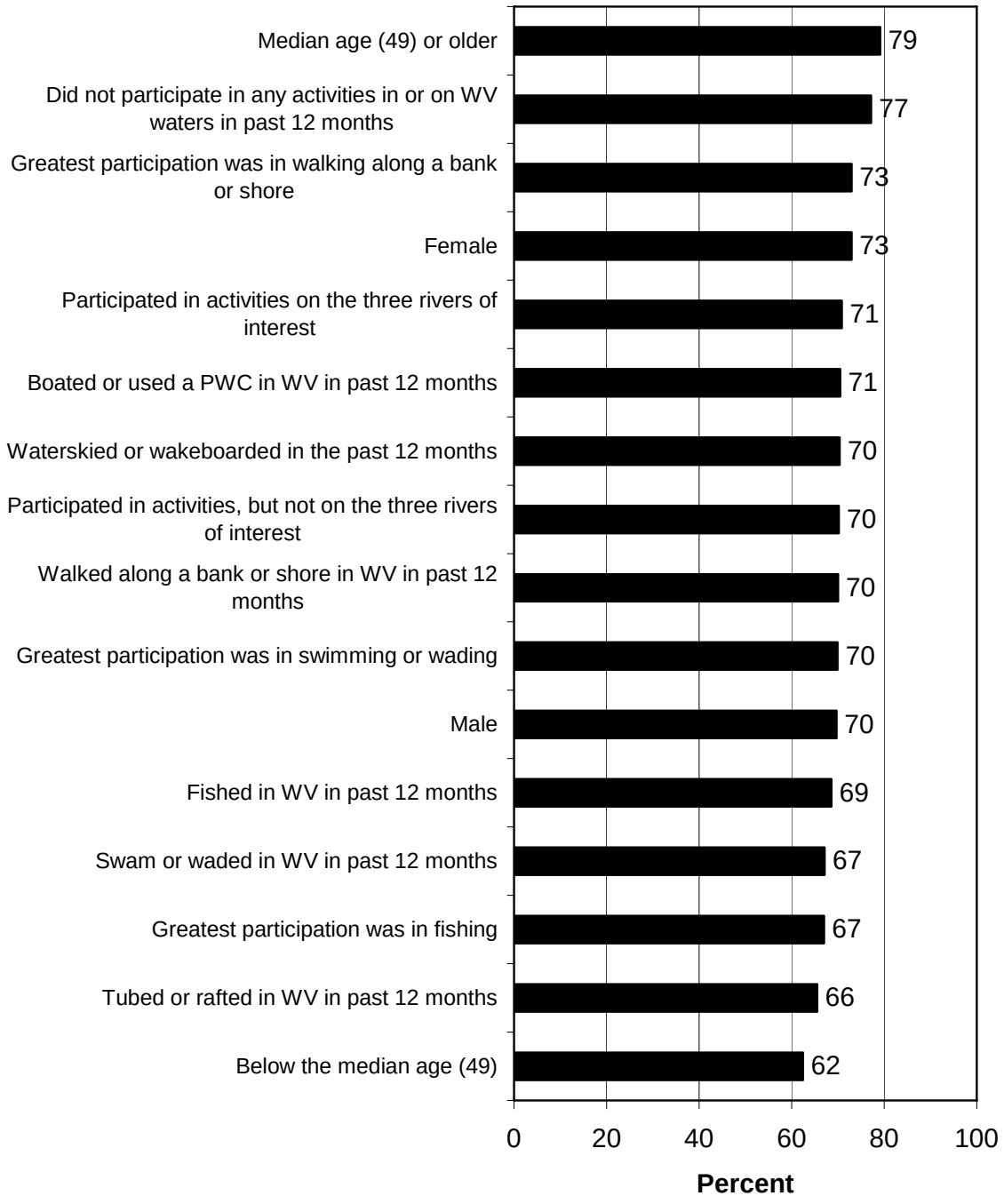
Percent of each of the following groups that considers 20 percent algae cover unacceptable:



Percent of each of the following groups that considers 26 percent algae cover unacceptable:



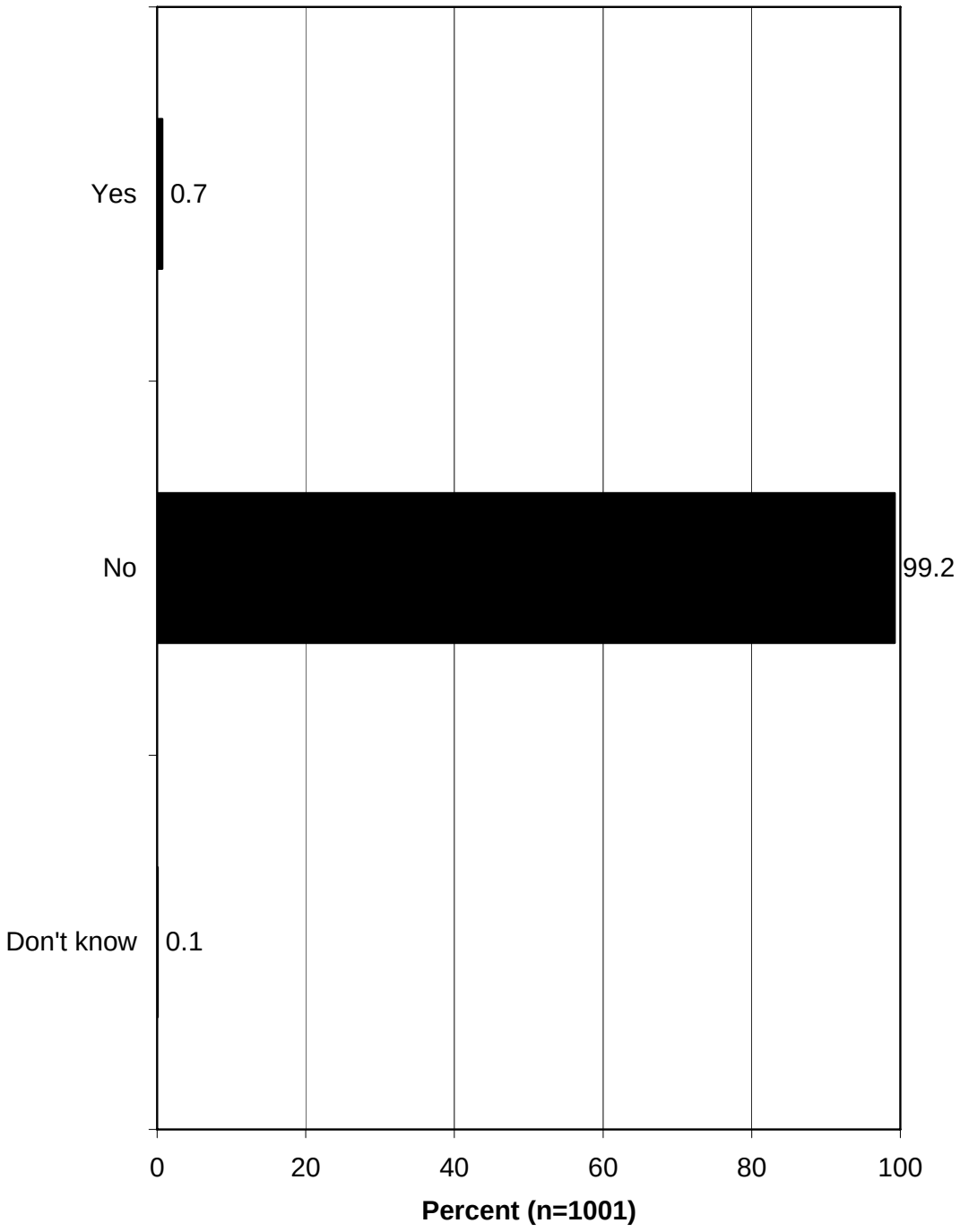
Percent of each of the following groups that considers 39 percent algae cover unacceptable:



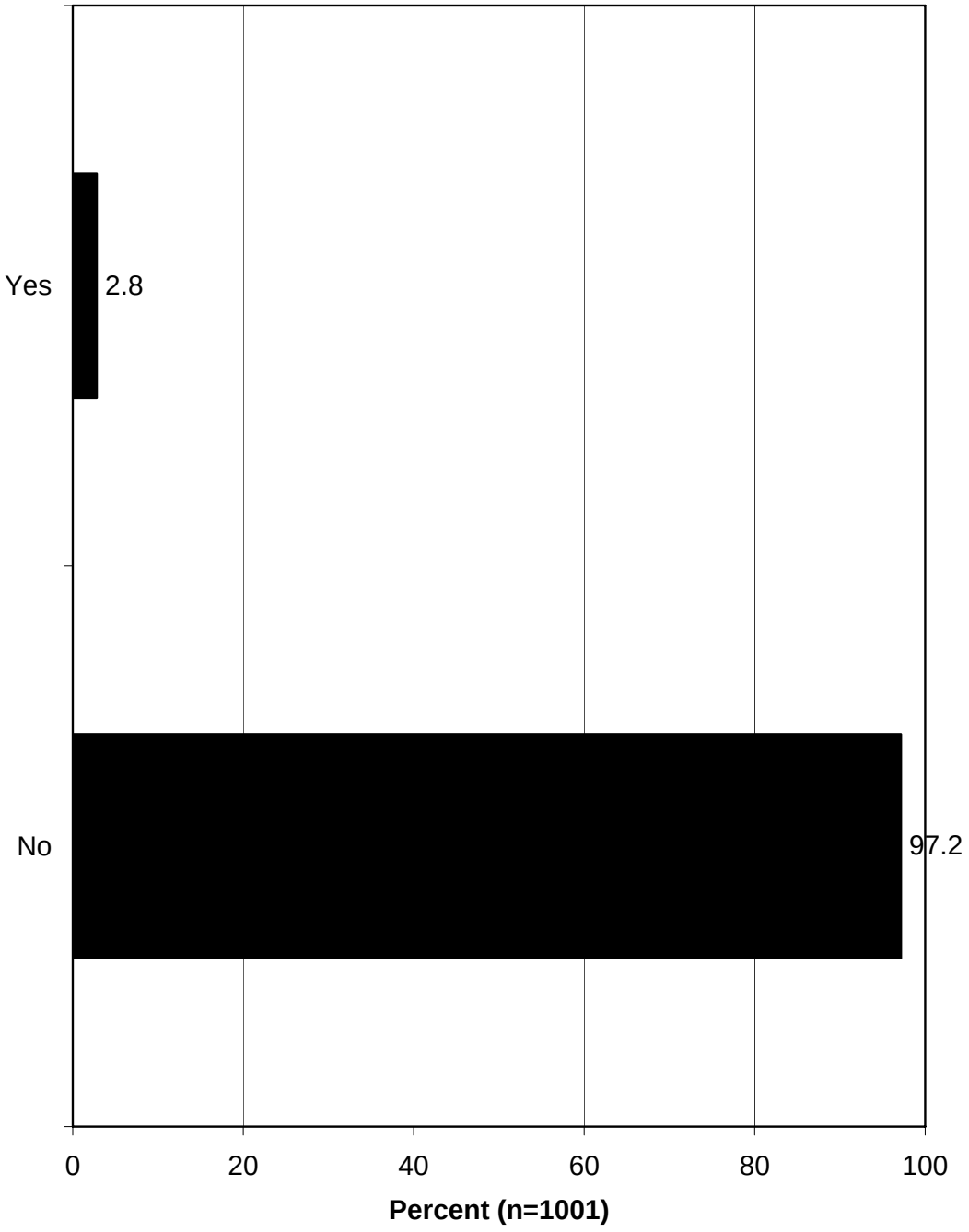
CONTACTING AGENCIES AND ORGANIZATIONS REGARDING ALGAE

- Less than 1% of West Virginia residents have contacted the DEP about algae in West Virginia waters in the past 2 years. Additionally, a little less than 3% contacted any other agency or organization about algae.
  - A follow-up question asked respondents to indicate why they had contacted the DEP or another agency or organization about algae (among those who had made contact). Most commonly, they contacted an agency or organization to obtain general information about algae, to complain about algae, or to ask specifically about safety and/or health hazards associated with algae.
  - Those who indicated that they had concerns about algae but did not indicate contacting the DEP about it were asked for their reasons for not contacting the DEP. Most commonly, it was because they did not consider algae much of a problem, because they did not know which agency to contact, did not know how to contact the DEP about it, assumed the problem was being addressed, or because they did not think it would help.

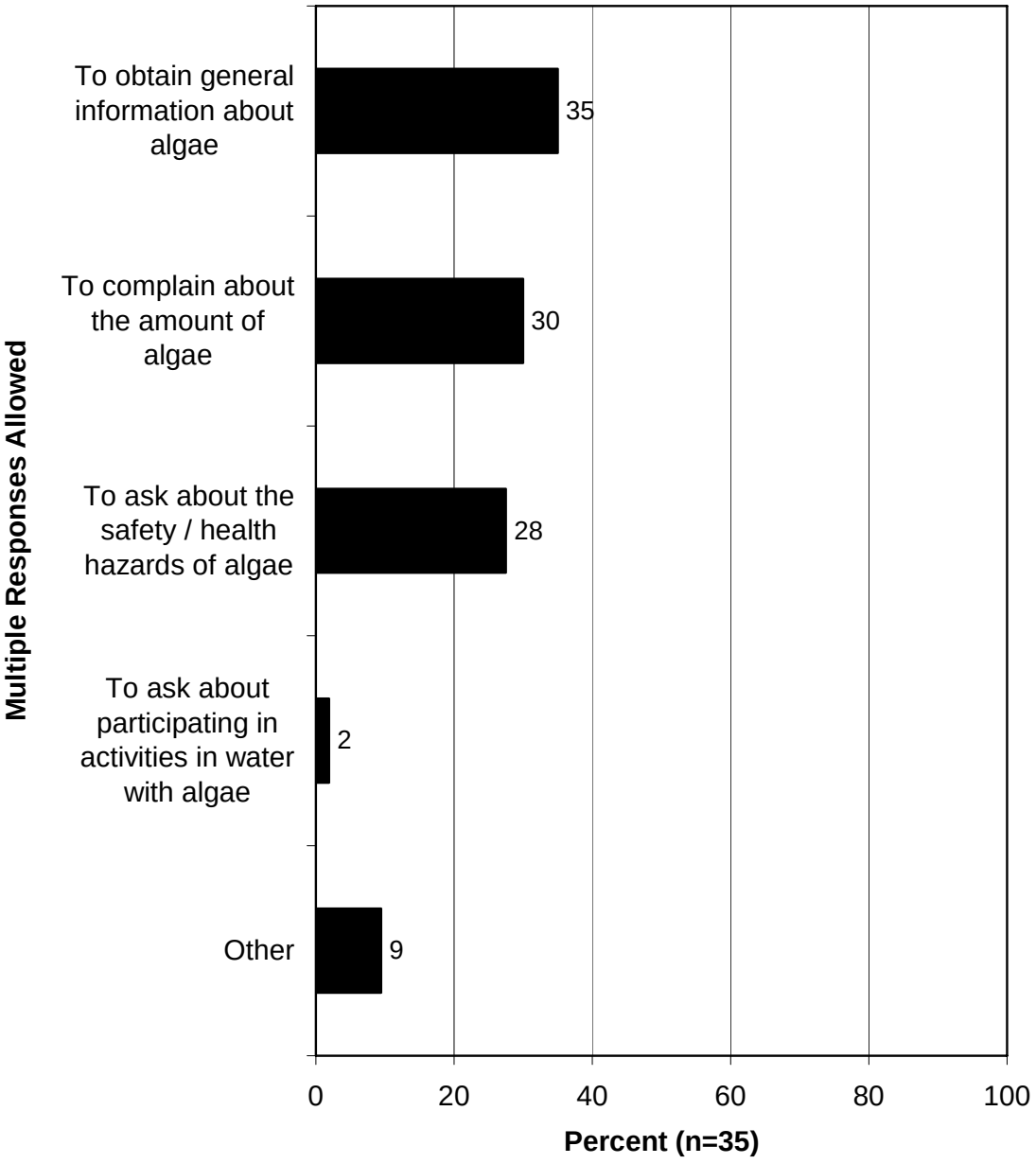
Q87. Have you contacted the DEP about algae in West Virginia waters in the past 2 years?



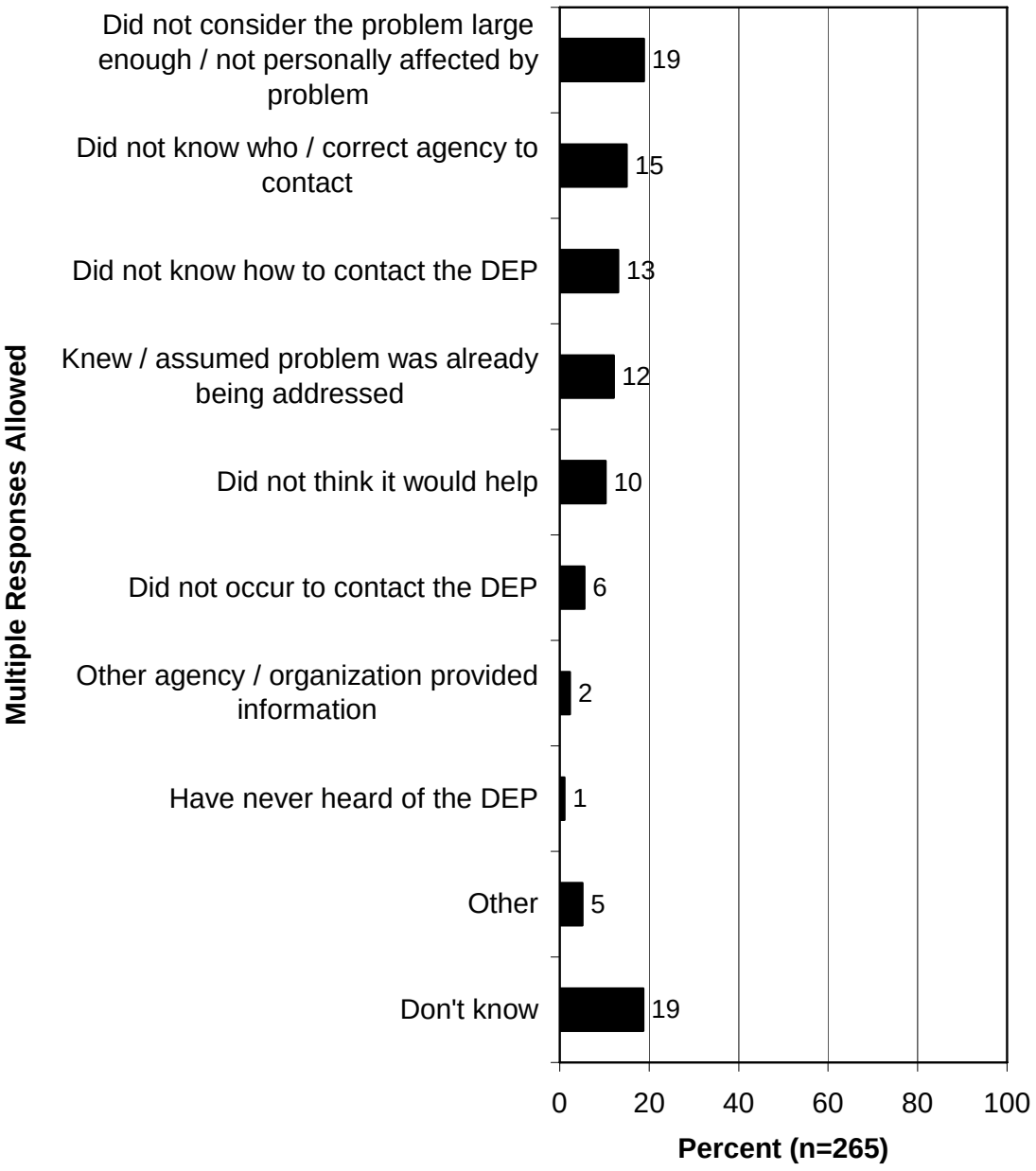
Q88. Have you contacted any other agency or organization about algae in West Virginia waters in the past 2 years?



Q91. What was your main reason for contacting an agency or organization about algae? (Asked of those who contacted the DEP or another agency or organization about algae in West Virginia waters in the past 2 years.)



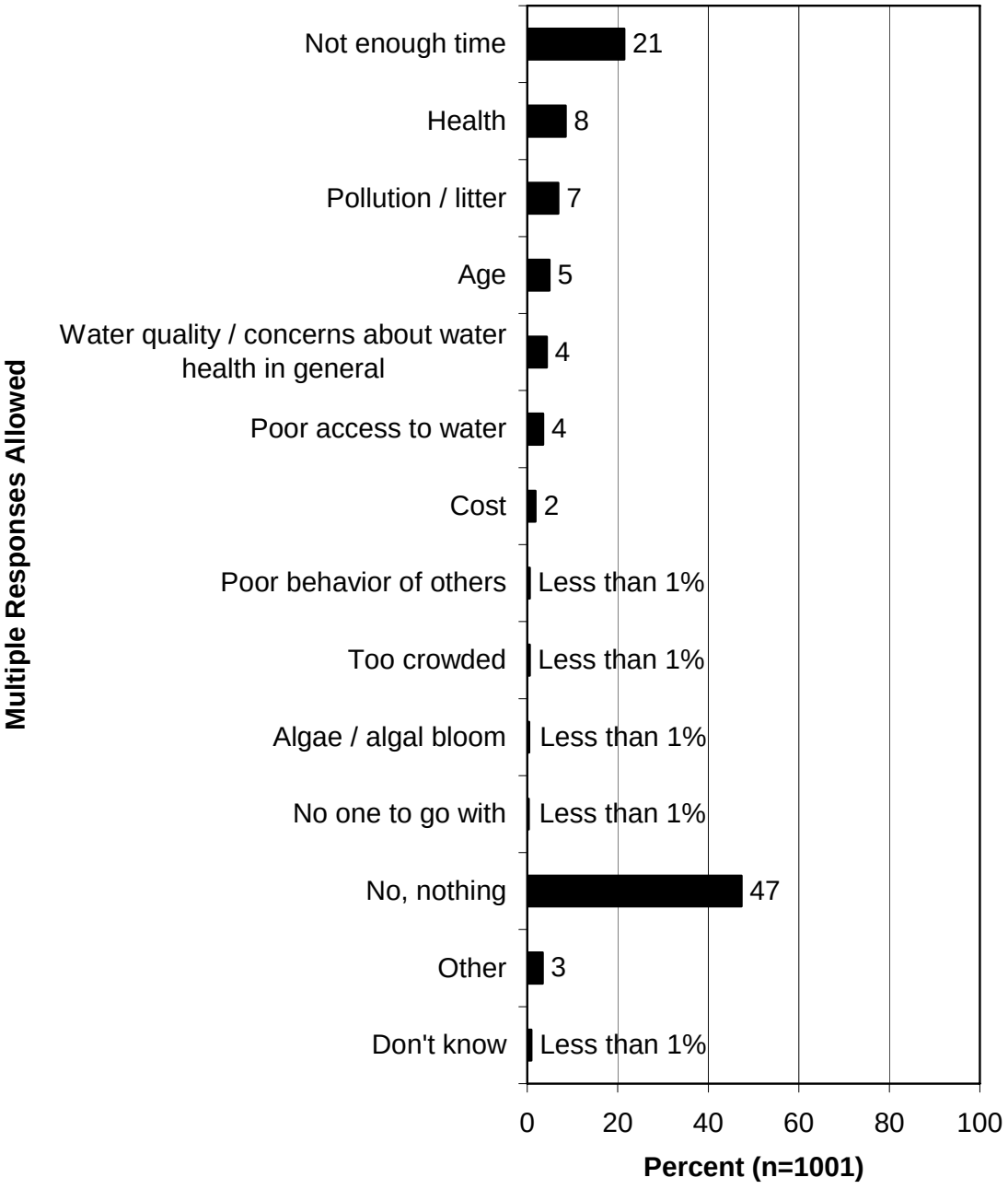
Q95. What was your main reason for NOT contacting the DEP about your concern regarding algae? (Asked of those who indicated being concerned about algae in West Virginia waters in the past 2 years but who did not contact the DEP about it.)



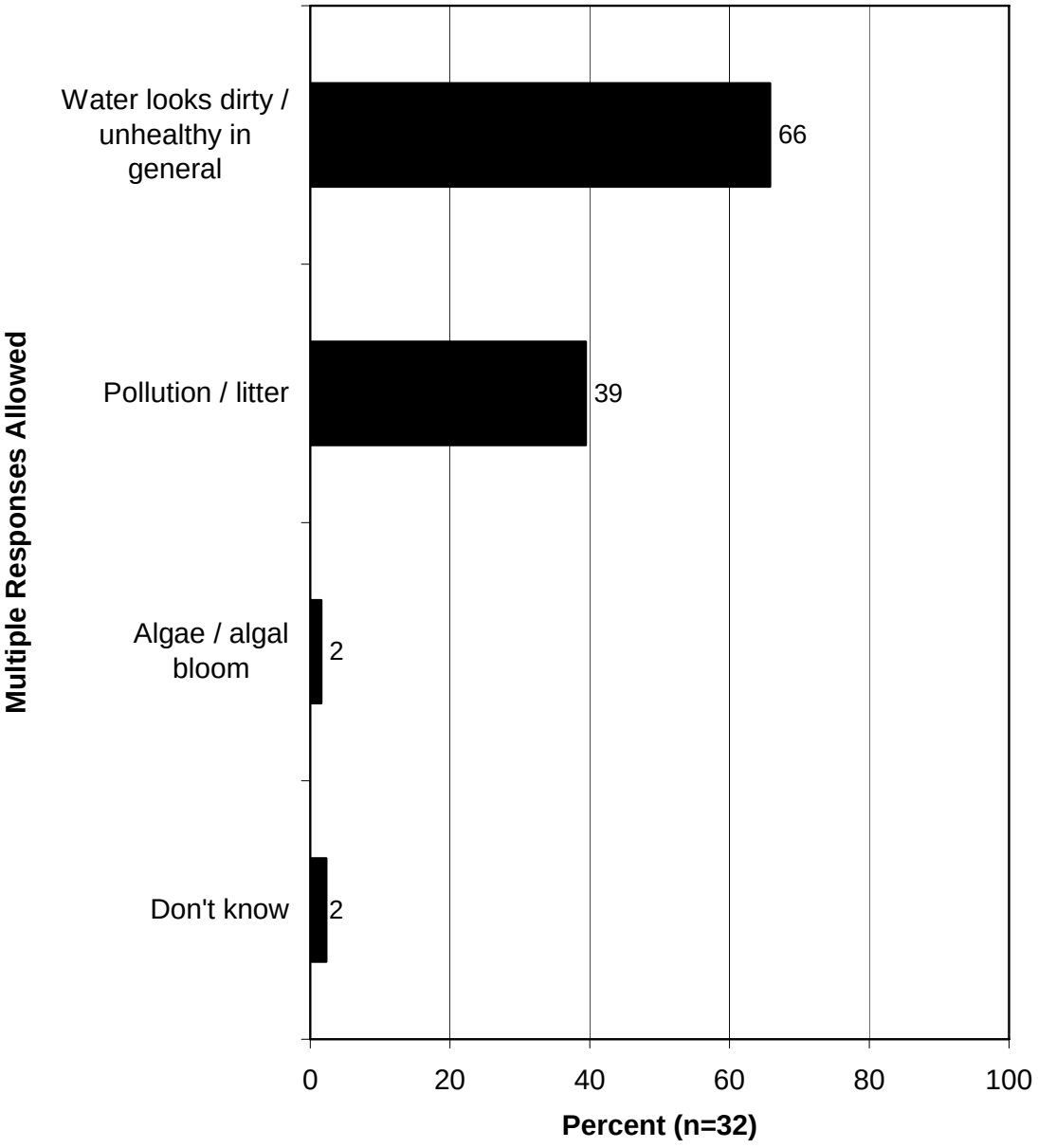
ALGAE-RELATED CONSTRAINTS TO PARTICIPATION IN WATER-BASED ACTIVITIES IN WEST VIRGINIA

- A general open-ended question asked respondents if there were things that took away from their participation in or their enjoyment of water-based recreation in West Virginia. Water quality was named by only 4%, and less than 1% named algae specifically. Those who answered "water quality" in this question were asked in follow-up to name the specific aspect of water quality that concerned them, and algae did not play much of a role in it, as shown in the graph.

Q36. In general, are there any things that take away from your participation in activities on or in West Virginia waters or cause you not to participate as much as you would like?



Q40. What are the main reasons you are concerned about water quality or water health in West Virginia? (Asked of those who indicated that water quality took away from their participation in activities on or in West Virginia waters.)

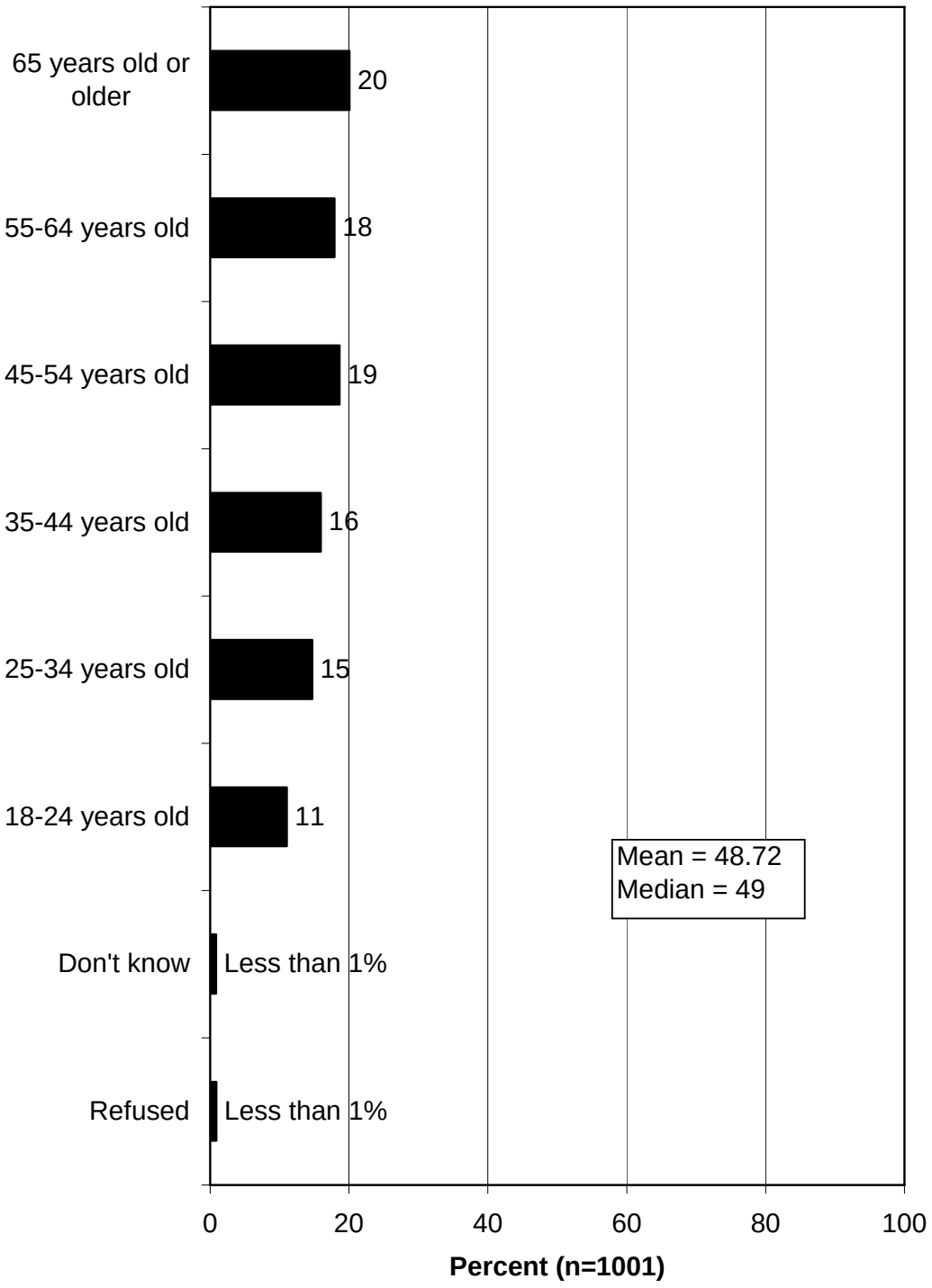


DEMOGRAPHIC DATA

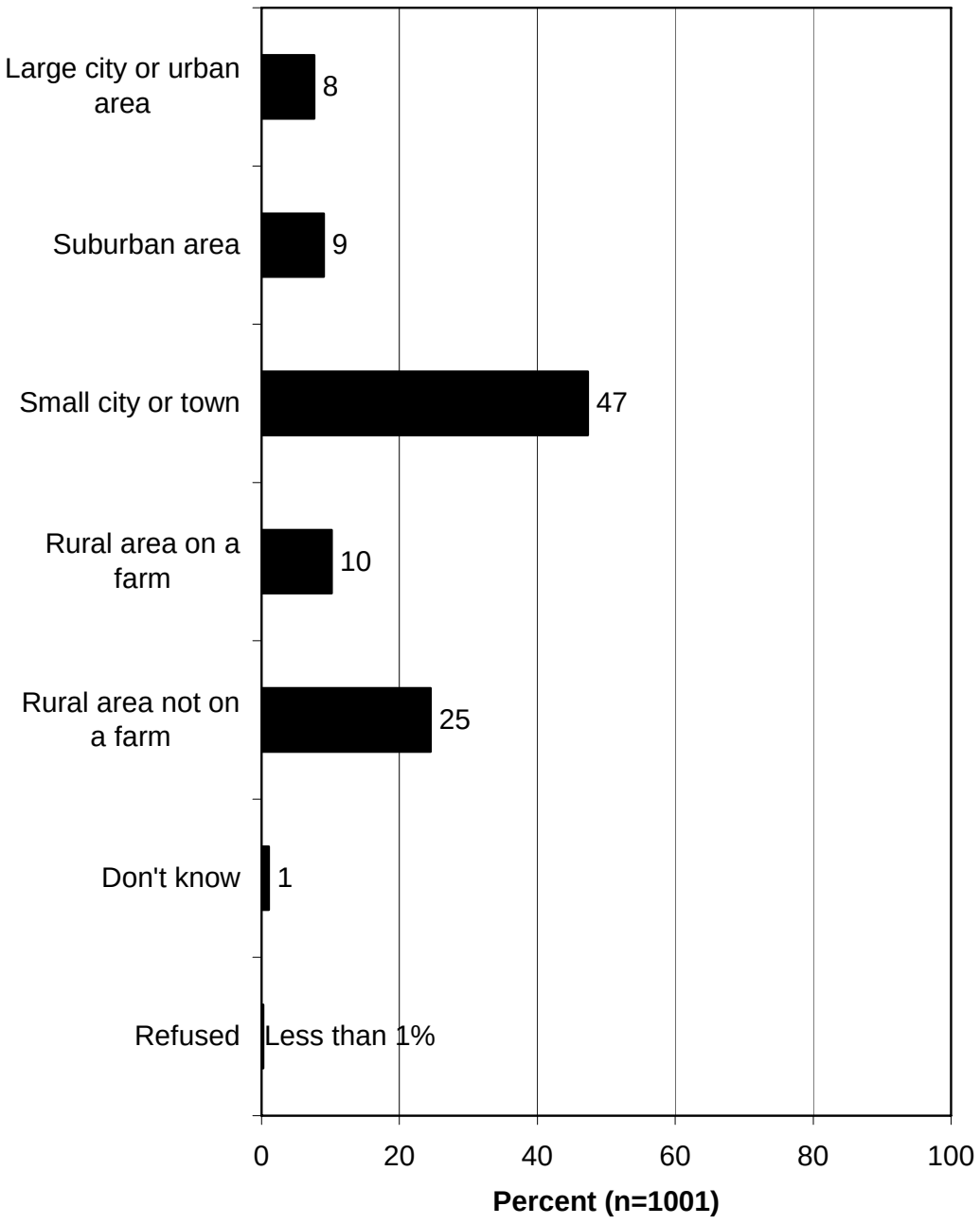
- Demographic data for the sample is shown.
  - Gender: the gender split, after weighting, exactly matches the U.S. Census data.
  - Age: the age breakdown, after weighting, exactly matches the U.S. Census data.
  - Residency: The breakdown by perceptions of residential character is shown.

Q109. Respondent's gender (not asked; observed by interviewer).

Q103. Respondent's age.



Q102. Do you consider your place of residence to be a large city or urban area, a suburban area, a small city or town, a rural area on a farm, or a rural area not on a farm?



IMPLICATIONS OF SURVEY RESULTS

PARTICIPATION IN WATER ACTIVITIES

Many West Virginia residents participate in water-based recreation, which means that there is likely to be wide exposure to algae issues. In particular, rivers and streams are commonly used, with somewhat less use being made of lakes/reservoirs, and much less use being made of ponds. The three rivers of interest (the Greenbrier, the South Branch of the Potomac, and the Cacapon, chosen for focus in the study because of the prevalence of algae issues on them) are widely used: about a third of water-based recreationists (32%) used one or more of these rivers for recreation.

KNOWLEDGE OF AND RATINGS OF THE WEST VIRGINIA DEPARTMENT OF ENVIRONMENTAL PROTECTION

Perceptions of credibility and trustworthiness of the DEP are positive, with only 4% saying that the DEP is *not at all* credible and 4% saying that the DEP is *not at all* trustworthy; however, the ratings are not uniformly positive, with substantial percentages saying that the DEP is *somewhat* credible (36%) or *somewhat* trustworthy (40%). Nonetheless, ratings of the DEP's efforts at protecting and restoring the environment are positive: 70% rate the efforts as excellent or good. Finally, in general, residents want the DEP to expend more effort on protecting and restoring the environment. In general, this suggests that the DEP will have credibility among residents regarding any communications relating to algae in West Virginia waters.

KNOWLEDGE AND AWARENESS OF ALGAE

In general, while West Virginia resident do not profess much knowledge of algae, they are aware of algae in West Virginia waters. About a third of residents (32%) think that algae has been increasing in West Virginia waters over the past 2 years. This perception of increasing algae levels is particularly strong among those who participate in water-based recreation on one of the three rivers of interest in this study (42% of those recreationists say that algae has been increasing over the past 2 years).

OPINIONS ON ALGAE IN GENERAL

General opinions of algae run from some mild concern ("Algae doesn't bother me, but I worry about problems it may cause") to more robust concern ("I generally regard algae as a nuisance"). When asked directly about whether algae is a problem in West Virginia, more than a third of residents indicate that algae is a problem. Furthermore, about a quarter indicate being concerned about algae in West Virginia waters.

A series of questions elicited opinions on various aspects of algae. Aesthetics plays a major role in feelings about algae, with 79% of West Virginia residents agreeing with the statement, "Algae is generally unattractive and unappealing." Safety also plays a role in opinions about algae, with well more than half of West Virginia residents (57%) agreeing that "algae causes safety hazards for people participating in activities on or in West Virginia waters." Those who participate in water-based recreation on the three rivers of interest have more negative opinions regarding algae, compared to those who do not engage in recreation on these rivers. The above should not be taken to mean that West Virginia residents misunderstand that some amount of algae in natural waters is part of a "normal" ecosystem. A large majority of residents (79%) agree that "algae is beneficial for the ecosystem in some situations."

LEVELS OF ACCEPTANCE OF ALGAE

The primary focus of the survey was to help determine the level of tolerance West Virginia residents have regarding algae in the state's natural waters. As discussed in the methodology, respondents examined photographs that showed varying levels of algae cover to determine at which point the level becomes unacceptable to them. The photograph that shows 20 percent coverage has only about a quarter of respondents (27%) thinking that this amount is unacceptable. However, the next photograph, showing 26 percent coverage, has nearly half of respondents (49%) thinking that the level is unacceptable. This suggests that waters with any more than a quarter coverage will be unacceptable to a majority of residents.

Crosstabulations of tolerance levels by participation in various activities and by demographic factors found that certain groups are less tolerant of algae than are other groups. In particular, people who go rafting or inner tubing, power boaters and users of personal watercraft, and those

who engage in walking along the banks and shores of natural waters are less tolerant of algae than are their counterparts. Demographically, those the median age or older have a lower tolerance of algae than do younger residents. Additionally, females have a lower tolerance of algae than do males.

CONTACTING AGENCIES AND ORGANIZATIONS REGARDING ALGAE

The substantial concern expressed about algae is not reflected in contacts with the DEP or other organizations about algae. The survey found that less than 1% of West Virginia residents contacted the DEP about algae in West Virginia waters in the past 2 years, and a little less than 3% contacted any other agency or organization about algae.

ALGAE-RELATED CONSTRAINTS TO PARTICIPATION IN WATER-BASED ACTIVITIES IN WEST VIRGINIA

At present, algae is not a major constraint to water-based recreation in the state. A general open-ended question asked respondents if there were things that took away from their participation in or their enjoyment of water-based recreation in West Virginia, and water quality was named by only 4%, and less than 1% named algae specifically. Nonetheless, if algae levels increase, the data suggest that water-based recreation could be affected. This would be particularly true if algae levels routinely reach more than about a quarter coverage in rivers and streams.

APPENDIX A: IMAGES

These algae coverage images were used in the survey (the "ruse" photographs are not shown).

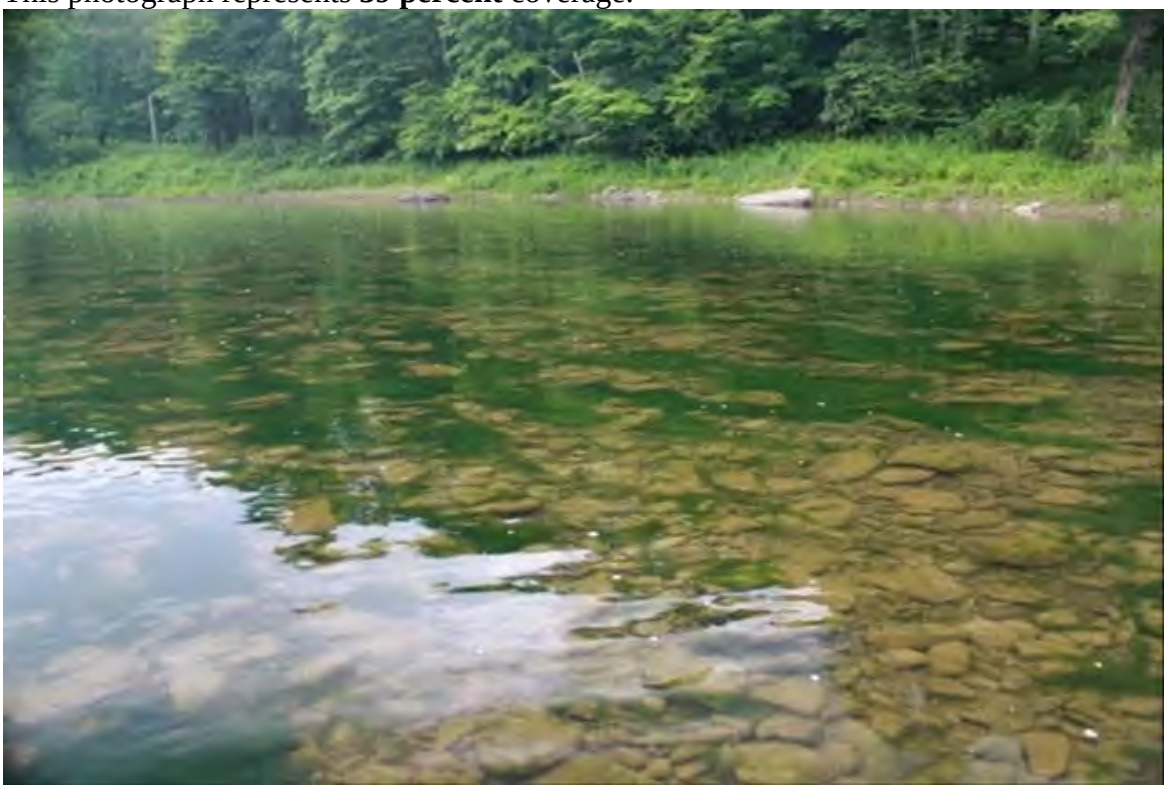
This photograph represents **4 percent** coverage.

This photograph represents **15 percent** coverage.

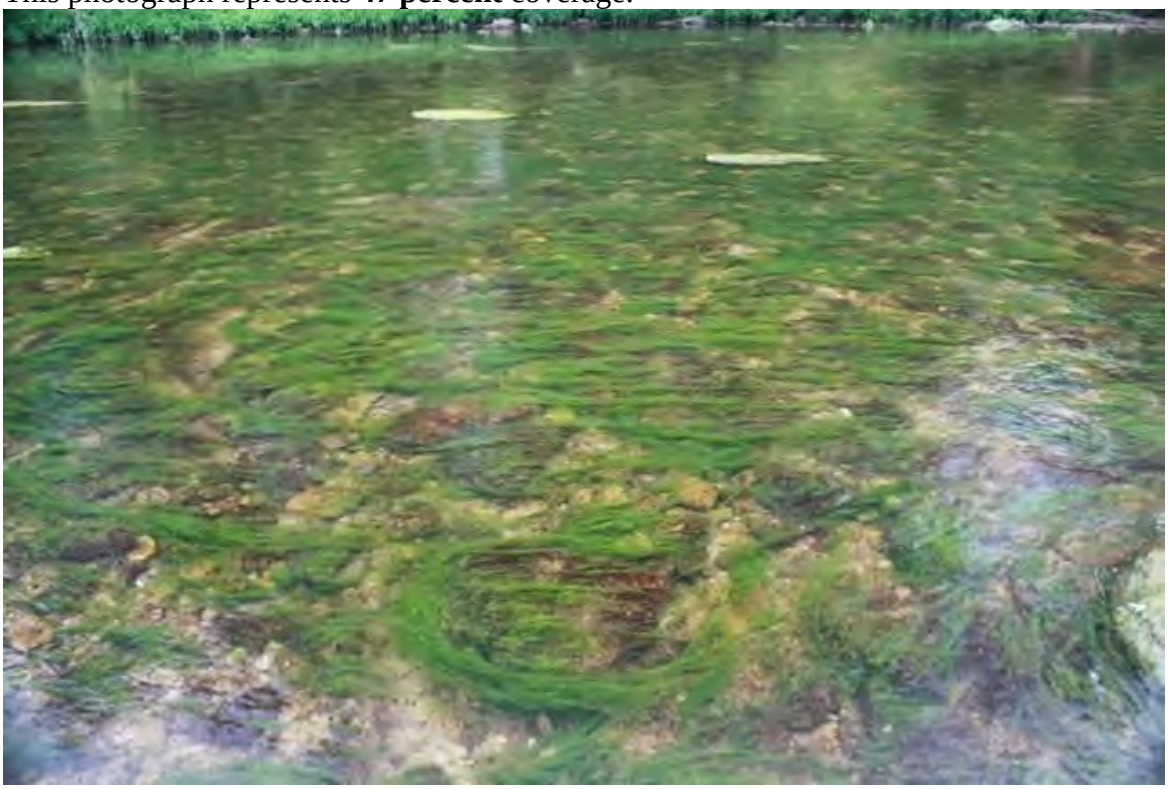
This photograph represents **20 percent** coverage.

This photograph represents **26 percent** coverage.

This photograph represents **39 percent** coverage.



This photograph represents **47 percent** coverage.



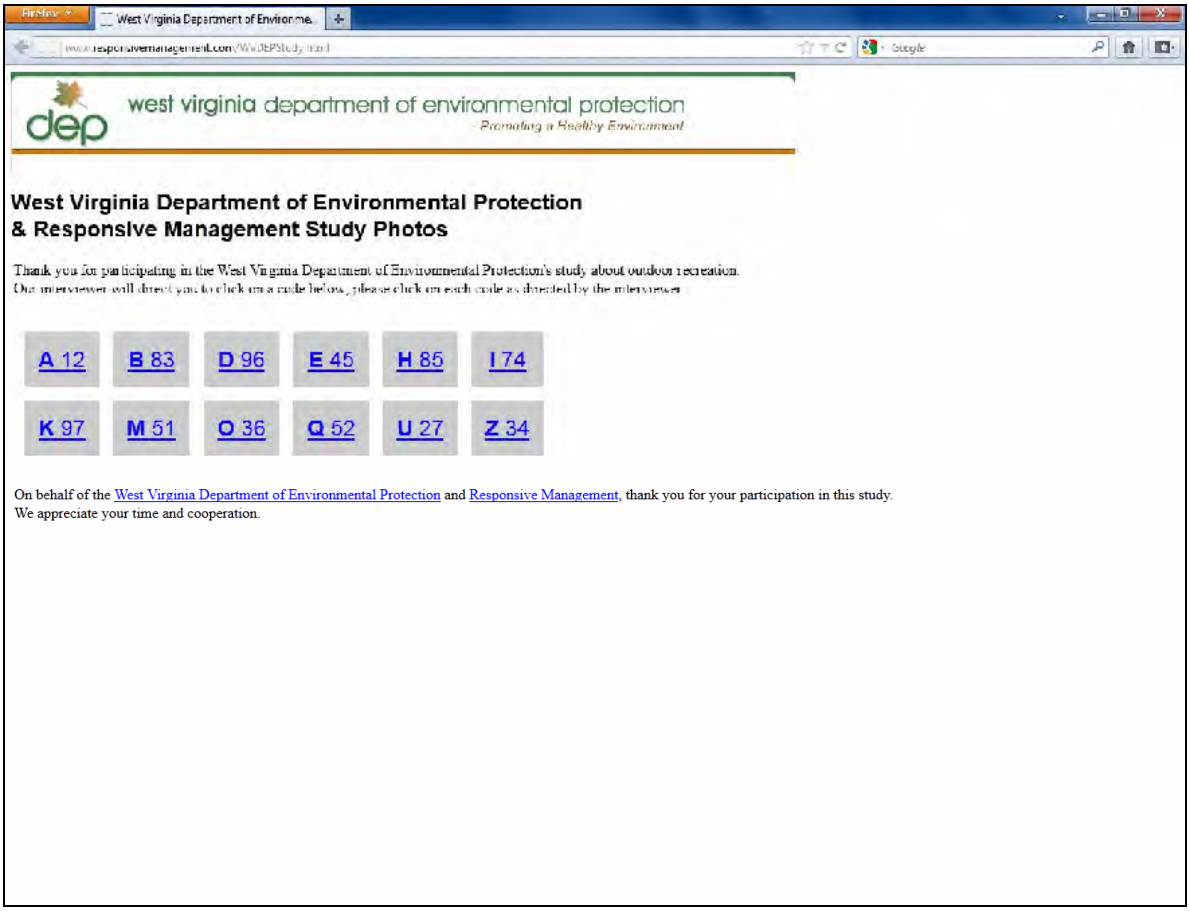
This photograph represents **65 percent** coverage.

APPENDIX B: IMAGE WEBSITE

The images were located on the web page at the address listed below; a screen shot of the web page is included. This web page will be left active for several months after the publication of the report for online viewing by interested readers. The alphanumeric code was meant to suggest a randomness to the viewers, thereby hiding the fact that the images include water photographs with algae coverage ranging from 4 percent to 65 percent.

In reality, the alphanumeric code can be used to view the photographs in order of increasing algae coverage. All photographs identified by a code starting with a vowel are ruse photographs and can be ignored. For those codes that start with a consonant, the last digit represents the rank order from least to most algae coverage. The photographs of algae, in order of increasing coverage, are M51, Q52, B83, Z34, H85, D96, and K97.

http://www.responsivemanagement.com/WVDEPStudy.html



ABOUT RESPONSIVE MANAGEMENT

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Utilizing our in-house, full-service telephone, mail, and web-based survey center with 50 professional interviewers, we have conducted more than 1,000 telephone surveys, mail surveys, personal interviews, and focus groups, as well as numerous marketing and communication plans, needs assessments, and program evaluations.

Clients include the federal natural resource and land management agencies, most state fish and wildlife agencies, state departments of natural resources, environmental protection agencies, state park agencies, tourism boards, most of the major conservation and sportsmen's organizations, and numerous private businesses. Responsive Management also collects attitude and opinion data for many of the nation's top universities.

Specializing in research on public attitudes toward natural resource and outdoor recreation issues, Responsive Management has completed a wide range of projects during the past 20 years, including dozens of studies of hunters, anglers, wildlife viewers, boaters, park visitors, historic site visitors, hikers, birdwatchers, campers, and rock climbers. Responsive Management has conducted studies on endangered species; waterfowl and wetlands; and the reintroduction of large predators such as wolves, grizzly bears, and the Florida panther.

Responsive Management has assisted with research on numerous natural resource ballot initiatives and referenda and has helped agencies and organizations find alternative funding and increase their membership and donations. Additionally, Responsive Management has conducted major organizational and programmatic needs assessments to assist natural resource agencies and organizations in developing more effective programs based on a solid foundation of fact.

Responsive Management has conducted research on public attitudes toward natural resources and outdoor recreation in almost every state in the United States, as well as in Canada, Australia, the United Kingdom, France, Germany, and Japan. Responsive Management has also conducted focus groups and personal interviews with residents of the African countries of Algeria, Cameroon, Mauritius, Namibia, South Africa, Tanzania, Zambia, and Zimbabwe.

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United States
Department of
Agriculture

Natural
Resources
Conservation
Service

National Water and Climate Center
Technical Note 99–1

Stream Visual Assessment Protocol

Issued December 1998

Cover photo: Stream in Clayton County, Iowa, exhibiting an impaired riparian zone.

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Preface

This document presents an easy-to-use assessment protocol to evaluate the condition of aquatic ecosystems associated with streams. The protocol does not require expertise in aquatic biology or extensive training. Least-impacted reference sites are used to provide a standard of comparison. The use of reference sites is variable depending on how the state chooses to implement the protocol. The state may modify the protocol based on a system of stream classification and a series of reference sites. Instructions for modifying the protocol are provided in the technical information section. Aternatively, a user may use reference sites in a less structured manner as a point of reference when applying the protocol.

The Stream Visual Assessment Protocol is the first level in a hierarchy of ecological assessment protocols. More sophisticated assessment methods may be found in the Stream Ecological Assessment Field Handbook. The field handbook also contains background information on basic stream ecology. Information on chemical monitoring of surface water and groundwater may be found in the National Handbook of Water Quality Monitoring.

The protocol is designed to be conducted with the landowner. Educational material is incorporated into the protocol. The document is structured so that the protocol (pp. 7–20) can be duplicated to provide a copy to the landowner after completion of an assessment. The assessment is recorded on a single sheet of paper (copied front and back).

Acknowledgments

This protocol was developed by the Natural Resources Conservation Service (NRCS) Aquatic Assessment Workgroup. The principal authors were **Bruce Newton**, limnologist, National Water and Climate Center, NRCS, Portland, OR; **Dr. Catherine Pringle**, associate professor of Aquatic Ecology, University of Georgia, Athens, GA; and **Ronald Bjorkland**, University of Georgia, Athens, GA. The NRCS Aquatic Assessment Workgroup members provided substantial assistance in development, field evaluation, and critical review of the document. These members were:

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Stream Visual Assessment Protocol

Introduction

This assessment protocol provides a basic level of stream health evaluation. It can be successfully applied by conservationists with little biological or hydrological training. It is intended to be conducted with the landowner and incorporates talking points for the conservationist to use during the assessment. This protocol is the first level in a four-part hierarchy of assessment protocols. Tier 2 is the NRCS Water Quality Indicators Guide, Tier 3 is the NRCS Stream Ecological Assessment Field Handbook, and Tier 4 is the intensive bioassessment protocol used by your State water quality agency.

This protocol provides an assessment based primarily on physical conditions within the assessment area. It may not detect some resource problems caused by factors located beyond the area being assessed. The use of higher tier methods is required to more fully assess the ecological condition and to detect problems originating elsewhere in the watershed. However, most landowners are mainly interested in evaluating conditions on their land, and this protocol is well suited to supporting that objective.

What makes for a healthy stream?

A stream is a complex ecosystem in which several biological, physical, and chemical processes interact. Changes in any one characteristic or process have cascading effects throughout the system and result in changes to many aspects of the system.

Some of the factors that influence and determine the integrity of streams are shown in figure 1. Often several factors can combine to cause profound changes. For example, increased nutrient loads alone might not cause a change to a forested stream. But when combined with tree removal and channel widening, the result is to shift the energy dynamics from an aquatic biological community based on leaf litter inputs to one based on algae and macrophytes. The resulting chemical changes caused by algal photosynthesis and respiration and elevated temperatures may further contribute to a completely different biological community.

Many stream processes are in a delicate balance. For example, stream power, sediment load, and channel roughness must be in balance. Hydrologic changes that increase stream power, if not balanced by greater channel complexity and roughness, result in "hungry" water that erodes banks or the stream bottom. Increases in sediment load beyond the transport capacity of the stream leads to deposition, lateral channel movement into streambanks, and channel widening.

Most systems would benefit from increased complexity and diversity in physical structure. Structural complexity is provided by trees fallen into the channel, overhanging banks, roots extending into the flow, pools and riffles, overhanging vegetation, and a variety of bottom materials. This complexity enhances habitat for organisms and also restores hydrologic properties that often have been lost.

Chemical pollution is a factor in most streams. The major categories of chemical pollutants are oxygen depleting substances, such as manure, ammonia, and organic wastes; the nutrients nitrogen and phosphorus; acids, such as from mining or industrial activities; and toxic materials, such as pesticides and salts or metals contained in some drain water. It is important to note that the effects of many chemicals depend on several factors. For example, an increase in the pH caused by excessive algal and aquatic plant growth may cause an otherwise safe concentration of ammonia to become toxic. This is because the equilibrium concentrations of nontoxic ammonium ion and toxic un-ionized ammonia are pH-dependent.

Finally, it is important to recognize that streams and flood plains need to operate as a connected system. Flooding is necessary to maintain the flood plain biological community and to relieve the erosive force of flood discharges by reducing the velocity of the water. Flooding and bankfull flows are also essential for maintaining the instream physical structure. These events scour out pools, clean coarser substrates (gravel, cobbles, and boulders) of fine sediment, and redistribute or introduce woody debris.

What's the stream type?

A healthy stream will look and function differently in different parts of the country and in different parts of the landscape. A mountain stream in a shale bedrock

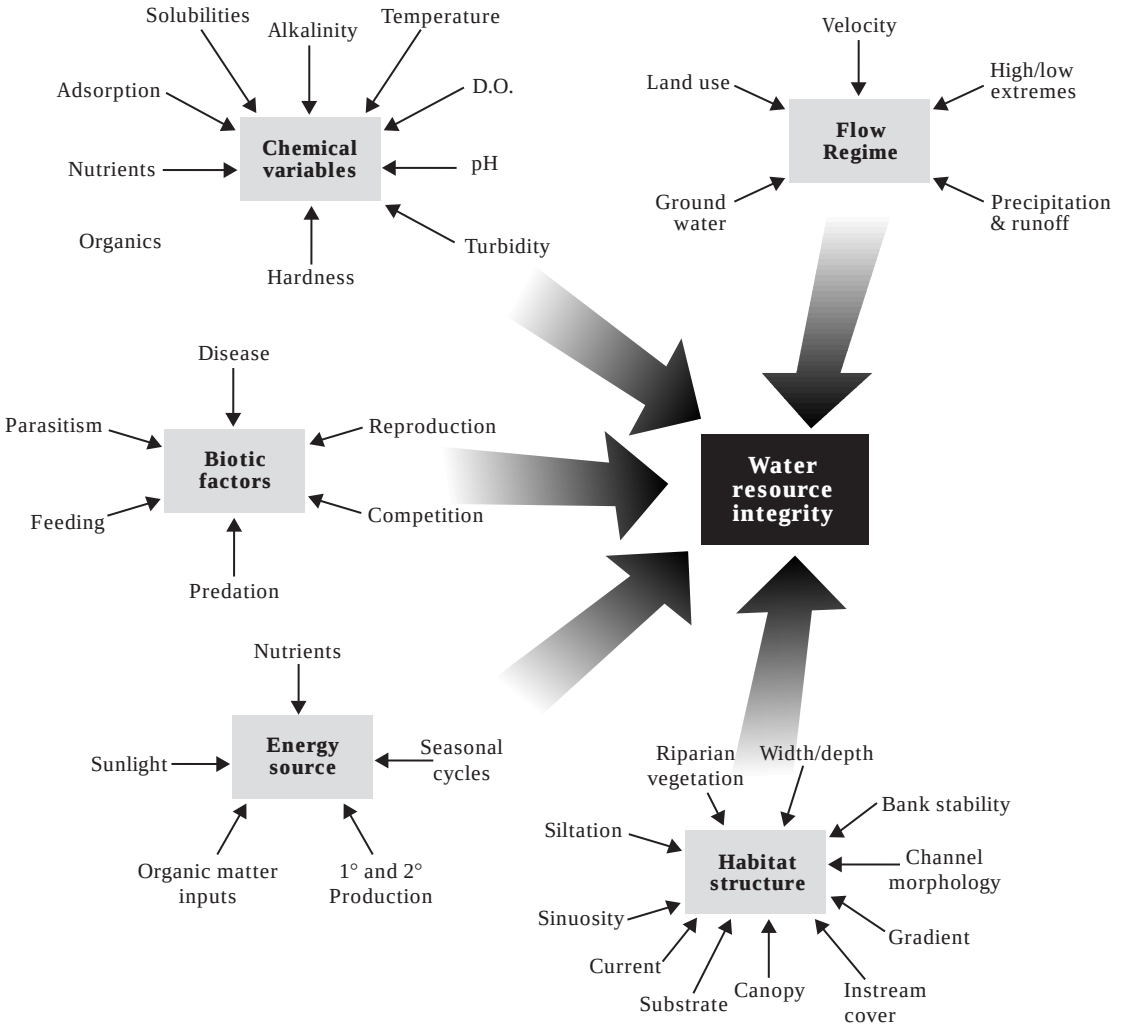
is different from a valley stream in alluvial deposits. Coastal streams are different from piedmont streams. Figuring out the different types of streams is called stream classification. Determining what types of streams are in your area is important to assessing the health of a particular stream.

There are many stream classification systems. For the purpose of a general assessment based on biology and habitat, you should think in terms of a three-level classification system based on ecoregion, drainage area, and gradient. *Ecoregions* are geographic areas in which ecosystems are expected to be similar. A national-level ecoregion map is available, and many states are working to develop maps at a higher level of resolution. *Drainage area* is the next most important factor to defining stream type. Finally, the slope or *gradient* of the reach you are assessing will help you determine the stream type. If you are familiar with another classification system, such as Rosgen or Montgomery/Buffington, you should use that system. This protocol may have been adjusted by your state office to reflect stream types common in your area.

Reference sites

One of the most difficult issues associated with stream ecosystems is the question of historic and potential conditions. To assess stream health, we need a benchmark of what the healthy condition is. We can usually assume that historic conditions were healthy. But in areas where streams have been degraded for 150 years or more, knowledge of historic conditions may have been lost. Moreover, in many areas returning to historic conditions is impossible or the historic conditions would not be stable under the current hydrology. Therefore, the question becomes what is the best we can expect for a particular stream. Scientists have grappled with this question for a long time, and the

Figure 1 Factors that influence the integrity of streams (modified from Karr 1986)



consensus that has emerged is to use reference sites within a classification system.

Reference sites represent the best conditions attainable within a particular stream class. The identification and characterization of reference sites is an ongoing effort led in most states by the water quality agency. You should determine whether your state has identified reference sites for the streams in your area. Such reference sites could be in another county or in another state. Unless your state office has provided photographs and other descriptive information, you should visit some reference sites to learn what healthy streams look like as part of your skills development. Visiting reference sites should also be part of your orientation after a move to a new field office.

Using this protocol

This protocol is intended for use in the field with the landowner. Conducting the assessment with the landowner gives you the opportunity to discuss natural resource concerns and conservation opportunities.

Before conducting the assessment, you should determine the following information in the field office:

- ecoregion (if in use in your State)
- drainage area
- stream gradients on the property
- overall position on the landscape

Your opening discussion with landowners should start by acknowledging that they own the land and that you understand that they know their operation best. Point out that streams, from small creeks to large rivers, are a resource that runs throughout the landscape—how they manage their part of the stream affects the entire system. Talk about the benefits of healthy streams and watersheds (improved baseflow, forage, fish, waterfowl, wildlife, aesthetics, reduced flooding downstream, and reduced water pollution). Talk about how restoring streams to a healthy condition is now a national priority.

Explain what will happen during the assessment and what you expect from them. An example follows:

This assessment will tell us how your stream is doing. We'll need to look at sections of the stream that are representative of different conditions. As we do the assessment we'll discuss how the functioning of different aspects of the stream work to keep the system healthy. After we're done, we can talk about the results of the assessment. I may recommend further assessment work to better understand what's going on. Once we understand what is happening, we can explore what you would like to accomplish with your stream and ideas for improving its condition, if necessary.

You need to assess one or more representative reaches. A reach is a length of stream. For this protocol, the length of the assessment reach is 12 times the active channel width. The reach should be representative of the stream through that area. If conditions change dramatically along the stream, you should identify additional assessment reaches and conduct separate assessments for each.

As you evaluate each element, try to work the talking points contained in the scoring descriptions into the conversation. If possible, involve the owner by asking him or her to help record the scores.

The assessment is recorded on a two-page worksheet. A completed worksheet is shown in figure 2. (A worksheet suitable for copying is at the end of this note.) The stream visual assessment protocol worksheet consists of two principal sections: reach identification and assessment. The identification section records basic information about the reach, such as name, location, and land uses. Space is provided for a diagram of the reach, which may be useful to locate the reach or illustrate problem areas. On this diagram draw all tributaries, drainage ditches, and irrigation ditches; note springs and ponds that drain to the stream; include road crossings and note whether they are fords, culverts, or bridges; note the direction of flow; and draw in any large woody debris, pools, and riffles.

The assessment section is used to record the scores for up to 15 assessment elements. Not all assessment elements will be applicable or useful for your site. Do not score elements that are not applicable. Score an element by comparing your observations to the descriptions provided. If you have difficulty matching descriptions, try to compare what you are observing to the conditions at reference sites for your area.

The overall assessment score is determined by adding the values for each element and dividing by the number of elements assessed. For example, if your scores add up to 76 and you used 12 assessment elements, you would have an overall assessment value of 6.3, which is classified as *fair*. This value provides a numerical assessment of the environmental condition of the stream reach. This value can be used as a general statement about the "state of the environment" of the stream or (over time) as an indicator of trends in condition.

Figure 2 Stream visual assessment protocol worksheet

Stream Visual Assessment Protocol

Owners name *Elmer Smith* Evaluator's name *Mary Soylkahn* Date *6-20-99*

Stream name *Camp Creek* Waterbody ID number _____

Reach location *About 2,000 feet upstream of equipment shed*

Ecoregion _____ Drainage area *2,200 acres* Gradient *1.2 % (map)*

Applicable reference site *Cherry Creek north of the Rt 310 bridge*

Land use within drainage (%): row crop *40* hayland *30* grazing/pasture *20* forest *10* residential _____

confined animal feeding operations _____ Cons. Reserve _____ industrial _____ Other: _____

Weather conditions-today *clear* Past 2-5 days *clear*

Active channel width *15 feet* Dominant substrate: boulder _____ gravel *X* sand *X* silt _____ mud _____

Site Diagram

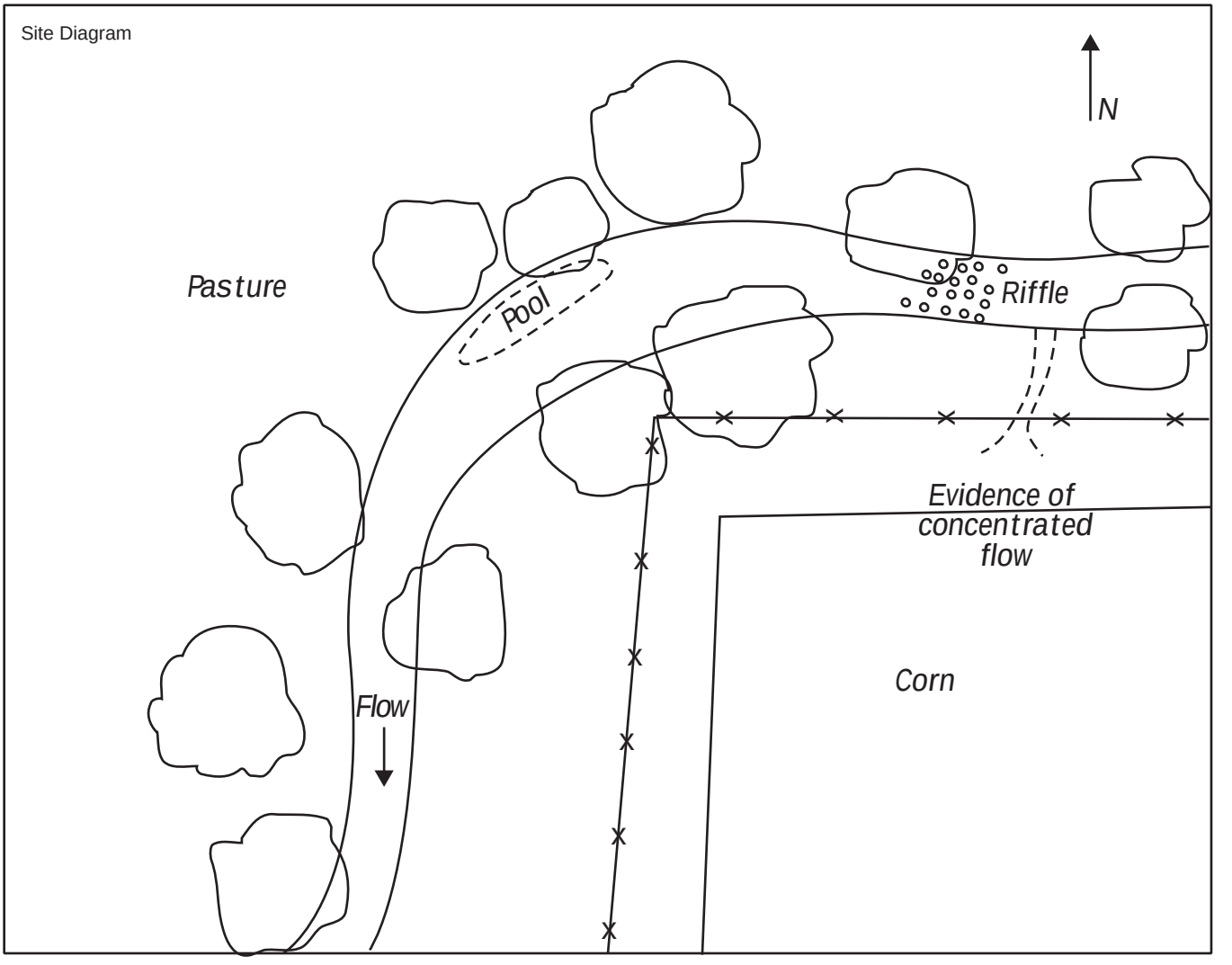


Figure 2 Stream visual assessment protocol worksheet—Continued

Assessment Scores

Channel condition	8
Hydrologic alteration	10
Riparian zone	1
Bank stability	5
Water appearance	3
Nutrient enrichment	7
Barriers to fish movement	10
Instream fish cover	3
Pools	3
Invertebrate habitat	7

Score only if applicable

Canopy cover	3
Manure presence	1
Salinity	
Riffle embeddedness	5
Marcroinvertebrates Observed (optional)	10

Overall score
(Total divided by number scored)
76/14 5.4

<6.0	**Poor**
6.1-7.4	**Fair**
7.5-8.9	**Good**
>9.0	**Excellent**

Suspected causes of observed problems *This reach is typical of the reaches on the property. Severely degraded riparian zones lack brush, small trees. Some bank problems from livestock access. Channel may be widening due to high sediment load. Does not appear to be downcutting.*

Recommendations *Install 391-Riparian Forest Buffer. Need to encourage livestock away from stream using water sources and shade or exclude livestock. Concentrated flows off fields need to be spread out in zone 3 of buffer. Relocate fallen trees if they deflect current into bank–use as stream barbs to deflect current to maintain channel.*

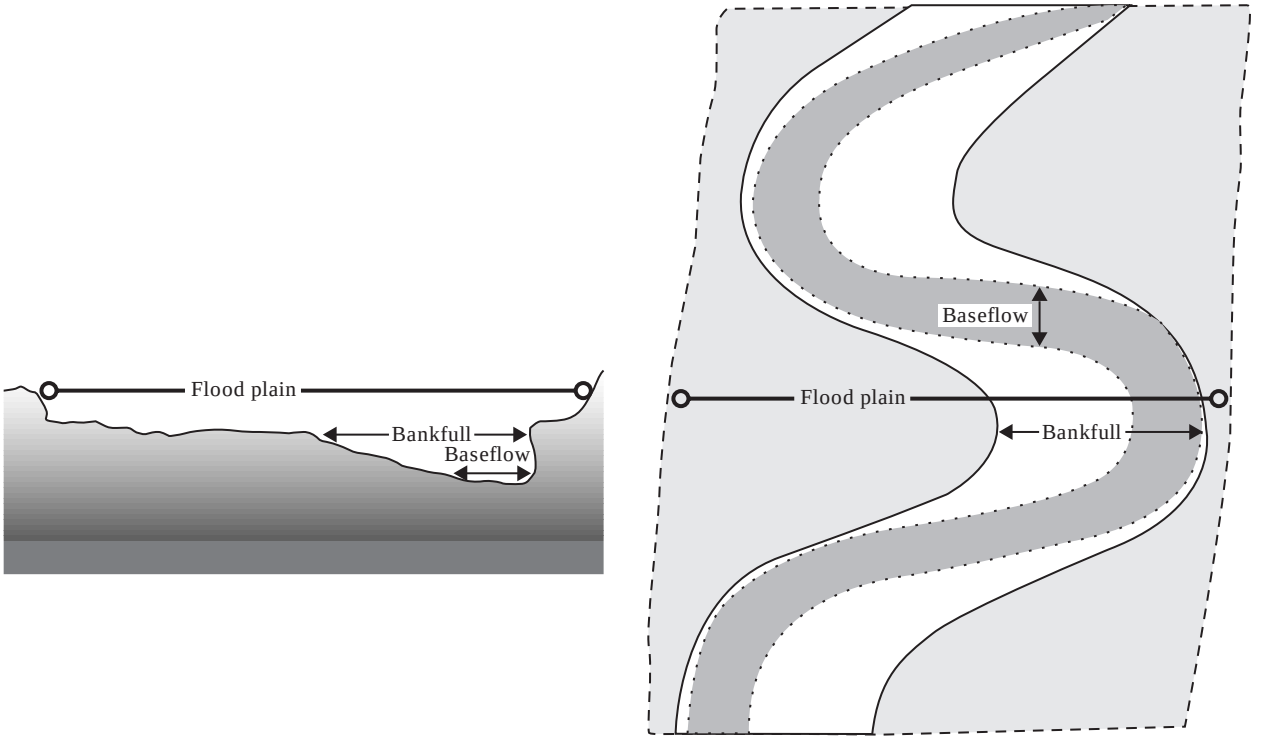
Reach description

The first page of the assessment worksheet records the identity and location of the stream reach. Most entries are self-explanatory. Waterbody ID and ecoregion should be filled out only if these identification and classification aids are used in your state.

Active channel width can be difficult to determine. However, active channel width helps to characterize the stream. It is also an important aspect of more advanced assessment protocols; therefore, it is worth becoming familiar with the concept and field determination. For this protocol you do not need to measure active channel width accurately — a visual estimate of the average width is adequate.

Active channel width is the stream width at the bankfull discharge. Bankfull discharge is the flow rate that forms and controls the shape and size of the active channel. It is approximately the flow rate at which the stream begins to move onto its flood plain if the stream has an active flood plain. The bankfull discharge is expected to occur every 1.5 years on average. Figure 3 illustrates the relationship between baseflow, bankfull flow, and the flood plain. Active channel width is best determined by locating the first flat depositional surface occurring above the bed of the stream (i.e., an active flood plain). The lowest elevation at which the bankfull surface could occur is at the top of the point bars or other sediment deposits in the channel bed. Other indicators of the bankfull surface include a break in slope on the bank, vegetation change, substrate, and debris. If you are not trained in locating the bankfull stage, ask the landowner how high the water gets every year and observe the location of permanent vegetation.

Figure 3 Baseflow, bankfull, and flood plain locations (Rosgen 1996)



Scoring descriptions

Each assessment element is rated with a value of 1 to 10. Rate only those elements appropriate to the stream. Using the Stream Visual Assessment Protocol worksheet, record the score that best fits the observations you make based on the narrative descriptions provided. Unless otherwise directed, assign the lowest score that applies. For example, if a reach has aspects of several narrative descriptions, assign a score based on the lowest scoring description that contains indicators present within the reach. You may record values intermediate to those listed. Some background information is provided for each assessment element, as well as a description of what to look for. The length of the assessment reach should be 12 times the active channel width.

Channel condition

Natural channel; no structures, dikes. No evidence of downcutting or excessive lateral cutting.	Evidence of past channel alteration, but with significant recovery of channel and banks. Any dikes or levies are set back to provide access to an adequate flood plain.	Altered channel; <50% of the reach with riprap and/or channelization. Excess aggradation; braided channel. Dikes or levees restrict flood plain width.	Channel is actively downcutting or widening. >50% of the reach with riprap or channelization. Dikes or levees prevent access to the flood plain.
10	7	3	1

Stream meandering generally increases as the gradient of the surrounding valley decreases. Often, development in the area results in changes to this meandering pattern and the flow of a stream. These changes in turn may affect the way a stream naturally does its work, such as the transport of sediment and the development and maintenance of habitat for fish, aquatic insects, and aquatic plants. Some modifications to stream channels have more impact on stream health than others. For example, channelization and dams affect a stream more than the presence of pilings or other supports for road crossings.

Active downcutting and excessive lateral cutting are serious impairments to stream function. Both conditions are indicative of an unstable stream channel. Usually, this instability must be addressed before committing time and money toward improving other stream problems. For example, restoring the woody vegetation within the riparian zone becomes increasingly difficult when a channel is downcutting because banks continue to be undermined and the water table drops below the root zone of the plants during their growing season. In this situation or when a channel is fairly stable, but already incised from previous downcutting or mechanical dredging, it is usually necessary to plant upland species, rather than hydrophytic, or to apply irrigation for several growing seasons, or both. Extensive bank-armoring of channels to stop lateral cutting usually leads to more problems (especially downstream). Often stability can be obtained by using a series of structures (barbs, groins, jetties, deflectors, weirs, vortex weirs) that reduce water velocity, deflect currents, or act as gradient controls. These structures are used in conjunction with large woody debris and woody vegetation plantings. Hydrologic alterations are described next.

What to look for: Signs of channelization or straightening of the stream may include an unnaturally straight section of the stream, high banks, dikes or berms, lack of flow diversity (e.g., few point bars and deep pools), and uniform-sized bed materials (e.g., all cobbles where there should be mixes of gravel and cobble). In newly channelized reaches, vegetation may be missing or appear very different (different species, not as well developed) from the bank vegetation of areas that were not channelized. Older channelized reaches may also have little or no vegetation or have grasses instead of woody vegetation. Drop structures (such as check dams), irrigation diversions, culverts, bridge abutments, and riprap also indicate changes to the stream channel.

Indicators of downcutting in the stream channel include nickpoints associated with headcuts in the stream bottom and exposure of cultural features, such as pipelines that were initially buried under the stream. Exposed footings in bridges and culvert outlets that are higher than the water surface during low flows are other examples. A lack of sediment depositional features, such as regularly-spaced point bars, is

normally an indicator of incision. A low vertical scarp at the toe of the streambank may indicate downcutting, especially if the scarp occurs on the inside of a meander. Another visual indicator of current or past downcutting is high streambanks with woody vegetation growing well below the top of the bank (as a channel incises the bankfull flow line moves downward within the former bankfull channel). Excessive bank erosion is indicated by raw banks in areas of the stream where they are not normally found, such as straight sections between meanders or on the inside of curves.

Hydrologic alteration

Flooding every 1.5 to 2 years. No dams, no water withdrawals, no dikes or other structures limiting the stream's access to the flood plain. Channel is not incised.	Flooding occurs only once every 3 to 5 years; limited channel incision. or Withdrawals, although present, do not affect available habitat for biota.	Flooding occurs only once every 6 to 10 years; channel deeply incised. or Withdrawals significantly affect available low flow habitat for biota.	No flooding; channel deeply incised or structures prevent access to flood plain or dam operations prevent flood flows. or Withdrawals have caused severe loss of low flow habitat. or Flooding occurs on a 1-year rain event or less.
10	7	3	1

Bankfull flows, as well as flooding, are important to maintaining channel shape and function (e.g., sediment transport) and maintaining the physical habitat for animals and plants. High flows scour fine sediment to keep gravel areas clean for fish and other aquatic organisms. These flows also redistribute larger sediment, such as gravel, cobbles, and boulders, as well as large woody debris, to form pool and riffle habitat important to stream biota. The river channel and flood plain exist in dynamic equilibrium, having evolved in the present climatic regime and geomorphic setting. The relationship of water and sediment is the basis for the dynamic equilibrium that maintains the form and function of the river channel. The energy of the river (water velocity and depth) should be in balance with the bedload (volume and particle size of the sediment). Any change in the flow regime alters this balance.

If a river is not incised and has access to its flood plain, decreases in the frequency of bankfull and out-of-bank flows decrease the river's ability to transport sediment. This can result in excess sediment deposition, channel widening and shallowing, and, ultimately, in *braiding* of the channel. Rosgen (1996) defines braiding as a stream with three or more smaller channels. These smaller channels are extremely unstable, rarely have woody vegetation along their banks, and provide poor habitat for stream biota. A *split channel*, however, has two or more smaller channels (called side channels) that are usually very stable, have woody vegetation along their banks, and provide excellent habitat.

Conversely, an increase in flood flows or the confinement of the river away from its flood plain (from either incision or levees) increases the energy available to transport sediment and can result in bank and channel erosion.

The low flow or baseflow during the dry periods of summer or fall usually comes from groundwater entering the stream through the stream banks and bottom. A decrease in the low-flow rate will result in a smaller portion of the channel suitable for aquatic organisms. The withdrawal of water from streams for irrigation or industry and the placement of dams often change the normal low-flow pattern. Baseflow can also

be affected by management and land use within the watershed — less infiltration of precipitation reduces baseflow and increases the frequency and severity of high flow events. For example, urbanization increases runoff and can increase the frequency of flooding to every year or more often and also reduce low flows. Overgrazing and clearcutting can have similar, although typically less severe, effects. The last description in the last box refers to the increased flood frequency that occurs with the above watershed changes.

What to look for: Ask the landowner about the frequency of flooding and about summer low-flow conditions. A flood plain should be inundated during flows that equal or exceed the 1.5- to 2.0-year flow event (2 out of 3 years or every other year). Be cautious because water in an adjacent field does not necessarily indicate natural flooding. The water may have flowed overland from a low spot in the bank outside the assessment reach.

Evidence of flooding includes high water marks (such as water lines), sediment deposits, or stream debris. Look for these on the banks, on the bankside trees or rocks, or on other structures (such as road pilings or culverts).

Excess sediment deposits and wide, shallow channels could indicate a loss of sediment transport capacity. The loss of transport capacity can result in a stream with three or more channels (braiding).

Riparian zone

Natural vegetation extends at least two active channel widths on each side.	Natural vegetation extends one active channel width on each side. or If less than one width, covers entire flood plain.	Natural vegetation extends half of the active channel width on each side.	Natural vegetation extends a third of the active channel width on each side. or Filtering function moderately compromised.	Natural vegetation less than a third of the active channel width on each side. or Lack of regeneration. or Filtering function severely compromised.
10	8	5	3	1

This element is the width of the natural vegetation zone from the edge of the active channel out onto the flood plain. For this element, the word *natural* means plant communities with (1) all appropriate structural components and (2) species native to the site or introduced species that function similar to native species at reference sites.

A healthy riparian vegetation zone is one of the most important elements for a healthy stream ecosystem. The quality of the riparian zone increases with the width and the complexity of the woody vegetation within it. This zone:

- Reduces the amount of pollutants that reach the stream in surface runoff.
- Helps control erosion.
- Provides a microclimate that is cooler during the summer providing cooler water for aquatic organisms.
- Provides large woody debris from fallen trees and limbs that form instream cover, create pools, stabilize the streambed, and provide habitat for stream biota.
- Provides fish habitat in the form of undercut banks with the "ceiling" held together by roots of woody vegetation.
- Provides organic material for stream biota that, among other functions, is the base of the food chain in lower order streams.
- Provides habitat for terrestrial insects that drop in the stream and become food for fish, and habitat and travel corridors for terrestrial animals.
- Dissipates energy during flood events.
- Often provides the only refuge areas for fish during out-of-bank flows (behind trees, stumps, and logs).

The type, timing, intensity, and extent of activity in riparian zones are critical in determining the impact on these areas. Narrow riparian zones and/or riparian zones that have roads, agricultural activities, residential or commercial structures, or significant areas of bare soils have reduced functional value for the stream. The filtering function of riparian zones can be compromised by concentrated flows. No evidence of concentrated flows through the zone should occur or, if concentrated flows are evident, they should be from land areas appropriately buffered with vegetated strips.

What to look for: Compare the width of the riparian zone to the active channel width. In steep, V-shaped valleys there may not be enough room for a flood plain riparian zone to extend as far as one or two active channel widths. In this case, observe how much of the flood plain is covered by riparian zone. The vegetation must be natural and consist of all of the structural components (aquatic plants, sedges or rushes, grasses, forbs, shrubs, understory trees, and overstory trees) appropriate for the area. A common problem is lack of shrubs and understory trees. Another common problem is lack of regeneration. The presence of only mature vegetation and few seedlings indicates lack of regeneration. Do not consider incomplete plant communities as natural. Healthy riparian zones on both sides of the stream are important for the health of the entire system. If one side is lacking the protective vegetative cover, the entire reach of the stream will be affected. In doing the assessment, examine both sides of the stream and note on the diagram which side of the stream has problems. There should be no evidence of concentrated flows through the riparian zone that are not adequately buffered before entering the riparian zone.

Bank stability

Banks are stable; banks are low (at elevation of active flood plain); 33% or more of eroding surface area of banks in outside bends is protected by roots that extend to the base-flow elevation.	Moderately stable; banks are low (at elevation of active flood plain); less than 33% of eroding surface area of banks in outside bends is protected by roots that extend to the baseflow elevation.	Moderately unstable; banks may be low, but typically are high (flooding occurs 1 year out of 5 or less frequently); outside bends are actively eroding (overhanging vegetation at top of bank, some mature trees falling into steam annually, some slope failures apparent).	Unstable; banks may be low, but typically are high; some straight reaches and inside edges of bends are actively eroding as well as outside bends (overhanging vegetation at top of bare bank, numerous mature trees falling into stream annually, numerous slope failures apparent).
10	7	3	1

This element is the existence of or the potential for detachment of soil from the upper and lower stream banks and its movement into the stream. Some bank erosion is normal in a healthy stream. Excessive bank erosion occurs where riparian zones are degraded or where the stream is unstable because of changes in hydrology, sediment load, or isolation from the flood plain. High and steep banks are more susceptible to erosion or collapse. All outside bends of streams erode, so even a stable stream may have 50 percent of its banks bare and eroding. A healthy riparian corridor with a vegetated flood plain contributes to bank stability. The roots of perennial grasses or woody vegetation typically extend to the baseflow elevation of water in streams that have bank heights of 6 feet or less. The root masses help hold the bank soils together and physically protect the bank from scour during bankfull and flooding events. Vegetation seldom becomes established below the elevation of the bankfull surface because of the frequency of inundation and the unstable bottom conditions as the stream moves its bedload.

The type of vegetation is important. For example, trees, shrubs, sedges, and rushes have the type of root masses capable of withstanding high streamflow events, while Kentucky bluegrass does not. Soil type at the surface and below the surface also influences bank stability. For example, banks with a thin soil cover over gravel or sand are more prone to collapse than are banks with a deep soil layer.

What to look for: Signs of erosion include unvegetated stretches, exposed tree roots, or scalloped edges. Evidence of construction, vehicular, or animal paths near banks or grazing areas leading directly to the water's edge suggest conditions that may lead to the collapse of banks. Estimate the size or area of the bank affected relative to the total bank area. This element may be difficult to score during high water.

Water appearance

Very clear, or clear but tea-colored; objects visible at depth 3 to 6 ft (less if slightly colored); no oil sheen on surface; no noticeable film on submerged objects or rocks.	Occasionally cloudy, especially after storm event, but clears rapidly; objects visible at depth 1.5 to 3 ft; may have slightly green color; no oil sheen on water surface.	Considerable cloudiness most of the time; objects visible to depth 0.5 to 1.5 ft; slow sections may appear pea-green; bottom rocks or submerged objects covered with heavy green or olive-green film. or Moderate odor of ammonia or rotten eggs.	Very turbid or muddy appearance most of the time; objects visible to depth < 0.5 ft; slow moving water may be bright-green; other obvious water pollutants; floating algal mats, surface scum, sheen or heavy coat of foam on surface. or Strong odor of chemicals, oil, sewage, other pollutants.
10	7	3	1

This element compares turbidity, color, and other visual characteristics with a healthy or reference stream. The depth to which an object can be clearly seen is a measure of turbidity. Turbidity is caused mostly by particles of soil and organic matter suspended in the water column. Water often shows some turbidity after a storm event because of soil and organic particles carried by runoff into the stream or suspended by turbulence. The water in some streams may be naturally tea-colored. This is particularly true in watersheds with extensive bog and wetland areas. Water that has slight nutrient enrichment may support communities of algae, which provide a greenish color to the water. Streams with heavy loads of nutrients have thick coatings of algae attached to the rocks and other submerged objects. In degraded streams, floating algal mats, surface scum, or pollutants, such as dyes and oil, may be visible.

What to look for: Clarity of the water is an obvious and easy feature to assess. The deeper an object in the water can be seen, the lower the amount of turbidity. Use the depth that objects are visible only if the stream is deep enough to evaluate turbidity using this approach. For example, if the water is clear, but only 1 foot deep, do not rate it as if an object became obscured at a depth of 1 foot. This measure should be taken after a stream has had the opportunity to "settle" following a storm event. A pea-green color indicates nutrient enrichment beyond what the stream can naturally absorb.

Nutrient enrichment

Clear water along entire reach; diverse aquatic plant community includes low quantities of many species of macrophytes; little algal growth present.	Fairly clear or slightly greenish water along entire reach; moderate algal growth on stream substrates.	Greenish water along entire reach; overabundance of lush green macrophytes; abundant algal growth, especially during warmer months.	Pea green, gray, or brown water along entire reach; dense stands of macrophytes clog stream; severe algal blooms create thick algal mats in stream.
10	7	3	1

Nutrient enrichment is often reflected by the types and amounts of aquatic vegetation in the water. High levels of nutrients (especially phosphorus and nitrogen) promote an overabundance of algae and floating and rooted macrophytes. The presence of some aquatic vegetation is normal in streams. Algae and macrophytes provide habitat and food for all stream animals. However, an excessive amount of aquatic vegetation is not beneficial to most stream life. Plant respiration and decomposition of dead vegetation consume dissolved oxygen in the water. Lack of dissolved oxygen creates stress for all aquatic organisms and can cause fish kills. A landowner may have seen fish gulping for air at the water surface during warm weather, indicating a lack of dissolved oxygen.

What to look for: Some aquatic vegetation (rooted macrophytes, floating plants, and algae attached to substrates) is normal and indicates a healthy stream. Excess nutrients cause excess growth of algae and macrophytes, which can create greenish color to the water. As nutrient loads increase the green becomes more intense and macrophytes become more lush and deep green. Intense algal blooms, thick mats of algae, or dense stands of macrophytes degrade water quality and habitat. Clear water and a diverse aquatic plant community without dense plant populations are optimal for this characteristic.

Barriers to fish movement

No barriers	Seasonal water withdrawals inhibit movement within the reach	Drop structures, culverts, dams, or diversions (< 1 foot drop) within the reach	Drop structures, culverts, dams, or diversions (> 1 foot drop) within 3 miles of the reach	Drop structures, culverts, dams, or diversions (> 1 foot drop) within the reach
10	8	5	3	1

Barriers that block the movement of fish or other aquatic organisms, such as fresh water mussels, must be considered as part of the overall stream assessment. If sufficiently high, these barriers may prevent the movement or migration of fish, deny access to important breeding and foraging habitats, and isolate populations of fish and other aquatic animals.

What to look for: Some barriers are natural, such as waterfalls and boulder dams, and some are developed by humans. Note the presence of such barriers along the reach of the stream you are assessing, their size, and whether provisions have been made for the passage of fish. Ask the landowner about any dams or other barriers that may be present 3 to 5 miles upstream or downstream. Larger dams are often noted on maps, so you may find some information even before going out into the field. Beaver dams generally do not prevent fish migration. Look for structures that may not involve a drop, but still present a hydraulic barrier. Single, large culverts with no slope and sufficient water depth usually do not constitute a barrier. Small culverts or culverts with slopes may cause high water velocities that prevent passage.

Instream fish cover

>7 cover types available	6 to 7 cover types available	4 to 5 cover types available	2 to 3 cover types available	None to 1 cover type available
10	8	5	3	1

Cover types: Logs/large woody debris, deep pools, overhanging vegetation, boulders/cobble, riffles, undercut banks, thick root mats, dense macrophyte beds, isolated/backwater pools, other: _____.

This assessment element measures availability of physical habitat for fish. The potential for the maintenance of a healthy fish community and its ability to recover from disturbance is dependent on the variety and abundance of suitable habitat and cover available.

What to look for: Observe the number of different habitat and cover types *within a representative subsection of the assessment* reach that is equivalent in length to *five times* the active channel width. Each cover type must be present in appreciable amounts to score. Cover types are described below.

Logs/large woody debris—Fallen trees or parts of trees that provide structure and attachment for aquatic macroinvertebrates and hiding places for fish.

Deep pools—Areas characterized by a smooth undisturbed surface, generally slow current, and deep enough to provide protective cover for fish (75 to 100% deeper than the prevailing stream depth).

Overhanging vegetation—Trees, shrubs, vines, or perennial herbaceous vegetation that hangs immediately over the stream surface, providing shade and cover.

Boulders/cobble—Boulders are rounded stones more than 10 inches in diameter or large slabs more than 10 inches in length; cobbles are stones between 2.5 and 10 inches in diameter.

Undercut banks—Eroded areas extending horizontally beneath the surface of the bank forming underwater pockets used by fish for hiding and protection.

Thick root mats—Dense mats of roots and rootlets (generally from trees) at or beneath the water surface forming structure for invertebrate attachment and fish cover.

Dense macrophyte beds—Beds of emergent (e.g., water willow), floating leaf (e.g., water lily), or submerged (e.g., riverweed) aquatic vegetation thick enough to provide invertebrate attachment and fish cover.

Riffles—Area characterized by broken water surface, rocky or firm substrate, moderate or swift current, and relatively shallow depth (usually less than 18 inches).

Isolated/backwater pools—Areas disconnected from the main channel or connected as a "blind" side channel, characterized by a lack of flow except in periods of high water.

Pools

Deep and shallow pools abundant; greater than 30% of the pool bottom is obscure due to depth, or the pools are at least 5 feet deep.	Pools present, but not abundant; from 10 to 30% of the pool bottom is obscure due to depth, or the pools are at least 3 feet deep.	Pools present, but shallow; from 5 to 10% of the pool bottom is obscure due to depth, or the pools are less than 3 feet deep.	Pools absent, or the entire bottom is discernible.
10	7	3	1

Pools are important resting and feeding sites for fish. A healthy stream has a mix of shallow and deep pools. A *deep* pool is 1.6 to 2 times deeper than the prevailing depth, while a *shallow* pool is less than 1.5 times deeper than the prevailing depth. Pools are abundant if a deep pool is in each of the meander bends in the reach being assessed. To determine if pools are abundant, look at a longer sample length than one that is 12 active channel widths in length. Generally, only 1 or 2 pools would typically form within a reach as long as 12 active channel widths. In low order, high gradient streams, pools are abundant if there is more than one pool every 4 channel widths.

What to look for: Pool diversity and abundance are estimated based on walking the stream or probing from the streambank with a stick or length of rebar. You should find deep pools on the outside of meander bends. In shallow, clear streams a visual inspection may provide an accurate estimate. In deep streams or streams with low visibility, this assessment characteristic may be difficult to determine and should not be scored.

Insect/invertebrate habitat

At least 5 types of habitat available. Habitat is at a stage to allow full insect colonization (woody debris and logs not freshly fallen).	3 to 4 types of habitat. Some potential habitat exists, such as overhanging trees, which will provide habitat, but have not yet entered the stream.	1 to 2 types of habitat. The substrate is often disturbed, covered, or removed by high stream velocities and scour or by sediment deposition.	None to 1 type of habitat.
10	7	3	1

Cover types: Fine woody debris, submerged logs, leaf packs, undercut banks, cobble, boulders, coarse gravel, other: _____.

Stable substrate is important for insect/invertebrate colonization. *Substrate* refers to the stream bottom, woody debris, or other surfaces on which invertebrates can live. Optimal conditions include a variety of substrate types within a relatively small area of the stream (5 times the active channel width). Stream and substrate stability are also important. High stream velocities, high sediment loads, and frequent flooding may cause substrate instability even if substrate is present.

What to look for: Observe the number of different types of habitat and cover within a representative subsection of the assessment reach that is equivalent in length to five times the active channel width. Each cover type must be present in appreciable amounts to score.

Score the following assessment elements only if applicable

Canopy cover (if applicable)

Coldwater fishery

> 75% of water surface shaded and upstream 2 to 3 miles generally well shaded.	>50% shaded in reach. or >75% in reach, but upstream 2 to 3 miles poorly shaded.	20 to 50% shaded.	< 20% of water surface in reach shaded.
10	7	3	1

Warmwater fishery

25 to 90% of water surface shaded; mixture of conditions.	> 90% shaded; full canopy; same shading condition throughout the reach.	(intentionally blank)	< 25% water surface shaded in reach.
10	7		1

Do not assess this element if active channel width is greater than 50 feet. Do not assess this element if woody vegetation is naturally absent (e.g., wet meadows).

Shading of the stream is important because it keeps water cool and limits algal growth. Cool water has a greater oxygen holding capacity than does warm water. When streamside trees are removed, the stream is exposed to the warming effects of the sun causing the water temperature to increase for longer periods during the daylight hours and for more days during the year. This shift in light intensity and temperature causes a decline in the numbers of certain species of fish, insects, and other invertebrates and some aquatic plants. They may be replaced altogether by other species that are more tolerant of increased light intensity, low dissolved oxygen, and warmer water temperature. For example, trout and salmon require cool, oxygen-rich water. Loss of streamside vegetation (and also channel widening) that cause increased water temperature and decreased oxygen levels are major contributing factors to the decrease in abundance of trout and salmon from many streams that historically supported these species. Increased light and the warmer water also promote excessive growth of submerged macrophytes and algae that compromises the biotic community of the stream. The temperature at the reach you are assessing will be affected by the amount of shading 2 to 3 miles upstream.

What to look for: Try to estimate the portion of the water surface area for the whole reach that is shaded by estimating areas with no shade, poor shade, and shade. Time of the year, time of the day, and weather can affect your observation of shading. Therefore, the relative amount of shade is estimated by assuming that the sun is directly overhead and the vegetation is in full leaf-out. First evaluate the shading conditions for the reach; then determine (by talking with the landowner) shading conditions 2 to 3 miles upstream. Alternatively, use aerial photographs taken during full leaf out. The following rough guidelines for percent shade may be used:

stream surface not visible ..... >90

surface slightly visible or visible only in patches .. 70 – 90

surface visible, but banks not visible ..... 40 – 70

surface visible and banks visible at times ..... 20 – 40

surface and banks visible ..... <20

Manure presence (if applicable)

(Intentionally blank)	Evidence of livestock access to riparian zone.	Occasional manure in stream or waste storage structure located on the flood plain.	Extensive amount of manure on banks or in stream. or Untreated human waste discharge pipes present.
	5	3	1

Do not score this element unless livestock operations or human waste discharges are present.

Manure from livestock may enter the water if livestock have access to the stream or from runoff of grazing land adjacent to the stream. In some communities untreated human waste may also empty directly into streams. Manure and human waste increase biochemical oxygen demand, increase the loading of nutrients, and alter the trophic state of the aquatic biological community. Untreated human waste is a health risk.

What to look for: Do not score this element unless livestock operations or human waste discharges are present. Look for evidence of animal droppings in or around streams, on the streambank, or in the adjacent riparian zone. Well-worn livestock paths leading to or near streams also suggest the probability of manure in the stream. Areas with stagnant or slow-moving water may have moderate to dense amounts of vegetation or algal blooms, indicating localized enrichment from manure.

Salinity (if applicable)

(Intentionally blank)	Minimal wilting, bleaching, leaf burn, or stunting of aquatic vegetation; some salt-tolerant streamside vegetation.	Aquatic vegetation may show significant wilting, bleaching, leaf burn, or stunting; dominance of salt-tolerant streamside vegetation.	Severe wilting, bleaching, leaf burn, or stunting; presence of only salt-tolerant aquatic vegetation; most streamside vegetation salt tolerant.
	5	3	1

Do not assess this element unless elevated salinity from anthropogenic sources is known to occur in the stream.

High salinity levels most often occur in arid areas and in areas that have high irrigation requirements. High salinity can also result from oil and gas well operations. Salt accumulation in soil causes a breakdown of soil structure, decreased infiltration of water, and potential toxicity. High salinity in streams affects aquatic vegetation, macroinvertebrates, and fish. Salts are a product of natural weathering processes of soil and geologic material.

What to look for: High salinity levels cause a "burning" or "bleaching" of aquatic vegetation. Wilting, loss of plant color, decreased productivity, and stunted growth are readily visible signs. Other indicators include whitish salt encrustments on the streambanks and the displacement of native vegetation by salt-tolerant aquatic plants and riparian vegetation (such as tamarix or salt cedar).

Riffle embeddedness (if applicable)

Gravel or cobble particles are < 20% embedded.	Gravel or cobble particles are 20 to 30% embedded.	Gravel or cobble particles are 30 to 40% embedded.	Gravel or cobble particles are >40% embedded.	Riffle is completely embedded.
10	8	5	3	1

Do not assess this element unless riffles are present or they are a natural feature that should be present.

Riffles are areas, often downstream of a pool, where the water is breaking over rocks or other debris causing surface agitation. In coastal areas riffles can be created by shoals and submerged objects. (This element is sensitive to regional differences and should be related to reference conditions.) Riffles are critical for maintaining high species diversity and abundance of insects for most streams and for serving as spawning and feeding grounds for some fish species. Embeddedness measures the degree to which gravel and cobble substrate are surrounded by fine sediment. It relates directly to the suitability of the stream substrate as habitat for macroinvertebrates, fish spawning, and egg incubation.

What to look for: This assessment characteristic should be used only in riffle areas and in streams where this is a natural feature. The measure is the depth to which objects are buried by sediment. This assessment is made by picking up particles of gravel or cobble with your fingertips at the fine sediment layer. Pull the particle out of the bed and estimate what percent of the particle was buried. Some streams have been so smothered by fine sediment that the original stream bottom is not visible. Test for complete burial of a streambed by probing with a length of rebar.

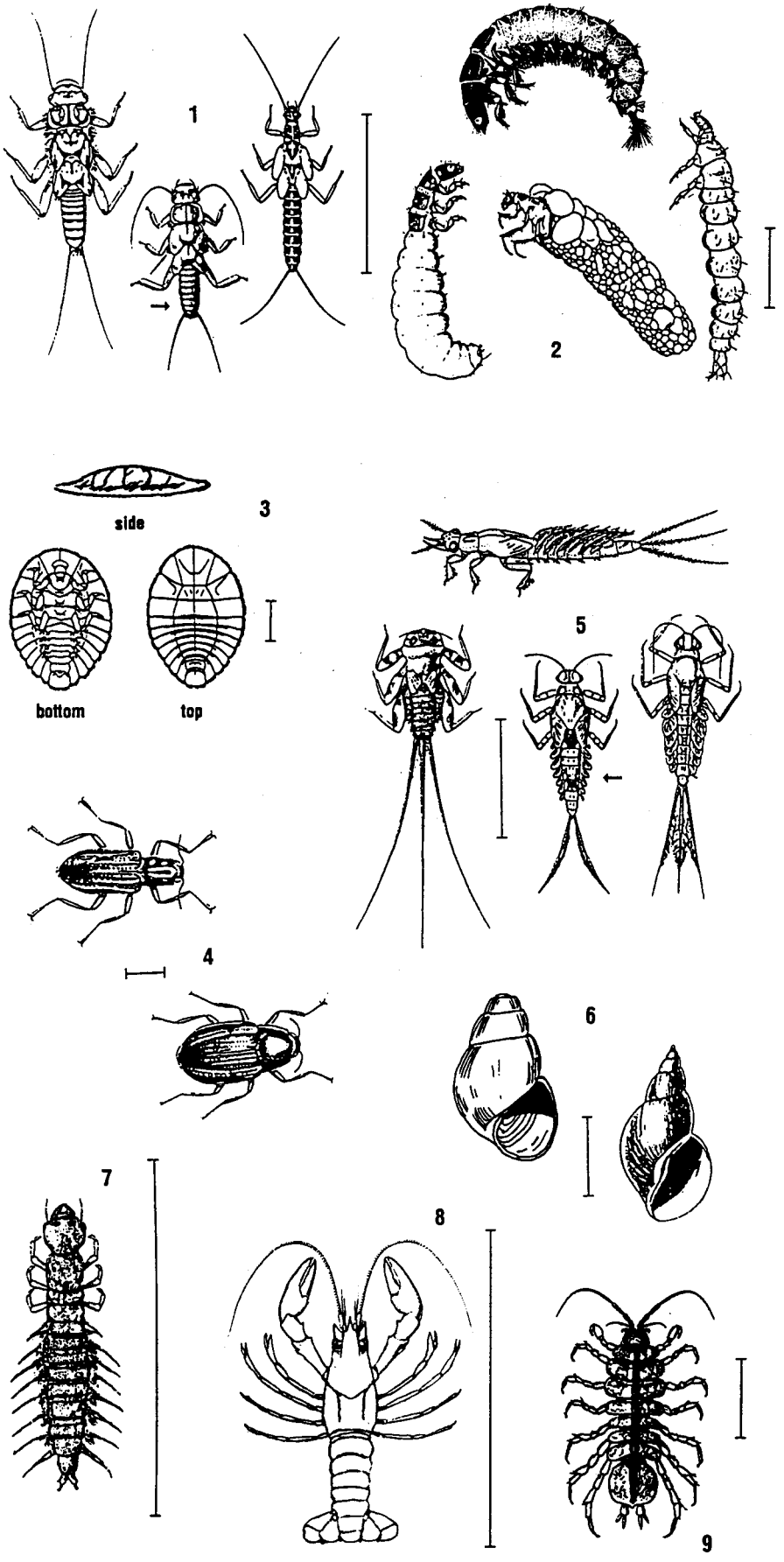
Macroinvertebrates observed

Community dominated by Group I or intolerant species with good species diversity. Examples include caddisflies, mayflies, stoneflies, hellgrammites.	Community dominated by Group II or facultative species, such as damselflies, dragonflies, aquatic sowbugs, blackflies, crayfish.	Community dominated by Group III or tolerant species, such as midges, craneflies, horseflies, leeches, aquatic earthworms, tubificid worms.	Very reduced number of species or near absence of all macroinvertebrates.
15	6	2	– 3

This important characteristic reflects the ability of the stream to support aquatic invertebrate animals. However, successful assessment requires knowledge of the life cycles of some aquatic insects and other macroinvertebrates and the ability to identify them. For this reason, this is an optional element. The presence of intolerant insect species (cannot survive in polluted water) indicates healthy stream conditions. Some kinds of macroinvertebrates, such as stoneflies, mayflies, and caddisflies, are sensitive to pollution and do not live in polluted water; they are considered Group I. Another group of macroinvertebrates, known as Group II or facultative macroinvertebrates, can tolerate limited pollution. This group includes damselflies, aquatic sowbugs, and crayfish. The presence of Group III macroinvertebrates, including midges, craneflies and leeches, suggests the water is significantly polluted. The presence of a single Group I species in a community does not constitute good diversity and should generally not be given a score of 15.

What to look for: You can collect macroinvertebrates by picking up cobbles and other submerged objects in the water. Look carefully for the insects; they are often well camouflaged and may appear as part of the stone or object. Note the kinds of insects, number of species, and relative abundance of each group of insects/macroinvertebrates. Each of the three classes of macroinvertebrates are illustrated on pages 19 and 20. ***Note that the scoring values for this element range from – 3 to 15.***

Stream Invertebrates



Bar line indicate relative size

Group One Taxa

Pollution sensitive organisms found in good quality water.

1 **Stonefly Order Plecoptera.** 1/2" to 1 1/2", 6 legs with hooked tips, antennae, 2 hair-line tails. Smooth (no gills) on lower half of body (see arrow).

2 **Caddisfly: Order Trichoptera.** Up to 1", 6 hooked legs on upper third of body, 2 hooks at back end. May be in a stick, rock, or leaf case with its head sticking out. May have fluffy gill tufts on underside.

3 **Water Penny: Order Coleoptera.** 1/4", flat saucer-shaped body with a raised bump on one side and 6 tiny legs and fluffy gills on the other side. Immature beetle.

4 **Riffle Beetle: Order Coleoptera.** 1/4", oval body covered with tiny hairs, 6 legs, antennae. Walks slowly underwater. Does not swim on surface.

5 **Mayfly: Order Ephemeroptera.** 1/4" to 1", brown, moving, plate-like or feathery gills on the sides of lower body (see arrow), 6 large hooked legs, antennae, 2 or 3 long hair-like tails. Tails may be webbed together.

6 **Gilled Snail: Class Gastropoda.** Shell opening covered by thin plate called operculum. When opening is facing you, shell usually opens on right.

7 **Dobsonfly (Hellgrammite): Family Corydalidae.** 3/4" to 4", dark-colored, 6 legs, large pinching jaws, eight pairs feelers on lower half of body with paired cotton-like gill tufts along underside, short antennae, 2 tails, and 2 pairs of hooks at back end.

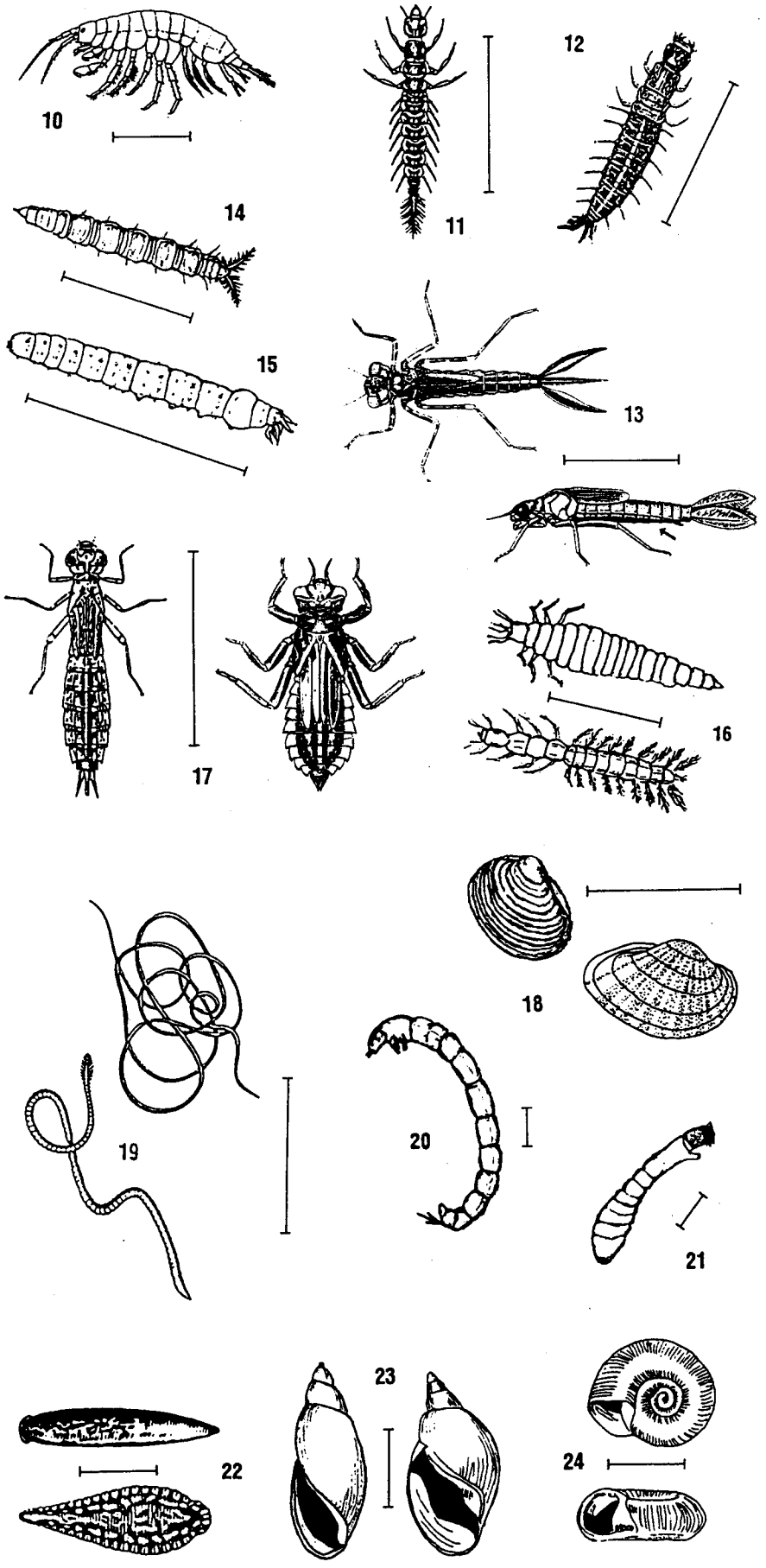
Group Two Taxa

Somewhat pollution tolerant organisms can be in good or fair quality water.

8 **Crayfish: Order Decapoda.** Up to 6", 2 large claws, 8 legs, resembles small lobster.

9 **Sowbug: Order Isopoda.** 1/4" to 3/4", gray oblong body wider than it is high, more than 6 legs, long antennae.

Source: Izaak Walton League of America, 707 Conservation Lane, Gaithersburg, MD 20878-2983. (800) BUG-IWLA



Bar line indicate relative size

Group Two Taxa

Somewhat pollution tolerant organisms can be in good or fair quality water.

10 **Scud: Order Amphipoda.** 1/4", white to gray, body higher than it is wide, swims sideways, more than 6 legs, resembles small shrimp.

11 **Alderfly Larva: Family Sialedae.** 1" long. Looks like small Hellgramite but has long, thin, branched tail at back end (no hooks). No gill tufts underneath.

12 **Fishfly Larva: Family Cordalidae.** Up to 1 1/2" long. Looks like small hellgramite but often a lighter reddish-tan color, or with yellowish streaks. No gill tufts underneath.

13 **Damselfly: Suborder Zygoptera.** 1/2" to 1", large eyes, 6 thin hooked legs, 3 broad oar-shaped tails, positioned like a tripod. Smooth (no gills) on sides of lower half of body. (See arrow.)

14 **Watersnipe Fly Larva: Family Athericidae (Atherix).** 1/4" to 1", pale to green, tapered body, many caterpillar-like legs, conical head, feathery "horns" at back end.

15 **Crane Fly: Suborder Nematocera.** 1/3" to 2", milky, green, or light brown, plump caterpillar-like segmented body, 4 finger-like lobes at back end.

16 **Beetle Larva: Order Coleoptera.** 1/4" to 1", light-colored, 6 legs on upper half of body, feelers, antennae.

17 **Dragon Fly: Suborder Anisoptera.** 1/2" to 2", large eyes, 6 hooked legs. Wide oval to round abdomen.

18 **Clam: Class Bivalvia.**

Group Three Taxa

Pollution tolerant organisms can be in any quality of water.

19 **Aquatic Worm: Class Oligochaeta.** 1/4" to 2", can be very tiny, thin worm-like body.

20 **Midge Fly Larva: Suborder Nematocera.** Up to 1/4", dark head, worm-like segmented body, 2 tiny legs on each side.

21 **Blackfly Larva: Family Simulidae.** Up to 1/4", one end of body wider. Black head, suction pad on other end.

22 **Leech: Order Hirudinea.** 1/4" to 2", brown, slimy body, ends with suction pads.

23 **Pouch Snail and Pond Snails: Class Gastropoda.** No operculum. Breath air. When opening is facing you, shell usually open to left.

24 **Other Snails: Class Gastropoda.** No operculum.Breath air. Snail shell coils in one plane.

Technical information to support implementation

Introduction

This section provides a guide for implementation of the Stream Visual Assessment Protocol (SVAP). The topics covered in this section include the origin of the protocol, development history, context for use in relation to other methods of stream assessment, instructions for modifying the protocol, and references.

Origin of the protocol

In 1996 the NRCS National Water and Climate Center surveyed the NRCS state biologists to determine the extent of activity in stream ecological assessment and the need for technical support. The survey indicated that less than a third of the NRCS states were active in supporting stream assessment within their state. Most respondents said they believed they should be more active and requested additional support from the National Centers and Institutes. In response to these findings, the NRCS Aquatic Assessment Workgroup was formed. In their first meeting the workgroup determined that a simple assessment protocol was needed. The Water Quality Indicators Guide (WQIG) had been available for 8 years, but was not being used extensively. The workgroup felt a simpler and more streamlined method was needed as an initial protocol for field office use.

The workgroup developed a plan for a tiered progression of methods that could be used in the field as conservationists became more skilled in stream assessment. These methods would also serve different assessment objectives. The first tier is a simple 2-page assessment — the Stream Visual Assessment Protocol (SVAP). The second tier is the existing WQIG. The third tier is a series of simple assessment methods that could be conducted by conservationists in the field. An example of a third tier method would be macroinvertibrate sampling and identification to the taxonomic level of Order. The fourth tier is fairly sophisticated methods used in special projects. Examples of fourth tier methods would be fish community sampling and quantitative sampling of macroinvertebrates with shipment of samples to a lab for identification.

The workgroup also found that introductory training and a field handbook that would serve as a comprehensive reference and guidance manual are needed. These projects are under development as of this writing.

Context for use

The Stream Visual Assessment Protocol is intended to be a simple, comprehensive assessment of stream condition that maximizes ease of use. It is suitable as a basic first approximation of stream condition. It can also be used to identify the need for more accurate assessment methods that focus on a particular aspect of the aquatic system.

The relationship of the SVAP to other assessment methods is shown in figure 4. In this figure a specific reference to a guidance document is provided for some methods. The horizontal bars indicate which aspects of stream condition (chemical, physical, or biological) are addressed by the method. The SVAP is the simplest method and covers all three aspects of stream condition. As you move upwards in figure 4 the methods provide more accuracy, but also become more focused on one or two aspects of stream condition and require more expertise or resources to conduct.

The SVAP is intended to be applicable nationwide. It has been designed to utilize factors that are least sensitive to regional differences. However, regional differences are a significant aspect of stream assessment, and the protocol can be enhanced by tailoring the assessment elements to regional conditions. The national SVAP can be viewed as a framework that can evolve over time to better reflect State or within-State regional differences. Instructions for modification are provided later in this document.

Development

The SVAP was developed by combining parts of several existing assessment procedures. Many of these sources are listed in the references section. Three drafts were developed and reviewed by the workgroup and others between the fall of 1996 and the spring of 1997. During the summer of 1997, the workgroup conducted a field trial evaluation of the third draft. Further field trials were conducted with the fourth draft in 1998. A report on the field trial results is appendix A of this document.

The field trials involved approximately 60 individuals and 182 assessment sites. The field trial consisted of a combination of replication studies (in which several individuals independently assessed the same sites) and accuracy studies (in which SVAP scores were compared to the results from other assessment methods). The average coefficient of variation in the replication studies was 10.5 percent. The accuracy results indicated that SVAP version 3 scores correlated well with

other methods for moderately impacted and high quality sites, but that low quality sites were not scoring correspondingly low in the SVAP. Conservationists in the field who participated in the trial were surveyed on the usability and value of the protocol. The participants indicated that they found it easy to use and thought it would be valuable for their clients.

Revisions were made to the draft to address the deficiencies identified in the field trial, and some reassessments were made during the winter of 1998 to see how the revisions affected performance. Performance was improved. Additional revisions were made, and the fifth draft was sent to all NRCS state offices, selected Federal agencies, and other partners for review and comment during the spring of 1998.

Comments were received from eight NRCS state offices, the Bureau of Land Management, and several NRCS national specialists. Comments were uniformly supportive of the need for the guidance and for the document as drafted. Many commenters provided improved explanatory text for the supporting descriptions accompanying the assessment elements. Most of the suggested revisions were incorporated.

Implementation

The SVAP is issued as a national product. States are encouraged to incorporate it within the Field Office Technical Guide. The document may be modified by States. The electronic file for the document may be downloaded from the National Water and Climate Center web site at http://www.wcc.nrcs.usda.gov.

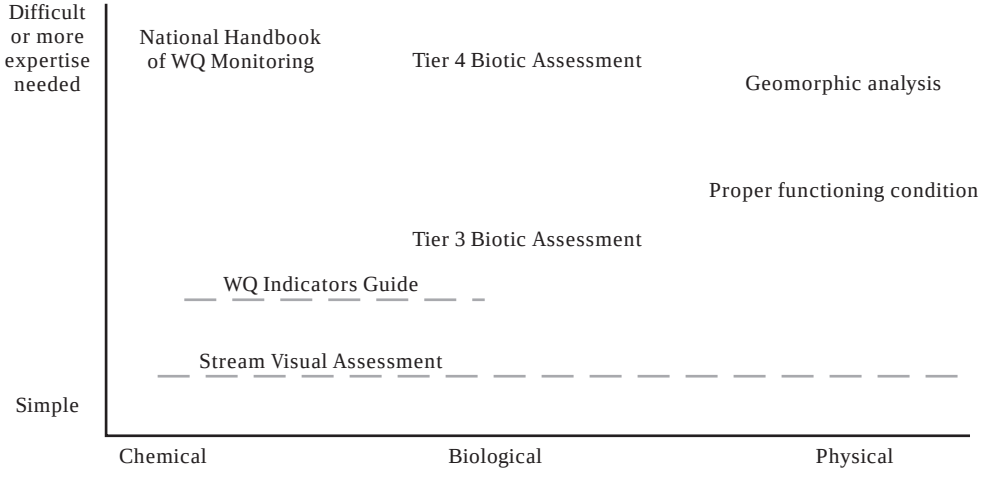
A training course for conservationists in the field suitable for use at the state or area level has been developed to facilitate implementation of the SVAP. It is designed as either a 1-day or 2-day session. The first day covers basic stream ecology and use of the SVAP. The second day includes an overview of several stream assessment methods, instruction on a macroinvertebrate survey method, and field exercises to apply the SVAP and macroinvertibrate protocols. The training materials consist of an instructor's guide, slides, video, a macroinvertebrate assessment training kit, and a student workbook. Training materials have been provided to each NRCS state office.

Instructions for modification

The national version of the Stream Visual Assessment Protocol may be used without modification. It has been designed to use assessment elements that are least sensitive to regional differences. Nonetheless, it can be modified to better reflect conditions within a geographic area. Modifying the protocol would have the following benefits:

- The protocol can be made easier to use with narrative descriptions that are closer to the conditions users will encounter.
- The protocol can be made more responsive to differences in stream condition.
- Precision can be improved by modifying elements that users have trouble evaluating.
- The rating scale can be calibrated to regionally-based criteria for excellent, good, fair, and poor condition.

Figure 4 Relationship of various stream condition assessment methods in terms of complexity or expertise required and the aspects of stream condition addressed



Two parts of the SVAP may be modified—the individual elements and their narrative descriptions, and the rating scale for assigning an overall condition rating of excellent, good, fair, or poor.

The simplest approach to modifying the SVAP is based on professional experience and judgment. Under this approach an interdisciplinary team should be assembled to develop proposed revisions. Revisions should then be evaluated by conducting comparison assessments at sites representing a range of conditions and evaluating accuracy (correlation between different assessment methods), precision (reproducibility among different users), and ease of use.

A second, more scientifically rigorous method for modifying the protocol is described below. This approach is based on a classification system for stream type and the use of reference sites.

Step 1 Decide on tentative number of versions.
Do you want to develop a revised version for your state, for each ecoregion within your state, or for several stream classes within each ecoregion?

Step 2 Develop tentative stream classification.
If you are developing protocols by stream class, you need to develop a tentative classification system. (If you are interested in a statewide or ecoregion protocol, go to step 3.) You might develop a classification system based on stream order, elevation, or landscape character. Do not create too many categories. The greater the number of categories, the more assessment work will be needed to modify the protocol and the more you will be accommodating degradation within the evaluation system. As an extreme example of the latter problem, you would not want to create a stream class consisting of those streams that have bank-to-bank cropping and at least one sewage outfall.

Step 3 Assess sites.
Assess a series of sites representing a range of conditions from highly impacted sites to least impacted sites. Try to have at least 10 sites in each of your tentative classes. Those sites should include several potential “least impacted reference sites.” Try to use sites that have been assessed by other assessment methods (such as sites assessed by state agencies or universities). As part of the assessments, be sure to record information on potential classification factors and if any particular elements are difficult to score. Take notes so that future revisions of the elements can be rescored without another site visit.

Step 4 Rank the sites.
Begin your data analysis by ranking all the sites from most impacted to least impacted. Rank sites according to the independent assessment results (preferred) or by the SVAP scores. Initially, rank all of the sites in the state data set. You will test classifications in subsequent iterations.

Step 5 Display scoring data.
Prepare a chart of the data from all sites in your state. The columns are the sites arranged by the ranking. The rows are the assessment elements, the overall numerical score, and the narrative rating. If you have independent assessment data, create a second chart by plotting the overall SVAP scores against the independent scores.

Step 6 Evaluate responsiveness.
Does the SVAP score change in response to the condition gradient represented by the different sites? Are the individual element scores responding to key resource problems? Were users comfortable with all elements? If the answers are yes, do not change the elements and proceed to step 7. If the answers are no, isolate which elements are not responsive. Revise the narrative descriptions for those elements to better respond to the observable conditions. Conduct a "desktop" reassessment of the sites with the new descriptions, and return to step 4.

Step 7 Evaluate the narrative rating breakpoints.
Do the breakpoints for the narrative rating correspond to other assessment results? The excellent range should encompass only reference sites. If not, you should reset the narrative rating breakpoints. Set the excellent breakpoint based on the least impacted reference sites. You must use judgment to set the other breakpoints.

Step 8 Evaluate tentative classification system.
Go back to step 4 and display your data this time by the tentative classes (ecoregions or stream classes). In other words, analyze sites from each ecoregion or each stream class separately. Repeat steps 5 through 7. If the responsiveness is significantly different from the responsiveness of the statewide data set or the breakpoints appear to be significantly different, adopt the classification system and revise the protocol for each ecoregion or stream class. If not, a single statewide protocol is adequate.

After the initial modification of the SVAP, the state may want to set up a process to consider future revisions. Field offices should be encouraged to locate and assess least impacted reference sites to build the data base for interpretation and future revisions. Ancillary data should be collected to help evaluate whether a potential reference site should be considered a reference site.

Caution should be exercised when considering future revisions. Revisions complicate comparing SVAP scores determined before and after the implementation of conservation practices if the protocol is substantially revised in the intervening period. Developing information to support refining the SVAP can be carried out by graduate students working cooperatively with NRCS. The Aquatic Assessment Workgroup has been conducting a pilot Graduate Student Fellowship program to evaluate whether students would be willing to work cooperatively for a small stipend. Early results indicate that students can provide valuable assistance. However, student response to advertisements has varied among states. If the pilot is successful, the program will be expanded.

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Glossary

Active channel width The width of the stream at the bankfull discharge. Permanent vegetation generally does not become established in the active channel.

Aggradation Geologic process by which a stream bottom or flood plain is raised in elevation by the deposition of material.

Bankfull discharge The stream discharge (flow rate, such as cubic feet per second) that forms and controls the shape and size of the active channel and creates the flood plain. This discharge generally occurs once every 1.5 years on average.

Bankfull stage The stage at which water starts to flow over the flood plain; the elevation of the water surface at bankfull discharge.

Baseflow The portion of streamflow that is derived from natural storage; average stream discharge during low flow conditions.

Benthos Bottom-dwelling or substrate-oriented organisms.

Boulders Large rocks measuring more than 10 inches across.

Channel A natural or artificial waterway of perceptible extent that periodically or continuously contains moving water. It has a definite bed and banks that serve to confine the water.

Channel roughness Physical elements of a stream channel upon which flow energy is expended including coarseness and texture of bed material, the curvature of the channel, and variation in the longitudinal profile.

Channelization Straightening of a stream channel to make water move faster.

Cobbles Medium-sized rocks which measure 2.5 to 10 inches across.

Confined channel A channel that does not have access to a flood plain.

Degradation Geologic process by which a stream bottom is lowered in elevation due to the net loss of substrate material. Often called downcutting.

Downcutting See Degradation.

Ecoregion A geographic area defined by similarity of climate, landform, soil, potential natural vegetation, hydrology, or other ecologically relevant variables.

Embeddedness The degree to which an object is buried in steam sediment.

Emergent plants Aquatic plants that extend out of the water.

Flood plain The flat area of land adjacent to a stream that is formed by current flood processes.

Forb Any broad-leaved herbaceous plant other than those in the Gramineae (Poceae), Cyperacea, and Juncaceae families (Society for Range Management, 1989).

Gabions A wire basket filled with rocks; used to stabilize streambanks and to control erosion.

Geomorphology The study of the evolution and configuration of landforms.

Glide A fast water habitat type that has low to moderate velocities, no surface agitation, no defined thalweg, and a U-shaped, smooth, wide bottom.

Gradient Slope calculated as the amount of vertical rise over horizontal run expressed as ft/ft or as percent (ft/ft * 100).

Grass An annual to perennial herb, generally with round erect stems and swollen nodes; leaves are alternate and two-ranked; flowers are in spikelets each subtended by two bracts.

Gravel Small rocks measuring 0.25 to 2.5 inches across.

Habitat The area or environment in which an organism lives.

Herbaceous Plants with nonwoody stems.

Hydrology The study of the properties, distribution, and effects of water on the Earth's surface, soil, and atmosphere.

Incised channel A channel with a streambed lower in elevation than its historic elevation in relation to the flood plain.

Intermittent stream A stream in contact with the ground water table that flows only certain times of the year, such as when the ground water table is high or when it receives water from surface sources.

Macrophyte bed A section of stream covered by a dense mat of aquatic plants.

Meander A winding section of stream with many bends that is at least 1.2 times longer, following the channel, than its straight-line distance. A single meander generally comprises two complete opposing bends, starting from the relatively straight section of the channel just before the first bend to the relatively straight section just after the second bend.

Macroinvertebrate A spineless animal visible to the naked eye or larger than 0.5 millimeters.

Nickpoint The point where a stream is actively eroding (downcutting) to a new base elevation. Nickpoints migrate upstream (through a process called headcutting).

Perennial stream A steam that flows continuously throughout the year.

Point bar A gravel or sand deposit on the inside of a meander; an actively mobile river feature.

Pool Deeper area of a stream with slow-moving water.

Reach A section of stream (defined in a variety of ways, such as the section between tributaries or a section with consistent characteristics).

Riffle A shallow section in a stream where water is breaking over rocks, wood, or other partly submerged debris and producing surface agitation.

Riparian The zone adjacent to a stream or any other waterbody (from the Latin word ripa, pertaining to the bank of a river, pond, or lake).

Riprap Rock material of varying size used to stabilize streambanks and other slopes.

Run A fast-moving section of a stream with a defined thalweg and little surface agitation.

Scouring The erosive removal of material from the stream bottom and banks.

Sedge A grasslike, fibrous-rooted herb with a triangular to round stem and leaves that are mostly three-ranked and with close sheaths; flowers are in spikes or spikelets, axillary to single bracts.

Substrate The mineral or organic material that forms the bed of the stream; the surface on which aquatic organisms live.

Surface fines That portion of streambed surface consisting of sand/silt (less than 6 mm).

Thalweg The line followed by the majority of the streamflow. The line connecting the lowest or deepest points along the streambed.

Turbidity Murkiness or cloudiness of water caused by particles, such as fine sediment (silts, clays) and algae.

Watershed A ridge of high land dividing two areas that are drained by different river systems. The land area draining to a waterbody or point in a river system; catchment area, drainage basin, drainage area.

Appendix A—1997 and 1998 Field Trial Results

Purpose and methods

The purpose of the field trials was to evaluate the accuracy, precision, and usability of the draft Steam Visual Assessment Protocol. The draft protocols evaluated were the third draft dated May 1997 and the fourth draft dated October 1997. A field trial workplan was developed with study guidelines and a survey form to solicit feedback from users. Accuracy was evaluated by comparison to other stream assessment methods. Precision was evaluated by replicate assessments conduced by different individuals at the same sites. In all studies an attempt was made to utilize sites ranging from high quality to degraded. Results consisted of the scoring data and the user feedback form for each site.

Results

Overall, 182 sites were assessed, and approximately 60 individuals participated in the field trials. The individual studies are summarized in table A–1.

Precision could be evaluated using data from the Colorado, New Jersey, Oregon, Virginia, and Georgia studies. Results are summarized in table A–2. The New Jersey sites had coefficients of variation of 9.0 (n=8), 14.4 (n=5), and 5.7 (n=4) percent. The Oregon site with three replicates was part of a course and had a coefficient of variation of 11.1 percent. One Georgia site was assessed using the fourth draft during a pilot of the training course. There were 11 replicates, and the coefficient of variation was 8.8 percent. In May 1998 the workgroup conducted replicate assessments of two sites in Virginia using the fifth draft of the protocol. Coefficients of variation were 14.7 and 3.6 percent. The average coefficient of variation of all studies in table A–2 is 10.5 percent.

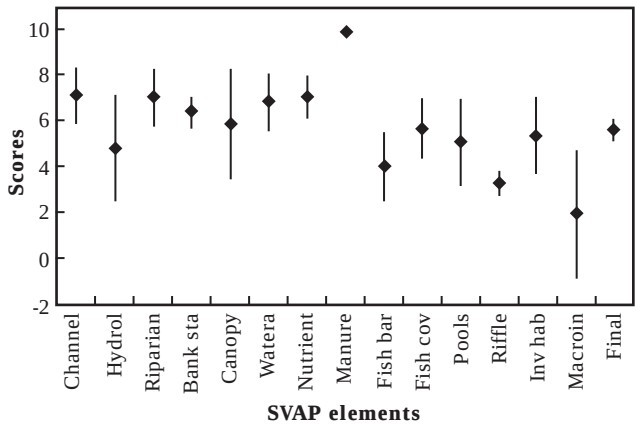
Variability within the individual elements of the SVAP was evaluated using the Georgia site with 11 replicates. The results of the individual element scores are presented in figure A–1. It should be noted that two individuals erroneously rated the "presence of manure" element.

Accuracy was evaluated by comparing the SVAP rating to other methods as noted in table A–1. Some of the comparisons involved professional judgment. In others the SVAP score could be compared with a quantitative evaluation. Figures A–2 through A–5 present data from the two studies that had larger numbers of sites. The Pearson's Correlation Coefficient is presented for these data. The results from other sites are presented in table A–3.

Table A–1 Summary of studies in the field trial

Location	Number of sites	Number of replicates	SVAP compared to	SVAP conducted by
VA	56	3, 5	IBI (fish) and Ohio QHEI	FO personnel
NC/SC	90	none	IBI, EPT	Soil scientists
MI	5	none	professional judgment	State biologist
NJ	3	4, 5, 8	NJDEP ratings	FO personnel
OR	3	none	IBI	NWCC scientist
CO	1	3	professional judgment	FO personnel
WA	3	none	professional judgment	State biologist
OR	2	3	no comparisons	FO personnel
GA	8	4-5	macroinvertebrates	FO personnel
GA	2	12, none	IBI, macroinvertebrate	FO personnel

Figure A–1 Means and standard deviations from the Parker's Mill Creek site in Americus, GA (n=11) (mean plus and minus one standard deviation is shown; SVAP version 4 used)



The SVAP version 3 scores correlated extremely well with the Ohio Qualitative Habitat Index and reasonably well with the fish community IBI in the Virginia study (fig. A–2 and A–3). However, the SVAP version 3 scores in the Carolinas study did not correlate well with either IBI or EPT Taxa (fig. A–4 and A–5). These results may reflect the fact that the SVAP primarily assesses physical habitat within the assessment reach whereas IBI and EPT Taxa are influenced by both physical habitat within the assessment reach and conditions within the watershed. Onsite physical habitat may have been a relatively more important factor at the Virginia sites than at the Carolina sites.

Overall, the field trial results for the third draft seemed to indicate that SVAP scores reflected conditions for sites in good to moderate condition. However, SVAP scores tended to be too high for poor quality sites.

Both the user questionnaires and verbal feedback indicated that users found the SVAP easy to use. Users reported that they thought it would be an effective tool to use with landowners. The majority indicated that they would recommend it to landowners.

Table A–2 Summary of replication results (version refers to the SVAP draft used; mean for overall score reported)

Site	SVAP version	No. replicates	Mean [1/]	Standard deviation	Coefficient of variation
Alloway Cr. NJ	3	5	3.6 F	0.52	14.4
Manasquan R. NJ	3	4	5.1 G	0.29	5.7
S. Br. Raritan R. NJ	3	8	5.9 G	0.53	9.0
Gales Cr. OR	3	3	5.5 G	0.61	11.1
Clear Cr. CO	3	3	5.4 G	0.74	13.7
Piscola Cr. GA #1	4	5	9.2 E	0.77	8.4
Piscola Cr. GA #2	4	5	9.0 E	0.85	9.4
Piscola Cr. GA #3	4	4	4.7 F	1.10	23.4
Piscola Cr. GA #4	4	4	7.4 G	0.96	13.0
Little R. GA # 1	4	4	8.3 E	0.73	8.8
Little R. GA # 2	4	4	7.4 E	0.83	11.2
Little R. GA # 3	4	4	8.1 E	0.41	5.1
Little R. GA # 4	4	4	7.3 G	0.60	8.2
Parker's Mill Cr. GA	4	11	5.7 F	0.50	8.8
Cedar Run (up), VA	5	5	7.7 G	1.1	14.7
Cedar R. (down), VA	5	5	6.6 F	.2	3.6

1/ Includes SVAP narrative ratings (P = poor, F = fair, G = good, E = excellent)

Table A–3 Accuracy comparison data from studies with too few sites to determine a correlation coefficient

Site	SVAP version	SVAP score and rating	Comparative rating	Comparative method
Alloway Cr. NJ	3	3.6* — fair	12 — mod. impaired	NJIS (macro.)
Manasquan R. NJ	3	5.1* — good	12 — mod. impaired	NJIS (macro.)
S. Br. Raritan R. NJ	3	5.9* — good	30 — not impaired	NJIS (macro.)
Site 1 OR	3	2.7 — fair	12 — very poor	IBI (fish)
Site 2 OR	3	4.6 — good	22 — poor	IBI (fish)
Site 3 OR	3	7.0 — excellent	44 — good	IBI (fish)
Muckalee Cr. GA	4	8.6 — good	good to excellent	mussel taxa

* Mean value of replicates

Figure A–2 Correlation between SVAP and IBI values in the Virginia study (n=56)

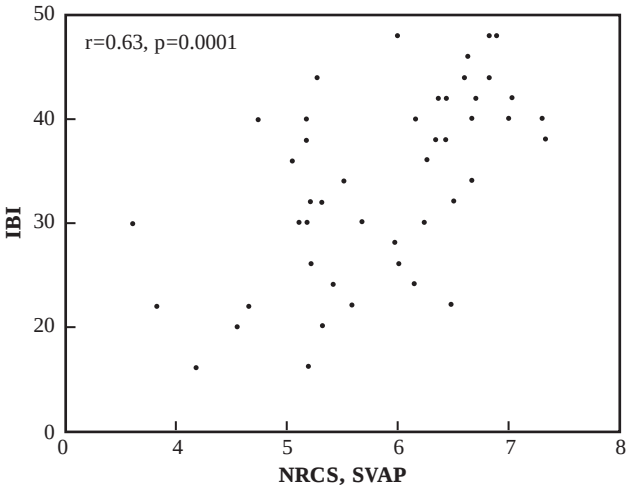


Figure A–3 Correlation between SVAP and Ohio Qualitative Habitat Evaluation Index values in the Virginia study (n=56)

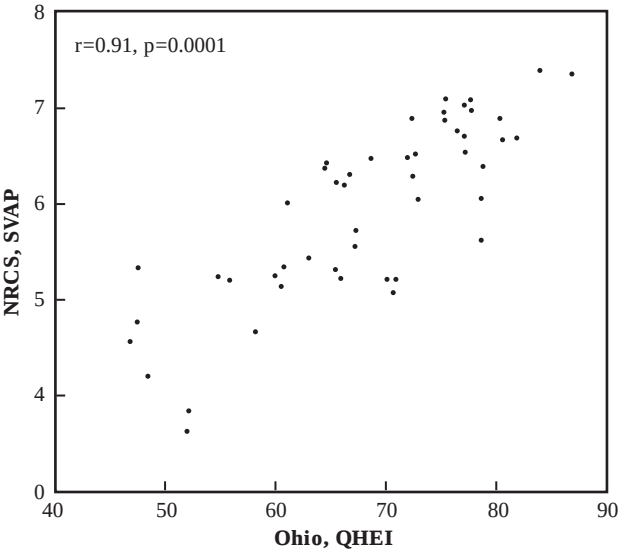


Figure A–4 Correlation between SVAP and IBI values in the Carolinas study (n=90)

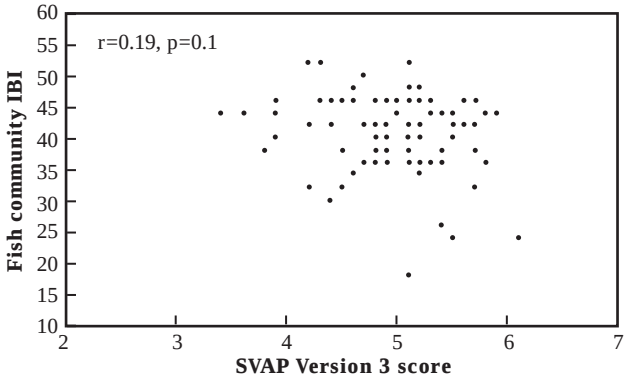
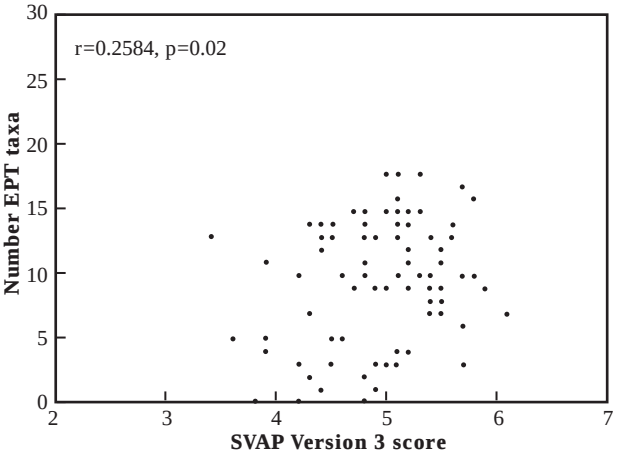


Figure A–5 Correlation between SVAP and macroinvertebrate index values in Carolinas study (n=90)



Discussion

Overall, the workgroup concluded from the first field trial that the SVAP could be used by conservationists in the field with reasonable reproducibility and a level of accuracy commensurate with its objective of providing a basic assessment of ecological condition provided the poor response to degraded streams could be corrected.

Several potential causes for the lack of accuracy with degraded sites were identified by the workgroup as follows:

- Because the overall score is an average of all assessed elements, the effect of low scoring elements can be damped out by averaging if the degradation is not picked up by many of the other assessed elements.
- Some of the elements needed to be adjusted to give lower scores for problems.
- The numerical breakpoints for the narrative ratings of poor/fair and fair/good were set too low.

To correct these problems the number of assessment elements was reduced and the instructions were modified so that certain elements are not scored if they do not apply. For example, the "presence of manure" element is not scored unless there are animal operations present. These changes reduced the potential for low scores to be damped out by the averaging process.

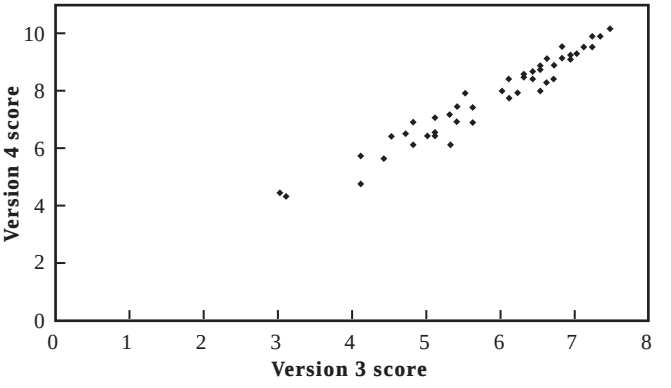
Several elements were also rewritten to reduce ambiguity at the low end of the rating scale. Additionally, several elements were rewritten to have five narrative descriptions instead of four to address a concern that users might err on the high side. The scoring scale was changed from a scale of 1 to 7 to a scale of 1 to 10 because it was felt that most people have a tendency to think in terms of a decimal scale.

The revisions were incorporated into a fourth draft and evaluated by the workgroup. Sites from the first field trial were rescored using the new draft. Response seemed to have improved as indicated by the greater separation of sites at lower scores in figure A–6.

During pilot testing of the training materials in March 1998, the fourth draft was used by 12 students independently at one site and collectively at another site. The coefficient of variation at the replication site was 8.8 percent. One of the sites had been previously assessed using other methods, and the SVAP rating corresponded well to the previous assessments.

After the evaluation of the fourth draft, minor revisions were made for the fifth draft. The breakpoints for the narrative rating of excellent, good, fair, and poor for the fifth draft were set using the Virginia data set. These breakpoints may be adjusted by the NRCS state office as explained in this document.

Figure A–6 Version 4 scores for VA plotted against version 3 scores (n=56)



Stream Visual Assessment Protocol

Owners name _____ Evaluator's name_____ Date _____

Stream name _____ Waterbody ID number _____

Reach location _____

_____

Ecoregion _____ Drainage area _____ Gradient_____

Applicable reference site _____

Land use within drainage (%): row crop _____ hayland _____ grazing/pasture _____ forest _____ residential _____

confined animal feeding operations _____ Cons. Reserve _____ industrial _____ Other: _____

Weather conditions-today _____ Past 2-5 days _____

Active channel width _____ Dominant substrate: boulder _____ gravel _____ sand _____ silt _____ mud _____

Site Diagram

Assessment Scores

Channel condition	
Hydrologic alteration	
Riparian zone	
Bank stability	
Water appearance	
Nutrient enrichment	
Barriers to fish movement	
Instream fish cover	
Pools	
Invertebrate habitat	

Score only if applicable

Canopy cover	
Manure presence	
Salinity	
Riffle embeddedness	
Marcroinvertebrates Observed (optional)	

Overall score (Total divided by number scored)	_____	<6.0	**Poor**
		6.1-7.4	**Fair**
		7.5-8.9	**Good**
		>9.0	**Excellent**

Suspected causes of observed problems_____

_____

_____

_____

Recommendations_____

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Phosphorus regulates stream injury by filamentous green algae, thresholds, DO, and pH

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Abstract

Nutrient concentrations, benthic algal biomass, dissolved oxygen, and pH were measured in 70 or more streams during spring and summer in the Illinois River Watershed (IRW) to determine injury to streams that was related to spreading poultry waste on fields. Nutrient concentrations were independently related to poultry house density in watersheds and percent urban land use in watersheds and were unusually high compared to regions with similar geology and hydrology. Molar N:P ratios were high and indicated that phosphorus was the most likely limiting nutrient. Phosphorus concentrations, as well as poultry house density and urban land use, were related to algal biomass during spring, but were less related during summer. A threshold response in cover of stream bottoms by filamentous green algae (FGA: *Cladophora*, *Rhizoclonium*, and *Oedogonium*) during spring was observed at 27 µg TP/L, with increases from averages of 4 to 36 percent cover in streams with TP less than and greater than the TP threshold. Average

concentrations of dissolved oxygen concentration (DO), variability in DO, and pH during spring were positively related to TP, chlorophyll a, and FGA cover. Minimum DO during spring and early morning DO during summer were negatively related to TP concentration. Spring pH and summer DO frequently violated water quality standards for protecting biodiversity. We conclude that poultry house operations as well as urban activities, independently and interactively, pollute IRW streams with phosphorus that results in an injury to aesthetic condition and potential for injury of biodiversity.

Keywords: phosphorus, poultry waste, benthic algae, *Cladophora*, streams

Introduction

Nutrient pollution has long been recognized as a widespread and important contaminant of streams and rivers (MacKenthun, 1968; Carpenter et al., 1998; Smith et al., 1999; USEPA, 2007). Low concentrations of nutrients occur naturally in water and are essential for the growth of algae, which supports the production of invertebrates and fish. Nutrient pollution, the loading of nutrients from human activities in streams and rivers, causes many instream as well as downstream problems. Recreation and biodiversity are two valued ecological attributes, more recently called ecosystem services (Millennium Assessment, 2005), that are affected by nutrient pollution and excessive growths of algae.

Excess algae resulting from nutrient pollution can dramatically reduce the desirability of recreational use of streams when algae on the stream bottom exceed 150 mg/m^2 of chlorophyll a (Suplee et al., 2008). Multiple lines of evidence show that the natural balance of stream flora and

fauna (*sensu* Karr and Dudley, 1981), herein referred to as biodiversity, are diminished by nutrient pollution. Correlations between human alterations of watersheds and nutrient concentrations measured in stream surveys, experiments, and process-based models show that nutrients from urban and agricultural activities move from land to waterways via surface and groundwater (Allan et al., 1997; Johnson et al., 1997; Sharpley et al. 2007; Tesoriero et al., 2009). Stream surveys, experiments, and models also confirm that nutrients stimulate growth (Bothwell, 1985; Dodds et al., 1997; Biggs, 2000; Rier and Stevenson, 2006) with both N and P being potential limiting factors in streams (Francoeur, 2001). The biodiversity of algae, invertebrates, and fish have also been related to nutrient concentrations (Miltner and Rankin, 1997; Wang et al., 2007; Stevenson et al., 2008).

Additional research is needed to sufficiently refine and quantify relationships among human activities, nutrients, and ecological response for application in environmental management. Quantitative relationships among these variables are needed to evaluate tradeoffs between costs and benefits of reducing nutrient pollution. Thresholds in ecological responses along environmental gradients help develop concensus among stakeholders for management actions (Muradian 2001). Thresholds in algal biomass and biodiversity along nutrient gradients have been hypothesized and observed (Dodds et al., 1997; Stevenson, 1997; Stevenson et al., 2008). The biodiversity and response of streams to nutrient pollution varies with climate, geology, stream size, and hydrogeomorphology (Biggs et al., 1995; Riseng et al., 2004; Dodds and Oakes, 2004). Thus, regional studies are needed for regional applications to better understand variability in these relationships caused by interactions with natural factors (e.g., climate and geology) and anthropogenic factors (e.g., nutrient sources and hydrologic modifications) that vary among regions and stream types (e.g., size, geomorphology).

This study was initiated because the Attorney General of the State of Oklahoma filed a lawsuit against numerous poultry companies. Results of this study were designed to quantitatively relate nutrient pollution to decreases in ecosystem services (i.e., injury) and evaluate whether poultry waste application to fields was a significant contributor to that injury. These evaluations would be used to determine whether the application of poultry wastes to fields should be stopped and to assign monetary damages for the ecological injury caused by poultry wastes. Additional studies were conducted to document sources and fates of nutrients in the IRW, model the proportion of nutrient pollution from different sources (e.g., municipal waste water treatment plants, septic systems, cattle, crop fertilization), determine downstream effects on a reservoir, and quantify the value of ecosystem services and injury to them.

The specific objectives of the research presented in this paper were to measure physical, chemical, and algal conditions in approximately 70 streams during spring and summer seasons, as well as land use in these watersheds. Then we evaluated injury of IRW streams resulting from phosphorus pollution, poultry manure application, and other phosphorus sources. We tested a set of hypotheses to evaluate the direct and indirect relationships among phosphorus sources (as indicated by land use), nutrient concentrations, algal biomass, and chemical stressors (DO and pH) of stream biodiversity. In particular, we were interested in evaluating relationships for thresholds which could be used to establish benchmarks for injury. Based on these relationships, the likely effects of poultry manure spreading on aesthetics and biodiversity were determined.

Methods

Study area

The Illinois River Watershed is a 4328 km^2 watershed in the Ozark Highlands ecoregion flowing from Arkansas to Oklahoma (Figure 2). The watershed is underlain by karst geology with gneiss and limestone bedrock covered by thin soils. Therefore, rainfall travels to streams via groundwater and surficial runoff resulting in a channel with riffle and pool sequences in which riffles are dominated by cobble or simply exposed bedrocks. Stream discharge is usually highest during winter and early spring and may drop to expose riffles from late spring through fall. Rains may occur anytime during the year and generate sufficient flow to fill the channel. Based on this flow regime, we would expect that spates and drought limit secondary production and allow accumulations of high biomasses of diatoms as well as filamentous green algae given sufficient nutrients and time for algal colonization (Riseng et al., 2004; Stevenson et al., 2006*)*.

Urban, agricultural, and forested land comprise 8.8, 43.7, and 46.0% respectively, of the land cover in the Illinois River Watershed. Most (99.7%) of the agricultural land is pasture, with a very small proportion planted with row crops. There are 3226 poultry houses in the Watershed in which poultry are or have been raised and manure is produced within the Illinois River Watershed. Sixty percent of these poultry houses are active, 26% are inactive, and others are abandoned (11%), removed (3%), or suspected to be poultry houses (9%)(Fisher, 2008). Three small urban centers are located at the head of two of the five major tributaries. Municipal wastewater treatment plants are co-located with these urban areas. A phosphorus mass balance (Engel 2008) indicates that 76% of the phosphorus additions to the IRW result from import of poultry feed for poultry operations with other sources greater than 1% being commercial fertilizers (7.5%), dairy cattle (5.2%), humans (3.2%), swine (2.9%), industrial sources (mostly poultry processing facilities, 2.7%), and beef cattle (1.7%).

Sampling, sample analysis, and map analysis

To evaluate the injury of the IRW resulting from poultry waste application to fields, we first had to quantify relationships among indicators of all major types of human activities, nutrient pollution, algal biomass, and chemical stressors of biodiversity (low DO and high pH) in the IRW (Figure 1). We used percentage of different urban and agricultural land uses in watersheds and poultry house density to characterize human activities and test hypotheses about relationships between sources of phosphorus and phosphorus concentration. We then tested the hypotheses that nutrient pollution, particularly phosphorus concentrations are related to poultry house operations, algal biomass, dissolved oxygen concentrations and pH. If relationships are statistically significant, we determine the magnitude of effects (injury) that are statistically related to poultry house operations and phosphorus pollution. Throughout this process, assumptions were tested statistically and alternative hypotheses were explored thoroughly.

Approximately 70 stream sites were selected for sampling to develop relationships illustrated in Figure 1 using a random stratified sampling design. Seventy sites were selected because in a previous study (Stevenson et al., 2006) using similar sampling methods, 70 sites were determined to be adequate for characterizing relationships among land use, nutrients, and algal biomass. Because our goal was to evaluate poultry house operations effects on nutrient pollution, and poultry operations were the dominant source of phosphorus in the watershed (Engel, 2008), we randomly selected sites from five groups of potential sampling sites (the strata). Sites assigned to these five groups based on poultry house density and geographic location in the IRW. The range in poultry houses was split evenly in five sub-ranges or quintiles.

All potential sampling sites were assigned to these groups. Then even numbers of sampling sites were selected from each group (Figure 2). Our goal in selecting sites by groups was to get a full range of nutrient conditions associated with poultry house operations and to have relatively even spacing geographically across the watershed. We expected to get a range of phosphorus and nitrogen concentrations that would bridge benchmarks for limiting concentrations of total phosphorus (TP), soluble reactive phosphate (SRP), total nitrogen (TN), and nitrate-nitrite (NO_x). Based on past experiments and surveys, we expected these limiting nutrient concentrations to be approximately 0.020-0.030 mg P/L for TP and SRP, 0.200 mg NO_x/L, and 0.500 mg TN/L (Bothwell, 1988; Dodds et al., 1997; Rier and Stevenson, 2006).

Two field sampling campaigns were conducted during summer 2006 and spring 2007. Spring and summer were selected as sampling periods because the potential for: 1) high algal biomass related to filamentous algal growth is greatest during the spring and 2) low dissolved oxygen is usually greatest during the warm-water, low-flow periods during summer. Land use, habitat characteristics, water chemistry, algal biomass, dissolved oxygen, and pH were determined for all sites. During the summer 2006 sampling campaigns, each site was visited and sampled one time. During the spring 2007 campaign, each site was sampled weekly for eight weeks. We sampled streams weekly during the spring 2007 campaign from mid-March through mid-May to characterize average conditions and the variation in conditions during a period when we expected spring rains to cause high temporal variability in water chemistry and algal biomass. In addition, we sampled streams in a haphazard order from week to week to eliminate bias that could be caused by diurnal variation in temperature, light, and nutrient concentrations. This repeated sampling of sites at times throughout the day provided the opportunity to examine

variability at a site that could be related to a systematic diurnal cycle associated with algal photosynthesis and respiration.

The sampling reach in each stream was defined as two or more riffles. Percent canopy cover was determined with a canopy densitometer. The pH, conductivity, and temperature of streams were measured in the field with an Oakton model 300® multimeter during each site visit. DO was measured colorimetrically in the field with a Chemetrics V-2000® photometer, vacu-vials, and reagents during each site visit. Water samples for analysis of TP and SRP concentrations were collected during each of the eight visits during the spring 2007 campaign. Water samples for TN, NO_x, and ammonia (NH_3-N) were collected once from each site during each season. Water samples were stored on ice and shipped via overnight courier to a laboratory for nutrient chemistry analysis. In the laboratory, standard methods (APHA 1998) were used to assay nutrient concentrations.

Benthic algal samples were collected one time during each field campaign to determine ash-free dry mass and chlorophyll a (chl a) concentrations of all algae in the streams during the summer 2006 and spring 2007. Algae were sampled by scrapping algae from known areas of rocks in five 3-rock clusters that were randomly selected from the stream bottom. Sampling occurred during base-flow periods after at least of week without high discharge events. Scrapings from the five 3-rock clusters were kept separate for chl a analysis to provide an estimate of the mean and variance in periphyton at sites. Samples from the 3-rock clusters were filtered and frozen the day of sampling and shipped to a commercial laboratory for chl a determination. There, chl a was measured fluorometrically (APHA 1998). Areas of rocks scraped and sub-sample volumes were recorded to enable calculation of chl a per unit area of stream bottom (μg chl a/cm^2).

Cover of the stream bottom by filamentous green algae is an indicator of algal biomass and aesthetic problems caused by these algae. We estimated the percent cover of stream bottoms with visual observations (Stevenson and Bahls, 1999; Stevenson et al., 2006). An area of stream was delimited using a viewing bucket or frame in which a grid of 50 points was arranged. The grid helped quantify percent cover of the stream bottom by filamentous algae. Twenty observations were made along 5 to 10 transects in riffles of the sampling reach. The percent cover of the stream bottom by filamentous algae and type of filamentous algae composing the cover was recorded. Based on a preliminary stream survey, field crews were trained to identify *Cladophora, Rhizoclonium,* Zygnematalean filaments *(Spirogyra, Mougeotia, and Zygnema) Tetraspora, Draparnaldia, Vaucheria,* and *Batrachospermum* based on growth form, color, and feel. Subsamples of macroalgae were collected for testing identifications and verification. Macroalgal testing showed that *Oedogonium* also occurred in streams and was difficult to distinguish from *Cladophora* and *Rhizoclonium*. They were difficult to distinguish from each other because they all feel rough in the field and branching patterns can be variable and difficult to observe. Since algae in these genera (*Oedogonium*, *Cladophora*, and *Rhizoclonium)* pose the greatest problems for nuisance accumulations in IRW streams and they were difficult to distinguish, we grouped them in a category that we refer to as filamentous green algae (FGA).

Land-use categories were determined as a percentage of basin area using land use classifications and maps from the 2001 National Landcover Dataset, which was assembled by the MultiResolution Land Characteristics Consortium and is derived from 30-meter resolution Landsat satellite imagery. We selected percent urban and agricultural land use in watersheds for indicators of the effects of those human activities on stream condition, which is a common approach for relating land uses to stream condition (Allan et al., 1997; Johnson et al., 1997). In

addition, poultry house density (houses/mi^2) was determined for each watershed plus a 2 mile buffer around watersheds. The 2 mile buffer around watersheds was included as a source of poultry waste to a watershed because poultry waste is transported relatively short distances from the house and may be across watershed boundaries (Bert Fisher, personal communication). Thus, poultry houses outside the watershed of a stream could be contributing to P loading. That hypothesis was tested by relating P concentration in a stream to the density of poultry houses in the watershed when poultry houses within the watershed only were counted and poultry houses within 2 miles of the watershed were counted as being in the watershed. The correlation with stream P was greatest when poultry houses within 2 miles of the watershed were included in the number of poultry houses in the watershed (Engel, 2008). We used total poultry house density as an indicator P contamination of watershed soils because inactive and abandoned houses were past sources of waste, and phosphorus can persist in soils long after waste application.

Statistical methods

To test hypotheses systematically, we grouped variables into four categories according to their causes and effects in the conceptual model (Figure 1). These categories were: 1) land use and land cover, 2) nutrients, 3) algal biomass, and 4) chemical stressors. The direct and indirect effects among the 4 categories of variables were then related by regression analysis to quantify relationships. For example, first we related direct effects of land use on nutrient concentrations and we characterized N:P ratios to determine the most limiting nutrient. Then we related algal biomass to nutrient concentrations, a direct relationship, followed by relating land use to algal

biomass, an indirect relationship. Finally we quantified relationships between DO and pH with algal biomass, nutrient concentrations, and land use, successively.

The rationale for this approach is direct relationships between cause-effect factors should be the most precise, and they help evaluate plausible causes. Indirect relationships were valuable for two reasons. One, some independent variables like land use vary less over time than nutrient concentrations in streams that vary with time of day and weather. Therefore, land use could provide a more precise and accurate characterization of nutrient concentrations affecting benthic algae than actual measured nutrient concentrations in waters of the stream. Two, independent variables help us determine the causal pathway. If poultry house density in the watershed is related to nutrients, algal biomass, and DO or pH, then it is plausible that poultry operations caused these alterations of IRW streams, even if they are not all related.

At each successive step in the causal pathway the range of conditions was characterized to provide a foundation for expected effects and for comparison with other regions. We used regression analyses to test hypotheses about relationships to determine whether they were statistically significant (unlikely to be observed by chance) and whether they were biologically significant (had a large effect).

All variables were transformed if necessary to meet the assumptions of parametric statistical techniques. Distribution of each variable was plotted in a results file and examined to determine whether they were skewed. We used logarithmic, square root, and positive power transformations (e.g. X^2 or X^{10}) to normalize distributions of variables. Distributions of transformed variables were also plotted and transformed using another power until their distributions were not skewed. Variables with too many zeros were not included in the statistical analyses. In addition to plotting distributions of variables before statistical tests, residuals were

plotted and examined after statistical tests to ensure they were near-normally distributed. Outliers were removed from analyses in some cases. In no case were results of the statistical tests substantially different before and after outlier removal. If they were, the cause of the outlier being unusual would be recorded and discussed.

Thresholds in relationships were identified using CART (classification and regression tree analyses) analysis. Thresholds were evaluated by comparing the variance explained by the non-linear model identified by CART analysis and linear model from regression. The ratio between variances explained by CART and linear models was used as a positive indicator of the biological and political significance of thresholds. All analyses were conducted using SYSTAT 11.0.

For data collected during spring, analyses were restricted to a single observation per site using a summary statistic, such as averages, minima, maxima, or standard deviations. The use of one estimate from each site maintained the independence in observations in the dataset, which can be a problem when repeated measures are collected from sites and used in statistical analyses.

Results

Land Use and Nutrient concentrations

A broad range of phosphorus concentrations was observed in rivers of the IRW, with slightly higher TP and SRP concentrations observed during summer than spring ($p<0.05$). TP concentrations ranged from less than 0.010 to more than 0.648 mg TP/L during both spring and

summer, with median concentrations of 0.057 and 0.076 mg TP/L during spring and summer, respectively (Figure 3). SRP concentrations ranged from less than or equal to 0.002 to more than 0.059 mg SRP/L during both spring and summer, with median concentrations of 0.034 and 0.057 mg SRP/L during spring and summer, respectively. Thus TP concentrations ranged from less than our benchmark for phosphorus limitation to greater than that benchmark (0.030 mg SRP/L).

Nitrogen concentrations as TN were higher during spring than summer ($p<0.05$), ranging from less 0.800 to more than 20.4 mg TN/L in both spring and summer, with median TN concentrations of 4.0 and 2.8 mg/L during spring and summer, respectively. NO_x concentrations were not different in spring and summer, ranging from 0.1 to more than 7.5 mg NO_x-N/L in both spring and summer, with median TN concentrations of 1.8 mg/L during both spring and summer. Where as TN concentrations were above concentrations that we would expect to be limiting benthic algal growth, NO_x concentrations were slightly below our benchmark for nitrogen limitation of algal growth.

N:P ratios indicated that phosphorus was more likely limiting than nitrogen, and were higher during spring than summer. N:P ratios ranged from 20.9 to 1890 during spring with a median of 261 and from 12.8 to 1024 during summer with a median of 152. Temperature and conductivity were also lower during spring than summer (Figure 3). Median water temperatures were 17ºC during spring and 25ºC during summer. Median conductivities were 294 and 449 mmhos/cm during spring and summer, respectively.

Regression analyses indicated that both TP and TN concentrations in IRW streams during spring were related to poultry house density as well as the percent urbanized land use in watersheds (Figure 4A & B). Forward stepwise multiple linear regression with land use attributes and watershed area produced a TP model with significant relationships between TP and both

poultry house density (PHD) and urban land use ($p \leq 0.001$, Table 2). The resulting TP model predicted that natural background TP concentration, when percent urban land use and poultry house density both equal 0, was 0.007 mg/L. Forward stepwise multiple linear regression with land use attributes and watershed area produced a TN model with significant relationships between TN and both poultry house density and urban land (p=0.019 and <0.001, respectively). The spring TN model predicted that natural background TN concentration, when urban and poultry house density both equal 0, was 1.15 mg/L.

TP concentrations in IRW streams during summer 2006 were related to poultry house density, but effects were masked statistically by high TP in waters downstream from urban land use (Figure 4C and D). TP concentrations were very weakly related to poultry house density in a multiple regression model (r^2=0.223) that included both poultry house density and the percent of watersheds that were urbanized (Table 2). The -4.292 intercept in this model indicated the natural background concentration of TP was 0.014 mg TP/L. If analysis was restricted to a subset of data in which urban land use was less than 10 percent of the watershed, poultry house density was significantly related to TP concentration (p=0.014, Figure 4D). Multiple regression showed that TN was not related to poultry house density during summer 2006, even if the dataset was constrained to streams with low urban activity (p=0.369); but TN during summer was significantly related to urban land use (p<0.001),

Algal biomass

Benthic algae in the IRW were composed mostly of diatoms, filamentous green algae, the red alga *Batrachospermum*, and the filamentous xanthophyte *Vaucheria*. *Cladophora*, *Rhizoclonium*,

and *Oedogonium* were the most common filamentous green algae observed in streams. *Spirogyra*, *Mougeotia*, *Zygnema*, and *Draparnaldia* were also observed, but less frequently.

Algal biomass was higher during spring 2007 than summer 2006. Benthic algal chl *a* was greater during spring 2007 than summer 2006 (Figure 5). During spring, chl *a* ranged from 0.2 to 33.5 μg/cm^2 with a median of 4.9 μg/cm^2. During summer, chl *a* ranged from 0.007 to 13.8 μg/cm^2 with a median of 3.79 μg/cm^2. During spring 2007, FGA cover ranged from 0 to 91 percent of the bottom of streams with a median of 20.8 percent FGA. During summer, FGA cover ranged from 0 to 85.8 percent of the bottom of streams with a median of 3.1 percent during the summer.

Benthic algal chl *a* and FGA during spring were significantly related to TP concentration ($p<0.001$) (Figure 5, Table 2). Chlorophyll *a* and FGA were more highly correlated with P concentrations than nitrogen (Table 2). Both measures of algal biomass were also more highly correlated with TP than with SRP or TDP. Benthic chl *a* and FGA during spring was significantly related to poultry house density ($p=0.001$) and percent urban land use ($p<0.001$) in a model determined by stepwise multiple linear regression (Figure 6, Table 2). For both measures of algal biomass, agricultural land use was also related to benthic algal biomass, but not after variation associated with poultry house density was accounted for.

Thresholds were indicated in chl *a* and FGA cover along the TP gradient, but only the FGA CART model explained substantially more variation than the linear model. A changepoint in benthic chlorophyll a was identified at 0.029 mg TP/L, with an average biomass of 1.3 and 5.4 μg chl a/cm^2 below and above the changepoint (Figure 5). The CART relationship explained 32.8 percent of the variance in benthic algal biomass. This compared to 39.1 percent of explained variance in benthic chlorophyll a using a linear regression model with TP concentration. A

CART changepoint in spring FGA cover was identified at 0.027 mg TP/L, with an average FGA cover of 4 and 36 percent in TP concentrations less than and greater than the TP changepoint, respectively (Figure 5). The CART relationship explained 42.7 percent of the variance in FGA cover compared to 28.7 percent of variance explained by a linear regression model with TP concentration.

The proliferations of benthic algale were related to canopy cover during the spring, but positively rather than negatively as predicted.

Benthic algal biomass was significantly related to TP concentrations during summer ($p<0.05$, Figure 5). Summer biomass of FGA was not related to TP concentrations in streams ($p=0.313$). Benthic algal biomass was related to urban land use ($p=0.013$), but not to poultry house density during summer 2006 ($p=0.429$, Figure 6). Cover of the stream bottom by FGA was not related to poultry house density or urban land use during summer 2006 ($p>0.190$, Figure 6). The lack of relationships between either measure of algal biomass and poultry house density was true even if data analyses were restricted to sites with low urban activity to reduce statistical effects of sites with high urban activity.

DO and pH

DO concentrations in streams over the 8-week spring 2007 sampling period were higher than during summer, with an average of 10.1 mg/L, a minimum of 7.6, and a maximum of 13.4 (Figure 7). Average DO concentration in streams during the summer 2006 sampling period was 5.2 mg/L with a minimum of 0.9 and a maximum of 9.2. DO concentration varied with time of day that sites were sampled during spring. Sites sampled early and late during the day had lower

DO than during mid-day. The standard deviation in stream DO during the 8-week spring 2007 sampling period averaged 1.8 mg/L with a minimum of 0.4 and a maximum of 3.7 mg/L. The minimum DO observed in each stream over the eight week period averaged 7.6 with the lowest value being 4.1 and the highest minimum being 9.7 mg/L.

Average DO measured in a stream during spring was positively related to benthic algal biomass (p=0.003), FGA cover (p<0.001), TP concentration (p=0.05), and poultry house density and percent urban land use in watersheds (p≤0.002) (Figure 8). The standard deviation in DO of streams during the eight weeks of sampling was positively related by multiple linear regression to chl *a* and FGA cover (p<0.001) as well as total phosphorus, poultry house density, and percent urban land use in watersheds upstream from the sites sampled (p<0.05, Figure 9). Minimum DO concentrations over the 8 week period were not clearly linked to higher algal biomass or poultry house density, but they were related negatively (p=0.001) to TP concentrations in streams (Figure 10).

DO in streams during summer was not related to TP concentration when all streams were included in the analysis (Figure 11). However, when we limited the streams in the data set to streams with a low percentage of urban land use in their watersheds, DO was significantly negatively related to TP concentration. Summer DO was complexly related to land use (Figure 11). In a two factor multiple regression analysis, DO was positively related to urban land use (p<0.05) and not significantly related to poultry house density. Again, the statistical masking of poultry house effects on DO by urban effects became evident; DO was negatively related (p=0.075) to poultry house density if the dataset was limited to streams with less than 10 percent urban land use in watersheds

The median of the 8-week spring average of pH in IRW streams was 7.8, with a 6.9 minimum, and a maximum of 9.1 (Figure 7). The maximum pH in an IRW stream during the spring 2007 had an average value of 8.8 with the lowest maximum of 7.2 and the highest pH maximum of 10.6. These maximum values of pH are higher than 9.0 more than 25 percent of the time. Median pH during the summer was 7.6 with a minimum of 6.1 and maximum of 8.3.

The high pH in IRW streams during spring was not due to natural causes. Average pH at sites increased with increasing benthic algal biomass ($p=0.118$), FGA cover ($p=0.001$), TP concentration ($p=0.013$), urban land use ($p=0.03$), and probably poultry house density ($p=0.166$) (Figure 12). Maximum pH observed at sites was better correlated with these causal variables than average pH. Maximum pH at sites increased with increasing benthic algal biomass ($p<0.001$), FGA cover ($p=0.005$), TP concentration ($p=0.004$), and urban land use ($p<0.001$), and poultry house density ($p=0.037$) (Figure 13). We did not observe significant variation in pH with time of day during spring as we did DO concentration. During summer, one time measurements of pH were not related to TP concentration, poultry house density, or urban land use.

Discussion

Nutrients and land use

Nutrient pollution was relatively high in the IRW watershed versus other watersheds in the US with similar geology and hydrogeomorphology. Nutrient concentrations in minimally disturbed watersheds (*sensu* Stoddard et al., 2006) were predicted to average 0.010 mg TP/L in the IRW based on regression models relating nutrient concentration to urban land use and poultry house

density in watersheds. Dodds and Oakes (2004) observed variation in predicted minimally disturbed conditions among ecoregions from 0.010 to 0.070 mg TP/L. Phosphorus concentrations in minimally disturbed watersheds in the Knobs ecoregion of Kentucky-Indiana and the mid-Atlantic Highlands, which had similar geology, soils, and stream hydrology as the IRW, were 0.010 mg TP/L (Stevenson et al. 2006, 2008), which is the same as the IRW. Given similar minimally disturbed conditions in these regions, we compared the 25th and 75th percentiles of TP concentrations in streams of the three regions. We calculated these percentiles using data from the previously studied regions and found for the streams of the IRW during spring, Kentucky-Indiana Knobs region during spring, and the mid-Atlantic Highlands during early summer, that the 25th percentiles of TP concentrations were respectively 0.030, 0.010, and 0.010 mg/L and the 75th percentiles were 0.127, 0.027 and 0.030 mg/L. We did not review TN concentrations in the three regions, because N:P ratios were greater than 16:1 in almost all streams. A 16:1 molar N:P ratio indicated that phosphorus was the most likely limiting nutrient (Redfield 1958), if nutrients were sufficiently low to limit algal growth.

Elevated phosphorus and nitrogen concentrations were related to poultry house operations in the IRW, as well as urban activities. First, a large portion of the phosphorus entering the IRW was related to poultry operations according to mass balance models (Engel 2008). Nutrients in poultry waste are readily transported via runoff and percolation to groundwater sources of stream flow in hydrogeological settings like the IRW (Sharpley et al., 2007). Many cattle occur in the IRW, but the phosphorus in their manure can be attributed to grass they eat, which used the phosphorus that has been applied to fields in the form of poultry waste (Tarkalson and Mikkelsen, 2003). Relatively little addition of phosphorus is imported into the IRW for fertilizing fields and feeding cattle. In addition, nutrients were statistically related to

poultry house density in watersheds and better related to poultry house density than percent agricultural lands in the watershed even though the litter is applied to pastures. Finally, many watersheds in the IRW have high poultry house density, high phosphorus, and low urban land use, leaving poultry waste as the only reasonable source of phosphorus in those streams.

Algal biomass

High phosphorus pollution associated stimulated accrual of benthic algae in IRW streams during spring and summer, but in different forms. Stimulation of algal growth across the range of phosphorus conditions in the IRW has been observed in numerous other studies (Dodds et al., 1997; Biggs, 2000; Stevenson et al., 2006). Chlorophyll a and FGA cover were both positively related to TP concentration, as well as TDP and SRP concentrations, during spring. Only benthic chlorophyll a was related to TP during summer. FGA densities were low and unrelated to TP during summer, probably because optimal temperatures for FGA growth occur during spring. *Cladophora*, the most common taxon observed in FGA, is widely recognized to have a spring growing season in this climatic zone (Dodd and Gudder, 1992).

A threshold in the relationship between FGA and TP was observed during spring at 27 µg TP/L. This threshold is supported by our comparison of the variance explained by non-linear and linear models with the CART model explaining 50% more variance than the linear model. Thresholds in algal biomass response to nutrients have often been observed close to this TP concentration. Peak biomass of diatoms and filamentous Zygnematales in stream-side experiments was saturated at 30 µg TP/L (Bothwell, 1988; Rier and Stevenson, 2006), i.e. biomass increased greatly in treatments with TP concentrations ranging near zero to 30 µg/L and

increased little when TP was greater than 30 µg/L. Dodds et al. (1997) found that accrual of benthic algae from streams throughout the world, measured as chlorophyll a, was also saturated at approximately 30 µg TP/L.

We re-evaluated data on *Cladophora* cover and TP concentrations in >100 streams from Kentucky and Michigan (Stevenson et al. 2006) using CART analysis; these streams were sampled using the same methods as in this IRW stream study, per stream rapid periphyton assessments and water chemistry sampling with 4-8 sampling times per stream in an 8-week period. CART analysis of the Kentucky-Michigan stream data showed a threshold in TP concentration at 0.023 mg TP/L (Figure 14). The variance explained by the CART model was twice as great as a linear model for the *Cladophora*-TP relationship. *Cladophora* cover was predicted to increase from 1 to 10 percent at this threshold, although the 10% underestimates potential problems with higher TP. Many streams with higher TP had no *Cladophora*, indicating that some other, non-nutrient factor prevented *Cladophora* from growing and reducing estimates of average *Cladophora* cover in high TP streams. If streams with zero cover were not included in the calculation, average *Cladophora* cover in streams with TP greater than 0.023 mg TP/L was 16 or 22 percent (depending upon whether cover was square-root transformed or not, respectively, when calculating the average). Note, *Cladophora* cover was almost always less than 5 percent in Michigan and Kentucky streams when TP concentration was less than 0.010 mg/L.

Phosphorus pollution stimulated an increase in algal biomass in IRW streams to what has been characterized to be a nuisance level. FGA cover in minimally disturbed, low P streams usually had less than 10 percent FGA cover. When TP was greater than 0.27 µg/L, FGA cover averaged 36% cover. Welch et al. (1988) synthesized results of literature and considered

nuisance algal biomass to be greater than 20 percent FGA cover or 100 to 150 mg chl *a*/cm^2. Surveys of Montana voters and recreational users of the Clark Fork River showed desirability for use decreased dramatically (threshold response) when chlorophyll a exceeded 150 mg /m^2 (=15 μg chl a/cm^2) (Suplee et al. 2008). In addition, the percent FGA cover of IRW streams with FGA was higher than in Kentucky and Michigan streams (Stevenson et al. 2006). Less than 3 percent of streams in Kentucky and Michigan streams had a seasonal average greater than 50 percent FGA cover. In the IRW, about 25 percent of the sampled streams had greater than 50 percent of the stream bottom covered by FGA.

We relied on FGA cover, rather than chl *a*, to assess the magnitude of impact of phosphorus pollution on benthic algae because we believe our chl *a* measurements of biomass were low compared to FGA cover measurements due to a laboratory error. Estimating FGA cover by visual assessment is relatively error-proof compared to assessing benthic algal chl a, so we put more credibility into the FGA measurements. In addition, conversations with the analytical laboratory after sample and data analyses uncovered that the subsampling techniques that the laboratory used before chl *a* assays probably under-sampled the FGA because a small bore pipette was used in subsampling and filaments were not cut into easily sampled pieces. In addition, we made a comparison of the relationships between chl *a* and FGA cover for the IRW and Kentucky streams, where similar diatom and FGA assemblages were observed to develop on substrata. The model for chl *a* as a function of FGA cover in the IRW was:

$$\ln(\text{chl } a) = -0.088 + 0.465 * \ln(\text{FGA}+1)$$

The model for chlorophyll a as a function of FGA cover in Kentucky streams was:

$$\ln(\text{chl } a) = 1.137 + 0.642 * \ln(\text{FGA}+1)$$

In both models, chlorophyll a (chl *a*) and FGA were natural log-transformed. 1 was added to FGA to accommodate zero values.. Chlorophyll a was measured as μg/cm^2 and the units for FGA are percent cover. Both intercept and slopes in the Kentucky model were significantly greater (p<0.05 and <0.10, respectively) than the IRW model. According to the IRW model, chl *a* with 35 percent FGA cover was 4.7 μg/cm^2 (= 47 mg/m^2), which is a relatively low value when compared to the potential range of benthic chl a for so much FGA. According to the Kentucky model, chl *a* with 35 percent FGA cover was 30 μg/cm^2 (=300 mg/m^2), which is well above the 150 mg/m^2 nuisance level established by Welch et al. (1988) and Suplee et al. (2008). Based on the more reasonable predictions of the Kentucky model than IRW model, we assume that laboratory error caused an underestimation of chl *a* in our samples. A reason that our chl *a* measures were correlated to urban land use, poultry house density, and TP is probably that the microalgal fraction assayed (not associated with FGA) was also related to TP and prevalence of its sources. For example, the density of microalgae in samples should increase with FGA, because many microalgae are epiphytes on FGA and would fall off during processing.

DO and pH

Nutrient pollution in the IRW indirectly increased average DO and the variability in DO at a site during spring and negatively affected DO concentration during summer. Few field surveys such as ours report the relationship between DO and nutrient concentrations, perhaps because diurnal variability makes resolving relationships difficult when sampling takes place at different times of day. In our study, we controlled for time of day by sampling early in the morning during summer. During spring, we sampled each site many times and at different times

of day, so we could characterize variability and the range in DO conditions over an eight week period.

The different effects of nutrients on DO during the two seasons were probably related to discharge levels and covarying factors. During spring, discharge was sufficiently high that reaeration of water may have prevented minimum DO from getting too low. At no time were dissolved oxygen concentrations less than 7.6 during spring. Variability in DO was related to TP and algal biomass, thus the potential for DO depletion was developing with increasing algal biomass. In one case during spring April 2006, discharge was low and an estimated 1,343 fish (*Campostoma anomalum*) were killed in a 2 km segment of the river (Oklahoma Water Resources Board, personal communication). Extensive growths of FGA were observed across the bottom of the Illinois River at a site with dead fish. We monitored the depletion of DO the day after the fish kill was reported and observed that DO was greater than 8 mg/L before 6 PM on April 19 and decreased to as low as 0.9 between 5:30 and 8:00 AM. It seems highly likely that the low DO concentrations killed the fish and were caused by FGA, which in turn resulted from high TP concentrations.

During summer, the negative relationships between DO and TP as well as poultry house density were only observed in streams with low urban activities upstream. DO was less than 5.0 mg/L, the Oklahoma state water quality criterion, in 45% of IRW streams during summer. Lack of flow augmentation by waste water treatment plants may explain why TP had a negative effect on DO during the low flow, high temperature summer period in streams with high TP and urban land use. It is also possible that allochthonous organic matter loading was correlated with TP loading; and during low flows of summer in rural streams, when reaeration could not compensate for the oxygen demand, low early morning DO concentrations developed.

pH was positively related to algal biomass during spring, but not during summer. Increases in pH with algal biomass are probably related to algal uptake of CO_2 and a shift in the carbonate equilibrium, thereby alkalizing the water. pH was elevated to greater than 9.0, which is the Oklahoma state water quality criterion for protection of biodiversity. Thus, elevated pH concentrations related to high algal accumulations may have had a negative effect on biodiversity during spring. No survey studies documenting relationships between nutrients and algae with pH are known.

Conclusions about causation

Both urban activities and poultry house operations had substantial effects on phosphorus pollution and benthic algal biomass during spring, resulting in injury to aesthetics and potentially to biodiversity. Spring pH was commonly elevated about 9.0, which may affect biodiversity of algae, which are highly sensitive to pH (Pan et al., 1996), as well as invertebrates and fish. Punctuated DO stress may result during spring when discharge is low. Altered physical habitat by FGA can affect species composition of invertebrates (Dudley et al., 1986). During summer, low DO related to elevated phosphorus and poultry house density may negatively affect biodiversity in streams with low urban activity.

Multiple lines of evidence (e.g. Beyer 1998) support attribution of injury to aesthetics and elevated chemical stressors to spreading of poultry wastes on fields. Phosphorus, the most likely limiting nutrient in these streams, was related to poultry house density, increases in algal biomass and pH during spring, and decreases in DO during summer in IRW streams. These observations are consistent with predictions of an *a priori* model and results of phosphorus enrichment in

other studies in which either phosphorus was manipulated experimentally or measured in large numbers of streams during surveys. Poultry operations are the dominant source of phosphorus in the IRW. Phosphorus concentrations and FGA were higher in the IRW than other regions in which similar minimally disturbed conditions would be expected, extensive alteration of lands by humans exists, and in which little poultry industry activity occurs. Finally, effects of phosphorus pollution occurred in many streams in which there was no other plausible source that could cause the elevated phosphorus concentrations.

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Table 1. Regression models relating land use, poultry house density, nutrient concentrations, and benthic algal biomass. The standard deviation and attained significance of the constant, first, and second dependent variables of models are reported. PCURB=percent urban land use. PHD=poultry house density (houses per mi^2). DV=dependent variable. Chlacm2= benthic algal biomass (chlorophyll a/cm^2).

Season	Model	LU Range	Standard Error			Attained Significance		
			Constant	1st DV	2nd DV	Constant	1st DV	2nd DV
Spring	ln(TP) = -4.924 + 0.581*ln(PCURB) + 0.580*ln(PHD)	all sites	0.366	0.118	0.159	<0.001	<0.001	0.001
Spring	ln(TN) = 0.14 + 0.367*ln(PCURB) + 0.273*ln(PHD)	all sites	0.266	0.084	0.114	0.602	<0.001	0.019
Summer	ln(TP)=-4.292+0.566*ln(PCURB)+0.278*ln(PHD)	all sites	0.453	0.125	0.184	<0.001	<0.001	0.134
Summer	ln(TP)=-3.90+0.548*ln(PHD)	low urban	0.287	0.208		<0.001	0.014	
Summer	ln(TN)=0.102+0.353*ln(PCURB)	all sites	0.192	0.069		0.596	<0.001	
Spring	sqr(FGA)=9.034+1.430*ln(TP)	all sites	0.833	0.273		<0.001	<0.001	
Spring	sqr(FGA)=0.288+1.515*ln(PHD)+1.220*ln(PCURB)	all sites	1.04	0.452	0.334	0.783	0.001	0.001
Spring	ln(chlacm2)=3.109+0.640*ln(TP)	all sites	0.327	0.107		<0.001	<0.001	
Spring	ln(chlacm2)=-1.024+0.560*ln(PHD)+0.706*ln(PCURB)	all sites	0.374	0.162	0.121	0.008	0.001	<0.001

Figure Captions

Figure 1. Variables grouped into four categories according to their causes and their effects in the conceptual model. Solid arrows indicate direct effects. Dashed arrows indicate indirect effects.

Figure 2. Sampling locations during the summer 2006 field sampling program.

Figure 3. Ranges in physical and chemical conditions in IRW streams during spring 2007 and summer 2007. Boxes delineate the interquartile range, median, and whiskers with 1.5 times the interquartile range.

Figure 4. Relationships between nutrient concentrations, poultry house density (PHD), and percent urban land use (PCURB). A and B) All sites during spring 2007. C) All sites during summer 2006. D) Sites with percent urban land use less than 10% during summer 2006.

Figure 5. Relationships between total phosphorus benthic algal biomass as chlorophyll a/cm2 and FGA cover for spring (A & C) and summer (B & D).

Figure 6. Relationships between benthic algal biomass (as chlorophyll a/cm2 and FGA cover) and total phosphorus benthic algal biomass during spring (A & C) and summer (B & D).

Figure 7. Ranges in chemical stressors in IRW streams during spring 2007 and summer 2007. Boxes delineate the interquartile range, median, and whiskers with 1.5 times the interquartile range.

Figure 8. Relationships between average and standard deviation in dissolved oxygen concentrations measured weekly in a stream for eight weeks as related to benthic algal biomass and filamentous green algal cover during spring 2007.

Figure 9. Relationships between the minimum of dissolved oxygen concentrations measured weekly in a stream for eight weeks and algal biomass, filamentous green algal cover, poultry house density, percent urban land use, and total phosphorus concentrations during spring 2007.

Figure 10. Relationships between average and maximum pH measured weekly in a stream for eight weeks and benthic algal biomass, filamentous green algal cover, total phosphorus concentration, urban land use, and poultry house density during the spring of 2007.

Figure 11. Changepoint determined by CART in the *Cladophora* cover of Kentucky and Michigan streams Redrawn from Stevenson et al. (2006).

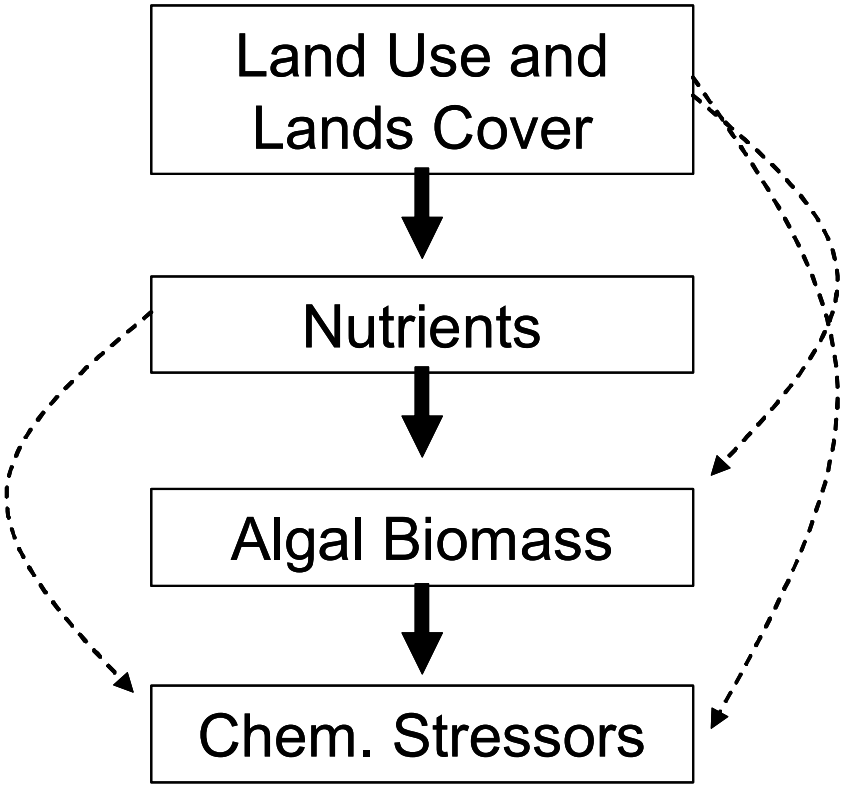
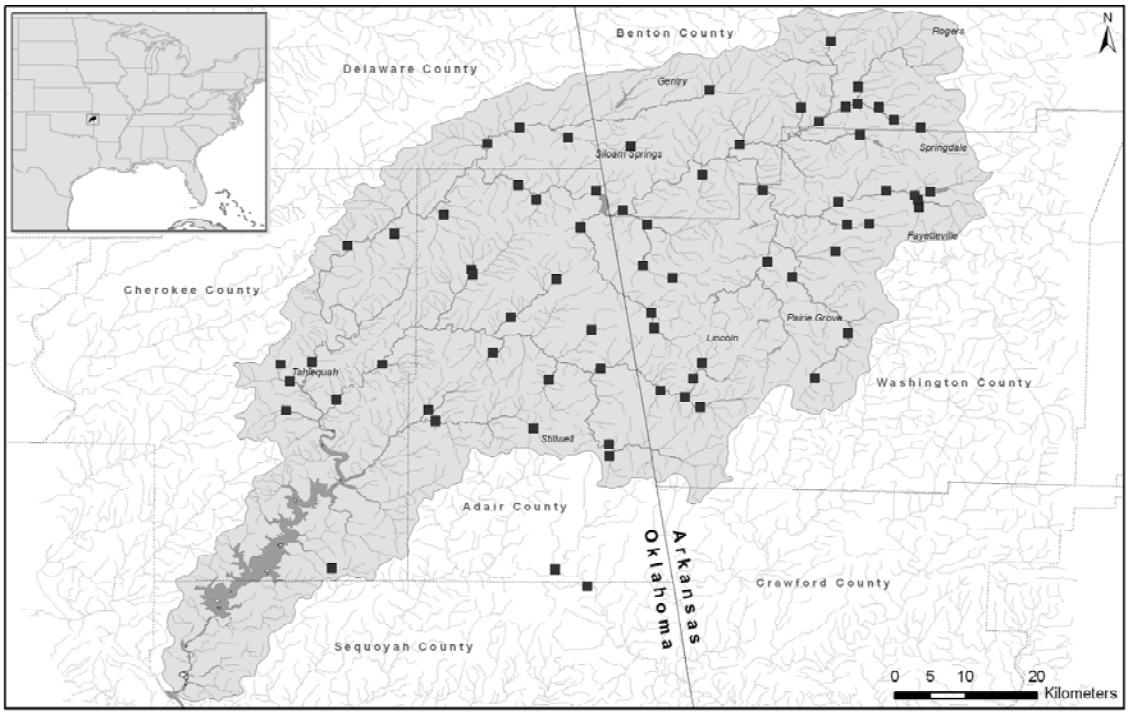

Land Use and
Lands Cover
Nutrients
Algal Biomass
Chem. Stressors

Figure 1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Washington County
Adair County
Crawford County
Sequoyah County
Arkansas
Oklahoma
Rogers
Gentry
Siloam Springs
Springdale
Fayetteville
Prairie Grove
Lincoln
Tahlequah
Stilwell
N
0 5 10 20
Kilometers

Figure 2

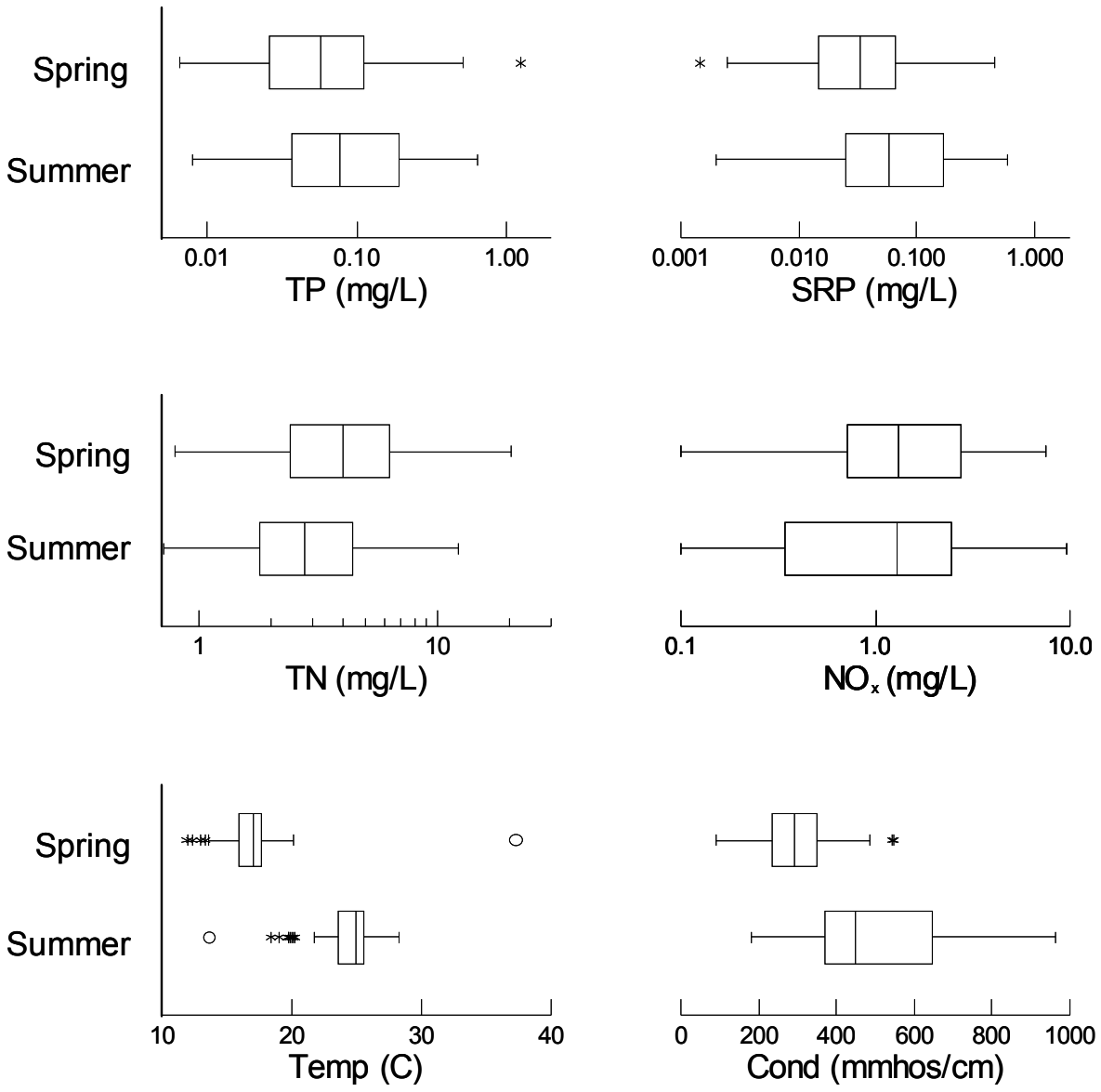

Spring
Summer
0.01
0.10
1.00
TP (mg/L)
0.001
0.010
0.100
1.000
SRP (mg/L)
Spring
Summer
1
10
TN (mg/L)
0.1
1.0
10.0
NOx (mg/L)
Spring
Summer
10
20
30
40
Temp (C)
0
200
400
600
800
1000
Cond (mmhos/cm)

Figure 3

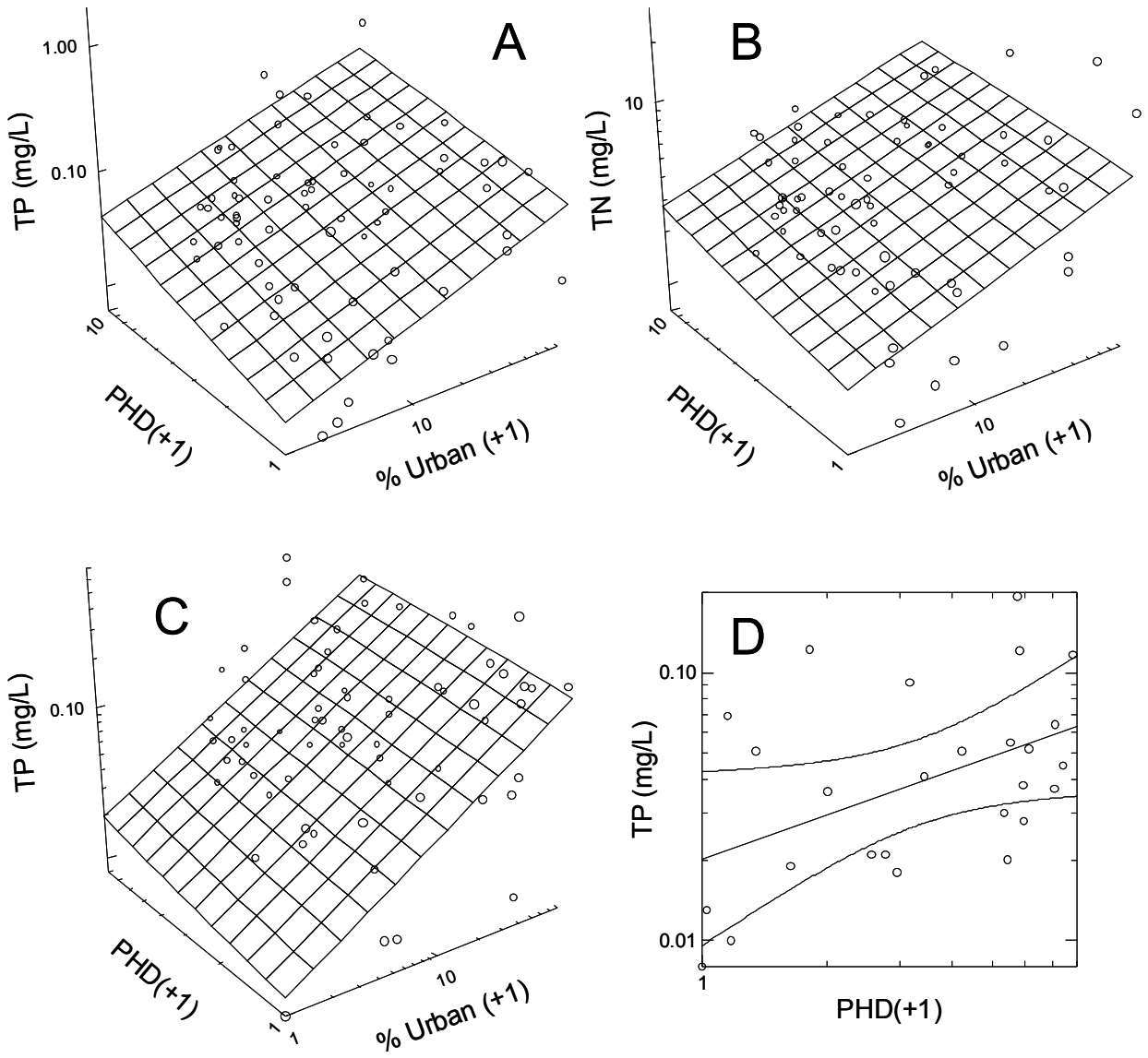

A
1.00
0.10
TP (mg/L)
10
PHD(+1)
1
10
% Urban (+1)
B
10
TN (mg/L)
10
PHD(+1)
1
10
% Urban (+1)
C
0.10
TP (mg/L)
PHD(+1)
1
1
10
% Urban (+1)
D
0.10
0.01
TP (mg/L)
1
PHD(+1)

Figure 4

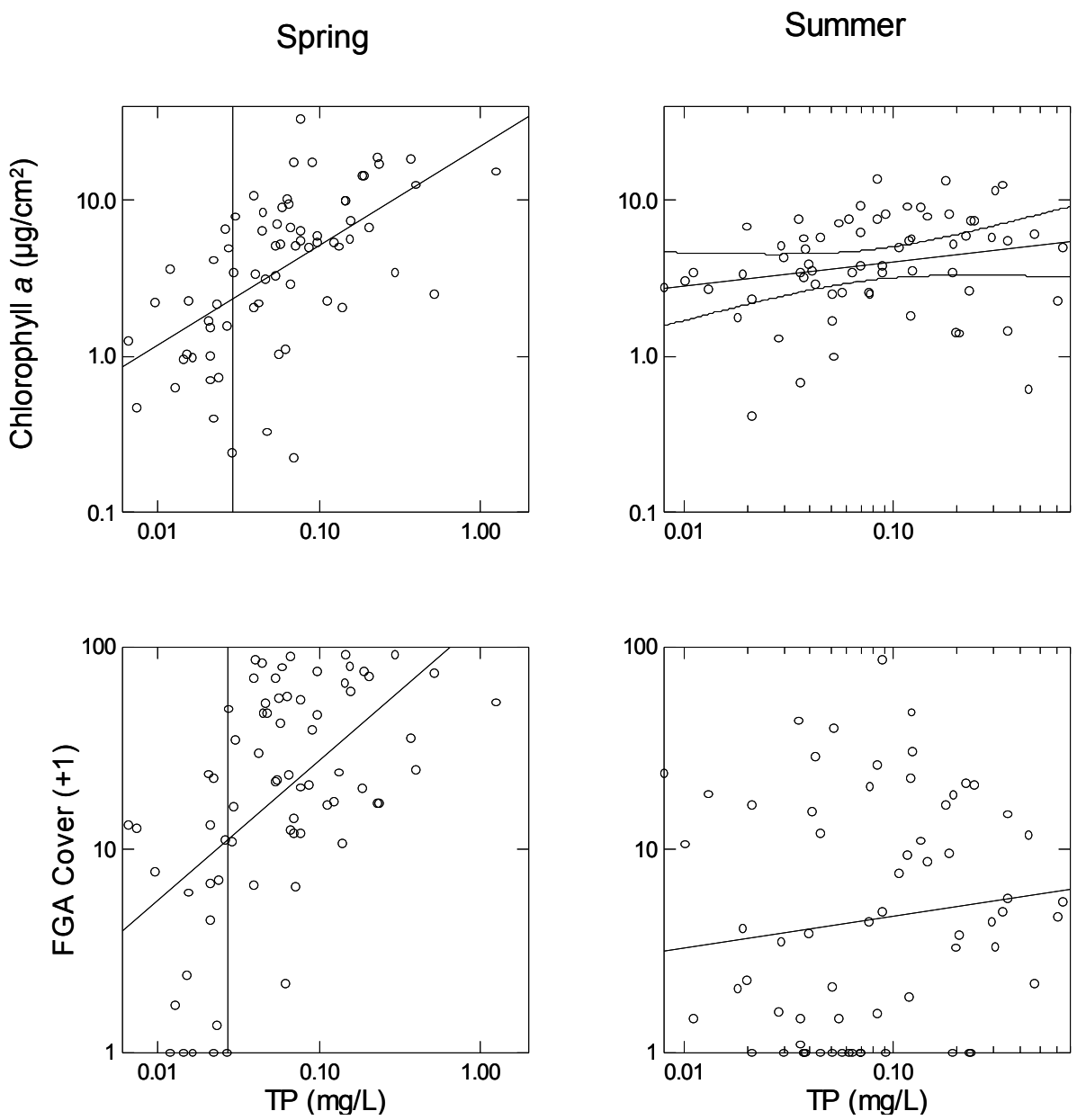

Spring
Summer
Chlorophyll a (µg/cm²)
10.0
1.0
0.1
0.01
0.10
1.00
FGA Cover (+1)
100
10
1
TP (mg/L)

Figure 5

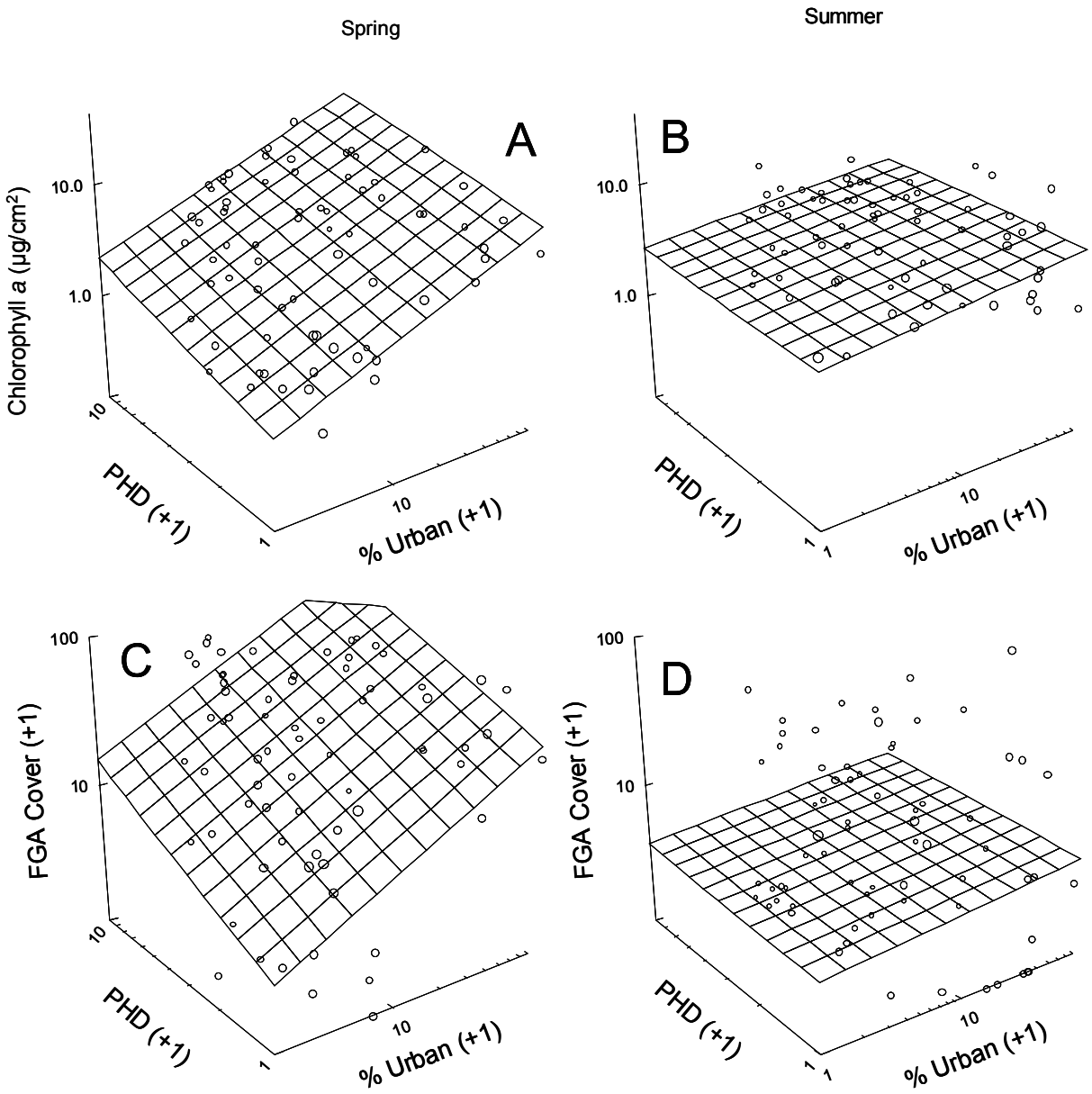

Spring
Summer
A
B
C
D
Chlorophyll a (µg/cm2)
10.0
1.0
FGA Cover (+1)
100
10
1
PHD (+1)
% Urban (+1)

Figure 6

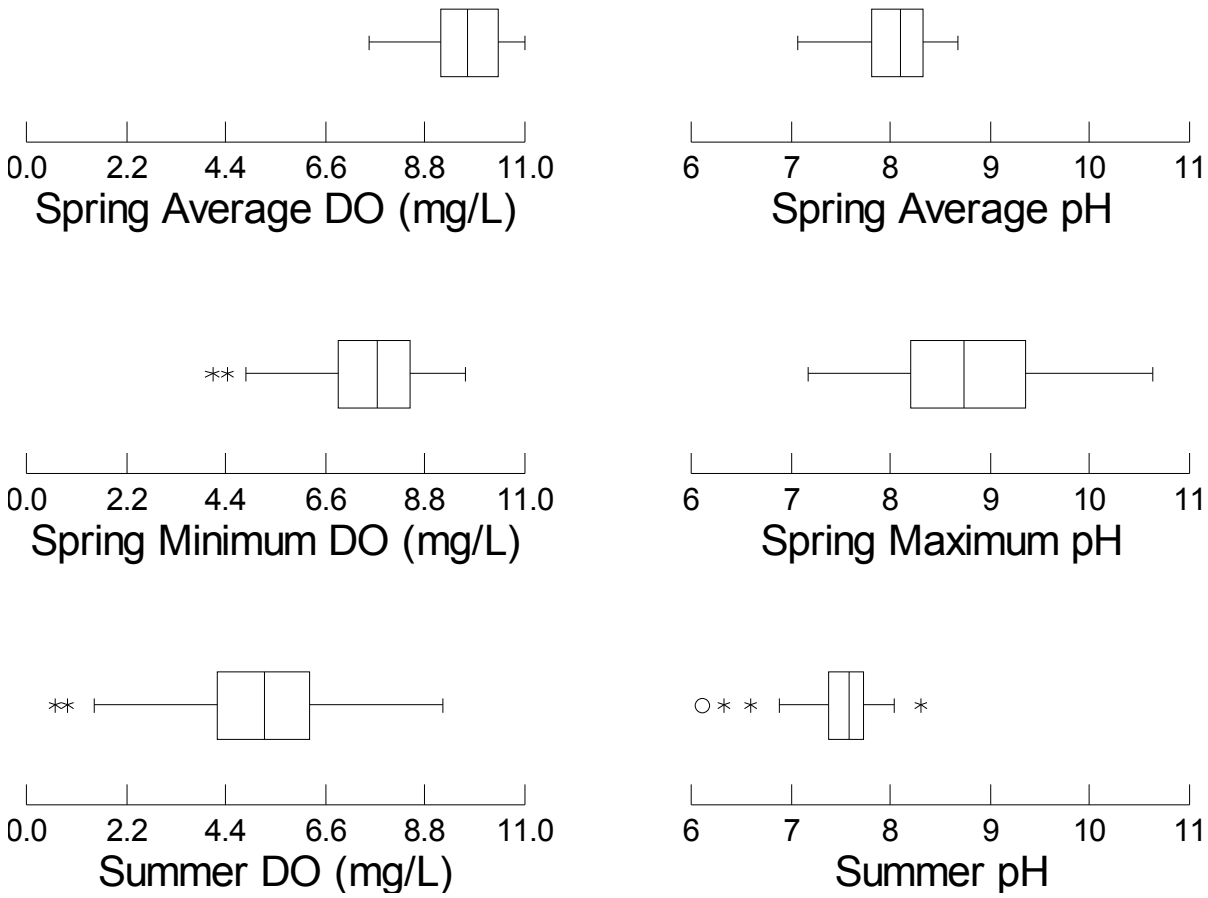

0.0
2.2
4.4
6.6
8.8
11.0
Spring Average DO (mg/L)
6
7
8
9
10
11
Spring Average pH
Spring Minimum DO (mg/L)
Spring Maximum pH
Summer DO (mg/L)
Summer pH

Figure 7

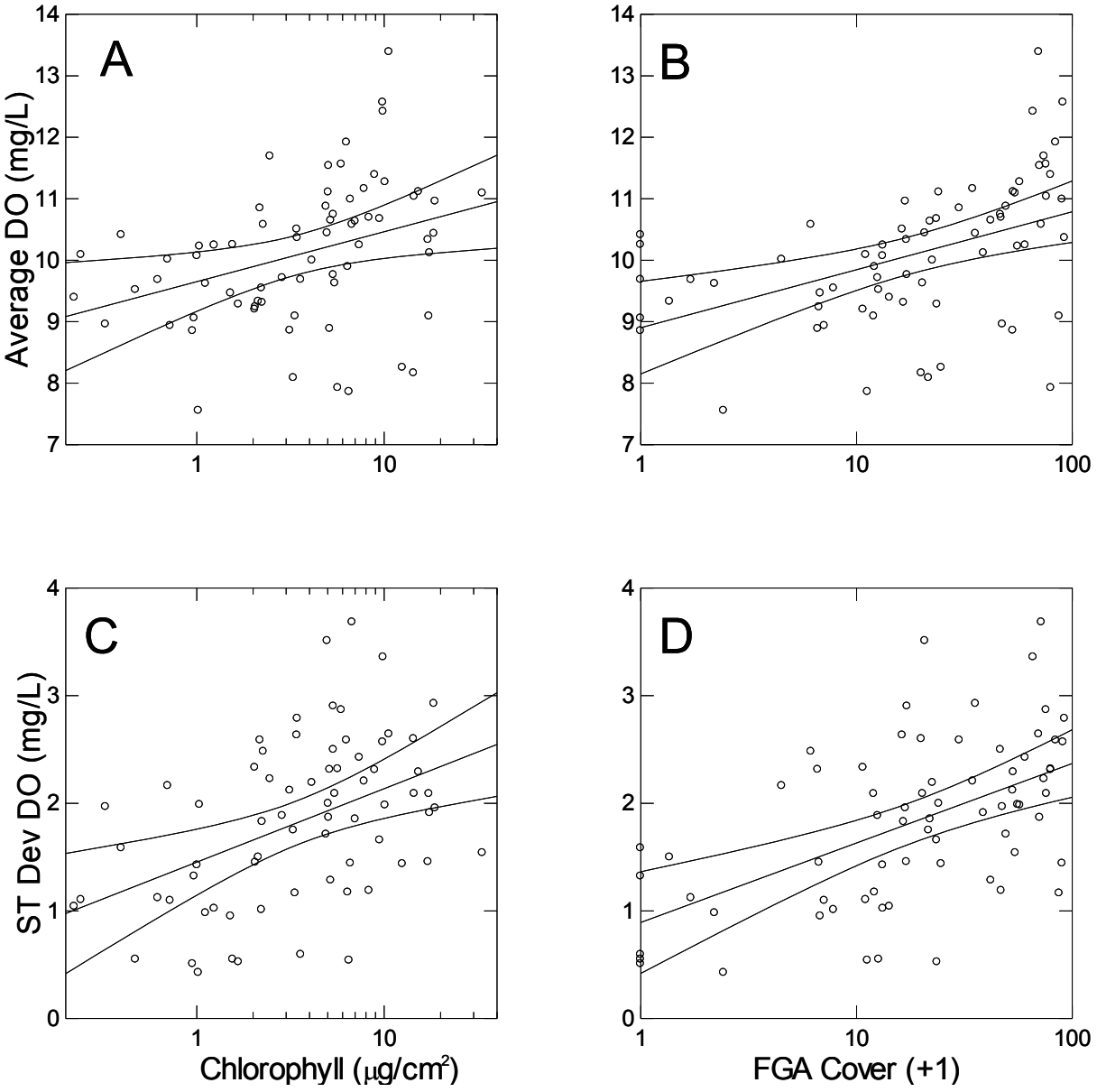

A
B
C
D
Average DO (mg/L)
ST Dev DO (mg/L)
Chlorophyll (μg/cm²)
FGA Cover (+1)

Figure 8

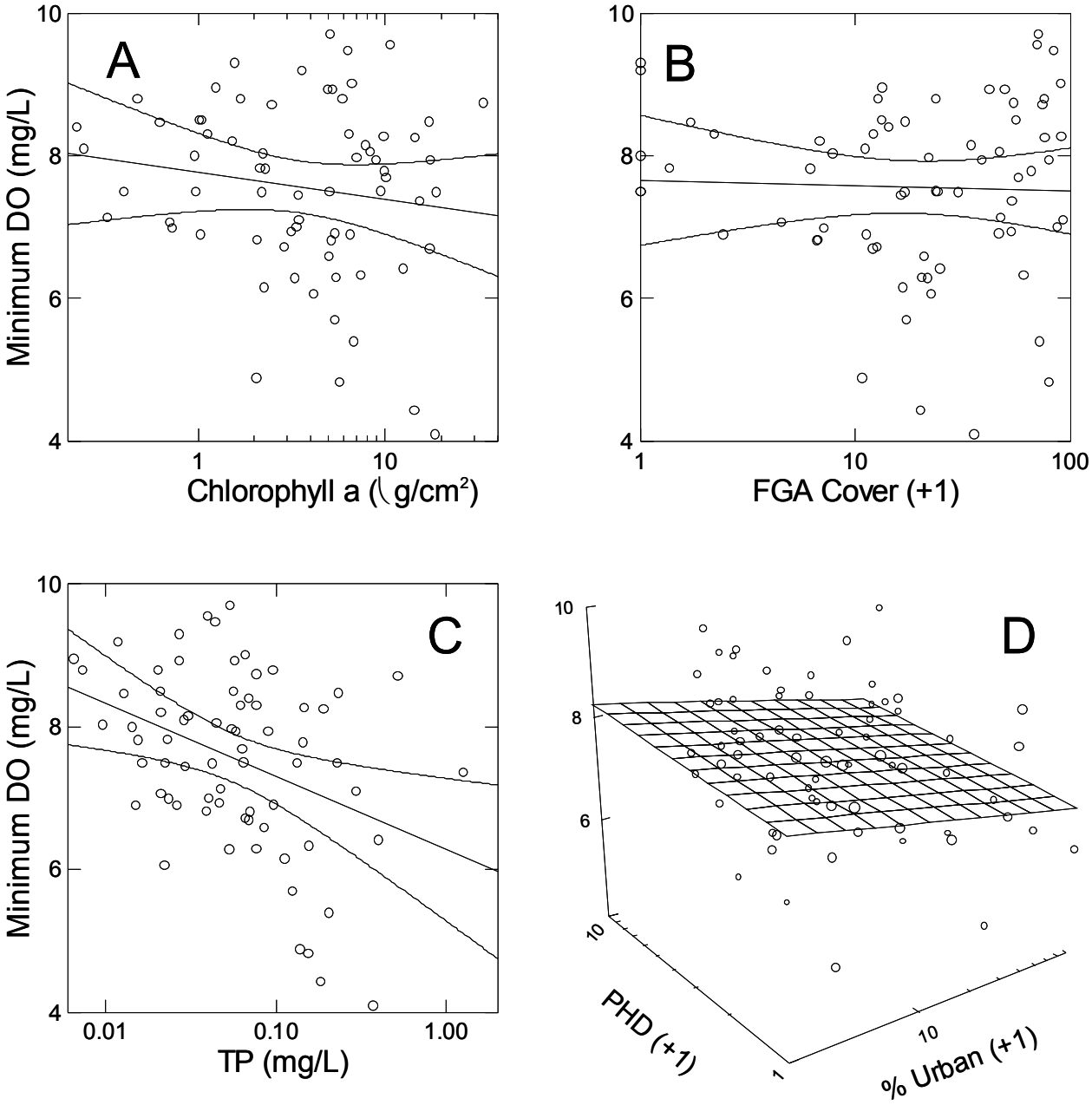

A
B
C
D
Minimum DO (mg/L)
Chlorophyll a (µg/cm²)
FGA Cover (+1)
TP (mg/L)
PHD (+1)
% Urban (+1)

Figure 9

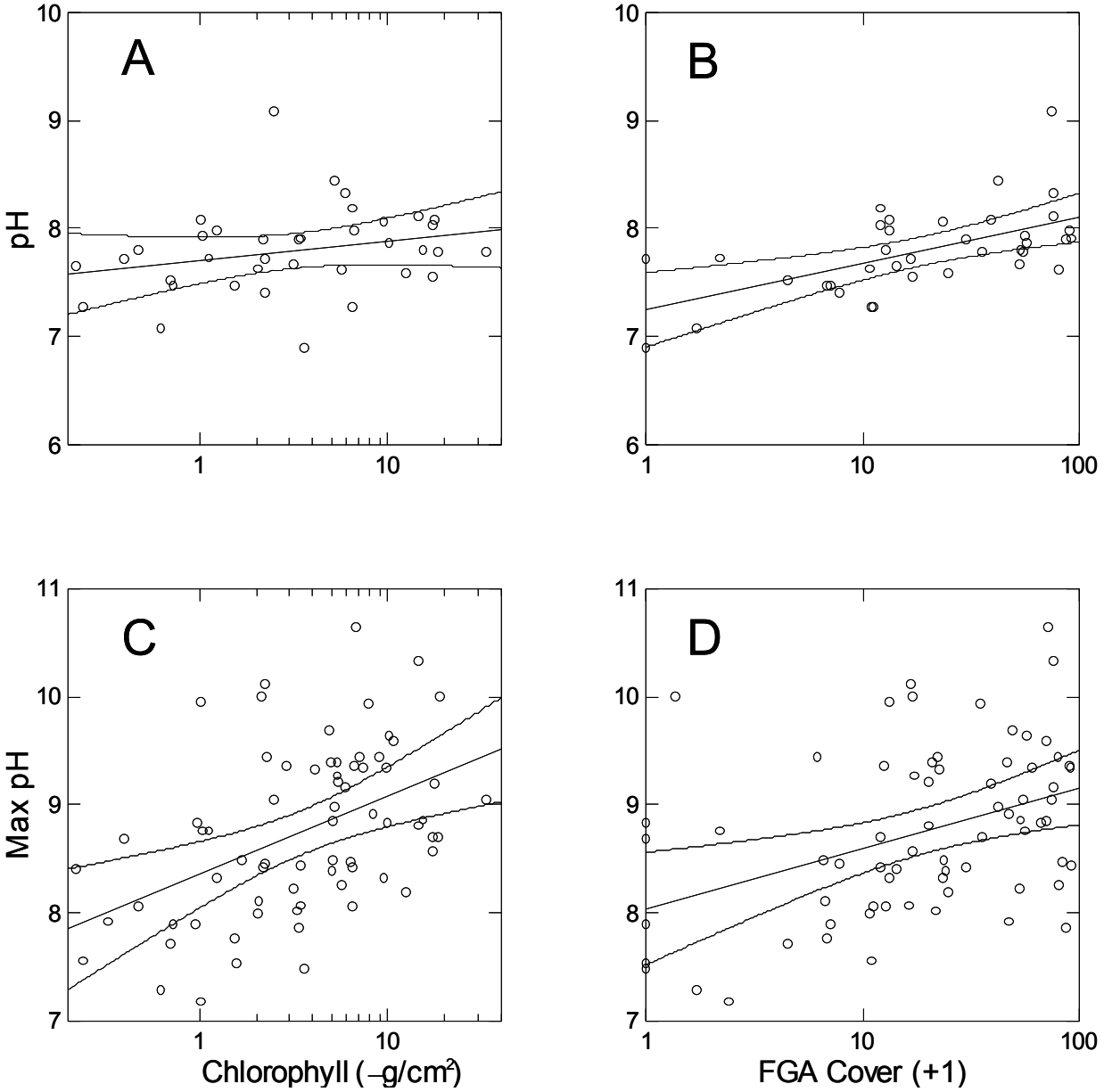
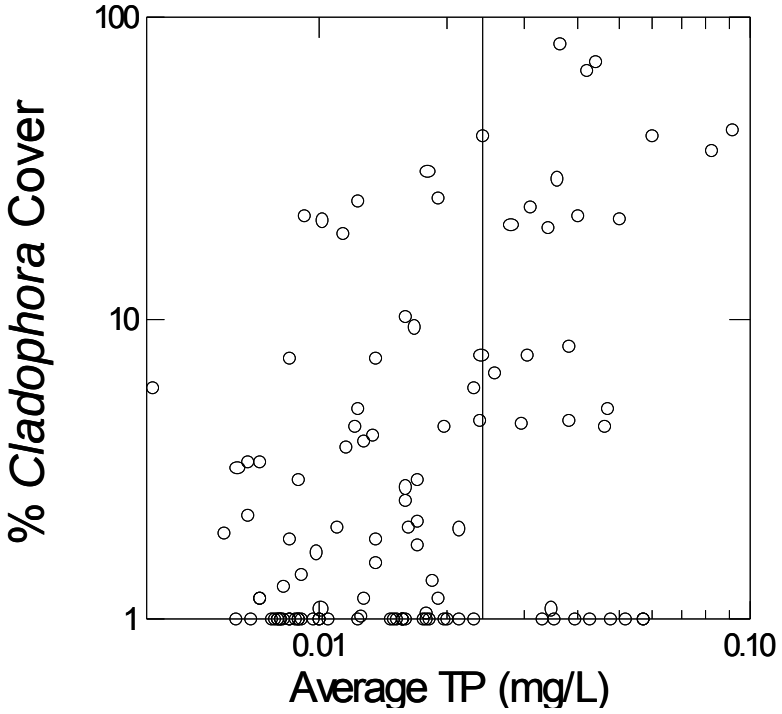


Figure 10



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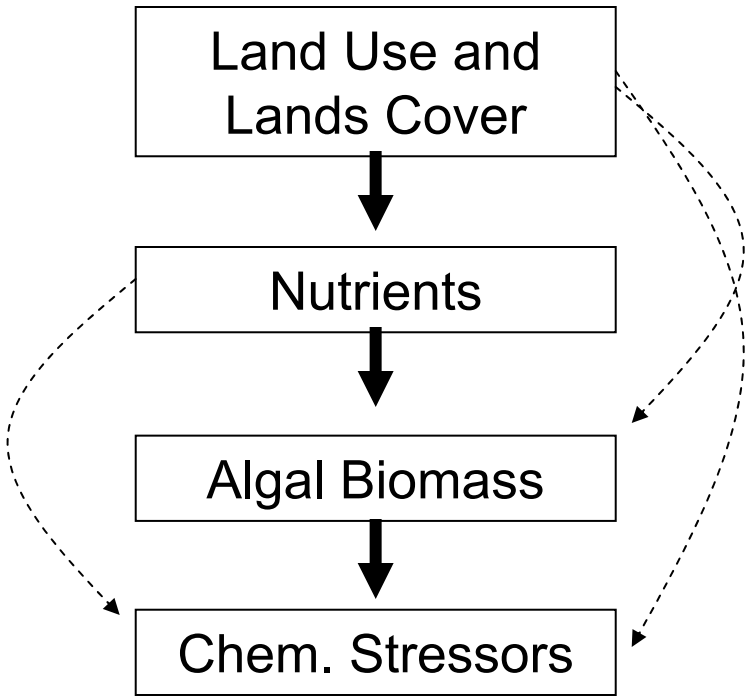
Land Use and
Lands Cover
Nutrients
Algal Biomass
Chem. Stressors

Figure 1

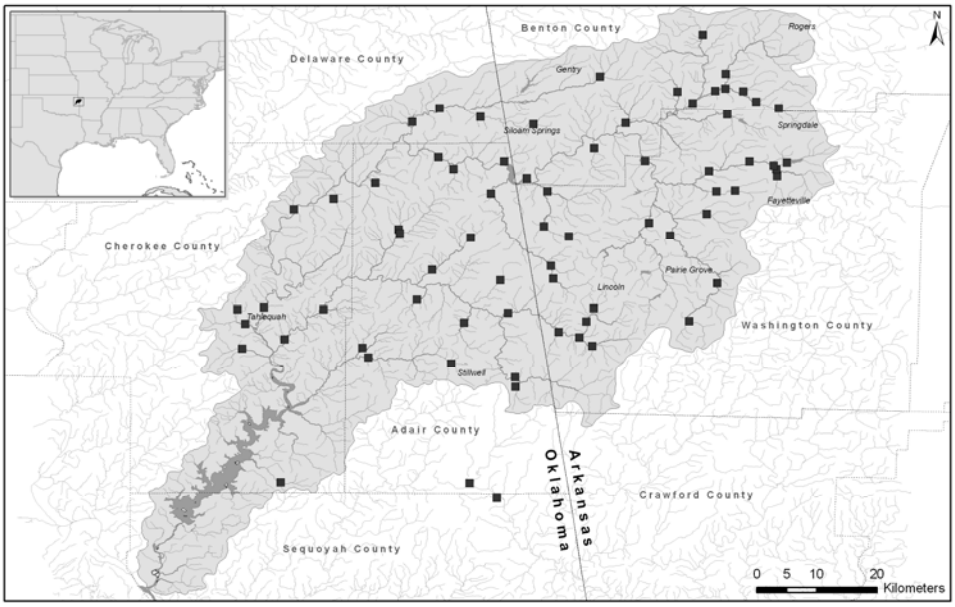
Benton County
Rogers
N
Delaware County
Gentry
Siloam Springs
Springdale
Fayetteville
Cherokee County
Pairie Grove
Lincoln
Tahlequah
Washington County
Stilwell
Adair County
Arkansas
Oklahoma
Crawford County
Sequoyah County
0 5 10 20
Kilometers

Figure 2

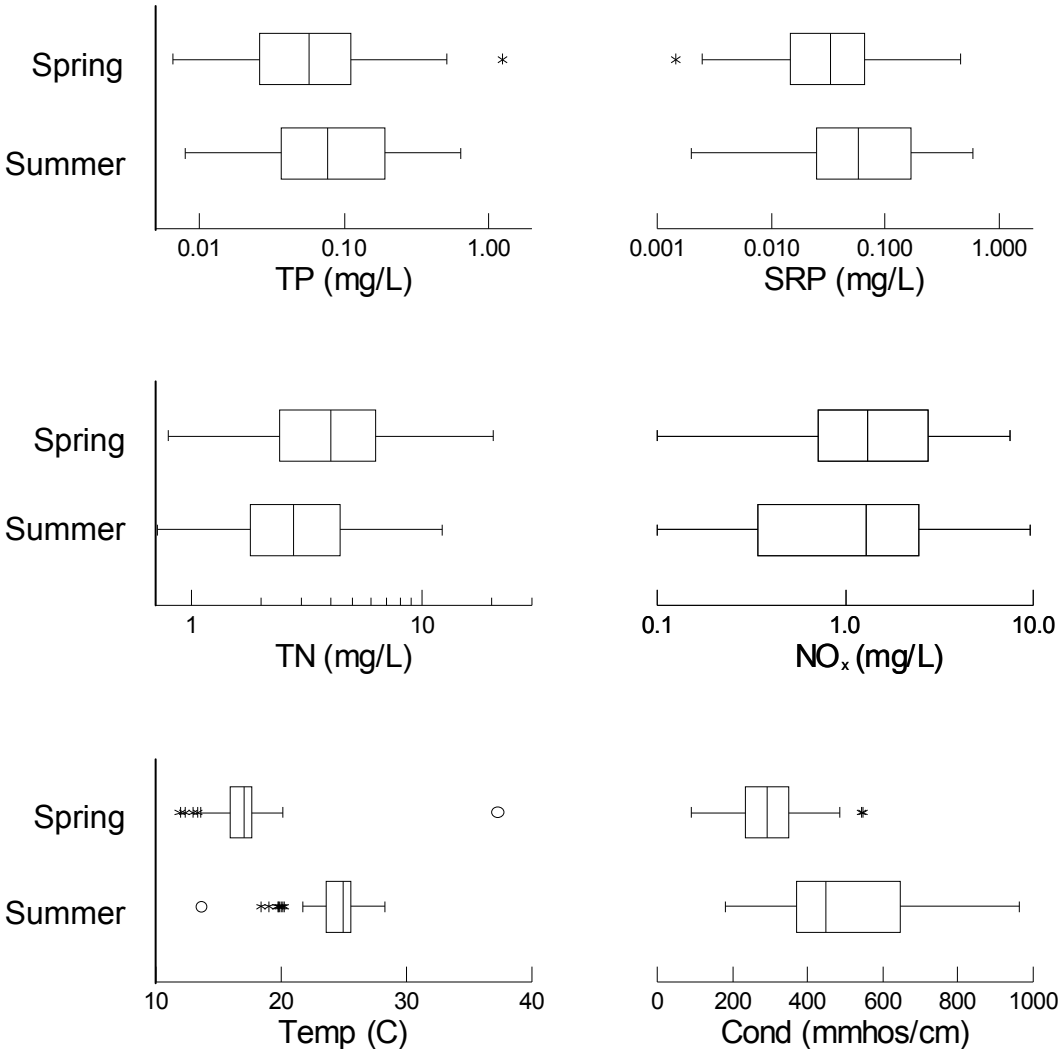
Spring
Summer
0.01
0.10
1.00
TP (mg/L)
0.001
0.010
0.100
1.000
SRP (mg/L)
Spring
Summer
1
10
TN (mg/L)
0.1
1.0
10.0
NOx (mg/L)
Spring
Summer
10
20
30
40
Temp (C)
0
200
400
600
800
1000
Cond (mmhos/cm)

Figure 3

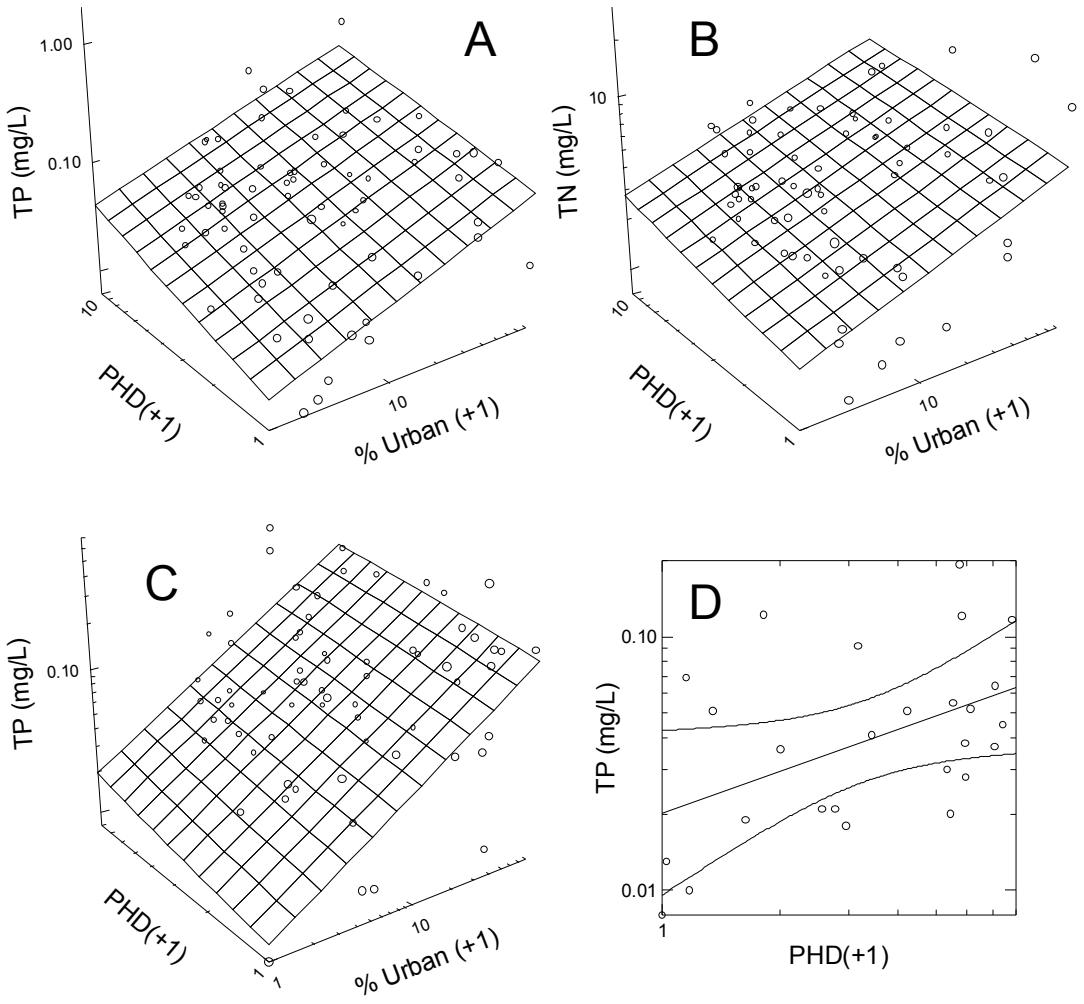
A
TP (mg/L)
1.00
0.10
10
PHD(+1)
1
10
% Urban (+1)
B
TN (mg/L)
10
10
PHD(+1)
1
10
% Urban (+1)
C
TP (mg/L)
0.10
PHD(+1)
1
1
10
% Urban (+1)
D
TP (mg/L)
0.10
0.01
1
PHD(+1)

Figure 4

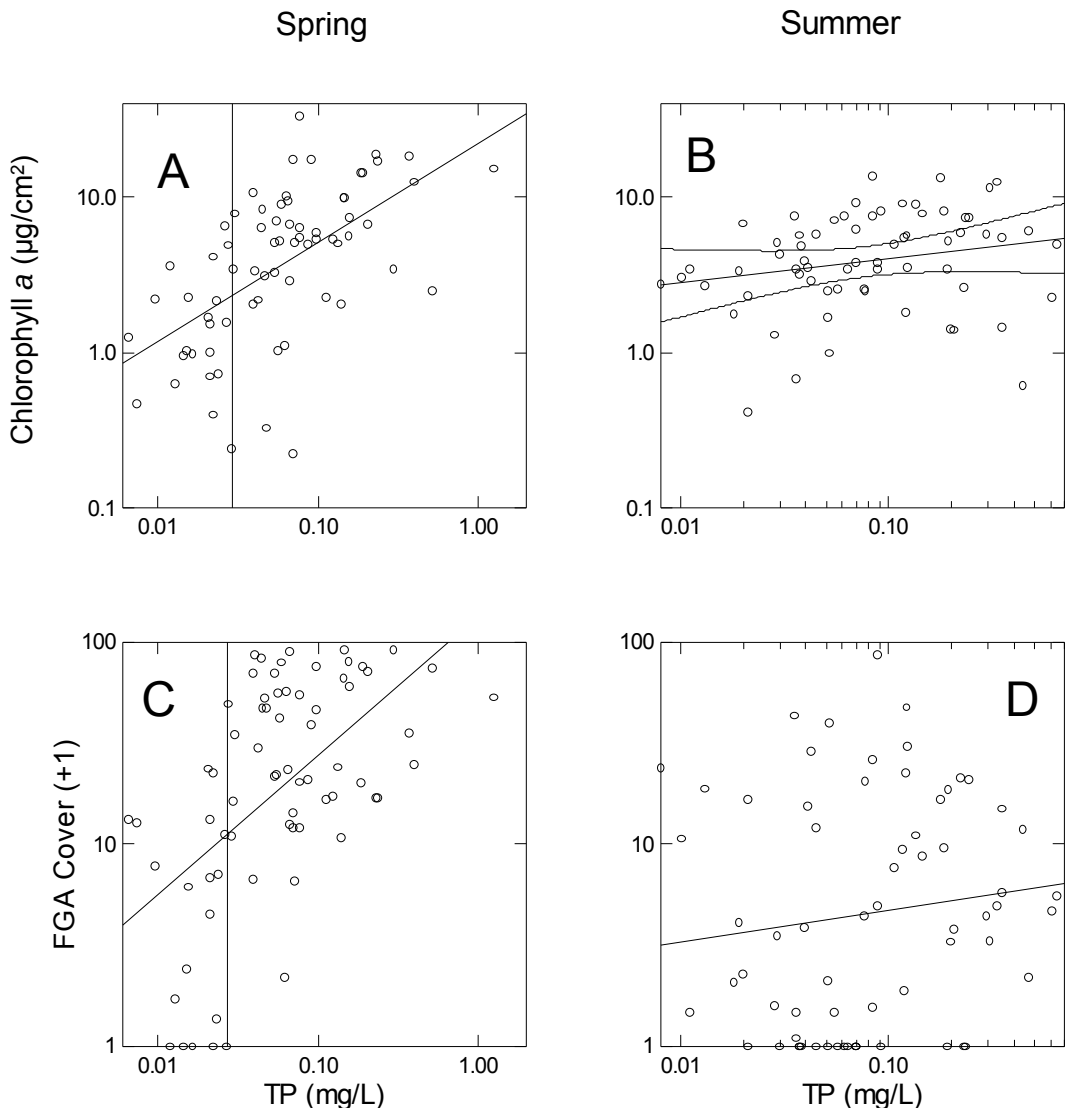
Spring
Summer
A
B
C
D
Chlorophyll a (µg/cm²)
FGA Cover (+1)
TP (mg/L)
TP (mg/L)
10.0
1.0
0.1
100
10
1
0.01
0.10
1.00

Figure 5

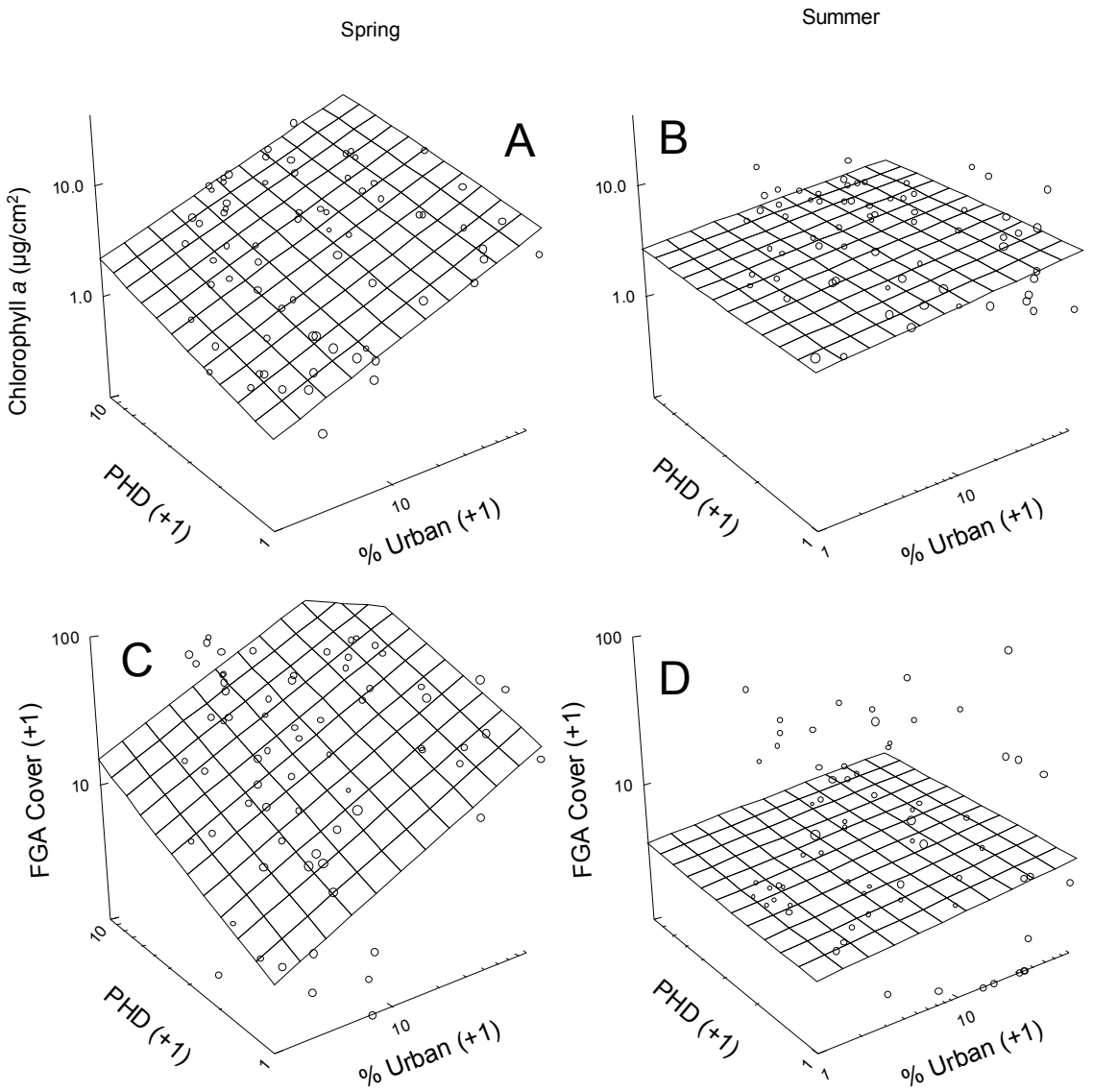
Spring
Summer
A
B
C
D
Chlorophyll a (µg/cm2)
10.0
1.0
FGA Cover (+1)
100
10
PHD (+1)
% Urban (+1)
1
10

Figure 6

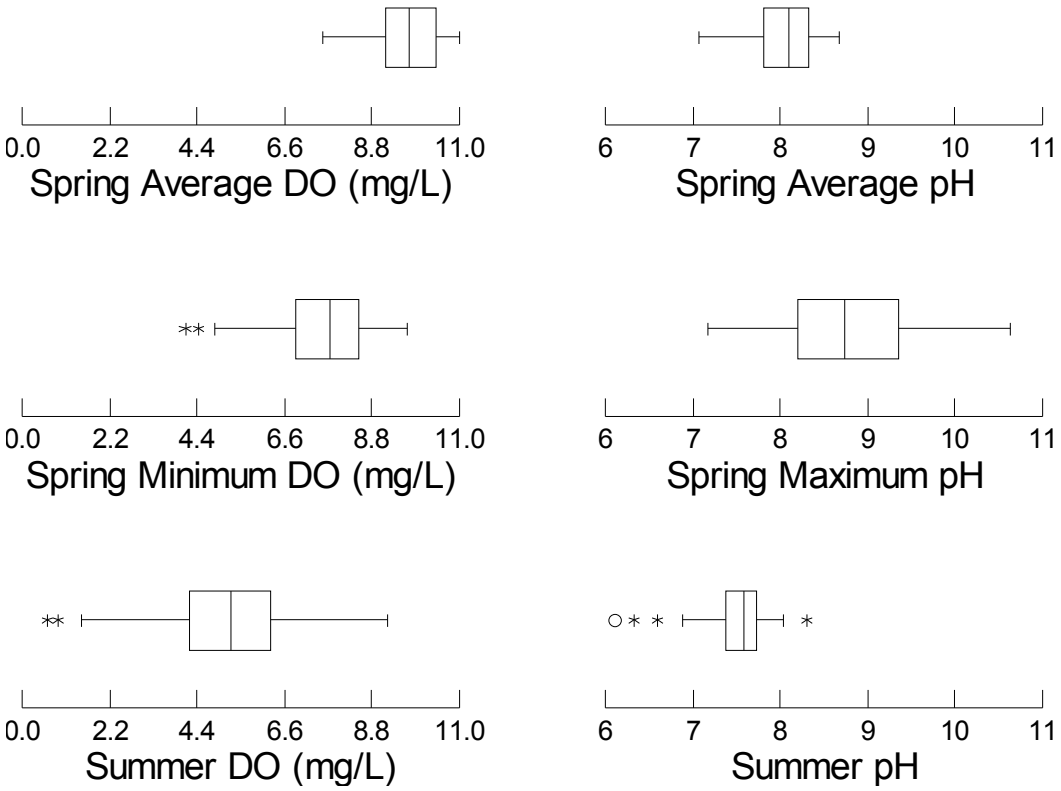
0.0
2.2
4.4
6.6
8.8
11.0
Spring Average DO (mg/L)
6
7
8
9
10
11
Spring Average pH
Spring Minimum DO (mg/L)
Spring Maximum pH
Summer DO (mg/L)
Summer pH

Figure 7

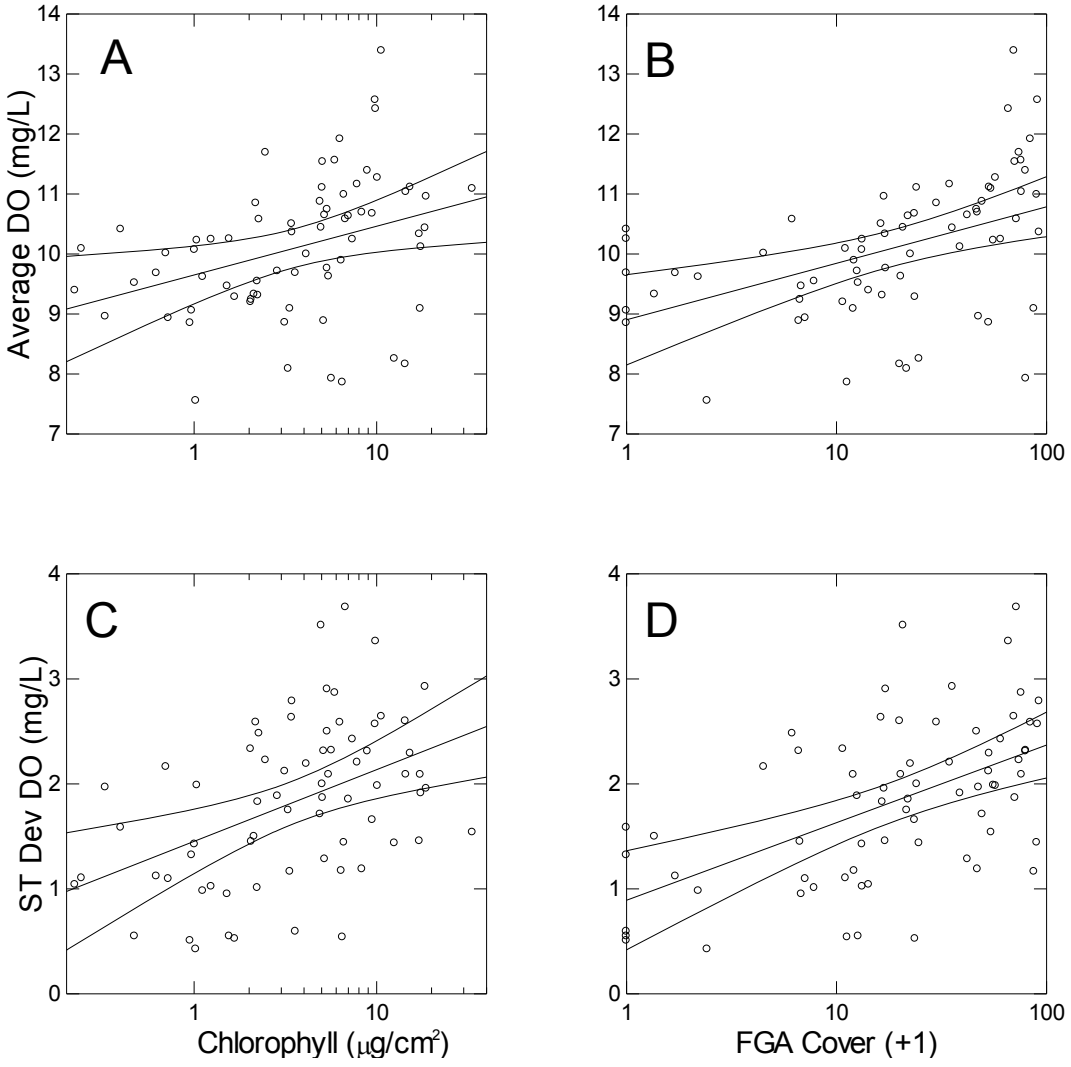
A
B
C
D
Average DO (mg/L)
ST Dev DO (mg/L)
Chlorophyll (μg/cm²)
FGA Cover (+1)
14
13
12
11
10
9
8
7
4
3
2
1
0
1
10
100

Figure 8

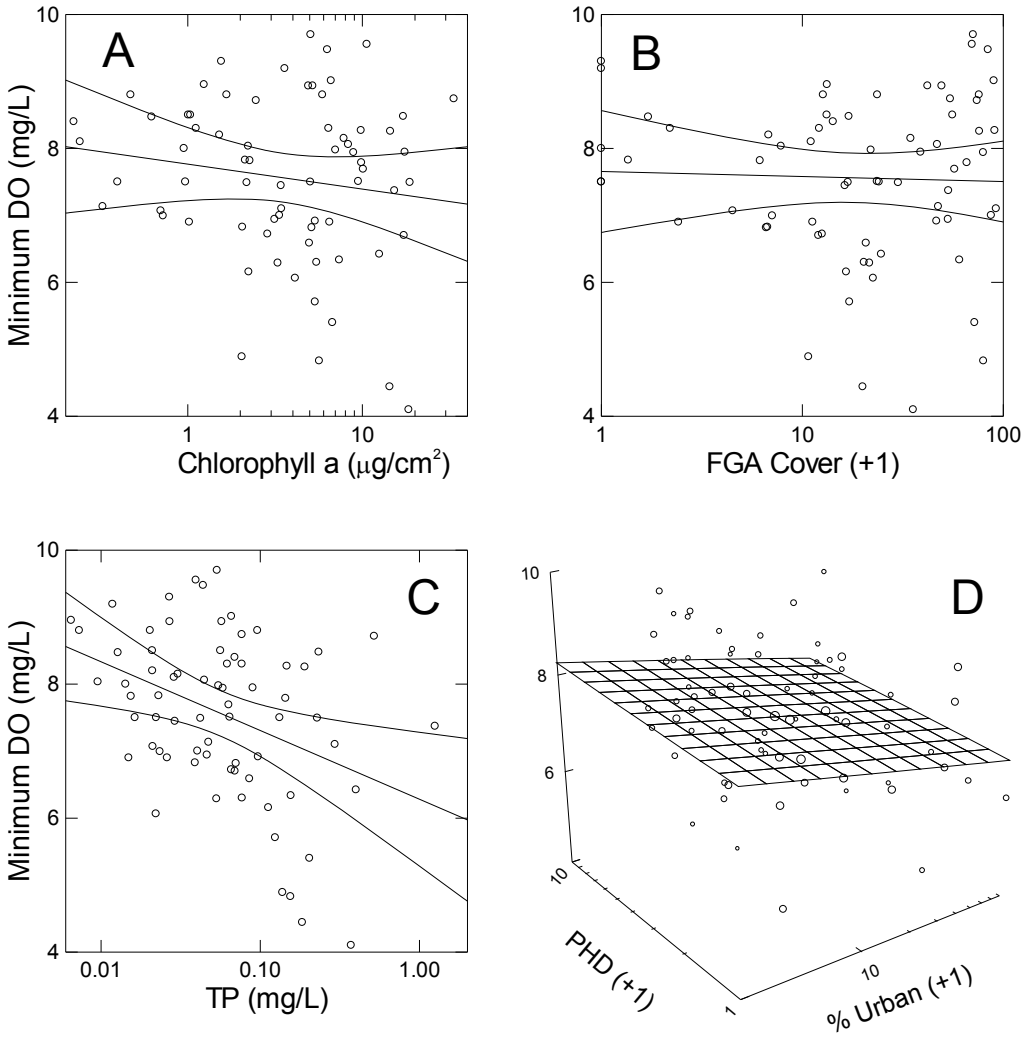
A
B
C
D
Minimum DO (mg/L)
Chlorophyll a (μg/cm²)
FGA Cover (+1)
TP (mg/L)
PHD (+1)
% Urban (+1)
10
8
6
4
1
10
100
0.01
0.10
1.00

Figure 9

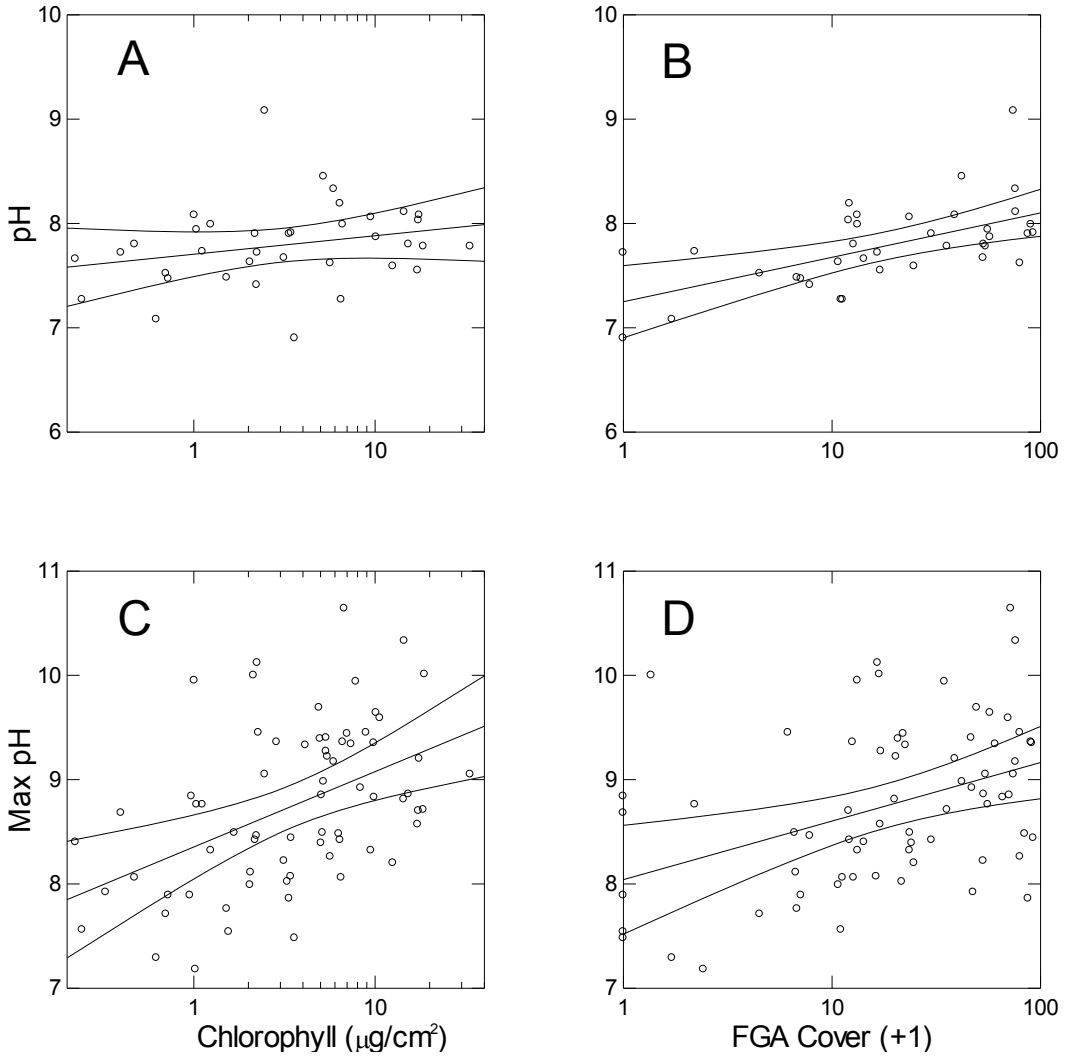
A
B
C
D
pH
Max pH
Chlorophyll (μg/cm²)
FGA Cover (+1)
10
9
8
7
6
11
1
10
100

Figure 10

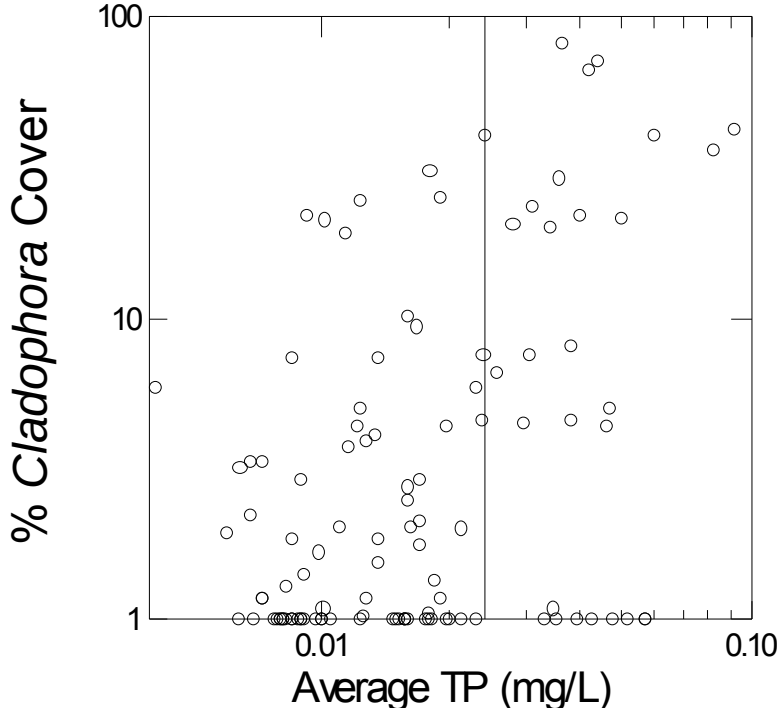

Figure 11

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The effects of plant growth on stream invertebrate communities during low flow: a conceptual model

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PERSPECTIVES

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The effects of plant growth on stream invertebrate communities during low flow: a conceptual model

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National Institute of Water and Atmospheric Research Ltd, P.O. Box 8602, Riccarton, New Zealand

T. Riis[2]
Department of Biological Sciences, Aarhus University, Ole Worms Allé, Building 1135, 8000 Århus C, Denmark

Abstract. Few studies have examined the effects of low flows on stream biota—a paradoxical situation considering the mounting pressure on streams as a result of increased human abstraction. We present a conceptual model describing processes operating in rivers during low flows and argue that such processes reflect both direct effects of reduced flow on benthic invertebrates and indirect effects of enhanced plant growth occurring during such periods. Our model suggests that the longer the duration of low flow, the more the plant community will change and, in turn, the more habitat quality will change, with consequences to benthic invertebrate communities and higher trophic levels. A fundamental part of the model is the recognition of different stream types, based on a habitat-template matrix of resource supply, interflood velocity, and substrate stability during flood events. This habitat template results in 12 stream types, each of which supports specific plant communities that set the antecedent plant conditions prior to a low-flow period. During low flows, hydraulic factors interact with the antecedent plant communities, which undergo specific responses. For example, in low-nutrient streams with gravel substrates and dominated by diatoms, low-flow conditions will cause little or no change to the antecedent plant community because this plant community is structured by top-down grazing pressure. Consequently, benthic invertebrate composition will not change. In contrast, cover and biomass of filamentous green algae in higher-nutrient gravel-bed streams will increase during low-flow events, and this increase will affect habitat suitability for invertebrates over and above those caused solely by hydraulic changes. The greatest changes to invertebrate communities are expected in macrophyte-dominated streams because these plants change instream habitat conditions the most during extended periods of low flow. Therefore, current flow-management techniques must consider the type of plant communities that exist prior to low flows and be cognizant of that fact that these plant communities can have dramatic influences on benthic communities during low-flow periods.

Key words: low flows, plant communities, diatoms, filamentous green algae, cyanobacteria, macrophytes, bryophytes, invertebrates, habitat change, conceptual model.

The importance of hydrology in influencing the composition and structure of stream biota has been increasingly recognized over the last 2 decades. In particular, much attention has focused on how aspects of high-flow regimes, such as flood frequency and intensity, affect biomass, abundance, and composition of biota, such as algae (Biggs and Close 1989, Biggs

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[2] tenna.riis@biology.au.dk

1995), macrophytes (Muotka and Virtanen 1995, Suren and Duncan 1999, Riis and Biggs 2001, 2003), and invertebrates (Scarsbrook and Townsend 1993, Gjerlov et al. 2003, Lamouroux et al. 2004). In contrast, fewer studies have examined the effects of low flows on stream biota (see review by Dewson et al. 2007). This knowledge gap is surprising considering that floods are stochastic events mostly outside human control, whereas low flows are a pervasive pressure facing streams and frequently result from human activities, such as abstraction of surface or ground water for irrigation, stock water, or human consumption (Baron et al. 2002, Poff et al. 2003, Petts et al. 2006). Low flows also are often defining features of rivers below hydroelectricity dams because these rivers are characterized by highly irregular and spiky hydrographs (Young et al. 2004). Predicted large-scale climatic changes also will almost certainly increase the frequency, duration, and magnitude of low flows (e.g., Cooper et al. 1995, Christensen and Christensen 2002, IPCC 2007).

Because of increased pressures to surface waters, managers are faced with the complex task of balancing in-stream and out-of-stream water needs. One way of managing these often conflicting demands is to set minimum flows (Baron et al. 2002, Poff et al. 2003), based on 3 common techniques: 1) historical analyses of hydrographs, 2) changes in hydraulic parameters with flow, 3) hydraulic-habitat-based methods. The latter approach, also known as Instream Flow Incremental Methodology (IFIM), is the most complex but arguably the most defensible approach because it links known habitat preferences (depth, velocity, and substrate size) of selected biota to changes in weighted usable area in response to flow changes (Bovee 1982, Jowett 1997). This approach is based on predetermined hydraulic and substrate size preferences of different taxa, and is considered one of the most sophisticated and widely used modeling approaches available (Richter et al. 1997). A common aim of setting minimum flows is to protect particular values deemed important by society. Such values most often include (but are not limited to) provision of habitat for maintenance of healthy salmonid populations.

The IFIM approach allows for detailed assessment of changes to the weighted useable area of a channel as flows decrease, but it does not consider how these changes might be influenced by other biological processes, such as plant growth, that occur in streams. Biological processes can influence in-stream habitat conditions (and thus, weighted usable area) over and above the influence caused by changes in hydraulics. Moreover, no 2 rivers are alike, and each supports unique biotic assemblages that might respond differently to low-flow events. Thus, river managers must be aware that dissimilar processes might operate in different streams during low flows, and that the nature and extent of these processes will vary among streams.

We present a conceptual model describing the interaction between physical conditions and plant growth and development in streams and how this plant growth influences other ecosystem components, such as benthic invertebrates. Our conceptual model is based on a habitat template describing the dominant plant community in a particular stream under normal flow conditions in combination with resource supply, interflood velocity, and substrate stability during flood. The interaction of these variables produces specific plant communities that result in specific antecedent conditions to a low-flow period. For the purposes of this paper, our focus is primarily on patch-scale habitats in unshaded perennial streams. In heavily shaded forested streams, aquatic plant growth is constrained by insufficient light (e.g., Keithan and Lowe 1985). In these systems, reductions in flow will not lead to enhanced plant growth, and any changes in the benthic invertebrate community will be the result of direct changes in stream hydraulics.

If the aim of resource managers is to minimize potential adverse effect of low flows, we contend that habitat changes caused by the indirect effects of reduced flow on aquatic plant growth must be considered as seriously as the direct effects of low flow on stream hydraulics. Because plant-induced changes to habitat conditions influence higher trophic levels, our conceptual model represents a useful complement to the more traditional IFIM approaches currently used to set minimum flows and predict the responses of biota to low flows. Our model might, for example, show that higher minimum flows than those derived from IFIM analyses are required in streams with a propensity for filamentous green algal (FGA) blooms if certain components of the fauna are to be protected. Such higher flows might minimize the chances of FGA blooms occurring. Alternatively, our model might suggest that flows lower than those derived from a purely hydrologically based IFIM approach are indeed sufficient to protect invertebrate communities in diatom-dominated streams.

Conceptual Low-Flow Model

Our model shows how the effects of low flows on stream invertebrate communities are controlled by direct hydraulic influences on benthic invertebrates

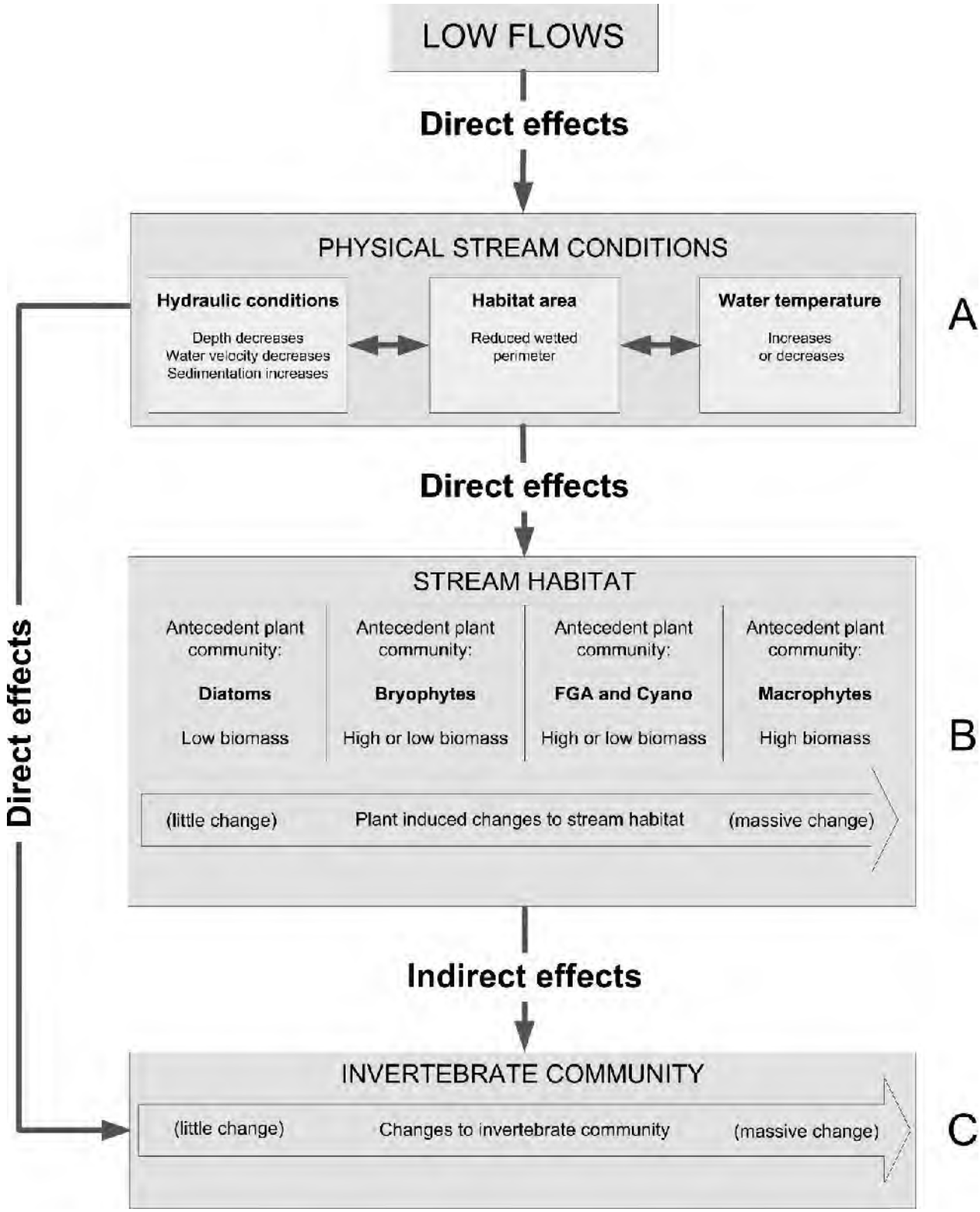

FIG. 1. Conceptual model describing processes operating during low flows in streams. Changes to the physical conditions in a stream (A) will have direct effects on the benthic invertebrate community (arrow from A to C). Changes in physical conditions also will have a direct effect on stream biotic habitat as a result of changes to the antecedent plant community (B). These changes will then have indirect effects on the benthic invertebrate community (C). FGA = filamentous green algae, Cyano = Cyanobacteria.

and by changes to in-stream plant communities (Fig. 1A–C). The model considers 4 different antecedent plant communities that occur in streams and how these plants influence stream habitat conditions during low flows. In the model, we assume that low flows will enhance plant biomass through changes to hydraulics, light, and temperature conditions and that these effects will be dependent on stream type.

A precise definition of what constitutes a *low flow* does not exist (Smakhtin 2001), but the World Meteorological Organization defines low flow as the "flow of water in a stream during prolonged dry weather" (WMO 1992). Flow statistics, such as the lowest 7-d average flow that occurs on average once every 10 y (7Q10; USEPA 1997), the 7-d mean annual low flow (Q_{7day}; Ministry for the Environment 2001), the flow equaled or exceeded 95% of the time (Q_{95}; Arthington et al. 2003), or flows at least 20% below hydrological base flow (Gordon et al. 1992) are commonly used to describe low flows. Our model is not based on a specific hydrological low-flow threshold, but describes processes that occur at any time when a river is in a continued flow recession. During these hydrologically quiescent times, plant biomass accrual processes dominate because loss processes associated with high velocities and shear stress, substrate movement, and abrasion do not occur. Our model also focuses on changes only to the benthic invertebrate communities because these animals occupy the same spatial scale as plants and so are likely to be affected by changes to these communities.

The model shows how invertebrate communities are influenced by direct and indirect effects that occur during a low-flow period (Fig. 1A–C). Direct effects occur with reductions in flow depth, velocity, and wetted perimeter, which change the usable hydraulic habitat area for invertebrates (Dewson et al. 2007) (Fig. 1A, C). Such changes (based on IFIM predictions) can be beneficial or detrimental depending on species-specific hydraulic habitat preference curves and the extent to which flow reduction affects weighted usable area of habitat (Jowett 1997, Milhous 1998). Other direct effects of reduced flows include changes to temperature regimes (Fig. 1A) that can affect invertebrates if lethal temperatures are reached (e.g., Quinn et al. 1994). Direct effects as a result of reductions in habitat area also might include increases in competitive interactions among species as a result of increases in relative abundance (Dewson et al. 2007).

Indirect effects of low flows on invertebrate communities occur as a result of alterations to habitat quality caused by plant communities, over and above actual changes to hydraulic conditions (Fig. 1B, C). These indirect changes are a direct result of natural biomass accrual processes exhibited by plants during low flows. This accrual leads to alterations to the physical environment that could, in turn, influence invertebrates. For example, increased sedimentation in macrophyte beds (Cotton et al. 2006, Wharton et al. 2006) is likely to reduce habitat suitability for aquatic insects that prefer larger substrates and also could lead to nighttime anoxia as a result of bacterial respiration within the sediments.

Our model hypothesizes that the greatest effect of low flows on invertebrate communities will occur where plant communities change the most as a result of natural biomass accrual. In diatom-dominated streams, the effects of low flows on invertebrate communities will be relatively minor. Stability of invertebrate abundance and community composition in diatom-dominated streams during prolonged low flows in streams has been observed repeatedly (Caruso 2002, Suren et al. 2003a, James et al. 2008, James and Suren 2009). Thus, the invertebrate community will persist relatively unchanged as long as water remains in the channel (Fig. 1B). In streams that become dominated by filamentous green algae (FGA) during low flows, larger changes to invertebrate communities are predicted following development of high FGA biomass. The largest potential change to invertebrate communities would occur in lowland macrophyte-dominated streams. Under low flows, macrophyte proliferations can occur, with subsequent reductions in velocity and increases in sedimentation (Cotton et al. 2006, Wharton et al. 2006). Densities of invertebrate taxa associated with gravel substrates will decline as these habitats become colonized by macrophytes, whereas densities of epiphytic taxa might increase. Further reductions in flow could cause macrophyte growth that chokes the entire stream bed and this effect can lead to low dissolved O_2 levels at night, which would stress any invertebrate taxa intolerant to slow-flowing, anoxic environments. Under this scenario, the fauna is expected to become dominated by invertebrate taxa, such as midges, snails, or worms, that are tolerant of such conditions. Bryophyte-dominated streams will undergo little or no change during low-flow periods because the growth of these plants is so slow (e.g., Englund 1991) that instream habitat conditions will not change over a period of a few weeks. However, under prolonged low flows that last months, or below hydro-impoundments on large rivers, bryophytes might be exposed to the air and subsequently die because many are not tolerant of desiccation (Glime 1971, Kimmerer and Allen 1982). The resultant loss of these plants would have potentially large consequences on the bryophyte-dwelling invertebrate community.

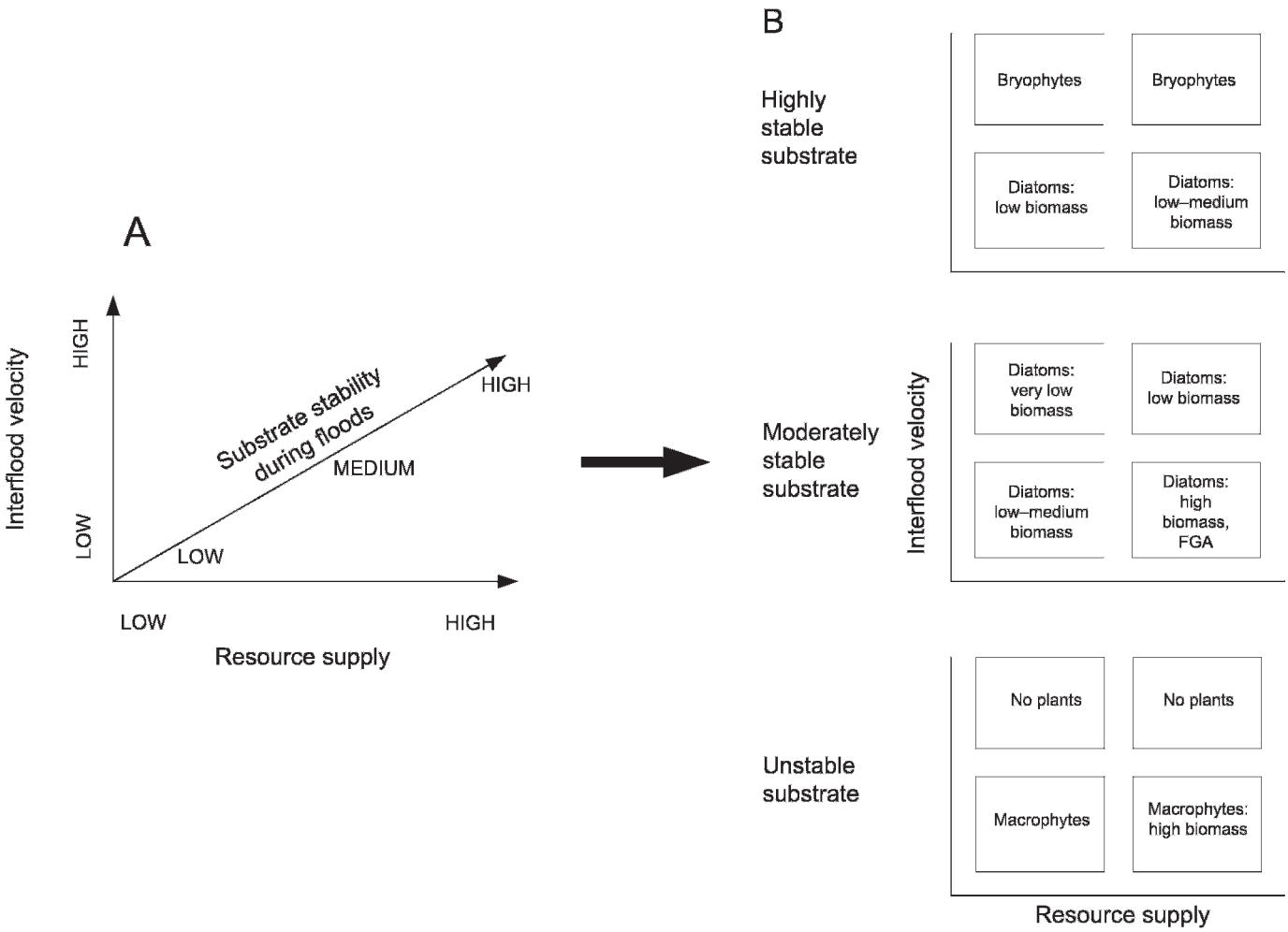

FIG. 2. Aquatic plant community types in relation to stream physical condition. A.—The 3 components of stream physical condition (resource supply, interflood velocity, and substrate stability) interact to produce 12 potential stream types. B.—Each cell represents one of the 12 potential stream types and shows the expected antecedent plant community (or average characteristic plant community) before the onset of a low-flow period. FGA = filamentous green algae.

The Importance of Stream Types

We assert that the indirect effects of low flows on instream habitats will be determined largely by the antecedent plant community that develops in the absence of floods (Fig. 1B, C). Many interlinked factors are responsible for controlling aquatic vegetation (Biggs 1996, 2000, Reeves et al. 2004), but we suggest that a 3-dimensional habitat template can be developed to predict the antecedent plant communities (Fig. 2A). The *x*-axis of our habitat template represents resource supply, ranging from oligotrophic systems (where nutrients and light might limit plant growth) to eutrophic streams (where nutrients and light do not). The *y*-axis of our habitat template represents interflood velocity, which influences both the dominant substrate size in the stream and the type of plant community (Biggs 1996). Streams with low interflood velocities will generally have smaller substrates and will be dominated by plant communities (e.g., broadleaved *Potamogeton* species) that are not resistant to high shear stresses associated with faster velocities. In contrast, streams with high interflood velocities will be dominated by bedrock or boulders and will have plant communities (e.g., bryophytes) that can tolerate high shear stress. The *z*-axis of our habitat template represents the overall substrate stability during floods. This axis ranges from fine substrates, such as sand and mud, that are constantly moving during floods to large, stable bedrock and boulders that never move during flood events.

Given 2 qualitative categories of both resource supply and interflood velocity (low and high), and 3 qualitative categories of substrate stability (low,

medium, and high), the result is a total of 12 different habitat types, 2 of which effectively will not support plants (Fig. 2B). In the 10 habitat types that can support plants, we suggest 4 different plant communities will develop: diatoms, FGA and cyanobacterial mats, macrophytes, and bryophytes. Below we describe these 4 plant communities and the habitat types where they most often dominate and explain how the plant communities have different habitat suitability for invertebrates.

Diatom communities

Diatoms will dominate in stream habitats with medium-to-high substrate stability during floods, low-to-high interflood velocity, and low-to-high nutrient supply (Stevenson et al. 1996, Biggs 2000). Such habitats are common in low-to-mid-order streams flowing from either mountain or hill catchments. In streams with moderately stable substrates, the eventual diatom biomass is controlled by a combination of resource supply and interflood velocity, with higher biomass being found in more resource-rich streams with slower velocities (Fig. 2B). The composition of diatom communities is a reflection of both interflood velocity and nutrients, with small, prostrate species such as *Cocconeis* being found in fast flowing water, and larger, stalked species, such as *Gomphoneis*, being found in faster flowing, more nutrient-rich water (Biggs and Hickey 1994). A notable characteristic of diatom-dominated habitats is the presence of often large numbers of grazing invertebrates, such as snails, mayfly, caddisfly, and midge larvae (McAuliffe 1983, Welch et al. 2000). These animals often can exert significant control of both diatom biomass and species composition (Hart 1985, Feminella et al. 1989). As a result of this grazing activity, high diatom biomass does not necessarily occur even during sustained low flows (Suren et al. 2003b, Dewson et al. 2007). Because of their relatively small size, diatoms do not usually act as physical habitat for invertebrates (e.g., Biggs 2000, Suren 2005), so they are thought to have only little influence in controlling their distributions. However, an exception to this rule is the recently discovered, invasive, stalked diatom *Didymosphenia geminata* in New Zealand rivers (Kilroy et al. 2009). *Didymosphenia geminata* can form very thick growths (up to 30 mm thick) of high biomass (up to 332 g ash-free dry mass/m^2), which are often associated with high invertebrate densities. Such high densities are attributable to a combination of increased surface area and provision of extra food in the form of *D. geminata* cells and other epiphytic diatoms (Kilroy et al. 2009).

FGA and cyanobacteria communities

Streams with a gravel substrate, low interflood velocity, and a moderate-to-high resource supply are typical of systems that can become dominated by FGA or cyanobacterial growths during periods of low flow (Borchardt 1996, Biggs 1990, Suren et al. 2003b). Such streams are typified by mid-order hill-fed rivers that rarely flood and have long interflood periods of stable flow. During this time, a gradual succession occurs from low-stature diatoms to high-stature FGA or thick cyanobacterial mats that often can form extremely high biomass and cover the entire stream bed (Stevenson et al. 1996, Suren et al. 2003b).

Both FGA and cyanobacterial mats are poor habitat for invertebrates, such as grazing mayflies and caddisflies (Quinn and Hickey 1990, Suren 2005). For example, Hart (1985) showed that the hydroptilid caddisfly *Leucotrichia* actively removed the filamentous cyanobacteria *Microcoleus* from its foraging area to encourage growths of favored diatom species. The most common invertebrates inhabiting FGA are the specialized algal piercing hydroptilid caddisflies, chironomid midges, and microcrustacea, such as ostracods and copepods (Suren et al. 2003a, Suren and Jowett 2006). FGA and cyanobacterial mats undergo autogenic sloughing when their biomass becomes too great. Sloughing is caused by senescence of older basal cells because of age and nutrient limitation (Biggs 2000). Sloughing sets the upper limits to biomass of these plants. Remnant communities following sloughing can regrow rapidly to attain high biomass values, often within weeks (AMS, personal observation).

Macrophyte communities

We define macrophytes as large rooted plants and do not include bryophytes and FGA. Macrophyte habitats are common in lowland rivers, which often have sand and gravel substrates. Despite the fine substrates, substrate stability is relatively high because floods are uncommon and interflood velocities low. For example, chalk rivers in the UK have gravel/cobble substrates and, being groundwater dominated, exhibit less flow variability than streams fed by surface water. These streams support *Ranunculus* plants that can cover up to 80% of the stream bed (Cotton et al. 2006, Wharton et al. 2006) because infrequent floods allow accumulation of fine sediments beneath the macrophytes, thereby facilitating further spread of the plant patch. Riis and Biggs (2003) found that macrophytes were absent from New Zealand streams with >13 high-flow disturbances/y. Prolonged periods of hydrological stability (10–20 wk)

also are required for macrophyte propagules (e.g., seeds, stem fragments) to settle into the stream bed and establish cover >1% of the stream bed (Riis et al. 2004). Macrophytes also require a long interflood period to develop to maximum possible biomass, so even a small number of floods within a year will decrease macrophyte abundance compared to abundance in streams without flood disturbances. Macrophytes require substrates that allow rooting in the stream bed, and thus, are absent from streams with bedrock substrate. Macrophyte cover also is regulated by nutrients and inorganic C, such that higher macrophyte biomass will develop in streams with a higher resource supply.

Macrophytes are important habitats for invertebrates (Rooke 1984, Collier et al. 1999, Cheruvelil et al. 2002) and provide them with shelter from currents, which are reduced within a macrophyte patch (Gregg and Rose 1985, Sand-Jensen and Pedersen 1999, Green 2005). Macrophytes also provide habitat for suspension-feeding blackfly larvae (Diptera:Simuliidae), which can be found at very high densities in regions of higher flow velocity on the outermost parts of the beds (Ladle et al. 1972, Wharton et al. 2006). These animals produce a large number of fecal pellets each day. The pellets are deposited in downstream patches of plants and have implications for C and nutrient dynamics in streams (Cotton et al. 2006, Wharton et al. 2006). Macrophytes also provide food for invertebrates and are consumed both as fresh tissue by some specialized invertebrates and, more generally, as detritus (Newman 1991, Lodge 1991). Given these diverse roles in stream ecosystems, macrophytes should be regarded as significant biogenic components of stream ecosystems.

Bryophyte communities

Aquatic bryophyte communities are restricted mostly to large, stable substrates in low-order mountain streams where interflood velocity is high. For aquatic bryophytes, disturbance occurs mainly in the form of substrate movement (Muotka and Virtanen 1995, Suren and Duncan 1999), which is important because of the slow growth and colonization rates of these plants. Their presence appears generally unrelated to nutrient supply. They are typically found in low-nutrient streams (Bowden et al. 1997, Suren 1993), but they also occur in highly enriched streams receiving organic effluent (e.g., Kelley and Huntley 1987). Many aquatic bryophytes are intolerant of desiccation (Craw 1976, Vitt and Glime 1984) and are found only on completely submerged substrates or substrates that are continually wetted by spray.

Aquatic bryophytes are very important habitats for stream invertebrates and support considerably higher densities than adjacent bare gravels (Percival and Whitehead 1929, Suren 1991, Bowden et al. 1997). Enhancement of invertebrate densities in bryophytes might be the result of accumulation of detritus and periphyton (Suren 1992) consequent to lower water velocities within bryophyte mats (Nikora et al. 1998) and the greatly expanded surface areas that these plants provide.

Interactions Between Low Flows and Plants

A central facet of our model is work by Grime (1979) and Biggs (1996), who emphasized that plant biomass in streams is a combination of plant growth and loss processes. The outcomes of these interactions are major driving factors influencing benthic invertebrate communities during times of low flow. Antecedent plant communities (a product of our 3-dimensional habitat template) interact with the hydraulic conditions experienced during low flows to produce a specific plant response. This response is a trajectory of growth and development that indirectly affects other aspects of stream ecosystems—in this case, the benthic invertebrate communities. Such mechanisms were first postulated by Suren et al. (2003a), who found that invertebrate communities during summer low flows changed more in a stream that became dominated by FGA than in a stream that remained diatom-dominated, despite the fact that relative flows were lower in the latter.

During low flows, hydraulic factors, physicochemical conditions, and grazing pressure change with corresponding effects on plant communities (Table 1). In diatom-dominated habitats, little or no successional change will occur, whereas in FGA- or macrophyte-dominated habitats, plant communities might change considerably as a result of succession or plant growth. The effects of variables known to influence biomass accrual during low flows in the 4 different plant communities and their habitat types are summarized in Table 1.

Based on our knowledge of the processes of biomass accrual that dominate during hydrologically quiescent times (in the absence of floods), we hypothesize that, in diatom-dominated habitats, top-down grazing generally will maintain algal biomass at low levels, and biomass will not accrue greatly (Feminella and Hawkins 1995; Fig. 3A). For example, Suren et al. (2003b) found that chlorophyll *a* levels in 5 foothill-fed rivers draining the east coast of New Zealand's South Island remained low over 3 mo in summer, and varied from 0.1 to only 3.5× the summer

TABLE 1. Summary of variables known to change during periods of low flow and their predicted effects on the different plant communities. FGA = filamentous green algae. Superscripts refer to literature sources (listed below this table).

Variable	Low-flow response	Diatoms	FGA	Macrophytes	Bryophytes
Velocity	Decrease	Growth might increase in eutrophic streams and decrease in oligotrophic streams[1, 2]	Growth might increase in eutrophic streams and decrease in oligotrophic streams[1, 2]	Cover might increase into areas where flow was previously too fast[3]	Little change because of slow growth rates[4]
Depth	Decrease	No effect as long as plants remain wet	No effect as long as plants remain wet	Plants might be exposed, resulting in reduced cover of aquatic plants and increased cover of terrestrial plants	Plants might be exposed, reducing their cover
Wetted perimeter	Decrease	No effect as long as plants remain wet	No effect as long as plants remain wet	Aquatic plant cover might decrease and terrestrial plants invade the channel	Plants might be exposed, reducing their cover
Water temperature	Increase[5, 6] in streams dominated by surface run-off OR might decrease as flows become dominated by groundwater inputs	Increased growth rates at increased temperature[7, 8]; possible change in life-cycle phenology[9, 10]	Increased growth rates at increased temperature[7, 8]; possible change in life-cycle phenology[9, 10]	Possible increased growth rates at increased temperature[7, 8]; possible change in life-cycle phenology[9, 10]	Possible increased growth rates at increased temperature[7, 8]; possible change in life-cycle phenology[9, 10]
Nutrients	Possible increase with lower dilution[11, 12, 13] OR decrease with less runoff[12, 14]	Possible increase with more nutrients, if nutrients are limiting	Possible increase with more nutrients, if nutrients are limiting	Little change because nutrients can be mainly absorbed from the roots[15]	Might show little change because of very low growth rates (but see [16])
Grazing pressure (via increased invertebrate density)	Possible increase with decreasing wetted perimeter[17]	Possible decrease, or maintenance of similar biomass[18, 19, 20]	Grazing pressure unlikely to lower FGA biomass[21], which might increase with increased duration of low flow	Grazing pressure unlikely to lower macrophyte biomass[22, 23], which might increase with increased duration of low flow	Grazing pressure unlikely to lower bryophyte biomass because of low palatability[24]

[1] Westlake 1975, [2] Madsen and Søndergaard 1983, [3] Sand-Jensen and Mebus 1996, [4] Englund 1991, [5] Cowx et al. 1984, [6] Cazaubon and Giudicelli 1999, [7] Glime and Acton 1979, [8] DeNicola 1996, [9] Hutchinson 1975, [10] Spencer et al. 2000, [11] Rader and Belish 1997, [12] Caruso 2002, [13] Dewson et al. 2003, [14] Dahm et al. 2003, [15] Carignan 1982, [16] Bowden et al. 1994, [17] Dewson et al. 2007, [18] Gregory 1983, [19] Welch et al. 2000, [20] Suren et al. 2003a, [21] Suren 2005, [22] Jacobsen and Sand-Jensen 1994, [23] Lodge 1991, [24] Bowden et al. 1997.

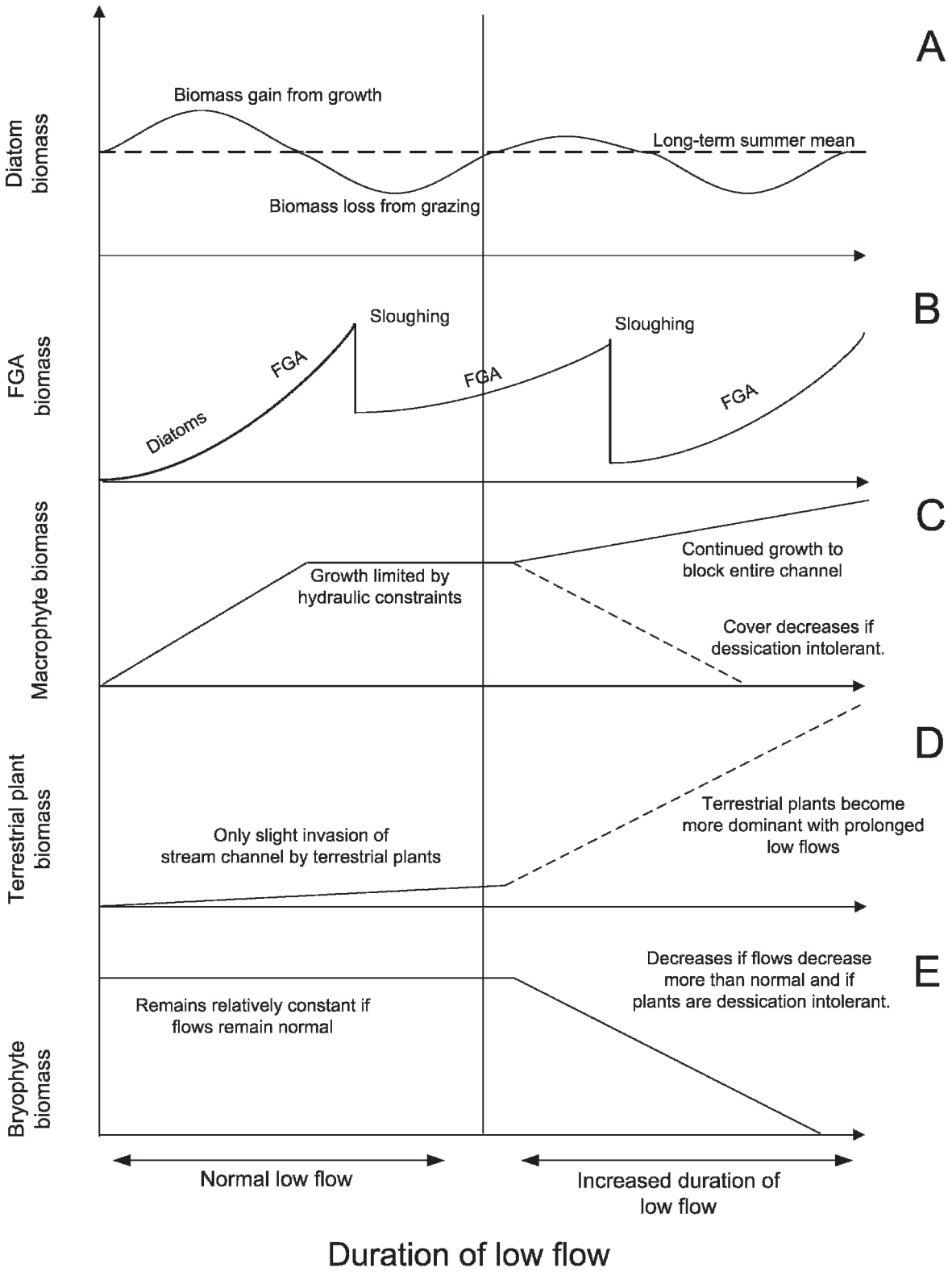

FIG. 3. Suggested changes to the biomass of diatoms (A), filamentous green algae (FGA) and cyanobacteria (B), macrophytes (C), terrestrial plant biomass (D), and bryophytes (E) as the duration of low flows increases in streams.

median chlorophyll *a* concentration, despite the fact that the rivers were near all-time record low flows. These rivers all had low nutrient levels and relatively high densities of grazing invertebrates. The algal communities were all dominated by diatoms, and the composition differed little over time. These results suggest that during summer low flows, periphyton biomass was regulated by a combination of top-down grazing pressure and potential nutrient limitation.

FGA or cyanobacteria-dominated habitats will experience greater changes to instream conditions during low flows than diatom-dominated habitats, especially when these plants cover the entire stream bed. Under such conditions, the substrate surface will change from cobbles and gravels to thick algae. Thus, although the hydraulic conditions and substrate size might not have changed, overall habitat suitability for invertebrates, such as mayflies or caddisflies would change as these thick algal communities develop (Hart 1985, Suren 2005). The natural propensity of mature FGA or cyanobacterial communities to slough will result in biomass fluctuations as algal biomass increases to a certain level, then decreases rapidly as material sloughs (Fig. 3B). The extent of biomass loss is unlikely to be consistent, and the result will be a heterogeneous habitat with patches of very different algal biomasses. Habitat conditions for invertebrates that avoid these high biomass blooms (e.g., mayflies) might decline during mat development, then improve briefly following sloughing, only to decline again as FGA biomass recovers. In contrast, autogenic sloughing of FGA or cyanobacteria will reduce densities of taxa, such as hydroptilid caddisflies, midges, and microcrustacea that favor this plant community. If low flows continue, the invertebrate communities will become dominated more by FGA tolerant species. In this case, sensitive invertebrate species will be faced with a multiple press disturbance (sensu Lake 2000) in that they will have to deal with both decreasing depths and velocities and deteriorating habitat conditions as algal biomass increases.

The effects of low flows in streams dominated by FGA or cyanobacteria might be dramatic, but we suggest that the greatest effects of low flows will be in streams dominated by macrophytes. In the absence of floods, macrophytes often grow across stream channels and reduce the area of free-flowing water (Fig. 3C). This constriction will increase local water velocities and prevent further growth into these areas (Champion and Tanner 2000, Wharton et al 2006). Considerable quantities of fine sediments can accumulate in and downstream from macrophyte beds, and this accumulation is likely to influence invertebrate communities. Continued low flows and sediment accumulation also is likely to lead to macrophyte colonization of previously fast flowing areas, and might eventually choke the entire stream bed. Terrestrial plants also might colonize the stream bed, especially in areas with increased sedimentation and reduced wetted perimeter (Fig. 3D). Habitat conditions for aquatic invertebrates under such a scenario would decline even further, and the system could slowly revert to one typical of slow-flowing lentic environments. Furthermore, large diurnal fluctuations in dissolved O_2 ranging from 40 to 130% air saturation have been observed in macrophyte-rich lowland streams (Wilcock et al. 1998, TR, personal observation). These fluctuations might influence invertebrate distributions at the patch scale. Unlike FGA or cyanobacteria, macrophytes do not autogenically slough but are relatively long-lived. Once macrophytes occupy the entire stream channel, the invertebrate community will change from one typical of a fast-flowing community to one characteristic of a slow-flowing community.

However, the effects of excessive macrophyte growth on streams might not be all negative. Macrophytes can often help maintain water levels in streams (Champion and Tanner 2000), a potentially beneficial effect during times of severe drought. Nevertheless, this benefit is tempered by the fact that luxuriant macrophyte growths will greatly alter available habitat for many invertebrates.

The effects of low flows in bryophyte-dominated streams will depend largely upon whether bryophyte cover is reduced (Fig. 3E). If bryophyte cover remains the same, then invertebrates are likely to continue to use bryophytes as places to live, feed, and oviposit. However, if bryophyte cover declines as a result of desiccation, loss of this important habitat might cause a subsequent decline in invertebrate density. In this sense, changes to invertebrate communities will be as a result of a loss of these plants as habitat and not as a result of increased plant growth as occurs with macrophytes and FGA.

In addition to the effect of low flow on biomass and composition of the dominant vegetation type, low flow also might result in a shift from dominance of one vegetation type to another. Diatom-dominated streams might change to FGA-dominated streams if resource supply is high or if the interflood period is prolonged (Fig. 2B). Furthermore, FGA-dominated streams might change to macrophyte dominated streams if the interflood period exceeds months and if sufficient soft substrate is present for these rooted plants to become established.

Conclusions

Our model applies to all unshaded streams during low flows where plant biomass can potentially increase because of increased resource availability. Such biomass increase might lead to large changes in in-stream habitat conditions depending on the dominant plant growth. We suggest that the effects of low flows on invertebrate communities will depend on the propensity of plant biomass to accrue (or to change from one plant type to another). The greater the accrual or change in plant type, the greater the change to overall habitat conditions. This conclusion means that benthic invertebrates will be faced with in-stream changes greater than predicted by traditional IFIM-based hydraulic habitat models. We suggest that managers need to take a more holistic approach when setting flow allocations in regulated rivers and should consider direct and indirect processes occurring during low flows. Adverse effects of low flows are more easily avoided or mitigated if such processes are identified and managed. Moreover, flow management techniques are likely to be more successful if they are not applied in a similar manner across different stream types but, rather, are applied with consideration of the nature of the antecedent plant communities present in streams prior to a low-flow event and how these communities might change in-stream habitat conditions as a low-flow event progresses.

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ARE BENTHIC CYANOBACTERIA INDICATORS OF NUTRIENT ENRICHMENT? RELATIONSHIPS BETWEEN CYANOBACTERIAL ABUNDANCE AND ENVIRONMENTAL FACTORS ON THE REEF FLATS OF GUAM

Robert W. Thacker and Valerie J. Paul

ABSTRACT

While benthic, filamentous cyanobacteria can be common on coral reefs, the factors influencing their distribution are poorly understood. Cyanobacterial blooms, like macroalgal blooms, may result from a combination of coastal eutrophication and reduced herbivory. Because benthic cyanobacteria are unpalatable to generalist herbivores, there may be little top-down control of cyanobacterial abundance. Since bottom-up factors may exert more influence on cyanobacterial populations, we hypothesized that high cyanobacterial abundance may be an indicator of high nutrient availability. In addition, cyanobacteria may compete with macroalgae for light and nutrients. Thus, we also hypothesized that cyanobacterial abundance may be negatively associated with macroalgal abundance. Since many strains of cyanobacteria wash ashore during periods of high wave action, we hypothesized that cyanobacterial abundance is negatively associated with wave height. We monitored cyanobacterial abundance, macroalgal abundance, nitrogen availability, phosphorus availability, salinity, and water temperature at nine reef flat locations around Guam. Average wave height for eastern and western shores, rainfall, minutes of sunshine, and wind speed were also monitored. Stepwise regression was used to determine which variable or combination of variables best explained variation in cyanobacterial abundance. Although nutrient availability was not significantly associated with cyanobacterial abundance, a positive association with macroalgal abundance explained 11.5% of the observed variation in total cyanobacterial abundance. At one site, negative relationships with macroalgal abundance and wave height explained 79.4% of the observed variation in the abundance of the cyanobacterium *Oscillatoria margaritifera*. Variation in cyanobacterial abundance can be best explained by examining individual strains of cyanobacteria, rather than by treating all cyanobacteria as a single ecological unit. Physical disturbance can be a more important influence on cyanobacterial abundance and distribution than either nutrient availability or interactions with macroalgae.

Most ecological studies of marine cyanobacteria have focused on planktonic forms (Kirk and Gilbert, 1992; Haney et al., 1995; Sellner, 1997), while benthic cyanobacteria have generally been grouped with turf or filamentous algae (Klumpp and McKinnon, 1992; Steneck and Dethier, 1994; Williams and Carpenter, 1997). Blooms of macroalgae are thought to result from a combination of nutrient enrichment associated with coastal eutrophication and decreased grazing by fishes and invertebrates due to overfishing and mass mortalities (Hughes, 1994; Littler and Littler, 1994; Lapointe, 1997). However, it is unclear whether cyanobacteria respond to these environmental factors in the same way as macroalgae (Fong and Zedler, 1993; Cowell and Botts, 1994). The nitrogen-fixing ability of some cyanobacteria may decrease their dependence on the availability of nitrogen in the water column (Larkum, 1988). Thus, nitrogen availability may not control the growth or abundance of some cyanobacteria (Cowell and Botts, 1994).

Cyanobacterial blooms are becoming more frequent around the island of Guam, and we often see mats of cyanobacteria covering thousands of square meters of reef flat and

washing up on shore (Limtiaco, 1994; Knuckles, 1995). These blooms can be single species or mixed-species assemblages, frequently containing *Oscillatoria* spp. and *Lyngbya* spp. These blooms can be unsightly for tourists and have been implicated in the death of juvenile siganids (rabbitfishes; Nagle et al., 1996). Secondary metabolites produced by benthic, marine cyanobacteria have also been cited in human poisonings (Carmichael et al., 1990; Nagai et al., 1996, 1997). To anticipate potentially harmful cyanobacterial blooms, we are studying top-down and bottom-up factors associated with spatial and temporal variation in cyanobacterial abundance. Chemical defenses render cyanobacteria highly unpalatable to generalist herbivores (Pennings et al., 1996, 1997; Thacker et al., 1997; Nagle and Paul, 1998). Caging experiments have shown that large, generalist herbivores play a limited role in determining cyanobacterial abundance (Thacker and Paul, unpublished data). However, some strains of cyanobacteria are the preferred foods of specialist sea hares (Nagle et al., 1998).

Since bottom-up factors may exert more influence on cyanobacterial populations than top-down factors, we hypothesized that (1) cyanobacterial abundance is positively associated with nutrient availability (Fong et al., 1993). In addition, cyanobacteria may compete with macroalgae for light and nutrients (Fong and Zedler, 1993). Thus, we also hypothesized that (2) cyanobacterial abundance is negatively associated with macroalgal abundance. Since many strains of cyanobacteria are fragile and do wash ashore during periods of high wave action, we hypothesized that (3) cyanobacterial abundance is highest when disturbance from wave action is lowest. We examined these three hypotheses by measuring cyanobacterial abundance and nine environmental variables at nine reef flat locations around Guam over 14 sampling periods. Associations among these variables and cyanobacterial abundance were examined using stepwise regression. Although previous studies of benthic, marine cyanobacteria have treated diverse assemblages as a single ecological unit (Klumpp and McKinnon,1992; Williams and Carpenter, 1997), all strains of cyanobacteria may not show the same responses to environmental conditions. Thus, we also examined the associations between environmental variables and the dominant strain of cyanobacteria at one site.

Methods

We monitored cyanobacterial abundance, macroalgal abundance, nitrogen availability (as nitrate and nitrite concentration), phosphorus availability (as ortho-phosphate concentration), salinity, and temperature at nine reef flat locations around Guam. Ammonium concentrations were not monitored regularly because a pilot study found that 70% of total nitrogen was represented by nitrate and nitrite, while only 8% was represented by ammonium, agreeing with previous reports of low ammonium concentrations around Guam (Matson, 1991). The nine locations were chosen to allow access in most weather conditions throughout the year and included Tanguisson Beach Park, Pago Bay, Togcha River, Cocos Lagoon, Fingers Reef, Piti Bombholes, and three sites in Tumon Bay: Sails, Pia, and Ypao Beach Park (Fig. 1). All sites were 0.5 to 2 m deep, depending on tidal height, with high light availability. We surveyed each site 14 times, approximately 6 wks apart.

We measured cyanobacterial and macroalgal abundance by sampling 0.5×0.5 m quadrats spaced 6 m apart along fixed transect lines. At the Tumon Bay and Tanguisson Beach Park sites, we sampled two 60 m transect lines, for a total of 20 quadrats. At the remaining sites, we sampled three 30 m transect lines, for a total of 15 quadrats. These sampling schemes were chosen to ensure an adequate representation of species diversity in each area. The quadrat was divided by strings into 100 cells, 5×5 cm each. We noted the presence or absence of each species in each cell of the quadrat

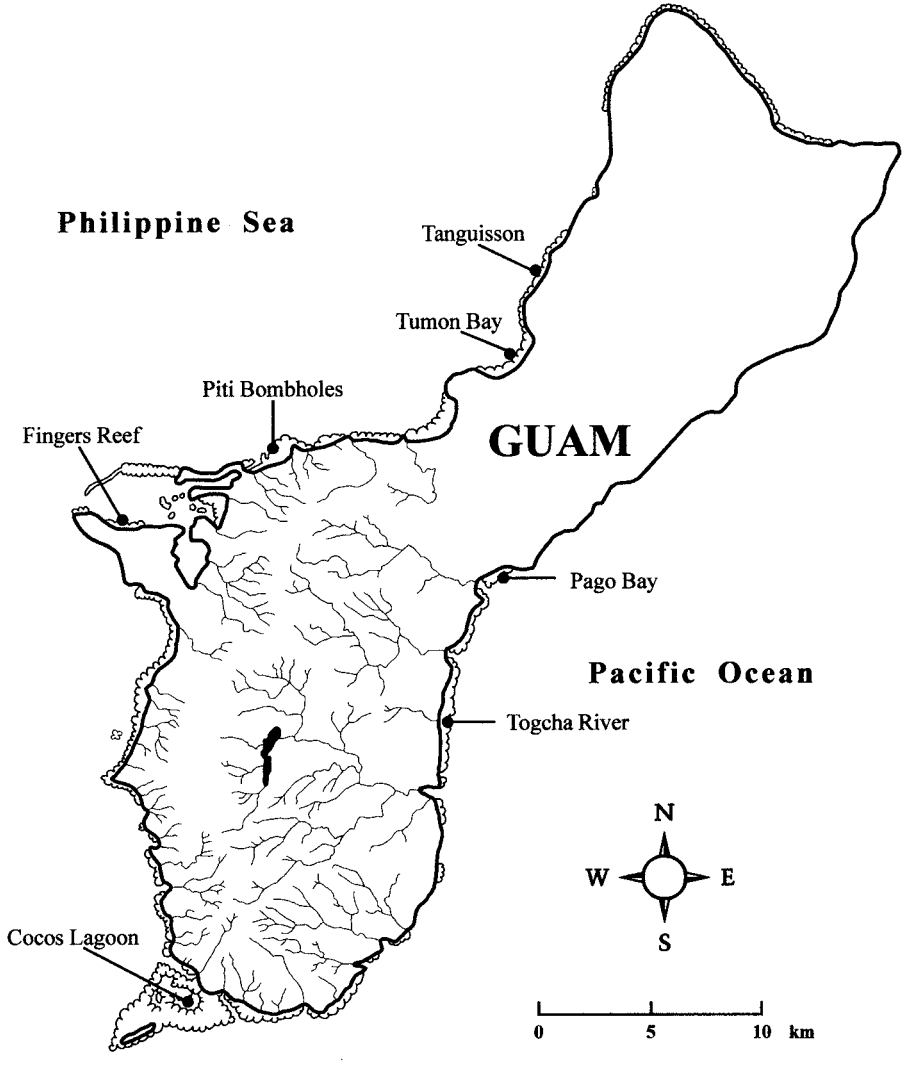

Figure 1. A map of Guam showing the nine sampling locations.

and scored the total as percent cover (Pennings and Callaway, 1996; Sutherland, 1996). The mean percent cover was then determined for each site. For this study, we summed the mean percent cover of all cyanobacterial species and all macroalgal species to estimate total cyanobacterial and macroalgal abundance at each site. Monthly total rainfall and monthly average minutes of sunshine, peak wind speed, and average wave height were obtained from a National Weather Service monitoring program.

Every 6 wks, we collected three replicate water samples 5 cm above the bottom from each study site. Nutrient analyses were contracted to the Guam Water and Environment Research Institute. Nitrate and nitrite were measured together in Jones's (1984) modification of the cadmium reduction method described in Parsons et al. (1984). Standards were run at 0, 7.14, 17.9 and 35.7 μM. A second source control (a control from a separate stock solution) was run at 3.57 μM. Ortho (reac-

Table 1. Correlation of environmental variables with cyanobacterial abundance at nine locations around Guam. n = 126. Bonferroni alpha = 0.006.

Variable	r	r^2	P
Phosphorus	0.034	0.001	0.706
Nitrogen	–0.196	0.038	0.028
Salinity	0.102	0.010	0.254
Water temperature	–0.029	0.001	0.748
Rainfall	–0.142	0.002	0.113
Minutes of sunshine	0.032	0.001	0.723
Peak wind speed	–0.042	0.002	0.640
Average wave height	0.007	<0.001	0.940
Macroalgal abundance	0.339	0.115	<0.001

tive) phosphate was measured by the method described in Parsons et al. (1984). Standards were run at 0, 1.61 and 3.23 μM, while a second source control was run at 0.81 μM. When water samples were collected, we also measured salinity and temperature using a Water Checker U-10 (Horiba Instruments, Irvine, California).

Pearson correlation coefficients were used to determine the relationships between the nine environmental variables and cyanobacterial abundance. Macroalgal abundance and cyanobacterial abundance were square-root transformed ($y = (x + 0.5)^{1/2}$) to meet the assumptions of normality (Sokal and Rohlf, 1995). Since many of the variables may be correlated with one another, stepwise regression (Wilkinson et al., 1992; Sokal and Rohlf, 1995) was used to determine which variable or combination of variables best explained the variation observed in cyanobacterial abundance. P-to-enter and P-to-leave the model were set at 0.05. Both forward and backward selection procedures were used to find the best model.

At the Togcha River reef flat, we studied a strain of *Oscillatoria margaritifera* in more detail. *O. margaritifera* was present at this site throughout this study, although it was not always present inside the sampling quadrats. Each week, we sampled the percent cover of *O. margaritifera*, other cyanobacteria, and macroalgae. Inorganic nitrogen availability, phosphorus availability, and salinity were measured every 6 wks, as described earlier. Water temperature was measured by a submerged StowAway Tidbit temperature logger (Onset Computer Corporation, Bourne, Massachusetts) located at a depth of 2 m in one transect. For these analyses, we noted the maximum temperature recorded on the day prior to the quadrat sampling. Rainfall, minutes of sunshine, peak wind speed, and wave height were obtained from a National Weather Service monitoring program. Rainfall and minutes of sunshine were summed for the 3 d prior to sampling, while peak wind speed and wave height were averaged for the 3 d prior to sampling. Pearson correlation coefficients were used to determine the relationships between the nine environmental variables and the abundance of *O. margaritifera* (Sokal and Rohlf, 1995). Stepwise regression (Wilkinson et al., 1992) was used to determine which variable or combination of variables best explained the variation observed in the abundance of *O. margaritifera*, with P-to-enter and P-to-leave the model set at 0.05. Both forward and backward selection procedures were used to find the best model.

Results

Cyanobacteria present at our study sites included *Oscillatoria* spp., *Lyngbya* spp., *Symploca* spp., and *Tolypothrix* sp. Our data revealed no relationship between phosphorus availability or nitrogen availability and cyanobacterial abundance (Table 1, Fig. 2A,B). Macroalgal abundance was positively associated with cyanobacterial abundance (Table 1, Fig. 2C). Only the positive association with macroalgal abundance significantly contributed to the stepwise regression model, which explained 11.5% of the variation in

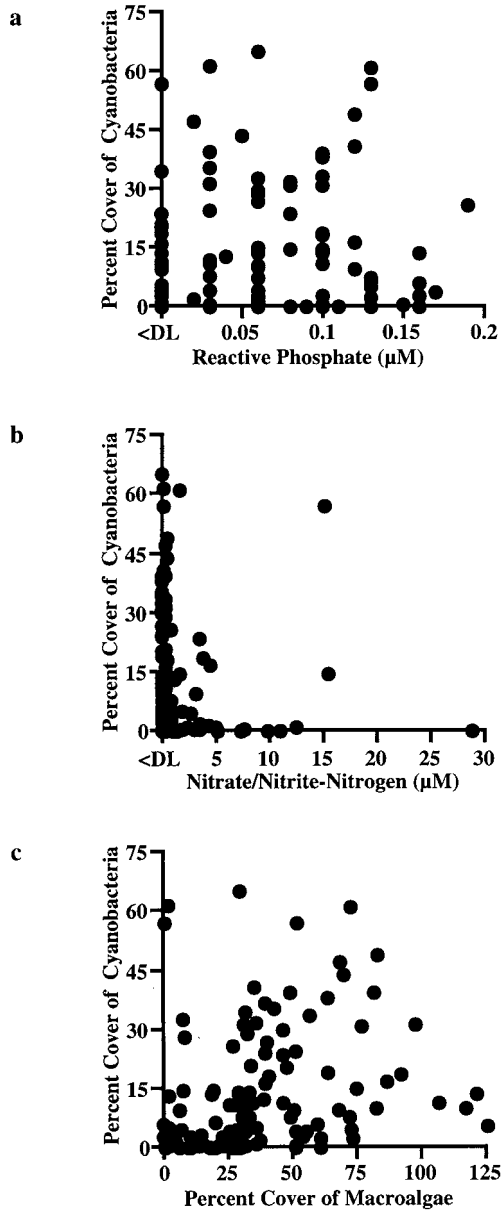

Figure 2. Associations between cyanobacterial abundance and (a) phosphorus availability (reactive phosphate), (b) nitrogen availability (nitrate and nitrite nitrogen), and (c) macroalgal abundance. Correlation coefficients and significance values are presented in Table 1.

cyanobacterial abundance ($n = 126$, $r^2 = 0.115$, $P < 0.001$, Table 2). Once macroalgal abundance was in the model, the partial correlations of the remaining variables were not significant (Table 2). Forward and backward selection procedures yielded the same model.

Although *O. margaritifera* was consistently present at the Togcha River reef flat, its abundance varied greatly and showed a general decline over time (Fig. 3). This

cyanobacterial strain spread over soft and hard substrates, and overgrew coral rubble, live coral, and macroalgae. Several environmental variables were significantly correlated with the abundance of *O. margaritifera* , including average wave height, water temperature, and macroalgal abundance (Table 3). A combination of negative relationships with wave height and macroalgal abundance significantly contributed to the stepwise regression model, which explained 79.4% of the observed variation in the abundance of *O. margaritifera* ($n = 25$, $r^2 = 0.794$, $P < 0.001$; Fig. 4, Table 4). Once these two variables were in the model, the partial correlations of the remaining variables were not significant (Table 4). Forward and backward selection procedures yielded the same model.

Table 2. Stepwise regression model and partial correlations for environmental variables associated with total cyanobacterial abundance.

Variables entered into stepwise regression model			
Variable	Coefficient	F	P
Constant	1.643	14.336	<0.001
Macroalgal abundance	0.281	16.142	<0.001

Variables excluded from stepwise regression model			
Variable	Partial Correlation	F	P
Phosphorus	–0.037	0.169	0.682
Nitrogen	–0.105	1.364	0.245
Salinity	0.012	0.017	0.895
Water temperature	0.019	0.044	0.835
Rainfall	–0.124	1.906	0.170
Minutes of sunshine	–0.027	0.091	0.763
Peak wind speed	–0.092	1.042	0.310
Average wave height	–0.076	0.710	0.401

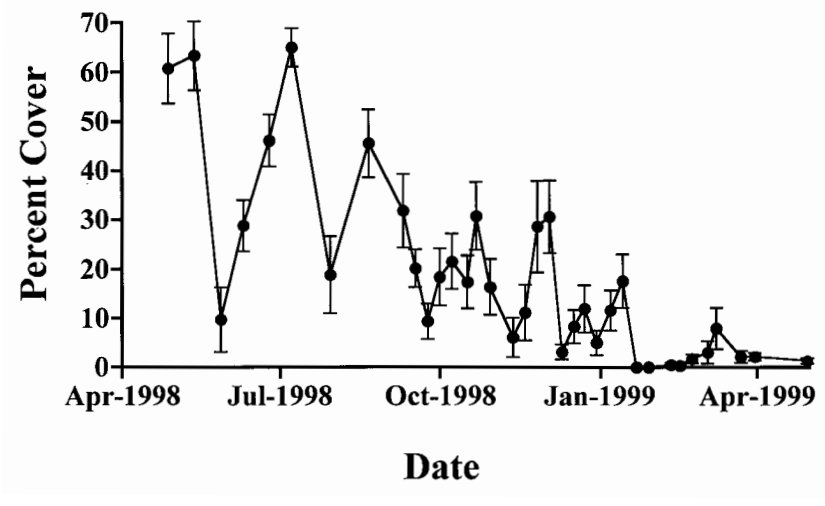

Figure 3. Variability in the abundance of *Oscillatoria margaritifera* at the Togcha River reef flat between April, 1998 and April, 1999.

Table 3. Correlation of environmental variables with the abundance of *Oscillatoria margaritifera* at the Togcha River reef flat. Bonferroni alpha = 0.006.

Variable	n	r	r^2	P
Phosphorus	8	–0.356	0.126	0.387
Nitrogen	8	0.405	0.164	0.320
Salinity	8	–0.188	0.035	0.655
Water temperature	36	0.605	0.366	0.001
Rainfall	36	–0.027	0.001	0.875
Minutes of sunshine	36	0.204	0.042	0.232
Peak wind speed	36	–0.362	0.131	0.030
Average wave height	36	–0.575	0.330	<0.001
Total macroalgae	25	–0.764	0.584	<0.001

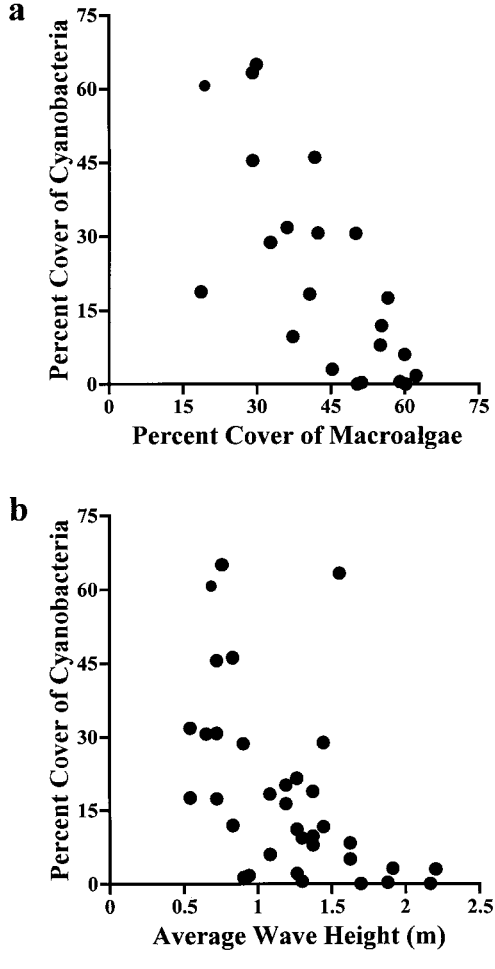

Figure 4. Associations between the abundance of *Oscillatoria margaritifera* and (a) macroalgal abundance and (b) average wave height at the Togcha River reef flat. Correlation coefficients and significance values are presented in Table 3.

Table 4. Stepwise regression model and partial correlations for environmental variables associated with the abundance of *Oscillatoria margaritifera* at the Togcha River reef flat.

Variables entered into stepwise regression model			
Variable	Coefficient	F	P
Constant	17.328	121.000	<0.001
Average wave height	–0.866	22.446	<0.001
Macroalgal abundance	–1.586	52.282	<0.001

Variables excluded from stepwise regression model			
Variable	Partial Correlation	F	P
Phosphorus	0.072	0.062	0.807
Nitrogen	0.192	0.458	0.511
Salinity	–0.225	0.642	0.439
Water temperature	–0.359	1.927	0.188
Rainfall	–0.185	0.460	0.510
Minutes of sunshine	–0.008	0.001	0.976
Peak wind speed	0.032	0.013	0.911

Discussion

Contrary to our first hypothesis, when we examined all cyanobacterial taxa, we found no significant relationships between nitrogen and phosphorus availability and cyanobacterial abundance. The concentrations of both nutrients sometimes exceeded the thresholds that may sustain macroalgal blooms on Caribbean reefs (approx. 1.0 μM nitrogen and 0.1 μM phosphorus; Lapointe, 1997). A negative trend between inorganic nitrogen availability and cyanobacterial abundance may be related to the measurements of higher inorganic nitrogen in low salinity areas. Since Guam's freshwater aquifer contains high nitrate concentrations (Matson, 1991, 1993), freshwater seeps located on reef flats may provide both high inorganic nitrogen and low salinity habitats. In addition, the nitrogen-fixation abilities of some cyanobacteria may remove their dependence on water-column nitrogen (Larkum, 1988). The common genera of cyanobacteria in our surveys included *Tolypothrix*, which contains heterocysts and can fix nitrogen, and *Oscillatoria* and *Lyngbya*, both of which lack heterocysts but can also fix nitrogen (Paerl, 1990). Cowell and Botts (1994) found that the biomass of the nitrogen-fixing, freshwater cyanobacterium *Lyngbya wollei* was negatively associated with alkalinity, conductivity, and ammonium concentrations, but positively associated with phosphate concentrations. We found no significant relationships with phosphate concentrations in our study, possibly because phosphate concentrations were low at all sites.

We also rejected our second hypothesis, since we found a strong, positive association between macroalgal abundance and total cyanobacterial abundance. The same factors that favor macroalgal growth may favor most cyanobacteria, indicating that these two taxonomic groups may respond to environmental conditions in the same way on tropical reefs. Studies in a freshwater estuary (Cowell and Botts, 1994) and a temperate marine lagoon (Fong and Zedler, 1993) found negative relationships between macroalgae and cyanobacteria, arguing that competition among these groups would be a major force in structuring their communities. Although many cyanobacteria can be epiphytes on macroalgae, the positive relationship that we found indicates that these two groups may

not necessarily compete with each other. Instead, cyanobacteria could facilitate the growth of macroalgae, through nitrogen fixation or associational defenses from herbivory (Pennings, 1997). However, macroalgal abundance explained only 11.5% of the variation in cyanobacterial abundance, indicating that other variables may be more important in determining the abundance and distribution of cyanobacteria, or that different strains of cyanobacteria respond to these variables in different ways.

When we examined the abundance of a single cyanobacterium, *O. margaritifera* , we found no significant associations with nutrient availability, again rejecting our first hypothesis. Since *O. margaritifera* was less abundant when there was greater macroalgal abundance, this cyanobacterium may compete with macroalgae, supporting our second hypothesis, and conflicting with our conclusions based on the abundance of all cyanobacterial species at all nine sites. However, since we made direct observations of *O. margaritifera* overgrowing several species of macroalgae, this association is not surprising. Wave height was also negatively associated with the abundance of *O. margaritifera*, indicating the importance of disturbance to the persistence of this cyanobacterial population and supporting our third hypothesis. Our best model for this single strain explained much more of the variation in abundance (79.4%) than the model for all strains (11.5%), which provides additional evidence that all cyanobacteria are not alike in their responses to environmental variables. For example, all cyanobacteria do not form the sprawling mats that are typical of *O. margaritifera*. Species that form more upright or ball-shaped colonies did not overgrow other substrates as extensively as *O. margaritifera*.

Although we found no relationships between cyanobacterial abundance and nutrient availability, measurements of nutrient availability were infrequent at each site. We may be unable to measure the possibly more important pulses of nutrient input from streams and shore run-off, e.g., after rainfall (Carpenter et al., 1998; Denton et al., 1998; Schaffelke and Klumpp, 1998). For example, the Togcha River reef flat is located near a river mouth that can have high levels of nitrate and phosphate due to a sewage outfall upstream (Guam Environmental Protection Agency, unpubl. data). These nutrients may be diluted before reaching our study site or occur in pulses that our sampling scheme was not able to detect. In addition, nutrient concentrations in sediment porewater may be more important than water column concentrations at our study sites (Larned, 1998). The use of biological indicators, such as tissue nutrient content (Frankovich and Fourqurean, 1997) and stable isotope concentrations in macroalgae and seagrasses (Mendes et al., 1997), may aid in determining the relative availability of nutrients among our sites.

In our future work, we will address the correlations we have found with manipulative experiments in laboratory aquaria and in the field (Thacker et al., in press). By examining both nitrogen-fixing and non-nitrogen-fixing strains of cyanobacteria, we may be able to determine which of these strains have positive or negative associations with macroalgae. In addition, studies of individual strains are needed to determine the different types of responses (including changing growth forms) that cyanobacteria may have to seasonal environmental conditions.

In contrast to our expectations, cyanobacteria are not necessarily associated with high nutrient availability and may not compete with macroalgae. The nitrogen fixation capabilities of some cyanobacteria may increase their abundance in low nutrient habitats. Both nitrogen fixation and associational defenses may create facilitative interactions with macroalgae. Although confirmed by only one site in this study, physical disturbance may be a more important influence on cyanobacterial populations than either nutrient avail-

ability or interactions with macroalgae. By combining a monitoring program with manipulative experiments, we hope to learn more about the factors influencing the distribution of cyanobacteria and to enhance our ability to anticipate harmful blooms.

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Long-term responses of aquatic macrophyte diversity and community structure to variation in nitrogen loading

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Abstract

In a chain of small lakes in southeastern Michigan, USA, macrophyte community dynamics have shown a strong relationship to variations in nitrogen (nitrate and ammonia) loading that appear to correlate with variations in regional annual precipitation. We examined the community structures of two of these lakes 9 years after a drought in 1987–1988 which had temporarily reduced nutrient loading in the system, and during which the macrophytes in normally more eutrophic Shoe Lake had shifted from a *Ceratophyllum*-dominated community of low diversity to a more diverse community similar to that in less productive East Graham Lake downstream. With a return to the normal precipitation rates and associated nitrogen loading, the macrophytes in Shoe Lake returned to a community dominated by the non-rooted species *Ceratophyllum demersum*. However, overall species diversity remained relatively high in Shoe Lake after the drought event.
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Keywords: Nitrogen loading; Macrophytes; Diversity; Community structure

1. Introduction

Nitrogen loading, especially in nitrogen limited aquatic environments, is of increasing concern because of its effects on the diversity, community structure, and overall health of the systems (Vitousek et al., 1997; Paerl, 1997; Bedford et al., 1999; Goldman, 2000; Sala et al., 2000). Annual wet deposition of dissolved inorganic nitrogen (DIN) from anthropogenic inputs is estimated to be 10 times higher than normal background levels for the eastern and

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mid-western regions of the United States and 100 times higher than background levels in heavily impacted European locations (reviewed in Vitousek et al., 1997). Although most freshwater aquatic systems are naturally phosphate limited, eutrophication from anthropogenic phosphorus inputs often causes many of these systems to become nitrogen limited. Under these conditions, elevated nitrogen deposition can bring such systems to higher levels of eutrophication, dramatically affecting the community structure, dynamics, and diversity of these communities (Schindler et al., 1990; Vitousek et al., 1997; Peterson et al., 2001). To address the question of how the performance of previously N-limited organisms and ecosystems is affected by inputs of nitrogen that have become larger than normal for their evolutionary background (Vitousek et al., 1997), site-specific analyses of non-point loading associated with eutrophication are needed (Carpenter et al., 1998). For example, Valiela et al. (2000) illustrated the local effects of nitrogen loading on estuarine plant communities.

Over the past two decades, we have studied aquatic plant diversity and community structure in a chain of small lakes in southeastern Michigan, which is within the region of northeastern US where atmospheric nitrogen deposition has been the highest in North America (Charles, 1991). A gradient of DIN concentrations exists in the surface water along this lake chain, and overall nitrogen levels fluctuate with major changes in annual precipitation. Earlier (Hough et al., 1989) we documented a relatively stable situation in which the most N-enriched of the lakes (Shoe Lake) was dominated by non-rooted macrophytes, phytoplankton, and periphyton with low diversity in rooted macrophytes. Later (Hough et al., 1991), we reported a relatively rapid shift in this community during a 2 year drought which lowered the loading rates and availability of DIN; non-rooted plants and phytoplankton declined markedly, and the rooted plant community increased in diversity to more closely resemble the N-limited lake (East Graham Lake) downstream.

Our hypothesis that high nitrogen loading and availability maintains low diversity in macrophyte communities primarily because of dominance by enhanced non-rooted plants and their shading effects (Hough et al. (1989, 1991)) suggested the prediction that, in the decade following the drought event, the previously N-enriched community structure would return in this system. Here, we report the outcome of this prediction, having carried out a reexamination of these macrophyte communities.

2. Study site and methods

The chain of small hard-water kettle lakes lies along the west branch of Stoney Creek located in the Bald Mountain State Recreation Area in southeastern Michigan USA (Fig. 1). From its source at the outflow of Bunny Run Lake in the village of Lake Orion, Stoney Creek flows eastward for ≈1 km through a wetland and meadow into Shoe Lake, followed closely by West Graham Lake and East Graham Lake. Below the Bald Mountain Recreational Area, the creek flows through additional lakes before it enters the Clinton River, a major tributary of Lake St. Clair in the Great Lakes.

Nutrient levels in the headwaters of this chain at Bunny Run Lake are moderate, and become enriched along the stream, especially with inorganic nitrogen, resulting in the highest levels at the Shoe Lake inflow (Fig. 2). This enrichment appears to be largely from precipitation runoff, as (1) there is no habitation or agriculture along this narrow watershed

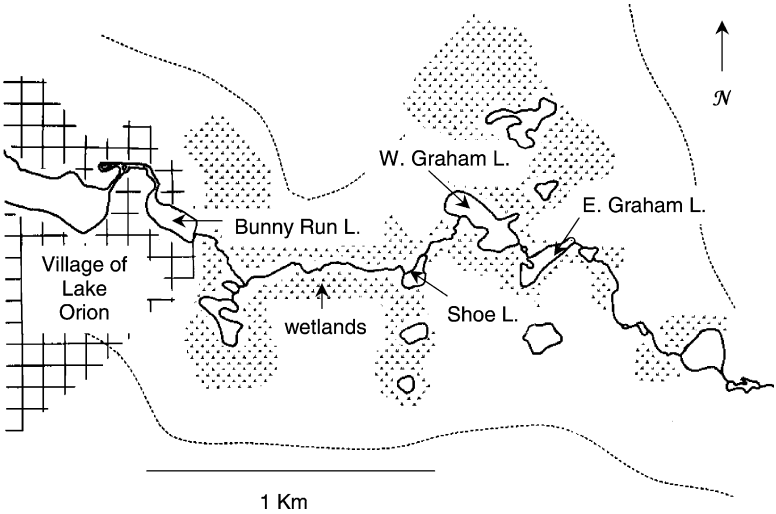

Fig. 1. Stoney Creek (West Branch) chain of lakes, Oakland County, MI, USA. Stream flow is from west to east.

below the outflow of Bunny Run Lake, and (2) nitrate and ammonia nitrogen concentrations in local precipitation generally are much higher than in the surface waters, by factors of three to eight-fold in 1985–1986 (Allenson, 1992) and by factors of 5 to >10 fold in 1997 (Montante, unpublished data). However, while this N-enrichment is readily apparent along the long stretch of stream from Bunny Run Lake to Shoe Lake, and sometimes in the short stretch between Shoe Lake and West Graham Lake (Fig. 2), the enrichment appears to be

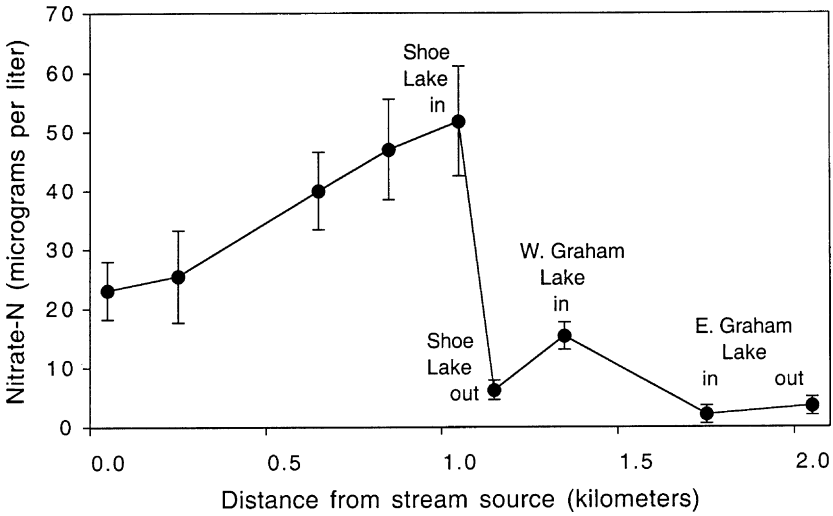

Fig. 2. Nitrate nitrogen concentrations along upper Stoney Creek and its chain of lakes during a year of normal annual precipitation. Mean ± S.D. of all biweekly measurements over the summer of 1984. From Allenson (1992).

reversed within the close succession of lakes including Shoe Lake and the Graham Lakes, presumably because the rate of removal in the lakes is more rapid than the enrichment rate.

Shoe Lake (1.9 ha surface area; 5.5 m maximum depth) is normally moderately eutrophic with abundant phytoplankton, periphyton, and non-rooted macrophytes but with a poorly developed rooted submersed macrophyte community that is light limited (Hough et al., 1989). After flowing through Shoe Lake, the water at its outflow is markedly lower in total DIN. DIN is kept very low in West Graham Lake and in the closely adjacent East Graham Lake (4.5 ha surface area; 11 m maximum depth). As a consequence, the phytoplankton, periphyton, and non-rooted macrophyte communities are N-limited in East Graham Lake (determined experimentally by Hough et al., 1989; Hough and Thompson, 1996), and the rooted macrophyte community is much more productive and diverse than in Shoe Lake.

Drought conditions began in the upper central USA in 1987, and became severe in 1988 with precipitation and surface flow generally reduced to 30% below normal. Stream discharge rates at the inflow of Shoe Lake during 1988 were 70% below those in 1986. Flow rates returned to normal after 1988.

During several different years over the past two decades, we monitored limnological conditions in this system every 3 weeks during the spring and summer using standard methods according to Wetzel and Likens (1991) as described in Hough et al. (1989). Macrophytes were collected from both Shoe Lake and East Graham Lake in mid-summer of 1979, 1988, and 1996. Transects perpendicular to the shore were spaced 15 m apart around the perimeter of the lake, similar to the method of Dubois et al. (1984). Along the transect, samples were taken using a 6-pronged macrophyte hook at depths of 0.25, 0.5, 1, 1.5, 2, and 2.5 m. Plants were identified according to Crow and Hellquist (2000a,b) and Gleason and Cronquist (1991). From the species frequency data for each lake in each year, we calculated the Shannon diversity (Zar, 1995). We also calculated indexes of dissimilarity and similarity of community structures in paired comparisons among lakes and years. In addition to the Bray–Curtis distance index that is commonly used in ecological studies (Zar, 1995), we also calculated chord distance index (CRD, one of the relative Euclidean distance group; Ludwig and Reynolds, 1988). CRD is an especially useful dissimilarity index for quantitative abundance data because with squared functions it puts more emphasis on relative proportions of species than on absolute quantities (Zar, 1995), which we believe is an important factor in our study. Finally, while our data were not appropriate for multivariate analyses, we calculated Pearson correlation coefficients as an index of similarity of community structures in paired comparisons, which enabled us to test for statistical significance of differences among multiple *r*-values (Zar, 1995).

3. Results

DIN and total phosphorus concentrations at the inflow and outflow of Shoe Lake generally have remained relatively consistent over time as shown by the 1982–1986 and 1996 data (Table 1). However, at the Shoe Lake inflow in 1988 the DIN concentrations were about 40% lower than in previous years, and at the Shoe Lake outflow DIN was virtually zero in 1988. Moreover, reduced stream discharge rates had resulted in an 80% reduction of both nitrogen and phosphorus loading rates at the Shoe Lake inflow (from 733 g N per day and

Table 1
Concentrations (mean ± S.D.) of total phosphorus and total dissolved inorganic nitrogen in stream water at Shoe Lake during summer

	Phosphorus[a] $\mu g\,l^{-1}$		Nitrogen[b] $\mu g\,l^{-1}$	
	Inflow	Outflow	Inflow	Outflow
1982	26 (3.7)	23 (4.1)	85 (14)	25 (5.0)
1984	19 (6.1)	12 (2.8)	74 (28)	18 (15)
1986	25 (15)	20 (2.5)	87 (19)	29 (15)
1988	20 (5.6)	17 (3.4)	**49** (25)	<**1***
1996	14 (6.3)	14 (6.9)	98 (27)	33 (24)

Data for years prior to 1996 appeared originally in Hough et al. (1989, 1991).

[a] Phosphorus concentrations at the inflow did not differ significantly among years (Kruskal–Wallis test, $P > 0.5$); concentrations at the outflow also did not differ among years (Kruskal–Wallis test, $P > 0.1$); concentrations in all years at the outflow were significantly lower than at the inflow (*t*-test, $P < 0.001$).

[b] Nitrogen concentrations at the inflow differed significantly among years (ANOVA, $P < 0.05$), with 1988 being different from all other years ($P < 0.01$); concentrations at the outflow differed significantly among years (ANOVA, $P < 0.05$), with 1988 being different from all other years ($P < 0.05$); concentrations in all years at the outflow were significantly lower than at the inflow (*t*-test, $P > 0.001$).

* Below detection limits.

211 g P per day in 1986 to 122 g N per day and 50 g P per day in 1988). By 1996, loading rates of both nutrients (1117 g N per day and 160 g P per day) had returned to levels similar to those in 1986.

In 1996, the majority of macrophyte species in Shoe Lake that were dominant in pre-drought years had returned to pre-drought levels of abundance (Table 2). Most notably, sample frequencies of *Ceratophyllum demersum* and *Utricularia vulgaris* were reduced by 63 and 39 ΔF (%), respectively in the drought of 1988, but since then they have increased to levels similar to or above pre-drought levels. *Najas flexilis* followed a similar pattern, whereby its frequency was reduced by 92 ΔF (%) during the drought but rebounded afterwards to 170 ΔF (%) of pre-drought levels. *Myriophyllum verticillatum*, which had decreased by 54 ΔF (%) during the drought, also returned but only to 60 ΔF (%) of its pre-drought frequency. Some species including *Chara vulgaris* and *Myriophyllum sibiricum* increased in frequency during the drought and then returned to lower levels. *M. sibiricum*, rare in pre-drought years, disappeared in 1996, while *C. vulgaris* retained a substantial part of the foothold gained in 1988 and remained present at 128 ΔF (%) of its 1979 frequency.

In general, by 1996 the macrophyte community structure in Shoe Lake appeared to be returning to one similar to that seen before the 1988 drought. Pearson correlation coefficients of the Shoe Lake communities among years suggest that the 1996 Shoe Lake community was most like that in 1979, and the chord distance (dissimilarity) was least for 1996 versus 1979 (Table 3). In comparing the two lakes, the 1988 Shoe Lake community was most similar to the East Graham Lake communities of 1979 and 1988. However, after the Shannon diversity index and species richness rose in Shoe Lake during the drought years (Table 2), they remained high since the drought.

In contrast to Shoe Lake, overall diversity in East Graham Lake was high and relatively stable throughout the two decades. However, some specific aspects of community structure

Table 2
Species frequencies (percentage of all samples in each lake containing each species[a])

	Shoe Lake			East Graham Lake		
	1979	1988	1996	1979	1988	1996
Non-rooted; submersed leaves						
Ceratophyllum demersum L.	91	34	84	30	4	32
Utricularia vulgaris L.	49	30	53	15	18	55
Rooted; submersed leaves						
Elodea canadensis (Michx.) Planchon	6	17	40	20	8	32
Myriophyllum sibiricum Komarov[b]	3	22	0	54	36	11
Myriophyllum verticillatum L.	35	16	21	0	1.5	35
Najas flexilis (Willd.) Rostk. and Schmidt	26	2	44	12	26	37
Potamogeton illinoensis Morong	8	0	0.88	24	17	0.6
Potamogeton filiformis Pers.[c]	2.6	4.4	0.44	1	4.6	2.1
Potamogeton foliosus Raf.	0	25	1.3	0	2.2	4.3
Potamogeton pectinatus L.[d]	0	0	2.6	28	13	30
Potamogeton pusillus L.	0	22	31	0	5.2	37
Potamogeton zosteriformis Fern.	36	24	35	39	12	32
Scirpus subterminalis Torr.	0	2	0	4	10	14
Rooted; submersed and floating leaves						
Potamogeton amplifolius Tuckerm.	0	0	0	11	0.2	1.4
Potamogeton natans L.	18	10	35	1	9	17
Potamogeton nodosus Poir.	0	5.5	8.8	0	3.5	0
Rooted; floating or emergent leaves						
Brasenia schreberi J.F. Gmel.	0	0	0	4	7	8.2
Eleocharis equisetoides (Ell.) Torr.	0	0	0.44	3	2.4	4.1
Nuphar variegata Engelm. ex Durand	2	10	12	5	8	13
Nymphaea odorata Ait.[e]	13	25	28	10	23	19
Pontederia cordata L.	1	3	21	2	5	10
Rhizoidal macroalgae						
Chara vulgaris L.	25	55	32	56	75	53
Nitella flexilis (L.) Ag.	0.6	2.3	0.44	14	6	0
Species richness (S)	15	18	22	21	25	24
Shannon diversity (H')	2.15	2.57	2.58	2.53	2.72	2.84

Species richness S = total number of species present; Shannon diversity $H' = -\Sigma p_i \ln p_i$. Data for years prior to 1996 appeared originally in Hough et al. (1989, 1991).

[a] Additional rare species included *Vallisneria americana* Michx. (1.8% SL'96, 0.3% EGL'79), *Utricularia intermedia* Hayne (0.3% EGL'79), *Potamogeton alpinus* Balb. (18% EGL'88, 19% EGL'96), *Zosterella dubia* (Jacq.) Small (formerly *Heteranthera dubia* (Jacq.) MacM. (Fassett, 1957)) (10% SL'96, 11% EGL'96), *Ranunculus longirostris* Godr. (3.9% SL'96, 0.7% EGL'96), *Potamogeton gramineus* L. (3.9% EGL'88).

[b] Formerly *Myriophyllum exalbescens* Fernald (Fassett, 1957).

[c] Recently renamed *Stuckenia filiformis* (Pers.) Börner (Crow and Hellquist, 2000a,b).

[d] Recently renamed *Stuckenia pectinata* (L.) Börner (Crow and Hellquist, 2000a,b).

[e] Formerly *Nymphaea tuberosa* Paine (Fassett, 1957).

Table 3
Coefficients of dissimilarity (B–C, CRD) and similarity (*r*) of community structures (from species frequencies in Table 2) among lakes and years (S: Shoe Lake; EG: East Graham Lake)

	S 96 vs. EG 79	S 79 vs. EG 79	S 79 vs. S 88	S 96 vs. S 88	S 88 vs. EG 79	S 88 vs. EG 88	S 96 vs. EG 96	S 79 vs. S 96
B–C	0.58	0.53	0.45	0.40	0.41	0.41	0.27	0.28
CRD	0.94	0.94	0.76	0.67	0.70	0.68	0.55	0.46
r	**0.23**	**0.27**	0.50*	0.52*	0.61**	0.61**	0.71**	**0.84****

B–C: Bray–Curtis dissimilarity index; CRD: chord distance; *r*: Pearson correlation coefficient.

* *r*-values individually significant at 0.05 level (2-tailed), but not significant in a table-wide sequential Bonferroni test (Rice, 1989).

** *r*-values significant at 0.01 level (2-tailed). Bold data denote that, in multiple comparisons of *r* (Zar, 1995), 0.84 is significantly different (0.05 level) from both 0.23 and 0.27.

did change over the years in East Graham Lake. *C. demersum* experienced the same pattern of decline during the drought and later resurgence as it did in Shoe Lake (Table 2), as did *Potamogeton zosteriformis*, *Potamogeton pectinatus*, and *Elodea canadensis*. A temporary increase in *C. vulgaris* during the drought years occurred in both lakes. Also, several species became much more abundant in 1996 than in either of the two previous decades.

4. Discussion

During the drought of 1988, the increase in the overall diversity of Shoe Lake macrophytes was a result of a decline in the dominant non-rooted macrophytes, and an increase in the more diverse benthic macrophyte community due to increased light availability (Hough et al., 1991). Similar drought effects during that period were reported elsewhere, including a temporary reversal in the trend of declining transparency of Lake Tahoe (Goldman, 2000), and an expansion of the distribution of submersed macrophytes in the Choptank river (Stevenson et al., 1993). In 1996, nutrient levels in Shoe Lake were comparable to the pre-drought years and correlated with a general reversal of many of the drought induced trends in the macrophyte community. Non-rooted macrophytes such as *Ceratophyllum* and *Utricularia* are dependent on available nitrogen in the water column (Best, 1980; Goulder and Boatman, 1971). These species had declined along with the nitrogen levels during the 1988 drought, as did phytoplankton chlorophyll *a* and epiphytic algae, and this allowed increased light penetration into the portion of the littoral zone occupied by the rooted macrophytes. As predicted, after the 1988 drought the non-rooted macrophytes returned to frequencies approaching or exceeding pre-drought levels, most likely because of the return of higher N-levels.

While most rooted macrophytes in Shoe Lake had increased in frequency with greater light availability during the drought, two rooted species that conversely had declined markedly at that time are *Najas flexilis* and *Myriophyllum verticillatum*. These two differ from most of the other species in that they both require free CO_2 as a carbon source, whereas their competitors can use bicarbonate. Bicarbonate use generally is advantageous in hardwater lakes, but less so under the limiting light conditions in Shoe Lake prior to

the drought, because the energy needed for active transport of bicarbonate requires higher photosynthetic rates (reviewed in Hough and Fornwall, 1988). Therefore, *N. flexilis* and *M. verticillatum* appear to have been able to compete well prior to 1988, but less so during the drought because the increase in light availability was favorable to the bicarbonate users (as demonstrated experimentally by Hough and Fornwall, 1988) including *M. sibiricum* and *C. vulgaris*, which increased during the drought. In 1996, the return of both of the free CO_2 requiring species to greater abundance, and the decline of both of the known bicarbonate users, was consistent with a predicted influence of a return to pre-drought levels of available light and the interaction of carbon source physiology.

While most macrophyte species returned to pre-drought abundance levels as predicted, the continuation of the elevated diversity in Shoe Lake after the drought was unexpected. Several factors contributed to the continuing high diversity in Shoe Lake, including colonization by previously absent species, increases in some previously rare species, and an overall shift to a more uniform frequency distribution. First, colonization by new species and an increase in previously rare species elevated the diversity (for example, without the newly introduced species such as *Ranunculus longirostris*, *Zosterella dubia*, *Eleocharis equisetoides*, *Vallisneria americana*, and *Potamogeton pectinatus* in 1996, the diversity would have been 2.40 rather than 2.58). Both cases may reflect the ability of certain species to quickly invade areas vacated by *C. demersum* during the drought. Second, the abundance of floating leaved or emergent plants including *Nuphar variegata*, *Nymphaea odorata* and *Pontederia cordata* increased both during and after the drought. Once established, these surface-leaved and emergent plants are less susceptible to shading by *Ceratophyllum*. Sýkora (1979) also noted that emergents proliferated in response to drought conditions, and were able to retain and expand on the foothold gained during the drought. Third, the high level of diversity maintained after 8 years suggests that, in disturbed systems, general community diversity may be more stable than are specific aspects of community structure. Finally, the climatic warming trend in the recent decades may be influencing the communities in all of the lakes; diversity appears to be increasing slightly in East Graham Lake as well. However, if N-loading remains relatively high, we expect that continued dominance and shading by *Ceratophyllum*, phytoplankton and periphyton will eventually reduce the macrophyte diversity again in Shoe Lake.

In conclusion, our observations here, along with the previous observations and experiments (Hough and Fornwall, 1988; Hough et al., 1991) over the past two decades in a relatively small and well defined watershed illustrate the sensitivity of freshwater macrophyte communities to the effects of variations in nutrient loading, particularly as influenced by atmospheric deposition of nitrogen compounds. Further work is needed here to quantify the sources of the nutrient loading and to determine the relative roles of the different pathways of nitrogen metabolism in the community.

Acknowledgements

We would like to acknowledge Bilal Bazzi, Ziad Bazzi, Magda Bucka, Michelle Cubler, Punkaj Guglani, Tim Neldrett, Matt Spangler, Jerri Ann Buxton, and Mike Whitton for technical assistance, and D. Carl Freeman for statistical assistance.

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Office of Water
Regulations and Standards
Washington, DC 20460

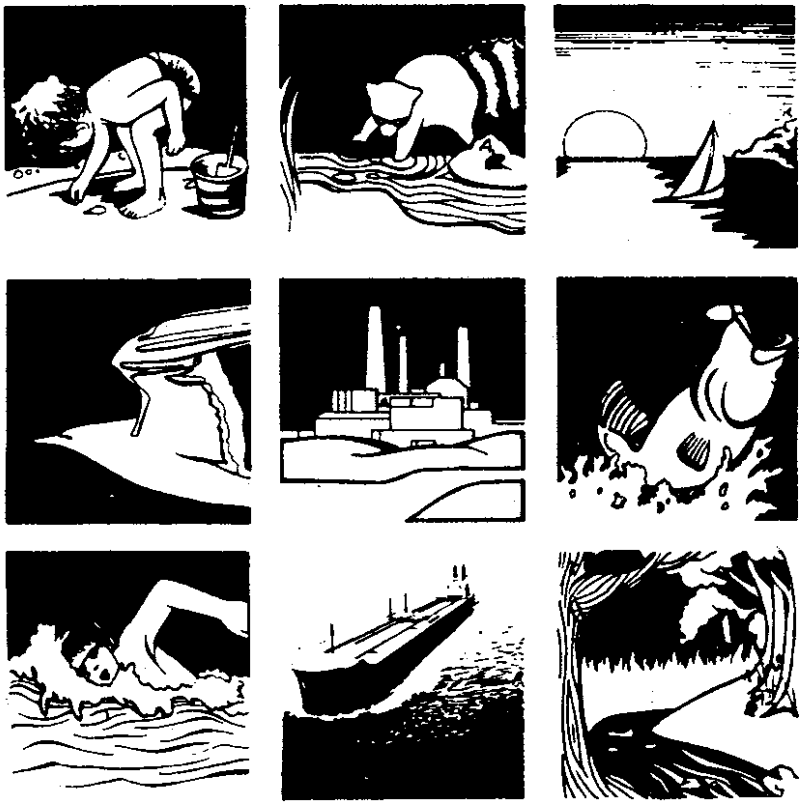
May 1, 1986

EPA

Water

EPA 440/5-86-001

QUALITY CRITERIA for WATER 1986



TO INTERESTED PARTIES

Section 304(a)(1) of the Clean Water Act (33 U.S.C. 1314(a)(1) requires the Environmental Protection Agency (EPA) to publish and periodically update ambient water quality criteria. These criteria are to accurately reflect the latest scientific knowledge (a) on the kind and extent of all identifiable effects on health and welfare including, but not limited to, plankton, fish shellfish, wildlife, plant life, shorelines, beaches, aesthetics, and recreation which may be expected from the presence of pollutants in any body of water including ground water; (b) on the concentration and dispersal of pollutants, or their byproducts, through biological, physical, and chemical processes; and (c) on the effects of pollutants on biological community diversity, productivity, and stability, including information on the factors affecting rates of eutrophication and organic and inorganic sedimentation for varying types of receiving waters. These criteria are not rules and they do not have regulatory impact. Rather, these criteria present scientific data and guidance of the environmental effects of pollutants which can be useful to derive regulatory requirements based on considerations of water quality impacts. When additional data has become available, these summaries have been updated to reflect the latest Agency recommendations on acceptable limits for aquatic life and human health protection.

Periodically EPA and its predecessor agencies has issued ambient water quality criteria, beginning in **1968** with the "Green Book" followed by the **1973** publication of the "Blue Book" (Water Quality Criteria **1972**). In **1976**, the "Red Book" (Quality

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Washington, DC 20402

Criteria for Water) was published. On November 28, 1980 (45 FR 79318), and February 15, 1984 (49 FR 5831), EPA announced through Federal Register notices, the publication of 65 individual ambient water quality criteria documents €or pollutants listed as toxic under section 307(a)(1) of the Clean Water Act. on July 29, 1985 (50 FR 30784), EPA published additional water quality criteria documents.

The development and publication of ambient water quality criteria has been pursued over the past 10 years and is an ongoing process. EPA expects to publish about 10 final criteria documents each year. Some of these will update and revise existing criteria recommendations and others will be issued for the first time.

In a continuing effort to provide those who use EPA's water quality and human health criteria with up-to-date criteria values and associated information, this document Quality Criteria €or Water 1986 was assembled. This document includes summaries of all the contaminants for which EPA has developed criteria recommendations (Appendix A-C). The appropriate appendix is identified at the end of each summary. A more detailed description of these procedures can be found in the appropriate Appendix. Copies of this document can be obtained by contacting the U.S. Government Printing Office at:

U.S. Government Printing Office
Superintendent of Documents
N. Capitol and H Street N.W.
Washington, D.C. 20401

A fee is charged for this document.

Copies of the complete background ambient water quality

criteria documents containing all the data used to develop the criteria recommendations summarized herein and the "Red Book", including complete bibliographies are available only from:

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161

Telephone: (703) 487-4650

The NTIS order numbers for the criteria documents can be found in the Index. A fee is charged for copies of these documents.

As new criteria are developed and existing criteria revised, updated criteria summaries will be made available once a year to those who purchase this document through the U.S. Government Printing office. You will automatically be placed on the mailing list to receive annual updates. The cost for receiving annual updates is included in the purchase price of the document.

Quality Criteria for Water, 1986 is designed to be easily updated to reflect EPA's continuing work to present the latest scientific information and practices, Our planned schedule €or future criteria development in the next few years is attached for your information.

The Agency is current1y developing Acceptable Daily Intake (ADI) or Verified Reference Dose (RfD) values on a number of chemicals for Agency-wide use. Based upon this new analysis the values have changed significantly for 5 chemicals from those used in the original human health criteria calculation done in 1980. The chemicals affected are as follows:

chemical	1980 WQC	Draft RfD
1. cyanide	200 ug/L	.02 mg/kg/day
2. Ethylbenzene	1.4 mg/L	.01 mg/kg/day
3. Nitrobenzene	19.8 mg/L	.0005 mg/kg/day
4. Phenol	3.5 mg/L	0.1 mg/kg/day
5. Toluene	14.3 mg/L	0.3 mg/kg/day

FOR FURTHER INFORMATION CONTACT:

Dr. Frank Gostomski at the above address or by phoning (202) 245-3030.

It is EPA's goal to continue to develop and make available ambient water quality criteria reflecting the latest scientific practices and information. In this way we can continue to improve and protect the quality of the Nation's waters.

James M. Conlon

James M Conlon

Acting Director,
Office of Water Regulations
and Standards

DRAFT CRITERIA DOCUMENTS TO BE PROPOSED

LATE FY 86/EARLY FY 87

Diethyhexylphthalate
1,2,4, Trichlorobenzene
Silver
Phenanthrene
2,4,5, Trichlorophenol
Organotins
Tributyltin
Selenium (no saltwater criteria)
Hexachlorobenzene
Antimony III
Acrolein (no saltwater criteria)

LATE FY 87/EARLY 88

Thallium (no saltwater criteria)
Tetrachloroethylene (no saltwater criteria)
Phenol
Toluene
Chloroform (no saltwater criteria)
Analine
Acrylontrile
Hexachlorocyclopentadiene (no saltwater criteria)
Dimethylphenol
Hexachlorobutadiene (no saltwater criteria)

- Both lists will incorporate aquatic and human health values.
- All above are toxic pollutants except for organotins and analine which are non-conventionals.

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Antimony	PB 81-117319
Arsenic	PB 85-227445
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Benzidine	PB 81-117343
Beryllium	PB 81-117350
Boron	PB-263943
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SUMMARY CHART

ACENAPHTHENE

CRITERIA:

Aquatic Life

The available data for acenaphthene indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 1,700 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of acenaphthene to sensitive freshwater aquatic animals but toxicity to freshwater algae occur at concentrations as low as 520 ug/L.

The available data for acenaphthene indicate that acute and chronic toxicity to saltwater aquatic life occurs at concentrations as low as 970 and 710 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested. Toxicity to algae occurs at concentrations as low as 500 ug/L.

Human Health

Sufficient data are not available for acenaphthene to derive a level which would protect against the potential toxicity of this compound. Using available organoleptic data, to control undesirable taste and odor quality of ambient water the estimated level is 0.02 mg/L. It should be recognized that organoleptic data, have limitations as a basis for establishing water quality criteria, and have no demonstrated relationship to potential adverse human health effects.

(45 F.R. 79318, November 28, 1980)

SEE APPENDIX B FOR METHODOLOGY

ACROLEIN

CRITERIA:

Aquatic Life

The available data for acrolein indicate that acute and chronic toxicity to freshwater aquatic life occurs at concentrations as low as 68 and 21 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for acrolein indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 55 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of acrolein to sensitive saltwater aquatic life.

Human Health

For the protection of human health from the toxic properties of acrolein ingested through contaminated aquatic organisms, the ambient water criterion is determined o be 320 ug/L.

For the protection of human health from the toxic properties of acrolein ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 780 ug/L.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

ACRYMNITRILE

CRITERIA:

Aquatic Life

The available, data for acrylonitrile indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 7,550 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No definitive data are available concerning the chronic toxicity of acrylonitrile to sensitive freshwater aquatic life but mortality occurs at concentrations as low as 2,600 ug/L with a fish species exposed for 30 days.

Only one saltwater species has been tested with acrylonitrile and no statement can be made concerning acute or chronic toxicity.

Human Health

For the maximum protection of human health from the potential carcinogenic effects resulting from exposure to acrylonitrile through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 0.58 ug/L, 0.058 ug/L, and 0.006 ug/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption

of water, the levels are 6.5 ug/L, 0.65 ug/L, and 0.065 ug/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

AESTHETIC QUALITIES

CRITERIA:

All waters free from substances attributable to wastewater or other discharges that:

(1) settle to form objectionable deposits;

(2) float as debris, scum, oil, or other matter to form nuisances;

(3) produce objectionable color, odor, taste, or turbidity;

(4) injure or are toxic or produce adverse physiological responses in humans, animals or plants: and,

(5) produce undesirable or nuisance aquatic life.

RATIONALE:

Aesthetic qualities of water address the general principles laid down in common law. They embody the beauty and quality of water and their concepts may vary within the minds of individuals encountering the waterway. A rationale for these qualities cannot be developed with quantifying definitions; however, decisions concerning such quality factors can portray the best in the public interest.

Aesthetic qualities provide the general rules to protect water against environmental insults: they provide minimal freedom requirements from pollution; they are essential properties to protect the Nation's waterways.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

ALKALINITY

CRITERION:

20 mg/L or more as $CaCO_3$ freshwater aquatic life except where natural concentrations are less.

INTRODUCTION:

Alkalinity is the sum total of components in the water that tend to elevate the pH of the water above a value of about 4.5. It is measured by titration with standardized acid to a pH value of about 4.5 and it is expressed commonly as milligrams per liter of calcium carbonate. Alkalinity, therefore, is a measure of the buffering capacity of the water, and since pH has a direct effect on organisms as well as an indirect effect on the toxicity of certain other pollutants in the water, the buffering capacity is important to water quality. Examples of commonly occurring materials in natural waters that increase the alkalinity are carbonates, bicarbonates, phosphates and hydroxides.

RATIONALE:

The alkalinity of water used for municipal water supplies is important because it affects the amounts of chemicals that need to be added to accomplish calculation, softening and control of corrosion in distribution systems. The alkalinity of water assists in the neutralization of excess acid produced during the addition of such materials as aluminum sulfate during chemical coagulation. Waters having sufficient alkalinity do not have to be supplemented with artificially added materials to increase the alkalinity. Alkalinity resulting from naturally occurring

materials such as carbonate and bicarbonate is not considered a health hazard in drinking water supplies, per se, and naturally occurring maximum levels up to approximately 400 mg/L as calcium carbonate are not considered a problem to human health (NAS, 1974).

Alkalinity is important for fish and other aquatic life in freshwater systems because it buffers pH changes that occur -naturally as a result of photosynthetic activity of the chlorophyll-bearing vegetation. Components of alkalinity such as carbonate and biocarbonate will complex some toxic heavy metals and reduce their toxicity markedly. For these reasons, the National Technical Advisory Committee (NATC, 1968) recommended a minimum alkalinity of 20 mg/L 'and the subsequent NAS Report (1974) recommended that natural alkalinity not be reduced by more than 25 percent but did not place an absolute minimal value for it. The use of the 25 present reduction avoids the problem of establishing standards on waters where natural alkalinity is at or below 20 mg/L. For such waters, alkalinity should not be further reduced.

The NAS Report recommends that adequate amounts of alkalinity be maintained to buffer the pH within tolerable limits for marine waters. It has been noted as a correlation that productive waterfowl habitats are above 25 mg/L with higher alkalinities resulting in better waterfowl habitats (NATC, 1968).

Excessive alkalinity can cause problems for swimmers by altering the pH of the lacrimal fluid around the eye, causing irritation.

For industrial water supplies, high alkalinity can be damaging to industries involved in food production, especially those in which acidity accounts for flavor and stability, such as the carbonated beverages. In other instances, alkalinity is desirable because water with a high alkalinity is much less corrosive.

A brief summary of maximum alkalinities accepted as a source of raw water by industry is included in Table 1. The concentrations listed in the table are for water prior to treatment and thus are only desirable ranges and not critical ranges for industrial use.

The effect of alkalinity in water used for irrigation may be important in some instances because it may indirectly increase the relative proportion of sodium in soil water. As an example, when bicarbonate concentrations are high, calcium and magnesium ions that are in solution precipitate as carbonates in the soil water as the water becomes more concentrated through evaporation and transpiration. As the calcium and magnesium ions decrease in concentration, the percentage of sodium increases and results in soil and plant damage. Alkalinity may also lead to chlorosis in plants because it causes the iron to precipitate as a hydroxide (NAS, 1974). Hydroxyl ions react with available iron in the soil

TABLE I*

Maximum Alkalinity In Waters Used As A Source Of Supply Prior To Treatment

Industry	Alkalinity mg/L as $CaCO_3$
Steam generation boiler makeup.....	350
Steam generation cooling.....	500
Textile mill products.....	50-200
Paper and allied products.....	75-150
Chemical and Allied Products.....	500
Petroleum refining.....	500
Primary metals industries.....	200
Food canning industries.....	300
Bottled and canned soft drinks.....	85

* NAS, 1974

water and make the iron unavailable to .plants. Such deficiencies induce chlorosis and further plant damage. Usually alkalinity must exceed 6 mg/L before such effects are noticed, however.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

*ALDRIN-DIELDRIN

CRITERIA:

Aquatic Life

Dieldrin

For dieldrin the criterion to protect freshwater aquatic life as derived using the Guidelines is **0.0019** ug/L as a 24-hour average, and the concentration should not exceed **1.0** ug/L at any time.

For dieldrin the criterion to protect saltwater aquatic life as derived using the Guidelines is **0.0019** ug/L as a 24-hour average, and the concentration should not exceed 0.71 ug/L at any time.

Aldrin

For freshwater aquatic life the concentration of aldrin should not exceed 4.0 ug/L at any time. No data are available concerning the chronic toxicity of aldrin to sensitive freshwater aquatic life.

For saltwater aquatic life the concentration of aldrin should not exceed 1.3 ug/L at any time. No data are available concerning the chronic toxicity of aldrin to sensitive saltwater aquatic life.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to aldrin through ingestion of contaminated water and contaminated aquatic organisms, the

*Indicates suspended, canceled or restricted by U.S. EPA Office of Pesticides and Toxic Substances

ambient water concentration should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6} and 10^{-7} The corresponding recommended criteria are 0.74 ng/L, 0.074 ng/L, and 0.0074 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 0.79 ng/L, 0.079 ng/L, and 0.0079 ng/L, respectively.

FoP the maximum protection of human health from the potential carcinogenic effects of exposure to dieldrin through ingestion of contaminated water and Contaminated aquatic organisms, the ambient water concentration should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the ,lifetime are estimated at 10^{-5}, 10^{-6} and 10^{-7}. The corresponding recommended criteria are 0.71 ng/L, 0.071 ng/L, and 0.0071 ng/L, respectively. If these above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 0.76 ng/L, 0.076 ng/L, and 0.0076 ng/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

AMMONIA

SUMMARY :

All concentrations used herein are expressed as un-ionized ammonia (NH_3), because NH_3, not the ammonium ion (NH_4^+) has been demonstrated to be the principal toxic form of ammonia. The data used in deriving criteria are predominantly from flow through tests in which ammonia concentrations were measured. Ammonia was reported to be acutely toxic to freshwater organisms at concentrations (uncorrected €or pH) ranging from 0.53 to 22.8 mg/L NH_3 for 19 invertebrate species representing 14 families and 16 genera and from 0.083 to 4.60 mg/L NH_3 €or 29 fish species from 9 families and 18 genera. Among fish species, reported 96-hour LC50 ranged from 0.083 to 1.09 mg/L for salmonids and from 0.14 to 4.60 mg/L NH_3 for nonsalmonids. Reported data from chronic tests on ammonia with two freshwater invertebrate species, both daphnids, showed effects at concentrations (uncorrected for pH) ranging from 0.304 to 1.2 mg/L NH_3, and with nine freshwater fish species, from five families and seven genera, ranging from 0.0017 to 0.612 mg/L NH_3.

Concentrations of ammonia acutely toxic to fishes may cause loss of equilibrium, hyperexcitability, increased breathing, cardiac output and oxygen uptake, and, in extreme cases, convulsions, coma, and death. At lower concentrations ammonia has many effects on fishes, including a reduction in hatching success, reduction in growth rate and morphological development, and pathologic changes in tissues of gills, livers, and kidneys.

Several factors have been shown to modify acute NH_3 toxicity in fresh water. Some factors alter the concentration of un-ionized ammonia in the water by affecting the aqueous ammonia equilibrium, and some factors affect the toxicity of un-ionized ammonia itself, either ameliorating or exacerbating the effects of ammonia. Factors that have been shown to affect ammonia toxicity include dissolved oxygen concentration, temperature, pH, previous acclimation to ammonia, fluctuating or intermittent exposures, carbon dioxide concentration, salinity, and the presence of other toxicants.

The most well-studied of these is pH; the acute toxicity of NH_3 has been shown to increase as pH decreases. Sufficient data exist from toxicity tests conducted at different pH values to formulate a mathematical expression to describe pH-dependent acute NH_3 toxicity. The very limited amount of data regarding effects of pH on chronic NH_3 toxicity also indicates increasing NH_3 toxicity with decreasing pH, but the data are insufficient to derive a broadly applicable toxicity/pH relationship. Data on temperature effects on acute NH_3 toxicity are limited and somewhat variable, but indications are that NH_3 toxicity to fish is greater as temperature decreases. There is no information available regarding temperature effects on chronic NH_3 toxicity.

Examination of pH and temperature-corrected acute NH_3 toxicity values among species and genera of freshwater organisms showed that invertebrates are generally more tolerant than fishes, a notable exception being the fingernail clam. There is no clear trend among groups of fish; the several most sensitive

tested species and genera include representatives from diverse families (Salmonidae, Cyprinidae, Percidae, and Centrarchidae). Available chronic toxicity data for freshwater organisms also indicate invertebrates (cladocerans, one insect species) to be more tolerant than fishes, again with the exception of the fingernail clam. When corrected for the presumed effects of temperature and pH, there is also no clear trend among groups of fish for chronic toxicity values, the most sensitive species including representatives from five families (Salmonidae, Cyprinidae, Ictaluridae, Centrarchidae, and Catostomidae) and having chronic values ranging by not much more than a factor or two. The range of acute-chronic ratios for 10 species from 6 families was 3 to 43, and acute-chronic ratios were higher for the species having chronic tolerance below the median. Available data indicate that differences in sensitivities between warm and coldwater families of aquatic organisms are inadequate to warrant discrimination in the national ammonia criterion between bodies of water with "warm" and "coldwater" fishes; rather, effects of organism sensitivities on the criterion are most appropriately handled by site-specific criteria derivation procedures.

Data for concentrations of NH_3 toxic to freshwater phytoplankton and vascular plants, although limited, indicate that freshwater plant species are appreciably more tolerant to NH_3 than are invertebrates or fishes. The ammonia criterion appropriate for the protection of aquatic animals will therefore in all likelihood be sufficiently protective of plant life.

Available acute and chronic data for ammonia with saltwater organisms are very limited, and insufficient to derive a saltwater criterion. A few saltwater invertebrate species have been tested, and the prawn Macrobrachiurn *rosenbergii* was the most sensitive. The few saltwater fishes tested suggest greater sensitivity than freshwater fishes. Acute toxicity of NH_3 appears to be greater at low pH values, similar to findings in freshwater. Data for saltwater plant species are limited to diatoms, which appear to be more sensitive than the saltwater invertebrates for which data are available.

More quantitative information needs to be published on the toxicity of ammonia to aquatic life. Several key research needs must be addressed to provide a more complete assessment of ammonia toxicity. These are: (1) acute tests with additional saltwater fish species and saltwater invertebrate species: (2) life-cycle and early life-stage tests with representative freshwater and saltwater organisms from different families, with particular attention to trends of acute-chronic ratios; (3) fluctuating and intermittent exposure tests with a variety of species and exposure patterns: (4) more complete tests of the individual and combined effects of pH and temperature, especially for chronic toxicity: (5) more histopathological and histochemical research with fishes, which would provide a rapid means of identifying and quantifying sublethal ammonia effects; and (6) studies on effects of dissolved and suspended solids on acute and chronic toxicity.

NATIONAL CRITERIA:

The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if:

(1) the 1-hour* average concentration of un-ionized ammonia (in mg/L NH_3) does not exceed, more often than once every 3 years on the average, the numerical value given by 0.52/FT/FPH/2,

where:

$$FT = 10^{-0.03(20-TCAP)}; \quad TCAP \leq T \leq 30$$

$$10^{-0.03(20-T)}; \quad 0 \leq T \leq TCAP$$

$$FPH = 1 \quad ; \quad 8 < pH < 9$$

$$\frac{1+10^{-7.4-PH}}{1.25} \quad ; \quad 6.5 < pH < 8$$

TCAP = 20 C: Salmonids or other sensitive coldwater species present

= 25 C; Salmonids and other sensitive coldwater species absent

(*An averaging period of 1 hour may not be appropriate if excursions of concentrations to greater than 1.5 times the average occur during the hour; in such cases, a shorter averaging period may be needed.)

(2) the 4-day average concentration of un-ionized ammonia (in mg/L NH_3) does not exceed, more often than once every 3 years on the average, the average* numerical value given by 0.80/FT/FPH/RATIO, where FT and FPH are as above and:

$$\text{RATIO} = 16 \quad ; \; 7.7 \leq pH \leq 9$$

$$= 24 \; \frac{10^{-7.7-ph}}{1+10^{-7.4ph}} \quad ; 6.5 \leq ph \leq 7.7$$

TCAP = 15 C; Salmonids or other sensitive coldwater species present

= 20 C: Salmonids and other sensitive coldwater species absent

(*Because these formulas are nonlinear in pH and temperature, the criterion should be the average of separate evaluations of the formulas reflective of the fluctuations of flow, pH, and temperature within the averaging period; it is not appropriate in general to simply apply the formula to average pH, temperature, and flow.)

The extremes for temperature (0, 30) and pH (6.5, 9) given in the above formulas are absolute. It is not permissible with current data to conduct any extrapolations beyond these limits. In particular, there is reason to believe that appropriate criteria at pH > 9 will be lower than the plateau between pH 8 and 9 given above.

Criteria concentrations for the pH range 6.5 to 9.0 and the temperature range 0 C to 30 C are provided in the following tables. Total ammonia concentrations equivalent to each un-ionized ammonia concentration are also provided in these tables. There are limited data on the effect of temperature on chronic toxicity. EPA will be conducting additional research on the effects of temperature on ammonia toxicity in order to fill perceived data gaps. Because of this uncertainty, additional site-specific information should be developed before these

criteria are used in wasteload allocation modeling. For example, the chronic criteria tabulated for sites lacking salmonids are less certain at temperatures much below 20 C than those tabulated at temperatures near 20 C. Where the treatment levels needed to meet these criteria below 20 C may be substantial, use of site-specific criteria is strongly suggested. Development of such criteria should be based upon site-specific toxicity tests.

Data available for saltwater species are insufficient to derive a criterion for saltwater.

The recommended exceedence frequency of 3 years is the Agency's best scientific judgment of the average amount of time it will take an unstressed system to recover from a pollution event in which exposure to ammonia exceeds the criterion. A stressed system, for example, one in which several outfalls occur in a limited area, would be expected to require more time for recovery. The resilience of ecosystems and their ability to recover differ greatly, however, and site-specific criteria may be established if adequate justification is provided.

The use of criteria in designing waste treatment facilities requires the selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria. Limited data or other factors may make their use impractical, in which case one should rely on a steady-state model. The Agency recommends the interim use of 1Q5 or 1Q10 for Criterion Maximum Concentration design flow and 7Q5 or 7Q10 for the Criterion Continuous Concentration design flow in steady-state models for unstressed and stressed systems respectively.

(1) One-hour average concentrations for ammonia.*

pH	0 C	5 C	10 C	15 C	20 C	25 C	30 C
A. Salmonids or Other Sensitive Coldwater Species Present							
Un-ionized Ammonia (mg/liter NH_3)							
6.54	0.0091	0.0129	0.0182	0.026	0.036	0.036	0.036
6.75	0.0149	0.021	0.030	0.042	0.059	0.059	0.059
7.00	0.023	0.033	0.046	0.066	0.093	0.093	0.093
7.25	0.034	0.048	0.068	0.095	0.135	0.135	0.135
7.50	0.045	0.064	0.091	0.128	0.181	0.181	0.181
7.75	0.056	0.080	0.113	0.159	0.22	0.22	0.22
8.00	0.065	0.092	0.130	0.184	0.26	0.26	0.26
8.25	0.065	0.092	0.130	0.184	0.26	0.26	0.26
8.54	0.065	0.092	0.130	0.184	0.26	0.26	0.26
8.15	0.065	0.092	0.130	0.184	0.26	0.26	0.26
9.00	0.065	0.092	0.130	0.184	0.26	0.26	0.26
Total Ammonia (mg/liter NH_3)							
6.50	35	33	31	30	29	20	14.3
6.75	32	30	28	27	27	18.6	13.3
7.00	28	26	25	24	23	16.4	11.6
7.25	23	22	20	19.7	19.2	13.4	9.5
7.50	17.4	16.3	15.5	14.9	14.6	10.1	7.3
7.75	12.2	11.4	10.9	10.5	10.3	7.2	5.2
8.00	8.0	7.5	7.1	6.9	6.8	4.8	3.5
8.25	4.5	4.2	4.1	4.0	3.9	2.8	2.1
8.50	2.6	2.4	2.3	2.3	2.3	1.71	1.28
8.75	1.47	1.40	1.37	1.38	1.42	1.07	0.83
9.00	0.86	0.83	0.83	0.86	0.91	0.72	0.58
B. Salmonids and Other Sensitive Coldwater Species Absent							
Un-ionized Ammonia (mg/liter NH_3)							
6.50	0.0091	0.0129	0.0182	0.026	0.036	0.051	0.051
6.75	0.0149	0.021	0.030	0.042	0.059	0.084	0.084
7.00	0.023	0.033	0.046	0.066	0.093	0.131	0.131
7.25	0.034	0.048	0.068	0.095	0.135	0.190	0.190
7.50	0.045	0.064	0.091	0.128	0.181	0.26	0.26
7.75	0.056	0.080	0.113	0.159	0.22	0.32	0.32
8.00	0.065	0.092	0.130	0.184	0.26	0.37	0.37
8.25	0.065	0.092	0.130	0.184	0.26	0.37	0.37
8.50	0.065	0.092	0.130	0.184	0.26	0.37	0.37
8.75	0.065	0.092	0.130	0.184	0.26	0.37	0.37
9.00	0.065	0.092	0.130	0.184	0.26	0.37	0.37
Total Ammonia (mg/liter NH_3)							
6.50	35	33	31	30	29	29	20
6.75	32	30	28	27	27	26	18.6
7.00	28	26	25	24	23	23	16.4
7.25	23	22	20	19.7	19.2	19.0	13.5
7.50	17.4	16.3	15.5	14.9	14.6	14.5	10.3
7.75	12.2	11.4	10.9	10.5	10.5	10.2	7.3
8.00	8.0	7.5	7.1	6.9	6.8	6.8	4.9
8.25	4.5	4.2	4.1	4.0	3.9	4.0	2.9
8.50	2.6	2.4	2.3	2.3	2.3	2.4	1.81
8.75	1.47	1.40	1.31	1.38	1.42	1.52	1.18
9.00	0.86	0.83	0.83	0.86	0.91	1.01	0.82

* To convert these values to mg/liter N, multiply by 0.822.

(2) 4-day average concentrations for ammonia.*

pH	0 C	5 C	10 C	15 C	20 C	is C	30 C
A. Salmonids or Other Sensitive Coldwater Species Present							
Un-ionized Ammonia (mg/liter NH_3)							
6.50	0.0007	0.0009	0.0013	0.0019	0.0019	0.0019	0.0019
6.75	0.0012	0.0017	0.0023	0.0033	0.0033	0.0033	0.0033
7.00	0.0021	0.0029	0.0042	0.0059	0.0059	0.0059	0.0059
7.25	0.0037	0.0052	0.0074	0.0105	0.0105	0.0105	0.0105
7.50	0.0066	0.0093	0.0132	0.0186	0.0186	0.0186	0.0186
7.75	0.0109	0.01 53	0.022	0.03 1	0.031	0.031	0.031
8.00	0.0126	0.0177	0.025	0.035	0.035	0.035	0.035
8.25	0.0126	0.0177	0.025	0.035	0.035	0.035	0.035
8.50	0.0126	0.0177	0.025	0.035	0.035	0.035	0.035
8.75	0.0126	0.0177	0.025	0.035	0.035	0.035	0.035
9.00	0.0126	0.0171	0.025	0.035	0.035	0.035	0.035
Total Ammonia (mg/liter NH_3)							
6.50	2.5	2.4	2.2	2.2	1.49	1.04	0.73
6.75	2.5	2.4	2.2	2.2	1.49	1.04	0.73
7.00	2.5	2.4	2 3	2.2	1-49	1.04	0.74
7.25	2.5	2.4	2.2	2.2	1.50	1.04	0.74
7.50	2.5	2.4	2.2	2.2	1.50	1.05	0.74
7.75	2.3	2.2	2.1	2.0	1.40	0.99	0.71
8.00	1.53	1.44	1.37	1.33	0.93	0.66	0.47
8.25	0.87	0.82	0.78	0.76	0.54	0.39	0.28
8.50	0.49	0.47	0.45	0.44	0.32	0.23	0.17
8.75	0.28	0.27	0.26	0.27	0.19	0.15	0.11
9.00	0.16	0.16	0.16	0.16	0.13	0.10	0.08
B. Salmonids and Other Sensitive Coldwater Species Absent†							
Un-ionized Ammonia (mg/liter NH_3)							
6.50	0.0007	0.0009	0.0013	0.0019	0.0026	0.0026	0.0026
6.75	0.0012	0.0017	0.0023	0.0033	0.0047	0.0047	0-0047
7.00	0.0021	0.0029	0.0042	0.0059	0.0083	0.0083	0.0083
7.25	0.0037	0.0052	0.0074	0.0105	0.0148	0.0148	0.0148
7.50	0.0066	0.0093	0.0132	0.0186	0.026	0.026	0.026
7.75	0.0109	0.0153	0.022	0.031	0.043	0.043	0.043
8.00	0.0126	0.0177	0.025	0.035	0.050	0.050	0.050
8.25	0.0126	0.0177	0.025	0.035	0.050	0.050	0.050
8.50	0.0126	0.0177	0.025	0.035	0.050	0.050	0.050
8.75	0.0126	0.0177	0.025	0.035	0.050	0.0%	0.050
9.00	0.0126	0.0177	0.025	0.035	0.050	0.050	0.050
Total Ammonia (mg/liter NH_3)							
6.50	25	2.4	2.2	2.2	2.1	1.46 1.20	1.03 0.85
6.75	25	2.4	2.2	23	21	1.47 1.21	1.04 0.85
7.00	2.5	2.4	2.2	2.2	21	1.47 1.21	1.04 0.85
7.25	25	2.4	2.2	2.2	2 I	1.48 1.22	1.05 0.86
7.50	25	2.4	2.2	2.2	2.1	1.49 1.22	1.06 0.87
7.75	23	2.2	2.1	20	1.98	1.39 1.14	1.00 0.82
8.00	153	1.44	1.37	1.33	1.31	0.93 0.76	0.67 0.55
8.25	0.87	0.82	0.78	0.76	0.76	0.54	0.40
8.50	0.49	0.47	0.45	0.44	0.45	0.33	03 5
8.75	0.28	03 7	0.26	0.27	0.27	0.21	0.16
9.00	0.16	0.16	0.16	0.16	0.17	0.14	0.1 I

* To convert these values to mg/liter N, multiply by 0.822.

† Site-specific criteria development is strongly suggested at temperatures above 20 C because of the limited data available to generate the criteria recommendation, and at temperatures below 20 C because of the limited data and because small changes in the criteria may have significant impact on the level of treatment required in meeting the recommended criteria.

The Agency acknowledges that the Criterion Continuous Concentration stream flow averaging period used for steady-state wasteload allocation modeling may be as long as 30 days in situations involving POTWs designed to remove ammonia where limited variability of effluent pollutant concentration and resultant concentrations in receiving waters can be demonstrated. In cases where low variability can be demonstrated, longer averaging periods for the ammonia Criterion Continuous Concentration (e.g., 30-day averaging periods) would be acceptable because the magnitude and duration of exceedences above the Criterion Continuous Concentration would be sufficiently limited. These matters are discussed in more detail in the Technical Support Document for Water Quality-Based Toxics Control (U.S. EPA, 1985a).

(50 F.R. 30784, July 29, 1985)
SEE APPENDIX A FOR METHODOLOGY

ANTIMONY

CRITERIA:

Aquatic Life

The available data for antimony indicate that acute and chronic toxicity to freshwater aquatic life occur at concentrations as low as 9,000 and 1,600 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested. Toxicity to algae occurs at concentrations as low as 610 ug/L.

No saltwater organisms have been adequately tested with antimony, and no statement can be made concerning acute or chronic toxicity.

Human Health

For the protection of human health from the toxic properties of antimony ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 146 ug/L.

For the protection of human health from the toxic properties of antimony ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 45 mg/L.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

ARSENIC

AQUATIC LIFE SUMMARY:

The chemistry of arsenic in water is complex and the form present in solution is dependent on such environmental conditions as Eh, pH, organic content, suspended solids, and sediment. The relative toxicities of the various forms of arsenic apparently vary from species to species. For inorganic arsenic(III) acute values for 16 freshwater animal species ranged from 812 ug/L for a cladoceran to 97,000 ug/L for a midge, but the three acute-chronic ratios only ranged from 4.660 to 4.862. The five acute values for inorganic arsenic(V) covered about the same range, but the single acute-chronic ratio was 28.71. The six acute values for MSMA ranged from 3,243 to 1,403,000 ug/L. The freshwater residue data indicated that arsenic is not bioconcentrated to a high degree but that lower forms of aquatic life may accumulate higher arsenic residues than fish. The low bioconcentration factor and short half-life of arsenic in fish tissue suggest that residues should not be a problem to predators of aquatic life.

The available data indicate that freshwater plants differ a great deal as to their sensitivity to arsenic(III) and arsenic(V). In comparable tests, the alga, Selenastrum capricornutum, was 45 times more sensitive to arsenic(V) than to arsenic(III), although other data present conflicting information on the sensitivity of this alga to arsenic(V). Many plant values for inorganic arsenic(III) were in the same range as the available chronic values for freshwater animals; several

plant values for arsenic(V) were lower than the one available chronic value.

The other toxicological data revealed a wide range of toxicity based on tests with a variety of freshwater species and endpoints. Tests with early life stages appeared to be the most sensitive indicator of arsenic toxicity. Values obtained from this type of test with inorganic arsenic(III) were lower than chronic values contained in Table 2. For example, an effect concentration of 40 ug/L was obtained in a test on inorganic arsenic(III) with embryos and larvae of a toad.

Twelve species of saltwater animals have acute values for inorganic arsenic(III) from 232 to 16,030 ug/L and the single acute-chronic ratio is 1.945. The only values available for inorganic arsenic(V) are for two invertebrate and are between 2,000 and 3,000 ug/L. Arsenic(III) and arsenic(V) are equally toxic to various species of saltwater algae, but the sensitivities of the species range from 19 ug/L to more than 1,000 ug/L. In a test with an oyster, a BCF of 350 was obtained for inorganic arsenic(III).

NATIONAL CRITERIA:

The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration of arsenic(III) does not exceed 190 ug/L more than once every 3 years on the

average and if the 1-hour average concentration does not exceed 360 ug/L more than once every 3 years on the average.

The procedures described in the Guidelines indicate that, except possibly where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration of arsenic(III) does not exceed 36 ug/L more than once every 3 years on the average and if the 1-hour average concentration does not exceed 69 ug/L more than once every 3 years on the average. This criterion might be too high wherever Skeletonema cosrarum or Thalassiosira aestivalis are ecologically important.

Not enough data are available to allow derivation of numerical national water quality criteria for freshwater aquatic life for inorganic arsenic(V) or any organic arsenic compound. Inorganic arsenic(V) is acutely toxic to freshwater aquatic animals at concentrations as low as 850 ug/L and an acute-chronic ratio of 28 was obtained with the fathead minnow. Arsenic(V) affected freshwater aquatic plants at concentrations as low as 48 ug/L. Monosodium methanearsenace (MSMA) is acutely toxic to aquatic animals at concentrations as low as 1,900 ug/L, but no data are available concerning chronic toxicity to animals or toxicity to plants.

Very few data are available concerning the toxicity of any form of arsenic other than inorganic arsenic(III) to saltwater aquatic life. The available data do show that inorganic arsenic(V) is acutely toxic to saltwater animals at concentrations as low as 2,319 ug/L and affected some saltwater

plants at 13 to 56 ug/L. No data are available concerning the chronic toxicity of any form of arsenic other than inorganic arsenic(III) to saltwater aquatic life.

EPA believes that a measurement such as "acid-soluble" would provide a more scientifically correct basis upon which to establish criteria for metals. The criteria were developed on this basis. However, at this time, no EPA approved methods for such a measurement are available to implement the criteria through the regulatory programs of the Agency and the States. The Agency is considering development and approval of methods for a measurement such as acid-soluble. Until available, however, EPA recommends applying the criteria using the total recoverable method. This has two impacts: (1) certain species of some metals cannot be analyzed directly because the total recoverable method does not distinguish between individual oxidation states, and (2) these criteria may be overly protective when based on the total recoverable method.

The recommended exceedence frequency of 3 years is the Agency's best scientific judgment of the average amount of time it will take an unstressed system to recover from a pollution event in which exposure to arsenic(III) exceeds the criterion. a stressed system, for example, one in which several outfalls occur in a limited area, would be expected to require more time for recovery. The resilience of ecosystems and their ability to recover differ greatly, however, and site-specific criteria may be established if adequate justification is provided.

The use of criteria in designing waste treatment facilities requires the selection of an appropriate wasteload allocation

model. Dynamic models are preferred for the application of these criteria. Limited data or other factors may make their use impractical, in which case one should rely on a steady-state model, The Agency recommends the interim use of 1Q5 or 1Q10 for Criterion Maximum Concentration design flow and 7Q5 or 7Q10 for the Criterion Continuous Concentration design flow in steady-state models for unstressed and stressed systems respective1y. These matters are discussed in more detail in the Technical Support Document fox Water Quality-Based Toxics Control (U.S. EPA, 1985).

HUMAN HEALTH CRITERIA:

For the maximum protection of human health from the potential carcinogenic effects due to exposure of arsenic through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero based on the non-threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-6}, and 10^{-7}. The corresponding criteria are 22 ng/L, 2.2 ng/L, and .22 ng/L, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 175 ng/L, 17.5 ng/L, and 1.75 ng/L, respectively. Other concentrations representing different risk levels may be calculated by use of the Guidelines. The risk estimate range is presented for information purpoes and does not represent an Agency judgment on an "acceptable" risk level.

(45 F.R. 79318 Nov. 28,1980) (50 F.R. 30784, July 29, 1985)
SEE APPENDIX A FOR METHODOLOGY

ASBESTOS

CRITERIA:

Aquatic Life

No freshwater organisms have been tested with any asbestiform mineral and no statement can be made concerning acute or chronic toxicity

No saltwater organisms have been tested with any asbestiform mineral and no statement can be made concerning acute or chronic toxicity.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to asbestos through ingestion of water and contaminated aquatic organisms, the ambient water concentration should be zero. The estimated levels which would result in increased lifetime cancer risks of 10^{-5}, 10^{-6}, and 10^{-7} are 300,000 fibers/L, 30,000 fibers/L, and 3,000 fibers/L, respectively. Estimates for consumption of aquatic organisms only, excluding the consumption of water cannot be made.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

BACTERIA

CRITERIA

Freshwater Bathing

Based on a statistically sufficient number of samples (generally not less than 5 samples equally spaced over a 30-day period), the geometric mean of the indicated bacterial densities should not exceed one or the other of the following:[1]

E. coli	126 per 100 ml; or
enterococci	33 per 100 ml;

no sample should exceed a one sided confidence limit (C.L.) calculated using the following as guidance:

designated bathing beach	75% C.L.
moderate use for bathing	82% C.L.
light use for bathing	90% C.L.
infrequent use for bathing	95% C.L.

based on a site-specific log standard deviation, or if site data are insufficient to establish a log standard deviation, then using 0.4 as the log standard deviation for both indicators.

Marine Water Bathing

Based on a statistically sufficient number of samples (generally not less than 5 samples equally spaced over a 30-day period), the geometric mean of the enterococci densities should not exceed 35 per 100 ml; no sample should exceed a one sided confidence limit using the following as guidance:

designated bathing beach	75% C.L.
moderate use for bathing	82% C.L.
light use for bathing	90% C.L.
infrequent use for bathing	95% C.L.

based on a site-specific log standard deviation, or if site data are insufficient to establish a log standard deviation, then using 0.7 as the log standard deviation.

Note (1) - Only one indicator should be used. The Regulatory agency should select the appropriate indicator for its conditions.

Shellfish Harvesting Waters

The median fecal coliform bacterial concentration should not exceed 14 MPN per 100 ml with not more than 10 percent of samples exceeding 43 MPN per 100 ml for the taking of shellfish.

RATIONALE

Bathing Waters

A recreational water quality criterion can be defined as a "quantifiable relationship between the density of an indicator in the water and the potential human health risks involved in the water's recreational use." From such a definition, a criterion can be adopted which establishes upper limits for densities of indicator bacteria in waters that are associated with acceptable health risks for swimmers.

The Environmental Protection Agency, in 1972, initiated a series of studies at marine and fresh water bathing beaches which were designed to determine if swimming in sewage-contaminated marine and fresh water carries a health risk for bathers; and, if so, to what type of illness. Additionally, the Agency wanted to determine which bacterial indicator is best correlated to swimming-associated health effects and if the relationship is strong enough to provide a criterion. (1)

The quantitative relationships between the rates of swimming-associated health effects and bacterial indicator densities were determined using regression analysis. Linear relationships were estimated from data grouped on the basis of summers or trials with similar indicator densities. The data for each summer were analyzed by pairing the geometric mean indicator density for a summer bathing season at each beach with the corresponding swimming-associated gastrointestinal illness rate for the same summer. The swimming-associated illness rate was determined by subtracting the gastrointestinal illness rate in nonswimmers from that for swimmers. Ihese two variables from multiple beach sites were used to calculate a regression coefficient, y-intercept and 95% confidence intervals for the paired data. In the marine studies the total number of points for use in regression analysis was increased by collecting trial days with similar indicator densities from each study location and placing them into groups. The swimming-associated illness rate was determined as before, by subtracting the nonswimmer illness rate of all the individuals included in the grouped trial days from the swimmer illness rate during these safe grouped trial days. The grouping by trial days with similar indicator densities approach was not possible with the freshwater data because the variation of bacterial indicator densities in freshwater samples was not larqe enouqh to allow such an adjustment to be made. For the saltwater studies the results of the regression analyses of illness rates against indicator density data was very similar using the "by summer" or "by grouped trial days" approaches.

The methods used to enumerate the bacterial indicator densities which showed the best relationship to swimming-associated gastroenteritis rates were specifically developed €or the recreational water quality studies.'

These membrane filter methods have successfully undergone precision and bias testing by the EPA Environmental Monitoring and Support Laboratory. (2)

Several monitoring situations to assess bacterial quality are encountered by regulatory agencies. The situation needing the most rigorous monitoring is the designated swimming beach. Such areas are frequently lifeguard protected, provide parking and other public access and are heavily used by the public. Public beaches of this type were used by EPA in developing the relationship described in this document.

Other recreational activities may involve bodies of water which are regulated by individual State water quality standards. These recreational resources may be natural wading ponds used by children or waters where incidential full body contact occurs because of water skiing or other similar activities.

EPA's evaluation of the bacteriological data indicated that using the fecal coliform indicator group at the maximum geometric mean of 200 per 100 ml, recommended in Quality Criteria for Water would cause an estimated 8 illness per 1,000 swimmers at fresh water beaches and 19 illness per 1,000 swimmers at marine beaches. These relationships are only approximate and are based on applying ratios of the geometric means of the various indicators from the EPA studies to the 200 per 100 ml fecal

coliform criterion. However, these are EPA's best estimates of the accepted illness rates for areas which apply the EPA fecal coliform criterion.

The E. coli and enterococci criteria presented in Table 1 were developed using these currently accepted illness rates. The equations developed by Dufour[3] and Cabelli[4] were used to calculate the geometric mean indicator densities corresponding to the accepted gastrointestinal illness rates. These densities are for steady state dry weather conditions. The beach is in noncompliance with the criteria if the geometric mean of several bacterial density samples exceeds the value listed in Table 1.

Noncompliance is also signaled by an unacceptably high value for any single bacterial sample. The maximum acceptable bacterial density for a single sample is set higher than that for the geometric mean, in order to avoid necessary beach closings based on single samples. In deciding whether a beach should be left open, it is the long term geometric mean bacterial density that is of interest. Because of day-to-day fluctuations around this mean, a decision based on a single sample (or even several samples) may be erroneous, i.e., the sample may exceed the recommended mean criteria even though the long-term geometric mean is protective, or may fall below the maximum even if this mean is in the nonprotective range.

To set the single sample maximum, it is necessary to specify the desired chance that the beach will be left open when the protection is adequate. This chance, or confidence level, was based on Agency judgment. For the simple decision rule considered here, a smaller confidence level corresponds to a more

stringent (i.e. lower) single sample maximum. Conversely, a greater confidence level corresponds to less stringent (i.e. higher) maximum values. This technique reduces the chances of single samples inappropriately indicating violations of the recommended criteria.

By using a control chart analogy [5] and the actual log standard deviations from the EPA studies, single sample maximum densities for various confidence levels were calculated. EPA then assigned qualitative use intensities to those confidence levels. A low confidence level (75%) was assigned to designated beach areas because a high degree of caution should be used to evaluate water quality for heavily used areas. Less intensively used areas would allow less restrictive single sample limits. Thus, 95% confidence might be appropriate for swimmable water in remote areas. Table 1 summarizes the results of these calculations. These single sample maximum levels should be recalculated for individual areas if significant differences in log standard deviations occur.

The levels displayed in Table 1 depend not only on the assumed standard deviation of log densities, but also on the chosen level of acceptable risk. While this level was based on the historically accepted risk, it is still arbitrary insofar as the historical risk was itself arbitrary.

Currently EPA is not recommending a change in the stringency of its bacterial criteria for recreational waters. Such a change does not appear warranted until more information based on greater experience with the new indicators can be accrued. EPA and the

State Agencies can then evaluate the impacts of change in terms of beach closures and other restricted uses.

Shellfish Harvesting Waters

The microbiological criterion for shellfish water quality has been accepted by international agreement to be 70 total coliforms per 100 ml, using a median MPN, with no more than 10 percent of the values exceeding 230 total coliforms. No evidence of disease outbreak from consumption of raw shellfish which were grown in waters meeting this bacteriological criterion has been demonstrated. This standard has proven to be a practical limit when supported by sanitary surveys of the growing waters, acceptable quality in shellfish meats, and good epidemiological evidence. However, evidence from field investigations suggests that not all total coliform occurrences can be associated with fecal pollution. Thus, attention has been directed toward the adoption of the fecal coliform test to measure more accurately the occurrence and magnitude of fecal pollution in shellfish-growing waters.

A series of studies was initiated by the National Shellfish Sanitation Program and data relating the occurrence of total coliforms to numbers of fecal coliforms were compiled. The data showthata 70 coliform MPN per 100 ml at the 50th percentile was equivalent to a fecal coliform MPN of 14 per 100 ml. The data, therefore, indicate that a median value for a fecal coliform standard is 15 and the 90th percentile shouldnot exceed 43 for a 5-tube, 3-dilution method.

EPA is currently (1986) co-sponsoring, with the National

Oceanic and Atmospheric Administration, research into the application of the enterococci and E. coli indicators for assessing the quality of shellfish harvesting waters. The Food and Drug Administration is also reviewing the results of these studies. A change to the new indicators may be forthcoming if the studies show a correlation between gastrointestinal disease and the consumption of raw shellfish from waters with defined densities of the new indicators. However, these studies have not sufficiently progressed to justify any change at this time. Thus, evaluation of the microbiological suitability of waters for recreational taking of shellfish should be based upon the fecal coliform bacterial levels. (6)

CRITERIA FOR INDICATOR FOR BACTERIOLOGICAL DENSITIES

	Acceptable Swimming Associated Gastro-enteritis Rate per 1000 swimmers	Steady State Geometric Mean Indicator Density	Single sample Maximum Allowable Density (4), (5)			
			Designated Beach Area (upper 75%C.L.)	Moderate Full Body contact Recreation (upper 82%C.L.)	Lightly Used Full m y Contact Recreation (upper 90%C.L.)	Infrequently Used Full m y Contact Recreation (upper 95%C.L.)
Freshwater						
enterococci	8	33(1)	61	89	108	151
E. coli	8	126(2)	235	298	406	576
Marine Water						
enterococci	19	35(3)	104	124	276	500

Notes:

(1) Calculated to nearest whole nunber using equation:

$$\text{(mean enterococci density)} = \text{antilog}_{10} \frac{\text{illness rate/1000 people} + 6.28}{9.40}$$

(2) Calculated to nearest whole number using equation:

$$\text{(mean } \underline{\text{E. coli}} \text{ density)} = \text{antilog}_{10} \frac{\text{illness rate/1000 people} + 11.74}{9.40}$$

(3) Calculated to nearest whole nunber using equation:

$$\text{(mean enterococci density)} = \text{antilog}_{10} \frac{\text{illness rate/1000 people} - 0.20}{12.17}$$

(4) Single sample limit=antilog$_{10}$ (Log$_{10}$ indicator geometric mean density/100 ml) + Factor determined from areas under the Normal probability curve for the assumed level of probability x (log$_{10}$ stand. deviation)

The appropriate factors for the indicated one sided confidence levels are:

75%C.L. - .675
82% CL - .935
90%C.L. - 1.28
95%C.L. - 1.65

(5) Based on the observed log standard deviations during the EPA studies: 0.4 for freshwater E coli and enterococci; and 0.7 for marine water enterococci. Each jurisdiction should establish its own standard deviation for its conditions which would then vary the single sample limit.

References

1. Ambient Water Quality Criteria for Bacteria - 1986, EPA 440/5-84-002, U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC. (NTIS access #: PB 86-158-045)

2. Test Methods for Escherichia coli and Enterococii in Water By The Membrane Filter procedure, EPA 600/4-85-076, U.S. Environmental Protection Agency, Cincinnati, OH. (NTIS access #: PB 86-158-052)

3. Dufour, A. P. 1983. Health Effects Criteria for Fresh Recreationa1 Waters. EPA-600/1-84-004, U.S. Environmental Protection Agency, Cincinnati, OH.

4. Cabelli, V. J. 1981. Health Effects Criteria for Marine Recreational Waters. EPA-600/1-80-031, U.S. Environxental Protection Agency, Cincinnati, OH.

5. ASTM. 1951. Manual on Quality Control of Materials. Special Technical Publication 15-C, American Society for Testing and Materials, Philadelphia, PA.

6. U.S. Environmental Protection Agency. 1976. Quality Criteria for Water. U.S. Environmental Protection Agency, Washington, DC.

BARIUM

CRITERION:

1 mg/L for domestic water supply (health).

INTRODUCTION:

Barium is a yellowish-white metal of the alkaline earth group. It occurs in nature chiefly as barite, $BaSO_4$ and witherite, $BaCO_3$, both of which are highly insoluble salts. The metal is stable in dry air, but readily oxidized by humid air or water.

Many of the salts of barium are soluble in both water and acid, and soluble barium salts are reported to be poisonous (Lange, 1965: NAS, 1974). However, barium ions generally are thought to be rapidly precipitated or removed from solution by absorption and sedimentation (McKee and Wolf, 1963 NAS, 1974).

While barium is a malleable, ductile metal, its major commercial value is in its compounds. Barium compounds are used in a variety of industrial applications including the metallurgic, paint, glass and electronics industries, as well as for medicinal purposes.

RATIONALE:

Concentrations of barium drinking water supplies generally range from less than 0.6 ug/L to approximately 10 ug/L with upper limits in a few midwestern and western States ranging from 100 to 3,000 ug/L (PHS, 1962/1963; Katz, 1970; Little, 1971). Barium enters the body primarily through air and water, since appreciable amounts are not contained in foods (NAS, 1974).

The fatal dose of barium for man is reported to be 550 to 600 mg. Ingestion of soluble barium compounds may also result in effects on the gastrointestinal tract, causing vomiting and diarrhea, and on the central nervous system, causing violent tonic and clonic spasms followed in some cases by paralysis (Browning, 1961; Patty, 1962, cited in Preliminary Air Pollution Survey of Barium and Its Compounds, 1969). Barium salts are considered to be muscle stimulants, especially for the heart muscle (Sollman, 1957). By constricting blood vessels, barium may cause an increase in blood pressure. On the other hand, it is not likely that barium accumulates in the bone, muscle, kidney or other tissues because it is readily excreted (Browning, 1961; McKee and Wolf, 1963).

Stokinger and Woodward (1958) developed a safe concentration for barium in drinking water based on the limiting values for industrial atmospheres, an estimate of the amount absorbed into the blood stream, and daily consumption of 2 liters of water. From other factors they arrived at a limiting concentration of 2 mg/L for a healthy adult human population, to which a safety factor was applied to allow for any possible accumulation in the body. Since barium is not removed by conventional water treatment processes and because of the toxic effect on the heart and blood vessels, a limit of 1 mg/L is recommended for barium in domestic water supplies.

Experimental data indicate that the soluble barium concentration in fresh and marine water generally would have to exceed 50 mg/L before toxicity to aquatic life would be expected. In most natural waters, there is sufficient sulfate or carbonate

to precipitate the barium present in the water as a virtually insoluble, non-toxic compound. Recognizing that the physical and chemical properties of barium generally will preclude the existence of the toxic soluble form under usual marine and fresh water conditions, a restrictive criterion for aquatic life appears unwarranted.

(QUALITY CRITERIA FOR WATER, JULY **1976)** **PB-263943**
SEE APPENDIX **C** FOR METHODOLOGY

BENZENE

CRITERIA:

Aquatic Life

The available data for benzene indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 5,300 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of benzene to sensitive freshwater aquatic life.

The available data for benzene indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 5,100 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No definitive data are available concerning the chronic toxicity of benzene to sensitive saltwater aquatic life, but adverse effects occur at concentrations as low as 700 ug/L with a fish species exposed for 168 days.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to benzene through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the non threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 6.6 ug/L, 0.66 ug/L, and

0.066 ug/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 400 ug/L, 40.0 ug/L, and 4.0 ug/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

BENZIDINE

CRITERIA:

Aquatic Life

The available data for benzidine indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 2,500 ug/L and would occur at lower concentrations among species that are more sensitive than those tested No data are available concerning the chronic toxicity of benzidine to sensitive freshwater aquatic life.

No saltwater organisms have been tested with benzidine and no statement can be made concerning acute and chronic toxicity.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to benzidine through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 1.2 ng/L, 0.12 ng/L, and 0.01 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 5.3 ng/L, 0.53 ng/L, and 0.05 ng/L, respectively.

(45 F.R. 79318, November 28. 1980)
SEE APPENDIX B FOR METHODOLOGY

BERYLLIUM

CRITERIA:

Aquatic Life

The available data for beryllium indicate that acute and chronic toxicity to freshwater aquatic life occur at concentrations as low as 130 and 5.3 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested. Hardness has a substantial effect on acute toxicity.

The limited saltwater data base available for beryllium does not permit any statement concerning acute or chronic toxicity.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to beryllium through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero, based on the non threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 37 ng/L, 3.7 ng/L, and 0.37 ng/L, respectively. If these estimates are made for

consumption of aquatic organisms only, excluding consumption of water, the levels are 641 ng/L, 64.1 ng/L, and 6.41 ng/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

BORON

CRITERION:

750 mg/L for long-term irrigation on sensitive crops.

INTRODUCTION:

Boron is not found in its elemental form in nature: it is usually found as a sodium or calcium borate salt. Boron salts are used in fire retardants, the production of glass, leather tanning and finishing industries, cosmetics, photographic materials, metallurgy and for high energy rocket fuels. Elemental boron also can be used in nuclear reactors for neutron absorption. Borates are used as "burnable" poisons.

RATIONALE :

Boron is an essential element for growth of plants but there is no evidence that it is required by animals, The maximum concentration found in 1,546 samples of river and lake waters from various parts of the United States was 5.0 mg/L; the mean value was 0.1 mg/L (Kopp and Kroner, 1967). Ground waters could contain substantially higher concentrations at certain places. The concentration in seawater is reported as 4.5 mg/L in the form of borate (NAS, 1974). Naturally occurring concentrations of boron should have no effects on aquatic life.

The minimum lethal dose for minnows exposed to boric acid at 20 °C for 6 hours was reported to be 18,000 to 19,000 mg/L in distilled water and 19,000 to 19,500 mg/L in hard water (Le Clerc and Devlaminck, 1955: Le Clerc, 1960).

In the dairy cow, 16 to 20 g/day of boric acid for 40 days produced no ill effects (McKee and Wolf, 1963).

Sensitive crops have shown toxic effects at 1000 ug/L or less of boron (Richards, 1954). Bradford (1966), in a review of boron deficiencies and toxicities, stated that when the boron concentration in irrigation waters was greater than 0.75 ug/L, some sensitive plants such as citrus began to show injury. Biggar and Fireman (1960) showed that with neutral and alkaline soils of high absorption capacities, water containing 2 ug/L boron might be used for some time without injury to sensitive plants. The criterion of 750 ug/L is thought to protect sensitive crops during long-term irrigation.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

CADMIUM

AQUATIC LIFE SUMMARY:

Freshwater acute values for cadmium are available for species in 44 genera and range from 1.0 ug/L for rainbow trout to 28,000 ug/L for a mayfly. The antagonistic effect of hardness on acute toxicity has been demonstrated with five species. Chronic tests have been conducted on cadmium with 12 freshwater fish species and 4 invertebrate species with chronic values ranging from 0.15 ug/L for *Daphnia magna* to 156 ug/L for the Atlantic salmon. Acute-chronic ratios are available for eight species and range from 0.9021 for the chinook salmon to 433.8 for the flagfish.

Freshwater aquatic plants are affected by cadmium at concentrations ranging from 2 to 7,400 ug/L. These values are in the same range as the acute toxicity values for fish and invertebrate species, and are considerably above the chronic values. Bioconcentration factors (BCFs) for cadmium in fresh water range from 164 to 4,190 for invertebrates and from 3 to 2,213 for fishes.

Saltwater acute values for cadmium and five species of fishes range from 577 ug/L for larval Atlantic silverside to 114,000 ug/L for juvenile mummichog. Acute values for 30 species of invertebrates range from 15.5 ug/L for a mysid to 135,000 ug/L for an oligochaete worm. The acute toxicity of cadmium generally increases as salinity decreases. The effect of temperature seems to be species-specific. Two life-cycle tests with *Mysidopsis bahia* under different test conditions resulted in similar chronic values of 8.2 and 7.1 ug/L, but the acute-chronic ratios were 1.9 and 15, respectively. The acute values appear to

reflect effects of salinity and temperature, whereas the few available chronic values apparently do not. A life-cycle test with Mysidopsis bigelowi also resulted in a chronic value of 7.1 ug/L and an acute-chronic ratio of 15. Studies with microalgae and macroalgae revealed effects at 22.8 to 860 ug/L.

BCFs determined with a variety of saltwater invertebrates ranged from 5 to 3,160. BCFs for bivalve molluscs were above 1,000 in long exposures, with no indication that steady-state had been reached. Cadmium mortality is cumulative for exposure periods beyond 4 days. Chronic cadmium exposure resulted in significant effects on the growth of bay scallops at 78 ug/L and on reproduction of a copepod at 44 ug/L.

NATIONAL CRITERIA:

The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration (in ug/L) of cadmium does not exceed the numerical value given by $e^{(0.7852[\ln(\text{hardness})]-3.490)}$ more than once every 3 years on the average and if the one-hour average concentration (in ug/L) does not exceed the numerical value given by $e^{(1.128[\ln(\text{hardness})]-3.828)}$ more than once every 3 years on the average. For example, at hardnesses of 50, 100, and 200 mg/L as $CaCO_3$ the 4-day average concentrations of cadmium are 0.66, 1.1, and 2.0 ug/L, respectively, and the 1-hour average concentrations are

1.8, 3.9 and 8.6 ug/L. If brook trout, brown trout, and striped bass are as sensitive as some data indicate, they might not be protected by this criterion.

The procedures described in the Guidelines indicate that, except possibly where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration of cadmium does not exceed 9.3 ug/L more than once every 3 years on the average and if the 1-hour average concentration does not exceed 43 ug/L more than once every 3 years on the average. The little information that is available concerning the sensitivity of the American lobster to cadmium indicates that this important species might not be protected by this criterion. In addition, data suggest that the acute toxicity of cadmium is salinity dependent: therefore, the 1-hour average concentration might be underprotective at low salinities and overprotective at high salinities.

EPA believes that a measurement such as "acid-soluble" would provide a more scientifically correct basis upon which to establish criteria for metals. The criteria were developed on this basis. However, at this time, no EPA-approved methods for such a measurement are available to implement the criteria through the regulatory programs of the Agency and the States. The Agency is considering development and approval of methods for a measurement such as acid-soluble. Until available, however, EPA recommends applying the criteria using the total recoverable method. This has two impacts: (1) certain species of some metals

cannot be analyzed directly because the total recoverable method does not distinguish between individual oxidation states, and (2) these criteria may be overly protective when based on the total recoverable method.

The recommended exceedence frequency of 3 years is the Agency's best scientific judgment of the average amount of time it will take an unstressed system to recover from a pollution event in which exposure to cadmium exceeds the criterion. A stressed system, for example, one in which several outfalls occur in a limited area, would be expected to require more time for recovery. The resilience of ecosystems and their ability to recover differ greatly, however, and site-specific criteria may be established if adequate justification is provided.

The use of criteria in designing waste treatment facilities requires the selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria. Limited data or other factors may make their use impractical, in which case one should rely on a steady-state model. The Agency recommends the interim use of 1Q5 or 1Q10 for Criterion Maximum Concentration design flow and 7Q5 or 7Q10 for the Criterion Continuous Concentration design flow in steady-state models for unstressed and stressed systems, respectively. These matters are discussed in more detail in the Technical Support Document for Water Quality-Based Toxics Control (U.S. EPA, 1985).

HUMAN HEALTH CRITERIA:

The ambient water quality criterion for cadmium is recommended to be identical to the existing drinking water

standard which is 10 ug/L. Analysis of the toxic effects data resulted in a calculated level which is protective of human health against the ingestion of contaminated water and contaminated aquatic organisms. The calculated value is comparable to the present standard. For this reason a selective criterion based on exposure solely from consumption of 6.5 grams of aquatic organisms was not derived.

(45 F.R. 79318 Nov. 28,1980) (50 F.R. 30784, July 29, 1985)
SEE APPENDIX A FOR METHODOLOGY

CARBON TETRACHLORIDE

CRITERIA:

Aquatic Life

The available data for carbon tetrachloride indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 35,200 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of carbon tetrachloride to sensitive freshwater aquatic life.

The available data for carbon tetrachloride indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 50,000 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of carbontetrachloride to sensitive saltwater aquatic life.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to carbon tetrachloride through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 4.0 ug/L, 0.40ug/L, and 0.04 ug/L, respectively. If these estimates are made for

consumption of aquatic organisms only, excluding consumption of water, the levels are 69.4 ug/L, 6.94 ug/L, and 0.69 ug/L respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

CHLORDANE

CRITERIA:

Aquatic Life

For chlordane the criterion to protect freshwat r aqu tic life as derived using the Guidelines is 0.0043 ug/L as a 24-hour average, and the concentration should not exceed 2.4 ug/L at any time.

For chlordane the criterion to protect saltwater aquatic life as derived using the Guidelines is 0.0040 ug/L as a 24-hour average, and the concentration should not exceed 0.09 ug/L at any time.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to chlordane through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 4.6 ng/L, 0.46 ng/L, and 0.046 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 4.8 ng/L, 0.48 ng/L, and 0.048 ng/L, respectively.

(45 F.R. 79318, November 28, 1980)

SEE APPENDIX B FOR METHODOLOGY

CHLORINATED BENZENES

CRITERIA:

Aquatic Life

The available data for chlorinated benzenes indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 250 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of the more toxic of the chlorinated benzenes to sensitive freshwater aquatic life, but toxicity occurs at concentrations as low as 50 ug/L for a fish species exposed for 7.5 days.

The available data for chlorinated benzenes indicate that acute and chronic toxicity to saltwater aquatic life occur at concentrations as low as 160 and 129 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

Human Health

Monochlorobenzene

For comparison purposes, two approaches were used to derive criterion levels for monochlorobenzene. Based on available toxicity data, for the protection of public health the derived level is 488 ug/L. Using available organoleptic data, to control undesirable taste and odor quality of ambient water the estimated level is 20 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing water quality criteria, and have no demonstrated relationship to potential adverse human health effects.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

CHLORINATED ETHANES

CRITERIA:

Aquatic Life

The available freshwater data for chlorinated ethanes indicate that toxicity increases greatly with increasing chlorination, and that acute toxicity occurs at concentrations as low as 118,000 ug/L for 1,2-dichloroethane, 18,000 ug/L for two trichloroethanes, 9,320 ug/L for two tetrachloroethanes, 7,240 ug/L for pentachloroethane, and 980 ug/L for hexachloroethane. Chronic toxicity occurs at concentrations as low as 20,000 ug/L for 1,2-dichloroethane, 9,400 ug/L for 1,1,2-trichloroethane, 2,400 ug/L for 1,1,2,2-tetrachloroethane, 1,100 ug/L for pentachloroethane, and 540 ug/L for hexachloroethane. Acute and chronic toxicity would occur at lower concentrations among species that are more sensitive than those tested.

The available saltwater data for chlorinated ethanes indicate that toxicity increases greatly with increasing chlorination and that acute toxicity to fish and invertebrate species occurs at concentrations as low as 113,000 ug/L for 1,2-dichloroethane, 31,200 ug/L for 1,1,1-trichloroethane, 9,020 ug/L for 1,1,2,2-tetrachloroethane, 390 ug/L for pentachloroethane, and 940 ug/L for hexachloroethane. Chronic toxicity occurs at concentrations as low as 281 ug/L for pentachloroethane. Acute and chronic toxicity would occur at lower concentrations among species that are more sensitive than those tested.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to 1,2-dichloroethane through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 9.4 ug/L, 0.94 ug/L, and 0.094 ug/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water,' the levels are 2,430 ug/L, 243 ug/L, and 24.3 ug/L, respectively.

For the maximum protection of human health from the potential carcinogenic effects of exposure to 1,1,2-trichloroethane through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 6.0 ug/L, 0.6 ug/L, and 0.06 ug/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 418 ug/L, 41.8 ug/L, and 4.18 ug/L, respectively.

For the maximum protection of human health from the potential carcinogenic effects of exposure to 1,1,2,2-tetrachloroethane through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 1.7 ug/L, 0.17 ug/L, and 0.017 ug/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 107 ug/L, 10.7 ug/L, and 1.07 ug/L, respectively.

For the maximum protection of human health from the potential carcinogenic effects of exposure to hexachloroethane through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 19 ug/L, 1.9 ug/L, and 0.19 ug/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 87.4 ug/L, 8.74 ug/L, and 0.87 ug/L, respectively.

For the protection of human health from the toxic properties of 1,1,1-trichloroethane ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 18.4 mg/L.

For the protection of human health from the toxic properties of 1,1,1-trichloroethane ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 1.03 ug/1.

Because of insufficient available data €or monochloroethane, 1,1-dichloroethane, 1,1,1,2-tetrachloroethane, and pentachloroethane, satisfactory criteria cannot be derived at this time, using the present guidelines.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

CHLORINATED NAPHTHALENES

CRITERIA:

Aquatic Life

The available data for chlorinated naphthalenes indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 1,600 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of chlorinated naphthalenes to sensitive freshwater aquatic life.

The available data for chlorinated naphthalenes,indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 7.5 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of chlorinated naphthalenes to sensitive saltwater aquatic life.

Human Health

Using the present guidelines, a satisfactory criterion cannot be derived at this time because of insufficient available data for chlorinated naphthalenes.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

CHLORINE

SUMMARY:

Thirty-three freshwater species in 28 genera have been exposed to TRC and the acute values range from 28 ug/L for *Daphnia magna* to 710 ug/L for the threespine stickleback. Fish and invertebrate species had similar ranges of sensitivity. Freshwater chronic tests have been conducted with two invertebrate and one fish species and the chronic values for these three species ranged from less than 3.4 to 26 ug/L, with acute-chronic ratios from 3.7 to greater than 78.

The acute sensitivities of 24 species of saltwater animals in 21 genera have been determined for CPO, and the LC50 range from 26 ug/L for the eastern oyster to 1,418 ug/L for a mixture of two shore crab species. This range is very similar to that observed with freshwater species, and fish and invertebrate species had similar sensitivities. Only one chronic test has been conducted with a saltwater species, *Menidia peninsulae*, and in this test the acute chronic ratio was 1.162.

The available data indicate that aquatic plants are more resistant to chlorine than fish and invertebrate species.

NATIONAL CRITERIA:

The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected

unacceptably if the 4-day average concentration of total residual chlorine does not exceed 11 ug/L more than once every 3 years on the average and if the 1-hour average concentration does not exceed 19 ug/L more than once every 3 years on the average.

The procedures described in the Guidelines indicate that, except possibly where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration of chlorine-produced oxidants does not exceed 7.5 ug/L more than once every 3 years on the average and if the one-hour average concentration does not exceed 13 ug/L more than once every 3 years on the average.

The recommended exceedence frequency of 3 years is the Agency's best scientific judgment of the average amount of time it will take an unstressed system to recover from a pollution event in which exposure to chlorine exceeds the criterion. A stressed system, for example, one in which several outfalls occur in a limited area, would be expected to require more time for recovery. The resilience of ecosystems and their ability to recover differ greatly, however, and site-specific criteria may be established if adequate justification is provided.

The use of criteria in designing waste treatment facilities requires the selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria. Limited data or other factors may make their use impractical, in which case one should rely on a steady-state model. The Agency recommends the interim use of 1Q5 or 1Q10 for Criterion Maximum Concentration design flow and 7Q5 or 7Q10 for

the Criterion Continuous Concentration design flow in steady-state models for unstressed and stressed systems, respectively. These matters are discussed in more detail in the Technical Support Document for Water Quality-Based Toxics Control (U.S. EPA, 1985).

(50 F.R. 30784, July 29, 1985)
SEE APPENDIX A FOR METHODOLOGY

CHLORINATED PHENOLS

CRITERIA:

Aquatic Life

The available freshwater data for chlorinated phenols indicate that toxicity generally increases with increasing chlorination, and that acute toxicity occurs at concentrations as low as 30 ug/L for 4-chloro-3-methylphenol to greater than 500,000 ug/L for other compounds. Chronic toxicity occurs at concentrations as low as 970 ug/L for 2,4,6-trichlorophenol. Acute and chronic toxicity would occur at lower concentrations among species that are more sensitive than those tested.

The available saltwater data for chlorinated phenols indicate that toxicity generally increases with increasing chlorination and that acute toxicity occurs at concentrations as low as 440 ug/L for 2,3,5,6-tetrachlorophenol and 29,700 ug/L for 4-chlorophenol. Acute toxicity would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of chlorinated phenols to sensitive saltwater aquatic life.

Human Health

Sufficient data are not available for 3-chlorophenol to derive a level which would protect against the potential toxicity of this compound. Using available organoleptic data, to control undesirable taste and odor qualities of ambient water, the estimated level is 0.1 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have no demonstrated

relationship to potential adverse human health effects.

Sufficient data are not available for 4-chlorophenol to derive a level which would protect against the potential toxicity of this compound. Using available organoleptic data, to control undesirable taste and odor qualities of ambient water the estimated level is 0.1 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have no demonstrated relationship to potential adverse human health effects.

Sufficient data are not available for 2,3-dichlorophenol to derive a level which would protect against the potential toxicity of this compound. Using available organoleptic data, to control undesirable taste and odor qualities of ambient water the estimated level is 0.04 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have no demonstrated relationship to potential adverse human health effects.

Sufficient data are not available for 2,5-dichlorophenol to derive a level which would protect against the potential toxicity of this compound. Using available organoleptic data, to control undesirable taste and odor qualities of ambient water the estimated level is 0.5 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have no demonstrated relationship to potential adverse human health effects.

Sufficient data are not available for 2,6-dichlorophenol to derive a level which would protect against the potential toxicity of this compound. Using available organoleptic data, to control

undesirable taste and odor qualities of ambient water the estimated level is 0.2 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have no demonstrated relationship to potential adverse human health effects.

Sufficient data are not available for 3,4-dichlorophenol to derive a level which would protect against the potential toxicity of this compound. Using available organoleptic data, to control undesirable taste and odor qualities of ambient water the estimated level is 0.3 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have no demonstrated relationship to potential adverse human health effects.

For comparison purposes, two approaches were used to derive criterion levels for 2,4,5-trichlorophenol. Based on available toxicity data, to protect public health the derived level is 2.6 mg/L. Using available organoleptic data, to control undesirable taste and odor quality of ambient water the estimated level is 1.0 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have no demonstrated relationship to potential adverse human health effects.

For the maximum protection of human health from the potential carcinogenic effects of exposure to 2,4,6-trichlorophenol through the ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero, based on the nonthreshold assumption for this

chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 12 ug/L, 1.2 ug/L, and 0.12 ug/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 36 ug/L, 3.6 ug/L, and 0.36 ug/L, respectively. Using available organoleptic data, to control undesirable taste and odor qualities of ambient water the estimated level is 2 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing. a water quality criterion, and have no demonstrated relationship to potential adverse human health effects.

Sufficient data are not available for 2,3,4,6-tetrachlorophenol to derive a level which would protect against the potential toxicity of this compound. Using available organoleptic data, to control undesirable taste and odor qualities of ambient water the estimated level is 1.0 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have demonstrated relationship to potential adverse human health effects.

Sufficient data are not available for 2-methyl-4-chlorophenol to derive a criterion level which would protect against any potential toxicity of this compound. Using available organoleptic data, to control undesirable taste and odor qualities of ambient water the estimated level is 1,800 ug/L. It

should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion and have no demonstrated relationship to potential adverse human health effects.

Sufficient data are not available for 3-methyl-4-chlorophenol to derive a criterion level which would protect against any potential toxicity of this compound. Using available organoleptic data, to control undesirable taste and odor qualities of ambient water the estimated level is 3,000 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have no demonstrated relationship to potential adverse human health effects.

Sufficient data are not available for 3-methyl-6-chlorophenol to derive a criterion level which would protect against any potential toxicity of this compound. Using available organoleptic data, to control undesirable taste and odor qualities of ambient water the estimated level is 20 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have no demonstrated relationship to potential adverse human health effects.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

CHLOROALKYL ETHERS

CRITERIA :

Aquatic ~~Life~~

The available data for chloroalkyl ethers indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 238,000 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. NO definitive data are available concerning the chronic toxicity of chloroalkyl ethers to sensitive freshwater aquatic life.

No saltwater organism has been tested with any chloroalkyl ether and therefore, no statement can be made concerning acute or chronic toxicity.

Human Health

For the protection of human health from the toxic properties of bis(2-chloroisopropyl) ether ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 34.7 ug/L.

For the protection of human health from the toxic properties of bis(2-chloroisopropyl) ether ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 4.36 mg/L.

For the maximum protection of human health from the potential carcinogenic effects of exposure to bis(chloromethyl) ether through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero

level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 37.6 x 10^{-6} ug/L, 3.76 x 10^{-6} ug/L, and 0.376 x 10^{-6} ug/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 18.4 x 10^{-3} ug/L, 1.84 x 10^{-3} ug/L, and 0.184 x 10^{-3} ug/L, respectively.

For the maximum protection of human health from the potential carcinogenic effects of exposure to bis(2-chloroethyl) ether through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 0.30 ug/L, 0.030 ug/L, and 0.003 ug/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 13.6 ug/L, 1.36 ug/L, and 0.136 ug/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

CHLOROFORM

CRITERIA:

Aquatic Life

The available data for chloroform indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as **28,900** ug/L, and would occur at lower concentrations among species that are more sensitive than the three tested species Twenty-seven-day **LC50** values indicate that chronic toxicity occurs at concentrations as low as 1,240 ug/L, and could occur at lower concentrations among species or other life stages that are more sensitive than the earliest life cycle stages of the rainbow trout. The data base for saltwater species is limited to one test and therefore, no statement can be made concerning acute or chronic toxicity.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to chloroform through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are **1.90** ug/L, **0.19** ug/L, and **0.019** ug/L, respectively. If these

estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 157 ug/L, 15.7 ug/L, and 1.57 ug/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

OROPHENOXY
2,4-D; 2

CRITERIA:

2,4-D 100 ug/L for domestic water supply (health)

2,4,5-TP 10 ug/L for domestic water supply (health)

RATIONALE:

Two widely used herbicides are 2,4-D (2, 4-dichlorophenoxyacetic acid) and 2,4,5-TP (silvex) [2-(2,4, 5-trichlorophenoxy) propionic acid. Each of these compounds is formulated in a variety of salts and esters that may have a marked difference in herbicidal properties, but all are hydrolyzed rapidly to the corresponding acid in the body.

The subacute oral toxicity of chlorophenoxy herbicides has been investigated in a number of species of experimental animals (Palmer and Radeleff, 1964; Lehman, 1965). The dog was found to be sensitive and often displayed mild injury in response to doses of 10 mg/kg/day for 90 days, and serious effects from a dose of 20 mg/kg/day for 90 days. Lehman (1965) reported that the no-effect level of 2,4-D is 0.5 mg/kg/day in the rat, and 8.0 mg/kg/day in the dog.

Data are available on the toxicity of 2,4-D to man. A daily dosage of 500 mg (about 7 mg/kg) produced no apparent ill effects in a volunteer over a 21-day period (Kraus, 1946). When 2,4-D was investigated as a possible treatment for disseminated coccidioidomycosis, the patient had no side effects from 18 intravenous doses during 33 days; each of the last 12 doses in

the series was 800 mg (about 15 mg/kg) or more, the last being 2000 mg (about 37 mg/kg) (Seabury, 1963). A 19th and final dose of 3600 mg (67 mg/kg) produced mild symptoms.

The long-term no-effects levels (mg/kg/day) are listed for the rat and the dog. Those values are adjusted by a factor of 1/500 for 2,4-D and 2,4,5-TP. The safe levels are then readjusted to reflect total allowable intake per person. Since little 2,4-D or 2,4,5-TP is expected to occur in foods, 20 percent of the safe exposure level can reasonably be allocated to water without jeopardizing the health of the consumer.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

CHROMIUM (VI)

AQUATIC LIFE SUMMARY:

Acute toxicity values for chromium(VI) are available for freshwater animal species in 27 genera and range from 23.07 ug/L for a cladoceran to 1,870,000 ug/L for a stonefly. These species include a wide variety of animals that perform a wide spectrum of ecological functions. All five tested species of daphnids are especially sensitive. The few data that are available indicate that the acute toxicity of chromium(VI) decreases as hardness and pH increase.

The chronic value for both rainbow trout and brook trout is 264.6 ug/L, which is much lower than the chronic value of 1,987 ug/L for the fathead minnow. The acute-chronic ratios for these three fishes range from 18.55 to 260.8. In all three chronic tests a temporary reduction in growth occurred at low concentrations. Six chronic tests with five species of daphnids gave chronic values that range from <2.5 to 40 ug/L and the acute-chronic ratios range from 1.130 to >9.680. Except for the fathead minnow, all the chronic tests were conducted in soft water. Green algae are quite sensitive to chromium(VI). The bioconcentration factor obtained with rainbow trout is less than 3. Growth of chinook salmon was reduced at a measured concentration of 16 ug/L.

The acute toxicity of chromium (VI) to 23 saltwater vertebrate and invertebrate species ranges from 2,000 ug/L €or a polychaete worm and a mysid to 105,000 ug/L for the mud snail. The chronic values €or a polychaete range from <13 to 36.74 ug/L, whereas

that for a mysid is 132 ug/L. The acute-chronic ratios range from 15.38 to >238.5. Toxicity to macroalgae was reported at 1,000 and 5,000 ug/L. Bioconcentration factors for chromium(VI) range from 125 to 236 for bivalve molluscs and polychaetes.

CHROMIUM (III)

Acute values for chromium(III) are available for 20 freshwater animal species in la genera ranging from 2,221 ug/L for a mayfly to 71,060 ug/L for caddisfly. Hardness has a significant influence on toxicity, with chromium(III) being more toxic in soft water.

A life-cycle test with *Daphnia magna* in soft water gave a chronic value of 66 ug/L. In a comparable test in hard water the lowest test concentration of 44 ug/L inhibited reproduction of *Daphnia magna*, but this effect may have resulted from ingested precipitated chromium. In a life-cycle test with the fathead minnow in hard water the chronic value was 1,025 ug/L. Toxicity data are available for only two freshwater plant species. A concentration of 9,900 ug/L inhibited growth of roots of Eurasian watermilfoil. A freshwater green alga was affected by a concentration of 397 ug/L in soft water. No bioconcentration factor has been measured for chromium(III) with freshwater organisms.

Only two acute values are available for chromium (III) in saltwater 10,300 ug/L for the eastern oyster and 31,500 ug/L for the mummichog. In a chronic test effects were not observed on a polychaete worm at 50,400 ug/L at pH = 7.9, but acute lethality occurred when pH = 4.5. Bioconcentration factors for saltwater

organisms and chromium(III) range from 86 to 153, similar to the bioconcentration factors for chromium(VI) and saltwater species.

NATIONAL CRITERIA:

CHROMIUM(VI)

The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration of chromium(VI) does not exceed 11 ug/L more than once every 3 years on the average and if the 1-hour average concentration does not exceed 16 ug/L more than once every 3 years on the average.

The procedures described in the Guidelines indicate that, except possibly where a locally important species is very sensitive, saltwater aquatic organisms, and their uses should not be affected unacceptably if the 4-day average concentration of chromium(VI) does not exceed 50 ug/L more than once every 3 years on the average and if the 1-hour average concentration does not exceed 1,100 ug/L more than once every 3 years on the average. Data suggest that the acute toxicity of chromium (VI) is salinity dependent; therefore, the 1-hour average concentration might be underprotective at low salinities.

CHROMIUM(III)

The procedures described in the Guidelines indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not

be affected unacceptably if the 4-day average concentration (in ug/L) of chromium(III) does not exceed the numerical value given by $e^{(0.8190[\ln(hardness)]+1.561)}$ more than once every 3 years on the average and if the 1-hour average concentration (in ug/L) does not exceed the numerical value given by $e^{(0.8190[\ln(hardness)]+3.688)}$ more than once every 3 years on the average. For example, at hardnesses of 50, 100, and 200 mg/L as $CaCO_3$ the 4-day average concentrations of chromium(III) are 120, 210, and 370 ug/L, respectively, and the 1-hour average concentrations are 980, 1,700, and 3,100 ug/L.

No saltwater criterion can be derived for chromium(III), but 10,300 ug/L is the EC50 for eastern oyster embryos, whereas 50,400 ug/L did not affect a polychaete worm in a life-cycle test.

EPA believes that a measurement such as "acid-soluble" would provide a more scientifically correct basis upon which to establish criteria for minerals. The criteria were developed on this basis. However, at this time, no EPA-approved methods for such a measurement are available to implement the criteria through the regulatory programs of the Agency and the States. The Agency is considering development and approval of methods for a measurement such as acid-soluble. Until available, however, EPA recommends applying the criteria using the total recoverable method. This has two impacts: (1) certain species of some metals cannot be analyzed directly because the total recoverable method does not distinguish between individual

oxidation states, and (2) these criteria may be overly protective when based on the total recoverable method.

The recommended exceedence frequency of 3 years is the 'Agency's best scientific judgment of the average amount of time it will take an unstressed system to recover from a pollution event in which exposure to chromium exceeds the criterion. A stressed system, for example, one in which several outfalls occur in a limited area, would be expected or require more time for recovery. The resilience of ecosystems and their ability to recover differ greatly, however, and site-specific criteria may be established if adequate justification is provided.

The use of criteria in designing waste treatment facilities requires the selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria. Limited data or other factors may make their use impractical, in which case one should rely on a steady-state model. The Agency recommends the interim use of 1Q5 or 1Q10 for Criterion Maximum Concentration design flow and 745 or 7Q10 or the Criterion Continuous Concentration design flow in steady-state models for unstressed and stressed systems, respectively. These matters are discussed in more detail in the Technical Support Document for water Quality-Based Toxics Control (U.S. EPA, 1985).

HUMAN HEALTH CRITERIA:

For the protection of human health from the toxic properties of Chromium III ingested through water and contaminated aquatic

organisms, the ambient water criterion is determined to be **170 mg/L.**

For the protection of human health from the toxic properties of Chromium III ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be **3433 mg/L.**

The ambient water quality criterion for total Chromium VI is recommended to be identical to the existing drinking water standard which is 50 ug/L. Analysis of the toxic effects data resulted in a calculated level which is protective of human health against the ingestion of contaminated water and contaminated aquatic organisms. The calculated value is comparable to the present standard. For this reason a selective criterion based on exposure solely from consumption of 6.5 grams of aquatic organisms was not derived.

(45 F.R. **79318 Nov. 28,1980) (50** F.R. **30784,** July **29, 1985)**
SEE APPENDIX A FOR METHODOLOGY

2-CHLOROPHENOL

CRITERIA:

Aquatic Life

The available data for 2-chlorophenol indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 4,380 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No definitive data are available concerning the chronic toxicity of 2-chlorophenol to sensitive freshwater aquatic life, but flavor impairment occurs in one species of fish at concentrations as low as 2,000 ug/L.

No saltwater organisms have been tested with 2-chlorophenol and therefore, no statement can be made concerning acute or chronic toxicity.

Human Health

Sufficient data are not available for 2-chlorophenol to derive a level which would protect against the potential toxicity of this compound. Using available organoleptic data, to control undesirable taste and odor qualities of ambient water the estimated level is 0.1 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have no demonstrated relationship to potential adverse human health effects.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

COLOR

CRITERIA:

Waters shall be virtually free from substances producing objectionable color for aesthetic purposes;

the source of supply should not exceed 75 color units on the platinum-cobalt scale for domestic water supplies; and

increased color (in combination with turbidity) should not reduce the depth of the compensation point for photosynthetic activity by more than 10 percent from the seasonally established norm for aquatic life.

INTRODUCTION:

Color in water principally results from degradation processes in the natural environment. Although colloidal forms of iron and manganese occasionally are the cause of color in water, the most common causes are complex organic compounds originating from the decomposition of naturally occurring organic matter (AWWA, 1971). Sources of organic material include human materials from the soil such as tannins, human acid and humates: decaying plankton: and other decaying aquatic plants. Industrial discharges may contribute similar compounds: for example, those from the pulp and paper and tanning industries. Other industrial discharges may contain brightly colored substances such as those from certain processes in textile and chemical industries.

Surface waters may appear colored because of suspended matter which comprises turbidity. Such color is referred to as apparent color and is differentiated from true color caused by colloidal human materials (Sawyer, 1960). Natural color is reported in

color "units" which generally are determined by use of the platinum-cobalt method (Standard Methods, 1971).

There is no general agreement as to the chemical composition of natural color, and in fact the composition may vary chemically from place to place (AWWA, 1971). Black and Christman (1963a) characterized color-causing colloids examined as aromatic, polyhydroxy, methoxy carboxylic acids. Shapiro (1964) characterized color-causing constituents as being dialyzable and composed of aliphatic, polyhydroxyl carboxylic acids with molecular weights varying from less than 200 to approximately 400. The colloidal fraction of color exists in the 3.5 to 10 mu diameter range (Black and Christman, 1963b). These same authors summarized other characteristics of color observed in laboratory studies of natural waters: color is caused by light scattering and fluorescence rather than absorption of light energy, and pH affects both particle size of the color-causing colloids and the intensity of color itself.

RATIONALE:

Color in water is an important constituent in terms of aesthetic considerations. To be aesthetically pleasing, water should be virtually free from substances introduced by man's activities which produce objectionable color. "Objectionable color" is defined to be a significant increase over natural background levels. Non-natural colors such as dyes should not be perceptible by the human eye as such colors are especially objectionable to those who receive pleasure by viewing water in its natural state. Because of the extreme variations in the

natural background amount of color, it is meaningless to attempt numerical limits. The aesthetic attributes of water depend on one's appreciation of the water setting.

The effects of col'or on public water supplies also are principally aesthetic. The 1962 Drinking Water Standards (PHS, 1962) recommended that color in finished waters should not exceed 15 units on the platinum-cobalt scale. Water consistently can be treated using standard coagulation, sedimentation and filtration processes to reduce color to substantially less than 15 color units when the source water does not exceed 75 color units AWWA, 1971; NAS, 1974).

The effects of color in water on aquatic life principally are to reduce light penetration and thereby generally reduce photosynthesis by phytoplankton and to restrict the zone for aquatic vascular plant growth.

The light supply necessary to support plant life is dependent on both intensity and effective wave lengths (Welch, 1952). In general, the rate of photosynthesis increases with the intensity of the incident light. Photosynthetic rates are most affected in the red region and least affected in the blue-violet region of incident light (Welch, 1952). It has been found that in colored waters the red spectrum is not a region of high absorption so that the effective penetration, and therefore the intensity for photosynthesis, is not as restricted as are other wave lengths. It should be emphasized that transmission of all parts of the spectrum is affected by color, but the greatest effect is on the standard or blue end of the spectrum (Birge and Juday, 1930). In

TABLE 2.

Maximum color of surface waters that have been used as sources for industrial water supplies.

Industry or Industrial Use	Color units
Boiler make up	1,200
cooling water	1,200
Pulp and paper	360
Chemical and allied products	500
Petroleum	25

highly colored waters (45 to 132 color units) Birge and Juday (1930) measured the light transmission as a percentage of the incident level and found very little blue, 50 percent or less yellow, and 100 to 120 percent red.

The light intensity required for some aquatic vascular plants to photosynthetically balance the oxygen used in respiration may be 5 percent of full sunlight during maximum summer illumination periods (NTAC, 1968). As much as 10 percent of the incident light may be required for plankton to likewise photosynthetically produce sufficient oxygen to balance their respiration requirements (NTAC, 1968). The depth at which such a compensation point is reached, calledthe compensation depth, delineates the zone of effective photosynthetic oxygen production. To maintain satisfactory biological conditions, this depth cannot be substantially reduced.

Industrial requirements as related to water color have been standardized (NAS, 1974). Table 2 lists the maximum value used as a source of water for various industries and industrial uses. Through treatment, such waters can be made to meet almost any industrial requirement.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

*COPPER

AQUATIC LIFE SUMMARY:

Acute toxicity data are available for species in 41 genera of freshwater animals. At a hardness of 50 mg/L the genera range in sensitivity from 16.74 ug/L for Ptychocheilus to 10,240 ug/L for Acroneuria. Data for eight species indicate that acute toxicity decreases as hardness increases. Additional data for several species indicate that toxicity also decreases with increases in alkalinity and total organic carbon.

Chronic values are available €or 15 freshwater species and range from 3.873 ug/L €or brook trout to 60.36 ug/L for northern pike. Fish and invertebrate species seem to be about equally sensitive to the chronic toxicity of copper.

Toxicity tests have been conducted on copper with a wide range of freshwater plants and the sensitivities are similar to those of animals. Complexing effects of the test media and a lack of good analytical data make interpretation and application of these results difficult. Protection of animal species, however, appears to offer adequate protection of plants. Copper does not appear to bioconcentrate very much in the edible portion of freshwater aquatic species.

The acute sensitivities of saltwater animals to copper range from 5.8 ug/L for the blue mussel to 600 ug/L for the green crab. A chronic life-cycle test has been conducted with a mysid, and adverse effects were observed at 77 ug/L but not at 38 ug/L, which resulted in an acute-chronic ratio of 3.346. Several

*Indicates suspended, canceled or restricted by U.S.EPA Office of Pesticides ahd Toxic Substances

saltwater algal species have been tested, and effects were observed between 5 and 100 ug/L. Oysters can bioaccumulate copper up to 28,200 times, and become bluish-green, apparently without significant mortality. In long-term exposures, the bay scallop was killed at 5 ug/L.

NATIONAL CRITERIA:

The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Uses indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration (in ug/L) of copper does not exceed the numerical value given by $e^{(0.8545[\ln(hardness)]-1.465)}$ more than once every 3 years on the average and if the 1-hour average concentration (in ug/L) does not exceed the numerical value given by $e^{(0.9422[\ln(hardness)]-1.464)}$ more than once every 3 years on the average. For example, at hardnesses of 50, 100, and 200 mg/L as $CaCO_3$ the 4-day average concentrations of copper are 6.5, 12, and 21 ug/L, respectively, and the 1-hour average concentrations are 9.2, 18, and 34 ug/L.

The procedures described in the Guidelines indicate that, except possibly where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the 1-hour average concentration of copper does not exceed 2.9 ug/L more than once every 3 years on the average.

EPA believes that a measurement such as "acid-soluble" would

provide a more scientifically correct basis upon which to establish criteria for metals. The criteria were developed on this basis. However, at this time, no EPA approved methods for such a measurement are available to implement the criteria through the regulatory programs of the Agency and the States. The Agency is considering development and approval of methods for a measurement such as acid-soluble. Until available, however, EPA recommends applying the criteria using the total recoverable method. This has two impacts: (1) certain species of some metals cannot be analyzed directly because the total recoverable method does not distinguish between individual oxidation states, and (2) these criteria may be overly protective when based on the total recoverable method.

The recommended exceedence frequency of 3 years is the Agency's best scientific judgment of the average amount of time it will take an unstressed system to recover from a pollution event in which exposure to copper exceeds the criterion. A stressed system, for example, one in which several outfalls occur in a limited area, would be expected to require more time for recovery. The resilience of ecosystems and their ability to recover differ greatly, however, and site-specific criteria may be established if adequate justification is provided.

The use of criteria in developing waste treatment facilities requires the selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria. Limited data or other factors may make their use impractical, in which case one should rely on a steady-state model. The Agency recommends the interim use of 1Q5 or 1Q10 for

Criterion Maximum Concentration design flow and 745 or 7Q10 for the Criterion Continuous Concentration (CCC) design flow in steady-state models for unstressed and stressed systems respectively. These matters are discussed in more detail in the Technical Support Document for Water Quality-Based Toxics Control (U.S. EPA, 1985).

HUMAN HEALTH CRITERIA:

Sufficient data is not available for copper to derive a level which would protect against the potential toxicity of this compound. Using available organoleptic data, for controlling undesirable taste and odor quality of ambient water, the estimated level is 1 mg/L. It should be recognized that organoleptic data as a basis for establishing a water quality criteria have 1imitations and have no demonstrated relationship to potential adverse human health effects.

(45 F.R. 79318 Nov. 28,1980) (50 F.R. 30784, July 29, 1985)
SEE APPENDIX A FOR METHODOLOGY

CYANIDE

AQUATIC LIFE SUMMARY:

Data on the acute toxicity of free cyanide (the sum of cyanide present as HCN and CN-, expressed as CN) are available for a wide variety of freshwater species that are involved in diverse community functions. The acute sensitivities ranged from **44.73** ug/L to **2,490** ug/L, but all of the species with acute sensitivities above **400** ug/L were invertebrates. A long-term survival, and a partial and life-cycle test with fish gave chronic values of **13.57, 7.849,** and **16.39** ug/L, respectively. Chronic values for two freshwater invertebrate species were **18.33** and **34.06** ug/L. Freshwater plants were affected at cyanide concentrations ranging from **30** ug/L to **26,000** ug/L.

The acute toxicity of free cyanide to saltwater species ranged from **4.893** ug/L to >10,000 ug/L and invertebrates were both the most and least sensitive species. Long-term survival in an early life-stage test with the sheepshead minnow gave a chronic value *of* **36.12** ug/L. Long-term survival in a mysid life-cycle test resulted in a chronic value of **69.71** ug/L. Tests with the red macroalga, Champia parvula, showed cyanide toxicity at **11** to **25** ug/L, but other species were affected at concentrations up to **3,000** ug/L.

NATIONAL CRITERIA:

The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their **Uses** indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected

unacceptably if the 4-day average concentration of cyanide does not exceed 5.2 ug/L more than once every 3 years on the average and if the 1-hour average concentration does not exceed 22 ug/L more than once every 3 years on the average.

The procedures described in the Guidelines indicate that, except possibly where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the 1-hour average concentration of cyanide does not exceed 1.0 ug/L more than once every 3 years on the average.

EPA believes that a measurement such as "acid soluble" would provide a more scientifically correct basis upon which to establish criteria for cyanide. The criteria were developed on this basis. However, at this time, no EPA-approved methods for such a measurement are available to implement the criteria through the regulatory programs of the Agency and the States.

The Agency is considering development and approval of methods for a measurement such as acid soluble. Until available, however, EPA recommends applying the criteria using the total recoverable method. These criteria may be overly protective when based on the total recoverable method.

The recommended exceedence frequency of 3 years is the Agency's best scientific judgment of the average amount of time it will take an unstressed system to recover from a pollution event in which exposure to cyanide exceeds the criterion. A stressed system, for example, one in which several outfalls occur in a limited area, would be expected to require more time for recovery. The resilience of ecosystems and their ability to

recover differ greatly, however, and site-specific criteria may be established if adequate justification is provided.

The use of criteria in designing waste treatment facilities requires the selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria. Limited data or other factors may make their use impractical, in which case one should rely on a steady-state model. The Agency recommends the interim use of 1Q5 or 1Q10 for Criterion Maximum Concentration design flow and 7Q5 or 7Q10 for the Criterion Continuous Concentration design flow in steady-state models for unstressed and stressed systems respectively. These matters are discussed in more detail in the Technical Support Document for Water Quality-Based Toxics Control (U.S. EPA, 1985).

HUMAN HEALTH CRITERIA

The ambient water quality criterion for cyanide is recommended to be identical to the existing drinking water standard which is 200 ug/L. Analysis of the toxic effects data resulted in a calculated level which is protective of human health against the ingestion of contaminated water and contaminated aquatic organisms. The calculated value is comparable to the present standard. For this reason a selective criterion based on exposure solely from consumption of 6.5 grams of aquatic organisms was not derived.

NOTE: The U.S. EPA is currently developing Acceptable Daily Intake (ADI) or Verified Reference Dose (RfD) values for Agency-wide use for this chemical. The new value should be substituted when it becomes available. The January, 1986, draft Verified Reference Dose document cites an RfD of .02 mg/kg/day for free cyanide.

~~DDT AND~~ METABOLITES

CRITERIA:

Aquatic Life

DDT

For DDT and its metabolites the criterion to protect freshwater aquatic life as derived using the Guidelines is 0.0010 ug/L as a 24-hour average and the concentration should not exceed 1.1 ug/L at any time.

For DDT and its metabolites the criterion to protect' saltwater aquatic life as derived using the Guidelines is 0.0010 ug/L as a 24-hour average and the concentration should not exceed 0.13 ug/L at any time.

TDE

The available data for TDE indicate that acute toxicity to freswater aquatic life occurs at concentrations as low as 0.6 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of TDE to sensitive freshwater aquatic life.

The available data for TDE indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 3.6 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of TDE to sensitive saltwater aquatic life.

DDE

The available data for DDE indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as

1,050 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of DDE to sensitive freshwater aquatic life.

The available data for DDE indicate that acute toxicity to saltwater aquatic life occurs in concentrations as low as 14 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of DDE to sensitive saltwater aquatic life.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to DDT through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6} and 10^{-7}. The corresponding recommended criteria are 0.24 ng/L, 0.024 ng/L, and 0.0024 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 0.24 ng/L, 0.024 ng/L, and 0.0024 ng/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

DEMETON

CRITERION:

0.1 ug/L for freshwater and marine aquatic life

RATIONALE:

Static LC50 bioassays yielded toxicity values for the organophosphorus pesticide demeton for carp, goldfish, fathead minnow, channel catfish, guppy, rainbow, trout and bluegill, ranging from 70 ug/L to 15,000 ug/L (Henderson and Pickering, 1958; Ludemann and Neumann, 1982; Macek and McAllister, 1970; McCann and Jasper, 1972; Pickering et al. 1962). Results of these tests demonstrate an apparent sharp division in species sensitivity, with bluegill (Lepomis macrochirus), rainbow trout (Salmo gairdneri) and guppy, (Poecilia reticulata), being susceptible to lower concentrations while the remaining species were comparatively resistant. In the 96-hour exposures toxicity did not increase significantly with time, indicating that concentrations close to nominal may not have been maintained for more than a few hours, Bluegills with a 24-hour LC50 of 70 ug/L were the most sensitive fish (McCann and Jasper, 1972).

When fish were exposed to acutely toxic levels of demeton €or 12 hours by Weiss (1959, 1961) the maximum inhibition of brain acetiylcholinesterase (AChE) was not reached. The lowest levels of AChE occurred after 24 to 48 hours. It was demonstrated that maximum inhibition could last as long as two weeks after exposure, and subsequent recovery to levels approaching normal took many more weeks. Weiss (1958) reported a significant increase in mortality of fathead minnows exposed for a second

time to the organophosphate, Sarin, before the fish had recovered normal brain AChE levels. The resistance of fully recovered fish was equal to that of previously unexposed controls. Weiss and Gakstatter (1964a) reported no significant inhibition of brain AChE in bluegills, goldfish and shiners (Notemigonus crysoleucas), following 15-day exposures to demeton at continuously replenished, nominal concentrations of 1 ug/L.

Acute toxicity values reported for invertebrates range from 10 to 100,000 ug/L (Ludemann and Neumann, 1962; Sanders, 1972). In general, molluscs and tubifex worms were very resistant while the smaller crustaceans and insect larvae were susceptible. Ludemann and Neumann (1962) reported that Chironomus plumosus larvae were the most sensitive species they tested. A 24-hour exposure at 10 ug/L produced undefined effects while 100 percent were killed at 1000 ug/L. Calculated LC50 data for invertebrates apparently are limited to a single, nominal concentration static exposure of Gammarus fasciatus (Sanders, 1972). These 24- and 96-hour LC50 values are reported as 500 and 27 ug/L, indicating a time-related effect not observed in the bioassays with fishes. As only a few of the sensitive species have been tested and great variance in response can result with different test methods, caution must be exercised in estimating the sub-acute concentration for aquatic fauna in general. It appears that no study has been made of possible residual effects other than AChE inhibition, which might result from short exposures to subacute concentrations of organophosphates.

There are few data on the toxicity of demeton to marine organisms. Butler (1964) reported a 48-hour EC50 of 63 ug/L for

the pink shrimp, Peneaus duorarum, and a 24-hour LC50 of 550 ug/L for the spot, Leiostomus xanthurus.

Chronic demeton toxicity data for freshwater organism are not currently available. Since no data are available at this time to indicate long-term no-effect levels for aquatic organisms, a criterion must be derived based partly on the fact that all organophosphates inhibit the production of the AChE enzyme. Demeton is unique, however, in that the persistence of its AChE-inhibiting ability is greater than that of 10 other common organophosphates, even though its acute toxicity is apparently less. The effective "half-life" of AChE inhibition for demeton is greater than one year (Weiss and Gakstatter, 1964b). Because such inhibition may be additive with repeated exposures and may be compounded by any of the organophosphates, it is recommended that a criterion for demeton be based primarily on its enzyme-inhibiting potential. A criterion of 0.1 ug/L demeton for freshwater and marine aquatic life is recommended since it will not be expected to significantly inhibit AChE over a prolonged period of time. In addition, the criteria recommendation is in close agreement with the criteria for the other organophosphates.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

DICHLOROBENZENES

CRITERIA:

Aquatic Life

The available data for dichlorobenzenes indicate that acute and chronic toxicity to freshwater aquatic life occur at concentrations as low as 1,120 and 763 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for dichlorobenzenes indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 1,970 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of dichlorobenzenes to sensitive saltwater aquatic life.

Human Health

For the protection of human health from the toxic properties of dichlorobenzene ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 400 ug/L.

For the protection of human health from the toxic properties of dichlorobenzenes ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 2.6 mg/L.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

DICHLOROBENZIDINE

CRITERIA:

Aquatic Life

The data base available for dichlorobenzidines and freshwater organisms is limited to one test on bioconcentration of 3,3-dichlorobenzidine, and therefore, no statement can be made concerning acute or chronic toxicity.

No saltwater organisms have been tested with any dichlorobenzidine, and therefore, no statement can be made concerning acute or chronic toxicity.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to dichlorobenzidine through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 0.103 ug/L, 0.010 ug/L, and 0.001 ug/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 0.204 ug/L, 0.020 ug/L, and 0.002 ug/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

DICHLOROETHYLENES

CRITERIA:

Aquatic Life

The available data for dichloroethylenes indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 11,600 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No definitive data are available concerning the chronic toxicity of dichloroethylenes to sensitive freshwater aquatic life.

The available data for dichloroethylenes indicate that acute and chronic toxicity to saltwater aquatic life occurs at concentrations as low as 224,000 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of dichloroethylenes to sensitive saltwater aquatic life.

Human Health

1,1-Dichloroethylene

For the maximum protection of human health from the potential carcinogenic effects of exposure to 1,1 dichloroethylene through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the non threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10-6, and 10^{-7}. The corresponding recommended criteria are 0.33 ug/L, 0.033 ug/L, and 0.003 ug/L, respectively. If

these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 18.5 ug/L, 1.85 ug/L, and 0.185 ug/L, respectively.

1,2-Dichloroethylene

Using the present guidelines, a satisfactory criterion cannot be derived at this tine because of insufficient available data for 1,2-dichloroethylene.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

2,4-DICHLOROPHENOL

CRITERIA:

Aquatic Life

The available data for 2,4-dichlorophenol indicate that acute and chronic toxicity to freshwater aquatic life occurs at concentrations as low as 2,020 and 365 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested. Mortality to early life stages of one species of fish occurs at concentrations as low as 70 ug/L.

Only one test has been conducted with saltwater organisms and 2,4-dichlorophenol and therefore, no statement can be made concerning acute or chronic toxicity.

Human Health

For comparison purposes, two approaches were used to derive criterion levels for 2,4-dichlorophenol. Based on available toxicity data, to protect public health the derived level is 3.09 mg/L. Using available organoleptic data, to control undesirable taste and odor qualities of ambient water the estimated level is 0.3 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have no demonstrated relationship to potential adverse human health effects.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

DICHLOROPROPANES/DICHLOROPROPENES

CRITERIA:

Aquatic Life

The available data for dichloropropanes indicate that acute and chronic toxicity to freshwater aquatic life occurs at concentrations as low as 23,000 and 5,700 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for dichloropropene indicate that acute and chronic toxicity to freshwater aquatic life occurs at concentrations as low as 6,060 and 244 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for dichloropropane indicate that acute and chronic toxicity to saltwater aquatic life occur at concentrations as low as 10,300 and 3,040 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for dichloropropene indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 790 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of dichloropropene to sensitive saltwater aquatic life.

Human Health

Using the present guidelines, a satisfactory criterion cannot be derived at this time because of insufficient available data for dichloropropanes.

(45 F.R. 79318, November 28, 1980)
SEE **APPENDIX B** FOR **METHODOLOGY**

2,4-DIMETHYLPHENOL

CRITERIA

Aquatic Life

The available data for 2,4-dimethylphenol indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as **2,120** ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of dimethylphenol to sensitive freshwater aquatic life.

No saltwater organisms have been tested with 2,4-dimethyl-phenol and therefore, no statement can be made concerning acute or chronic toxicity.

Human Health

Sufficient data are not available for 2,4-dimethylphenol to derive a level which would protect against the potential toxicity of this compound. Using available organoleptic data, to control undesirable taste and odor quality of ambient water the estimated level is 400 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have no demonstrated relationship to potential adverse human health effects.

(45 F.R. **79318,** November **28, 1980)**
SEE APPENDIX B FOR METHODOLOGY

DINITROTOLUENE

CRITERIA:

Aquatic Life

The available data for dinitrotoluenes indicate that acute and chronic toxicity to freshwater aquatic life occurs at concentrations as low as 330 and 230 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for dinitrotoluenes indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 590 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of dinitrotoluenes to sensitive saltwater aquatic life but a decrease in algal cell numbers occurs at concentrations as low as 370 ug/L.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to 2,4-dinitrotoluene through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6} and 10^{-7}. The corresponding recommended criteria are 1.1 ug/L, 0.11 ug/L, and 0.011 ug/L, respectively. If these estimates are made for consumption of aquatic organ-isms only, excluding consumption

of water, the levels are 91 ug/L, 9.1 ug/L, and 0.91 ug/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

DIPHENYLHYDRAZINE

CRITERIA:

Aquatic Life

The available data for 1,2-diphenylhydrazine indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 270 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of 1,2-diphenylhydrazine to sensitive freshwater aquatic life.

No saltwater organisms have been tested with 1,2-diphenylhydrazine and therefore, no statement can be made concerning acute or chronic toxicity.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to diphenylhydrazine through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 422 ng/L, 42 ng/L, and 4 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 5.6 ug/L, 0.56 ug/L, and 0.056 ug/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

ENDOSULFAN

CRITERIA:

Aquatic Life

For endosulfan the criterion to protect freshwater aquatic life as derived using the Guidelines is 0.056 ug/L as a 24-hour average and the concentration should not exceed 0.22 ug/L at any time.

For endosulfan the criterion to protect saltwater aquatic life as derived using the Guidelines is 0.0087 ug/L as a 24-hour average and the concentration should not exceed 0.034 ug/L at any time.

Human Health

For the protection of human 'health from the toxic properties of endosulfan ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 74 ug/L.

For the protection of human health from the toxic properties of endosulfan ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 159 ug/L.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

*ENDRIN

CRITERIA:

Aquatic Life

For endrin the criterion to protect freshwater aquatic life as derived using the Guidelines is 0.0023 ug/L as a 24-hour average, and the concentration should not exceed 0.18 ug/L at any time.

For endrin the criterion to protect saltwater aquatic life as derived using the Guidelines is 0.0023 ug/L as a 24-hour average, and the concentration should not exceed 0.037 ug/L at any time.

Human Health

The ambient water quality criterion for endrin is recommended to be identical to the existing water standard which is 1.0 ug/L. Analysis of the toxic effects data resulted in a calculated level which is protective of human health against the ingestion of contaminated water and contaminated aquatic organisms. The calculated value is comparable to the present standard. For this reason a selective criterion based on exposure solely from assumption of 6.5 g of aquatic organisms was not derived.

*Indicates suspended, canceled or restricted by U.S. EPA Office of Pesticides and Toxic Substances

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

ETHYLBENZENE

CRITERIA:

Aquatic Life

The available data for ethylbenzene indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 32,000 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No definitive data are available concerning the chronic toxicity of ethylbenzene to sensitive freshwater aquatic life.

The available data for ethylbenzene indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 430 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of ethylbenzene to sensitive saltwater aquatic life.

Human Health

For the protection of human health from the toxic properties of ethylbenzene ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 1.4 mg/L.

For the protection of human health from the toxic properties of ethylbenzene ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 3.28 mg/L.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

NOTE: The U.S. EPA is currently developing Acceptable Daily Intake (ADI) or Verified Reference Dose (RfD) values for Agency-wide use for this chemical. The new value should be substituted when it becomes available. The January, 1986, draft Verified Reference Dose document cites an RfD of 0.1 mg/kg/day for ethylbenzene.

FLUORANTHENE

CRITERIA:

Aquatic Life

The available data for fluoranthene indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 3,980 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of fluoranthene to sensitive freshwater aquatic life.

The available data for fluoranthene indicate that acute and chronic toxicity to saltwater aquatic life occur at concentrations as low as 40 and 16 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

Human Health

For the protection of human health from the toxic properties of fluoranthene ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 42 ug/L.

For the protection of human health from the toxic properties of fluoranthene ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 54 ug/L.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

GASES, TOTAL DISSOLVED

CRITERION:

To protect freshwater and marine aquatic life, the total dissolved gas concentrations in water should not exceed 110 percent of the saturation value for gases at the existing atmospheric and hydrostatic pressures.

RATIONALE:

Fish in water containing excessive dissolved gas pressure or tension are killed when dissolved gases in their circulatory system come out of solution to form bubbles (emboli) which block the flow of blood through the capillary vessels. In aquatic organisms this is commonly referred to as "gas bubble disease". External bubbles (emphysema) also appear in the fins, on the opercula, in the skin and in other body tissues. Aquatic invertebrates are also affected by gas bubble disease, but usually at supersaturation levels higher than those lethal to fish.

The standard method of analyzing for gases in solutions has been the Van Slyke method (Van Slyke et al. 1934); now, gas chromatography also is used for determination of individual and total gases. For determination of total gas pressure, Weiss has developed the saturometer, a device based upon a thin-wall silicone rubber tube that is permeable to gases but impermeable to water. Gases pass from the water through the tube, thus raising the internal gas pressure which is measured by a

manometer or pressure gauge connected to the tube (NAS, 1974). This method alone does not separate the total gas pressure into the separate components, but Winkler oxygen determinations can be run simultaneously, and gas concentrations can be calculated.

Total dissolved gas concentrations must be determined because analysis of individual gases may not determine with certainty that gas supersaturation exists. For example, water could be highly supersaturated with oxygen, but if nitrogen were at less than saturation, the saturation as measured by total gas pressure might not exceed 100 percent. Also, if the water was highly supersaturated with dissolved oxygen, the oxygen alone might be sufficient to create gas pressures or tensions greater than the Criterion limits, but one would not know the total gas pressure or tension, or by how much the criterion was exceeded. The rare and inert gases such as argon, neon and helium are not usually involved in causing gas bubble disease as their contribution to total gas pressures is very low. Dissolved nitrogen (N_2), which comprises roughly 80 percent of the earth's atmosphere, is nearly inert biologically and is the most significant cause of gas bubble disease in aquatic animals. Dissolved oxygen, which is extremely bioactive, is consumed by the metabolic processes of the organism and is less important in causing serious gas bubble disease though it may be involved in initiating emboli formation in the blood (Nebeker et al. 1976a).

Percent saturation of water containing a given amount of gas varies with the absolute temperature and with the pressure. Because of the pressure changes, percent saturation with a given

amount of gas changes with depth of the water. Gas supersaturation decreases by 10 percent per meter of increase in water depth because of hydrostatic pressure; a gas that is at 130 percent saturation at the surface would be at 100 percent saturation at 3 meters' depth. Compensation €or altitude may be needed because a reduction in atmospheric pressure changes the water/gas equilibria, resulting in changes in solubility of dissolved gases.

There are several ways that total dissolved gas supersaturation can occur:

1. Excessive biological activity--dissolved oxygen concentrations often reach supersaturation because of excessive algal photosynthesis. Renfro (1963) reported gas bubble disease in fishes resulting, in part, from algal blooms. Algal blooms often accompany an increase in water temperature and this higher temperature further contributes to supersaturation.

2. Lindroff (1957) reported that water spillage at hydropower dams caused supersaturation. When excess water is spilled over the face of a dam it entrains air as it plunges to the stilling or plunge pool at the base of the dam. The momentum of the fall carries the water and entrained gases to great depths in the pool; and, under increased hydrostatic pressure, the entrained gases are driven into solution, causing supersaturation of dissolved gases.

3. Gas bubble disease may be induced by discharges from power-generating and other thermal sources (Marcello et al. 1975). Cool, gas-saturated water is heated as it passes through the condenser or heat exchanger. As the temperature of the water

rises, percent saturation increases because of the reduced solubility of gases at higher temperatures. Thus, the discharged water becomes supersaturated with gases and fish or other organisms living in the heated water may exhibit gas bubble disease (DeMont and Miller, 1972: Malouf et al. 1972; Keup, 1975).

In recent years, gas bubble disease has been identified as a major problem affecting valuable stocks of salmon and trout in the Columbia River system (Rulifson and Abel, 1971). The disease is caused by high concentrations of dissolved atmospheric gas which enter the river's water during heavy spilling at hydroelectric dams. A report by Ebel et al. (1975) presents results from field and laboratory studies on the lethal, sublethal and physiological effects of gas on fish, depth distribution of fish in the river (fish can compensate for some high concentrations of gas by moving deeper into the water column), detection and avoidance of gas concentrations by fish, intermittent exposure of fish to gas concentrations, and bioassays of many species of fish exposed to different concentrations of gas. Several conclusions resulting from these studies are:

1. When either juvenile or adult salmonids are confined to shallow water (1 m), substantial mortality occurs at and above 115 percent total dissolved gas saturation.

2. When either juvenile or adult salmonids are free to sound and obtain hydrostatic compensation either in the laboratory or in the field, substantial mortality still occurs when saturation

levels (of total dissolved gases) exceed 120 percent saturation.

3. On the basis of survival estimates made in the Snake River from 1966 to 1975, it is concluded that juvenile fish losses ranging from 40 to 95 percent do occur and a major portion of this mortality can be attributed to fish exposure to supersaturation by atmospheric gases during years of high flow.

4. Juvenile salmonids subjected to sublethal periods of exposure to supersaturation can recover when returned to normally saturated water, but adults do not recover and generally die from direct and indirect effects of the exposure.

5. Some species of salmon and trout can detect and avoid supersaturated water; others may not.

6. Higher survival was 'observed during periods of intermittent exposure than during continuous exposure.

7. In general, in acute bioassays, salmon and trout were less tolerant than the nonsalmonids.

Dawley and Ebel (1975) found that exposure of juvenile spring chinook salmon, Oncorhynchus tshawy~~tsch~~a, and steelhead trout, Salmo gairdneri, to 120 percent saturation for 1.5 days resulted in over 50 percent mortality; 100 percent mortality occurred in less than 3 days. They also determined that the threshold level where significant mortalities begin occurring is at 115 percent nitrogen saturation (111 percent total gas saturation in this test).

Rucker (1974), using juvenile coho salmon, Oncorhynchus kisutch, determined the effect of individual ratios of oxygen and nitrogen and established that a decrease in lethal effect occurred when the nitrogen content fell below 109 percent

saturation even though total gas saturation remained at 119 percent saturation, indicating the importance of determining the concentration of the individual components (O_2 and N_2) of the atmospheric supersaturation. Nebeker et al. (1976a), using juvenile sockeye salmon, Oncorhynchus nerka, also showed that there was a significant increase in fish mortality when the nitrogen concentration was increased while holding the total percent saturation constant. They also showed that there was no significant difference in fish mortality at different CO_2 concentrations.

Research collected by Bouck et al. (1975) showed that gas supersaturated water at and above 115 percent total gas saturation is acutely lethal to most species of salmonids, with 120 percent saturation and above rapidly lethal to all salmonids tested. Levels as low as 110 percent will produce emphysema in most species. Steelhead trout were most sensitive to gas-supersaturated water followed by sockeye salmon, Oncorhyncnus nerka. Chinook salmon, Oncorhynchus tshawytscha, were intermediate in sensitivity. Coho salmon, Oncorhyncnus kisutch, were significantly the more tolerant of the salmonids though still much more susceptible than non-salmonids like bass or carp.

Daphnia magna exhibited a sensitivity to supersaturation similar to that of the salmonids (Nebeker et al. 1975), with 115 percent saturation lethal within a few days. Stoneflies exhibited an intermediate sensitivity similar to bass with mortality at 130 percent saturation. Crayfish were very tolerant, with levels near 140 percent total gas saturation resulting in mortality.

No differences are proposed in the criteria €or freshwater and marine aquatic life as the data available indicate that there probably is little difference in overall tolerances between marine and freshwater species.

The development of gas bubble disease in menhaden, Brevoortia sp., and their tolerance to gas saturation in laboratory bioassays and in the field (Pilgrim Nuclear Power Station Discharge Canal) are discussed by Clay et al. (1975) and Marcello et al. (1975). At 100 percent and 105 percent nitrogen saturation, no gas bubbles developed externally or in any of the internal organs of menhaden. At 105 percent nitrogen saturation, however, certain behavioral changes became apparent. Fish sloughed of€ mucus, swam erratically, were more excitable, and became darker in color. Menhaden behavioral changes observed at 110 percent nitrogen saturation were similar to those noted at 105 percent. In addition, at 110 percent gas emboli were found in the intestines, the pyloric caeca, and occasionally the operculum. The behavioral changes described were also observed at 115 percent, and clearly defined subcutaneous emphysema was observed in the fins and occasionally in the eye. At 120 percent and 130 percent nitrogen saturation, menhaden developed within a few hours classic symptoms of gas bubble disease. Externally, emboli were evident in all fins, the operculum and within the oral cavity.

Exophthalmia also occurred and emboli developed in internal organs. The bulbous arteriosis and swim bladder were severely distended, and emboli were found along the length of the gill arterioles, resulting in hemostasis. At water temperatures of 30

°C, menhaden did not survive, regardless of gas saturation level. At water temperatures of 15 , 22 , and 25 °C 100 percent of the menhaden died within 24 hours at 120 percent and 130 percent gas saturation. Fifty percent died after 96 hours at 115 percent (22 °C) Menhaden survival after 96 hours at 110 percent nitrogen saturation ranged from 92 percent at 22° and 25° to 83 percent at 15 °C. Observations on the relationship between the mortality rate of menhaden and gas saturation levels at Pilgrim Station during the April 1975, incident suggest that the fish may tolerate somewhat higher gas saturation levels in nature.

It has been shown by Bouck et al. (1975) and Dawley et al. (1975) that survival of salmon and steelhead smolts in seawater is not affected by prior exposure to gas supersaturation while in fresh water. No significant mortality of juvenile coho and sockeye salmon occurred when they were exposed to sublethal concentrations of supersaturated water and then transferred to seawater (Nebeker et al. 1976b).

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX *c* FOR METHODOLOGY

GUTHION

CRITERION:

.01 ug/L for freshwater and marine aquatic life.

RATIONALE:

Ninety-six-hour LC50 values for fish exposed to the organophosphorus pesticide guthion range from 4 to 4270 ug/L (Katz, 1961: Pickering et al. 1962; Lahav and Sarig, 1969; Macek et al. 1969; Macek and McAllister, 1970). The only long-term fish exposure data available are those obtained recently by Adelman and Smith (unpublished data). Decreased spawning (eggs produced per female) was observed in fathead minnows, *Pimephales promelas*, exposed during a complete life cycle. An estimated "safe" long-term exposure concentration for fathead minnows lies between 0.3 and 0.5 ug/L. survival of larvae was reduced at approximately 0.7 ug/L.

An investigation of the persistence of guthion in fish revealed that 50 percent of the chemical was lost in less than one week (Meyer, 1965). Analysis of plankton and pond water in the same study indicated a 50 percent loss of guthion in about 48 hours. Flint et al. (1970) determined the half-life of guthion at 30C in pond water and in a phosphate buffer protected from light in the laboratory. The half-life in pond water was 1.2 days whereas that in the laboratory solution was 10 days. The more rapid degradation in pond water was attributed to the effect of sunlight and microorganisms.

Organophosphate pesticides are toxic because they inhibit the

enzyme acetylcholinesterase (AChE) which is essential to nerve impulse conduction and transmission (Holland et al. 1967). Weiss (1958, 1959, 1961) demonstrated that a 40 to 70 percent inhibition of fish brain AChE usually is lethal. Centrarchids generally are considered one of the more sensitive groups of fish to guthion (Pickering et al. 1962; Weiss and Gakstatter, 1964; Meyer, 1965). Weiss and Gakskatter (1964) found that over a 15-day period bluegills, Lepomis macrochirus, exhibited AChE inhibition at 1.0 ug/L guthion but not at 0.1 ug/L. Exposure at 0.05 ug/L for 30 days also failed to produce inhibition below the range of normal variation, but the authors stated that it appeared there was a downward trend in brain enzyme activity and that if exposure was continued a definite reduction might develop. Weiss (1961) found that about 30 days were required for fathead minnow and bluegill brain AChE levels to recover after 8 to 24 hours exposure to 10 ug/L guthion.

Benke and Murphy (1974) showed that repetitive injection of fish with guthion caused cumulative inhibition of brain AChE and mortality. After substantial inhibition by guthion exposure, it takes several weeks for brain AChE of fishes to return to normal even though exposure is discontinued (Weiss, 1959, 1960; Carter, 1971). Inhibition of brain AChE of fishes by 46 percent or more has been associated with harmful effects in exposures to there organophosphate pesticides for a life cycle (Eaton, 1970) and for shorter periods (Carter, 1971; Coppage and Duke, 1971; Coppage, 1972; Coppage and Matthews, 1974: Post and Leasure, 1974; Coppage et al. in press). In static tests, similar inhibition of AChE and mortality were caused in the sheepshead minnow, Cyprinodon

variegatus, in 2, 24, 48 and 72 hours at concentrations of 50, 7, 3.5 and 3 ug/L, respectively (Coppage, 1972). These data indicate that reduction of brain AChE activity of marine fishes by 70 to 80 percent or more in short-term exposures to guthion may be associated with some deaths.

There is no evidence to indicate that guthion would cause adverse effects through the food chain. Tissue residue accumulation for whole fish calculated from the data of Meyer (1965) indicate no more than a twentyfold accumulation. LC50 toxicity values for birds are relatively high and range from 70 to 2,000 mg/kg (Tucker and Crabtree, 1970).

Ninety-six-hour LC50 values for aquatic invertebrates range from 0.10 to 22.0 ug/L (Nebeker and Gaufin, 1964; Gaufin et al. 1965: Jensen and Gaufin, 1966: Sanders and Cope, 1968: Sanders, 1969, 1972). Sanders (1972) exposed the grass shrimp, Paleomonetes kadiakensis, to guthion in a continuous flow bioassay for up to 20 days and found that the 5- and 20-day LC50 values were 1.2 and 0.16 ug/L, respectively. He found that the amphipod, Gammarus fasciatus, was the most sensitive aquatic organism tested, with a 96-hour LC50 of 0.10 ug/L. Jensen and Gaufin (1966), also using a continuous flow system, exposed two species of stonefly naiads in 4- and 30-day studies. They observed 96-hour and 30-day LC50 values for Acroneuria pacifica of 2.0 and 0.24 ug/L, respectively, whereas for Pteronarcys californica the values were 4.6 and 1.3 ug/L, respectively.

Results of other toxicity studies on marine organisms have been reported. The 24-hour LC50 for the white mullet, Mugil

curema, was found to be 5.5 ug/L guthion (Butler, 1963). The 96-hour LC50 for the striped mullet, Mugil cephalus, was determined by Lahav and Sarig (1969) to be 8 ug/L guthion. Portman (1972) reported the 48-hour LC50 for the fish, Pleuronectes limanda, to be 10 to 30 ug/L. The 48-hour LC50 for the European shrimp, Cranqon crangon, was found to be 0.33 ug/L guthion (Portman, 1972). Butler (1963) found that the 24-hour EC50 €or blue crab, Callinectes sapidus, was 550 ug/L and the 48-hour EC50 €or pink shrimp, Penaeus duorarum, was 4.4 ug/L guthion. The 48-hour TLm was estimated to be 620 ug/L €or fertilized oyster eggs, Crassostrea virginica, and 860 ug/L for fertilized clam eggs, Mercenaria mercenaria (Davis and Hidu, 1969).

A criterion level of .01 ug/L for guthion is based upon use of an 0.1 application factor applied to the 96-hour LC50 of 0.1 ug/L for Gammarus and a similar value of 0.3 ug/L for the European shrimp.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

HALOETHERS

CRITERIA:

Aquatic Life

The available data for haloethers indicate that acute and chronic toxicity to freshwater aquatic life occurs at concentrations as low as 360 and 122 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

No saltwater organisms have been tested with any haloether and therefore, no statement can be made concerning acute or chronic toxicity.

Human Health

Using the present guidelines, a satisfactory criterion cannot be derived at this time because of insufficient available data for ha1oethers.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

HALOMETHANES

CRITERIA:

Aquatic Life

The available data for halomethanes indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low a 11,000 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of halomethanes to sensitive freshwater aquatic life.

The available data for halomethanes indicate that acute and chronic toxicity to saltwater aquatic life occurs at concentrations as low as 12,000 and 6,400 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested. A decrease in algal cell numbers occurs at concentrations as low as 11,500 ug/L.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to chloromethane, bromomethane, dichloromethane, bromodichloromethane, tribromomethane, dichlorodifluoromethane, trichlorofluoromethane, or combinations of these chemicals through ingestion of contaminated water and aquatic organisms, the ambient water concentration should be zero, based on the nonthreshold assumption for this chemical, However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime

are estimated at 10^{-5}, 10^{-6} and 10^{-7}. The corresponding recommended criteria are 1.9 ug/L, 0.19 ug/L, and 0.019 ug/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 157 ug/L, 15.7 ug/L, and 1.57 ug/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

HARDNESS

INTRODUCTION:

Water hardness is caused by the polyvalent metallic ions disolved in water. In fresh water these are primarily calcium and magnesium although other metals such as iron, strontium and manganese contribute to the extent that appreciable concentrations are present. Hardness commonly is reported as an equivalent concentration of calcium carborate ($CaCO_3$).

The concept of hardness comes from water supply practice. It is measured by soap requirements for adequate lather formation and as an indicator of the rate of scale formation in hot water heaters and low pressure boilers. A commonly used classification is given in the following table (Sawyer, 1960).

TABLE 3.

Classification of Water by Hardness Content

Conc. mg/L $CaCO_3$	Description
0 - 75	soft
75 - 150	moderately hard
150 - 300	hard
300 and up	very hard

Natural sources of hardness principally are limestones which are dissolved by percolating rainwater made acid by dissolved carbon dioxide. Industrial and industrially related sources include the inorganic chemical industry and discharges from operating and abandoned mines.

Hardness in fresh water frequently is distinguished in carbonate and non-carbonate fractions. The carbonate fraction is chemically equivalent to the bicarbonates present in water.

Since bicarbonates generally are measured as alkalinity, the carbonate hardness usually is considered equal to the alkalinity.

RATIONALE:

The determination of hardness in raw waters subsequently treated and used for domestic water supplies is useful as a parameter to characterize the total dissolved solids present and for calculating dosages where lime-soda softening is practiced. Because hardness concentrations in water have not been proven health related, the final level achieved principally is a function of economics. Since hardness in water can be removed with treatment by such processes as lime-soda softening and zeolite or ion exchange systems, a criterion for raw waters used for public water supply is not practical.

The effects of hardness on freshwater fish and other aquatic life appear to be related to the ions causing the hardness rather than hardness. Both the NTAC (1968) and NAS **(1974)** panels have recommended against the use of the term hardness but suggest the inclusion of the concentrations of the specific ions. This procedure should avoid confusion in future studies but is not helpful in evaluating previous studies. For most existing data, it is difficult to determine whether toxicity of various metal ions is reduced because of the formation of metallic hydroxides and carbonates caused by the associated increases in alkalinity, or because of an antagonistic effect of one of the principal cations contributing to hardness, e.g., calcium, or a combination of both effects. Stiff **(1971)** presented a theory (without proof)

that if cupric ions were the toxic form of copper whereas copper carbonate complexes were relatively non-toxic, then the observed difference in toxicity of copper between hard and soft waters can be explained by the difference in alkalinity rather than hardness. Doudoroff and Katz (1953), in their review of the literature on toxicity, presented data showing that increasing calcium in particular reduced the toxicity of other heavy metals. Under usual conditions in fresh water and assuming that other bivalent metals behave similarly to copper, it is reasonable to assume that both effects occur simultaneously and explain the observed reduction of toxicity of metals in waters containing carbonate hardness. The amount of reduced toxicity related to hardness, as measured by a 40-hour LC50 for rainbow trout, has been estimated to be about four times for copper and zinc when the hardness was increased from 10 to 100 mg/L as $CaCO_3$ (NAS, 1974).

Limits on hardness for industrial uses are quite variable. Table 4 lists maximum values that have been accepted by various industries as a source of raw water (NAS, 1974). Subsequent treatment generally can reduce hardness to tolerable limits although costs of such treatment are an important factor in determining its desirability for a particular water source.

Hardness is not a determination of concern for irrigation use of water. The concentrations of the cations calcium and magnesium, which comprise hardness, are important in determining the exchangeable sodium in a given water. This particular calculation will be discussed under total dissolved solids rather

TABLE 4.

Maximum Hardness Levels Accepted
By Industry as a Raw Water Source*

Industry	Maximum Concentration mg/L as $CaCO_3$
Electric utilities	5,000
Textile	120
Pulp and paper	475
Chemical	1,000
Petroleum	900
Primary metals	1,000

* Requirements for final use within a process may be essentially zero, which requires treatment for concentration reductions.

than hardness.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

HEFTACHLOR

CRITERIA:

Aquatic Life

For heptachlor the criterion to protect freshwater aquatic life as derived using the Guidelines is 0.0038 ug/L as a 24-hour average, and the concentration should not exceed 0.52 ug/L at any time.

For heptachlor the criterion to protect saltwater aquatic life as derived using the Guidelines is 0.0036 ug/L as a 24-hour average, and the concentration should not exceed 0.053 ug/L at any time.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to heptachlor through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero, based on the non threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 2.00 ng/L, 0.20 ng/L, and 0.020 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 2.04 ng/L, 0.20 ng/L, and 0.020. ng/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

HEXACHLOROBUTADIENE

CRITERIA:

Aquatic Life

The available data for hexachlorobutadiene indicate that acute and chronic toxicity to freshwater aquatic life occur at concentrations as low as 90 and 9.3 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for hexachlorobutadiene indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 32 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of hexachlorobutadiene to sensitive saltwater aquatic life.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to hexachlorobutadiene through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime 'are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are **4.47** ug/L, **0.45** ug/L, and **0.045** ug/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding

consumption of water, the levels are 500 ug/L, 50 ug/L, and 5.0 ug/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

HEXACHLOROCYCLOHEXANE

CRITERIA:

Aquatic Life

Lindane

For lindane the criterion to protect freshwater aquatic life as derived using the Guidelines is 0.080 ug/L as a 24-hour average and the concentration should not exceed 2.0 ug/L at any time.

For saltwater aquatic life the concentration of lindane should not exceed 0.16 ug/L at any time. No data are available concerning the chronic toxicity of lindane to sensitive saltwater aquatic life.

BHC

The available data for a mixture of isomers of BHC indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 100 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of a mixture of isomers of BHC to sensitive freshwater aquatic life.

The available data for a mixture of isomers of BHC indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 0.34 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of a mixture of isomers of BHC to sensitive saltwater aquatic life.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to hexachlorocyclohexane through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 22 ng/L, 2.2 ng/L, and .22 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 74 ng/L, 7.4 ng/L, and .74 ng/L, respectively.

For the maximum protection of human health from the potential carcinogenic effects of exposure to hexachlorocyclohexane through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 134 ng/L, 13.4 ng/L, and 1.34 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 450 ng/L, 45.0 ng/L, and 4.50 ng/L, respectively.

For the maximum protection of human health from the potential carcinogenic effects due to exposure of α-hexachlorocyclohexane through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 186 ng/L, 18.6 ng/L, and 1.86 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 625 ng/L, 62.5 ng/L, and 6.25 ng/L, respectively.

For the maximum protection of human health from the potential carcinogenic effects of exposure to technical-hexachlorocyclohexane through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 52 ng/L, 5.2 ng/L, and .52 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 174 ng/L, 17.4 ng/L, and 1.74 ng/L, respectively.

Using the present guidelines, satisfactory criteria cannot be derived at this time for d- and e- hexachlorocyclohexane because of insufficient available data.

(45 F.R. 79318, November 28, 1980)

SEE APPENDIX B FOR **METHODOLOGY**

HEXACHLOROCYCLOPENTADIENE

CRITERIA:

Aquatic Life

The available data for hexachlorocyclopentadiene indicate that acute and chronic toxicity to freshwater aquatic life occurs at concentrations as low as 7.0 and 5.2 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for hexachlorocyclopentadiene indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 7.0 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of hexachlorocyclopentadiene to sensitive saltwater aquatic life.

Human Health

For comparison purposes, two approaches were used to derive criterion levels for hexachlorocyclopentadiene. Based on available toxicity data, to protect public health the derived level is 206 ug/L. Using available organoleptic data, to control undersirable taste and odor quality of ambient water the estimated level is 1 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing water quality criteria, and have no demonstrated relationship to potential adverse human health effects.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

IRON

CRITERIA:

0.3 mg/L for domestic water supplies (welfare).

1.0 mg/L for freshwater aquatic life.

INTRODUCTION:

Iron is the fourth most abundant, by weight, of the elements that make up the earth's crust. Common in many rocks, it is an important component of many soils, especially the clay soils where usually it is a major constituent. Iron in water may be present in varying quantities dependent upon the geology of the area and other chemical components of the waterway.

Iron is an essential trace element required by both plants and animals. In some waters it may be a limiting factor for the growth of algae and other plants; this is true especially in some marl lakes where it is precipitated by the highly alkaline conditions. It is a vital oxygen transport mechanism in the blood of all vertebrate and some invertebrate animals.

The ferrous, or bivalent (Fe^{++}), and the ferric, or trivalent (Fe^{+++}) irons, are the primary forms of concern in the aquatic environment, although other forms may be in organic and inorganic wastewater streams. The ferrous (Fe^{++}) form can persist in waters void of dissolved oxygen and originates usually from groundwaters or mines when these are pumped or drained. For practical purposes the ferric (Fe^{+++}) form is insoluble. Iron can exist in natural organometallic or humic compounds and colloidal forms. Black or brown swamp waters may contain iron concentrations of several mg/L in the presence or absence of dissolved oxygen, but this iron form has little effect on aquatic

life.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

ISOPHORONE

CRITERIA:

Aquatic Life

The available data for isophorone indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 117,000 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of isophorone to sensitive freshwater aquatic life.

The available data for isophorone indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 12,900 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of isophorone to sensitive saltwater aquatic life.

Human Health

For the protection of human health from the toxic properties of isophorone ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 5.2 mg/L.

For the protection of human health from the toxic properties of isophorone ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 520 mg/L.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

LEAD

AQUATIC LIFE SUMMARY:

The acute toxicity of lead to several species of freshwater animals has been shown to decrease as the hardness of water increases. At a hardness of 50 mg/L the acute sensitivities of 10 species range from 142.5 ug/L for an amphipod to 235,900 ug/L for a midge. Data on the chronic effects of lead on freshwater animals are available for two fish and two invertebrate species. The chronic toxicity of lead also decreases as hardness increases and the lowest and highest available chronic values (12.26 and 128.1 ug/L) are both for a cladoceran, but in soft and hard water, respectively. Acute-chronic ratios are available for three species and range from 18 to 62. Freshwater algae are affected by concentrations of lead above 500 ug/L, based on data for four species. Bioconcentration factors are available for four invertebrate and two fish species and range from 42 to 1,700.

Acute values are available for 13 saltwater animal species and range from 315 ug/L for the mummichog to 27,000 ug/L for the soft shell clam. A chronic toxicity test was conducted with a mysid; unacceptable effects were observed at 37 ug/L but not at 17 ug/L and the acute-chronic ratio for this species is 124.8. A species of macroalgae was affected at 20 ug/L. Available bioconcentration factors range from 17.5 to 2,570.

NATIONAL CRITERIA:

The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of

Aquatic Organisms and Their Uses indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration (in ug/L) of lead does not exceed the numerical value given by $e^{(1.273[\ln(hardness)]-4.705)}$ more than once every 3 years on the average and if the 1-hour average concentration (in ug/L) does not exceed the numerical value given by $e^{(1.273[\ln(hardness)]-1.460)}$ more than once every 3 years on the average. For example, at hardnesses of 50, 100, and 200 mg/L as $CaCO_3$ the 4-day average concentrations of lead are 1.3, 3.2, and 7.7 ug/L, respectively, and the 1-hour average concentrations are 34, 82, and 200 ug/L.

The procedures described in the Guidelines indicate that, except possibly where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration of lead does not exceed 5.6 ug/L more than once every 3 years on the average and if the 1-hour average concentration does not exceed 140 ug/L more than once every three years on the average.

EPA believes that a measurement such as "acid-soluble" would provide a more scientifically correct basis upon which to establish criteria for metals. The criteria were developed on this basis. However, at this time, no EPA-approved methods for such a measurement are available to implement the criteria through the regulatory programs of the Agency and the States. The Agency is considering development and approval of methods for a measurement such as acid-soluble. Until available, however, EPA recommends applying the criteria using the total recoverable

method. This has two impacts: (1) Certain species of some metals cannot be analyzed directly because the total recoverable method does not distinguish between individual oxidation states, and (2) these criteria may be overly protective when based on the total recoverable method.

The recommended exceedence frequency of 3 years is the Agency's best scientific judgment of the average amount of time it will take an unstressed system to recover from a pollution event in which exposure to lead exceeds the criterion. A stressed system, for example, one in which several outfalls occur in a limited area, would be expected to require more time for recovery. The resilience of ecosystems and their ability to recover differ greatly, however, and site-specific criteria may be established if adequate justification is provided.

The use of criteria in designing waste treatment facilities requires the selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria. Limited data or other factors may make their use impractical, in which case one should rely on a steady-state model. The Agency recommends the interim use of 1Q5 or 1Q10 for Criterion Maximum Concentration design flow and 7Q5 or 7Q10 for the Criterion Continuous Concentration design flow in steady-state models for unstressed and stressed systems, respectively. These matters are discussed in more detail in the Technical Support Document for Water Quality-Based Toxics Control (U.S. EPA, 1985).

HUMAN HEALTH CRITERIA:

The ambient water quality criterion for lead is recommended to be identical to the existing drinking water standard which is 50 ug/L. Analysis of the toxic effects data resulted in a calculated level whic is protective to human health against the ingestion of contaminated water and contaminated aquatic organisms. The calculated value is comparable to the present standard. For this reason a selective criterion based on expoeure soley from consumption of 6.5 grams of aquatic organisms was not derived.

(45 F.R. 79318 Nov. 28,1980) (50 F.R. 30784, July 29, 1985)
SEE APPENDIX A FOR METHODOLOGY

MALATHION

CRITERION:

0.1 ug/L for freshwater and marine aquatic life.

RATIONALE:

The freshwater fish most sensitive to malathion, an organophosphorus pesticide, appear to be the salmonids and centrarchids. Post and Schroeder (1971) report a 96-hour LC50 between 120 and 265 ug/L for 4 species of salmonids. Macek and McAllister (1970) found a 96-hour LC50 range between 101 and 285 ug/L for 3 species of centrarchids and 3 species of salmonids. Other 96-hour LC50's are: rainbow trout, ~~Salmo~~ gairdneri, 68 ug/L (Cope, 1965); largemouth bass, Micropterus ~~salmoides~~, 50 ug/L (Pickering et al. 1962); and chinook salmon, Oncorhynchus tshawytscha, 23 ug/L (Katz, 1961). All of the above tests were in static systems. Eaton (1970) determined a 96-hour LC50 for bluegill, Lepomis ~~macrochirus~~, in a flow-through system at 110 ug/L. Macek and McAllister (1970) reported a similar 96-hour LC50 for the bluegill in a static exposure. Static 96-hour LC50s of 120 and 160 ug/L were reported by Post and Schroeder (1971) for brook trout, Salvelinus fontinalis. Bender (1969) indicated that the acute toxicity to fathead minnows, Pimephales promelas, is slightly greater (about 2.0 times) in a static system than in a flow-through system. The flow-through acute toxicity to fathead minnows reported by Mount and Stephan (1967) approximated the static acute toxicity reported by Henderson and Pickering (1958) and Bender (1969).

Many aquatic invertebrates appear to be more sensitive than fish to malathion. The 96-hour LC50 for Gammarus lacustris was 1.0 ug/L (Sanders, 1969); for Pteronarcella badia, 1.1 ug/L (Sanders and Cope, 1968); and for Gammarus fasciatus, 0.76 ug/L (Sanders, 1972). The 48-hour LC50 for Simocephalus serrulatus was 3.5 ug/L and for Daphnia pulex, 1.8 ug/L (Sanders and Cope, 1966). Daphnia were immobilized in 50 hours in 0.9 ug/L (Anderson, 1960). The 24-hour LC50s for two species of midge larvae were 2.1 ug/L (Mulla and Khasawinah, 1969) and 2.0 ug/L (Karnak and Collins, 1974).

Safe life cycle exposure concentrations for the more sensitive invertebrates are not known. The most sensitive aquatic organisms probably have not yet been tested; safe concentrations for the most sensitive invertebrates exposed through a complete life cycle have not been determined; and effects of low concentrations on invertebrate behavior are unknown.

The stability of malathion in water is dependent on the chemical and biological conditions of the water (Paris et al. 1975). Weiss and Gakstatter (1964) have shown that the half-life of malathion was reduced from about 5 months at pH 6 to 1 to 2 weeks at pH 8. Eichelberger and Lichtenberg (1971) found that only 10 percent remained in the Little Miami River (pH 7.3-8.0) after 2 weeks. Bender (1969) states that one of the malathion breakdown products may be more toxic than the parent compound.

It has been shown that a measured concentration of 575 ug/L malathion in flowing seawater kills 40 to 60 percent of the

marine fish, Lagodon rhomboides, in 3.5 hours and causes about 75 percent brain acetylcholinesterase (AChE) inhibition (Coppage et al. 1975). Similar inhibition of AChE and mortality were caused in pinfish in 24, 48, and 72 hours'at measured concentrations of 142, 92 and 58 ug/L, respectively. A concentration of 31 ug/L caused 34 percent AChE inhibition in pinfish but no deaths in 72 hours. Coppage and Matthews (1974) demonstrated that death may be associated with reductions of brain AChE activity of four marine fishes by 70 to 80 percent or more in short-term exposures to malathion. Coppage and Duke (1971) found that moribund mullet, Mugil cephalus, in an estuary sprayed with malathion (3 oz./acre) during a large-scale mosquito control operation had about 98 percent inhibition of brain AChE. This is in agreement with 70 to 80 percent or more inhibition of brain AChE levels at and below which some deaths are likely to occur in short-term exposure. Spot, Leiostomus xanthurus, and Atlantic croaker, Micropogon undulatus, also had substantial inhibition of brain during the spray operation (70 percent or more inhibition).

Toxicity studies have been made on a number of marine animals. Eisler (1970) studied the 96-hour LC50 for several marine fishes at 20 °C in static, aerated seawater. The 96-hour LC50 values (in ug/L) were: Menidia menidia, 125: Mugil cephalus, 550; Fundulus majalis, 250; Fundulus heteroclitus, 240; Sphaeroides maculatus, 3250: Anguilla rostrata, 82; and Thalassoma bifasciatum, 27. Katz (1961) reported the static 24-hour LC50 for Gasterosteus aculeatus in 25 o/oo saltwater as 76.9 ug/L active ingredient. The 96-hour LC50 for striped bass,

Morone saxatilis, in intermittent flowing seawater has been reported as 14 ug/L (U.S. BSFW, 1970).

Reporting on studies of the toxicity of malathion on marine invertebrates, Eisler (1969) found the 96-hour LC50 (static, 24 o/oo salinity aerated) to be 33 ug/L for sand shrimp, Crangon septemspinosa; 82 ug/L for grass shrimp, Palaemonetes vulgaris; and 83 ug/L for hermit crab, Pagurus longicarpus. Growth of oyster, Crassostrea virginica, was reduced 32 percent by 96-hour exposure to 1 mg/L (Butler, 1963). The 48-hour LC50 for fertilized eggs of oysters was estimated by Davis and Hidu (1969) to be 9.07 mg/L and the 14-day LC50 for larvae, 2.66 mg/L.

Malathion enters the aquatic environment primarily as a result of its application as an insecticide. Because it degrades quite rapidly in most waters, depending on pH, its occurrence is sporadic rather than continuous. Because the toxicity is exerted through inhibition of AChE and because such inhibition may be additive with repeated exposures and may be caused by any of the organophosphorus insecticides, inhibition of AChE by more than 35 percent may be expected to result in damage to aquatic organisms.

An application factor of 0.1 is applied to the 96-hour LC50 data for Gammarus lacustris, G. fasciatis and Daphnia, which are all approximately 1.0 ug/L, yielding a criterion of 0.1 ug/L.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

MANGANESE

CRITERIA:

50 ug/L for domestic water supplies (welfare):

100 ug/L for protection of consumers of marine molluscs.

INTRODUCTION:

Manganese does not occur naturally as a metal but is found in various salts and minerals, frequently in association with iron compounds. The principal manganese-containing substances are manganese dioxide (MnO_2), pyrolusite, manganese carbonate (rhodocrosite) and manganese silicate (rhodonite). The oxides are the only important minerals mined. Manganese is not mined in the United States except when manganese is contained in iron ores that are deliberately used to form ferro-manganese alloys.

The primary uses of manganese are in metal alloys, dry cell batteries, micro-nutrient fertilizer additives, organic compounds used in paint driers and as chemical reagents. Permanganates are very strong oxidizing agents of organic materials.

Manganese is a vital micro-nutrient for both plants and animals. When manganese is not present in sufficient quantities, plants exhibit chlorosis (a yellowing of the leaves) or failure of the leaves to develop properly. Inadequate quantities of manganese in domestic animal food results in reduced reproductive capabilities and deformed or poorly maturing young. Livestock feeds usually have sufficient manganese, but beef cattle on a high corn diet may require a supplement.

RATIONALE:

Although inhaled manganese dusts have been reported to be toxic to humans, manganese normally is ingested as a trace nutrient in food. The average human intake is approximately 10 mg/day (Sollman, 1957). Very large doses of ingested manganese can cause some disease and liver damage but these are not known to occur in the United States. Only a few manganese toxicity problems have been found throughout the world and these have occurred under unique circumstances, i.e., a well in Japan near a deposit of buried batteries (McKee and Wolf, 1963).

It is possible to partially sequester manganese with special treatment but manganese is not removed in the conventional treatment of domestic waters (Riddick et al. 1958: Illig, 1960). Consumer complaints arise when manganese exceeds a concentration of 150 ug/L in water supplies (Griffin, 1960). These complaints are concerned primarily with the brownish staining of laundry and objectionable tastes in beverages. It is possible that the presence of low concentrations of iron may intensify the adverse effects of manganese. Manganese at concentrations of about 10 to 20 ug/L is acceptable to most consumers. A criterion for domestic water supplies of 50 ug/L should minimize the objectionable qualities.

McKee and Wolf (1963) summarized data on toxicity of manganese to freshwater aquatic life. Ions of manganese are found rarely at concentrations above 1 mg/L. The tolerance values reported range from 1.5 mg/L to over 1000 mg/L. Thus, manganese is not considered to be a problem in fresh waters. Permanganates have been reported to kill fish in 8 to 18 hours at

concentrations of 2.2 to 4.1 mg/L, but permanganates are not persistent because they rapidly oxidize organic materials and are thereby reduced and rendered nontoxic.

Few data are available on the toxicity of manganese to marine organisms. The ambient concentration of manganese is about 2 ug/L (Fairbridge, 1966). The material is rapidly assimilated and bioconcentrated into nodules that are deposited on the sea floor. The major problem with manganese may be concentration in the edible portions of molluscs, as bioaccumulation factors as high as 12,000 have been reported (NAS, 1974). In order to protect against a possible health hazard to humans by manganese accumulation in shellfish, a criterion of 100 ug/L is recommended for marine water.

Manganese is not known to be a problem in water consumed by livestock. At concentrations of slightly less than 1 mg/L to a few milligrams per liter, manganese nay be toxic to plants from irrigation water applied to soils with pH values lower than 6.0. The problem may be rectified by liming soils to increase the pH. Problems may develop with long-term (20 year) continuous irrigation on other soils with water containing about 10 mg/L of manganese (NAS, 1974). But, as stated above, manganese is rarely found in surface waters at concentrations greater than 1 mg/L. Thus, no specific criterion for manganese in agricultural waters is proposed. In select areas, and where acidophilic crops ate cultivated and irrigated, a criterion of 200 ug/L is suggested for consideration.

Most industrial users of water can operate successfully where the criterion proposed for public water supplies is observed. Examples of industrial tolerance of manganese in water are summarized for industries such as dyeing, milk processing, paper, textiles, photography and plastics (McKee and Wolf, 1963). A more restrictive criterion may be needed to protect or ensure product quality.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

*MERCURY

AQUATIC LIFE SUMMARY:

Data are available on the acute toxicity of mercury(II) ,to 28 genera of freshwater animals. Acute values for invertebrate species range from 2.2 ug/L for Daphnia pulex to 2,000 ug/L for three insects. Acute values for fishes range from 30 ug/L for the guppy to 1,000 ug/L for the Mozambique tilapia. Few data are available for various organomercury compounds and mercurous nitrate, and they all appear to be 4 to 31 times more acutely toxic than mercury(II).

Available chronic data indicate that methylmercury is the most chronically toxic of the tested mercury compounds. Tests on methylmercury with Daphnia magna and brook trout produced chronic values less than 0.07 ug/L. For mercury(II) the chronic value obtained with Daphnia magna was about 1.1 ug/L and the acute-chronic ratio was 4.5. In both a life-cycle test and an early life-stage test on mercuric chloride with the fathead minnow, the chronic value was less than 0.26 ug/L and the acute-chronic ratio was over 600.

Freshwater plants show a wide range of sensitivities to mercury, but the most sensitive plants appear to be less sensitive than the most sensitive freshwater animals to both mercury(II) and methylmercury. A bioconcentration factor of 4,994 is available for mercury(II), but the bioconcentration factors for methylmercury range from 4,000 to 85,000.

*Indicates suspended, canceled or restricted by U.S. EPA Office of Pesticides and Toxic Substances

Data on the acute toxicity of mercuric chloride are available for 29 genera of saltwater animals, including annelids, molluscs, crustaceans, echinoderms, and fishes. Acute values range from 3.5 ug/L for a mysid to 1,678 ug/L for winter flounder. Fishes tend to be more resistant and molluscs and crustaceans tend to be more sensitive to the acute toxic effects of mercury(II). Results of a life-cycle test with the mysid show that mercury(II) at a concentration of 1.6 ug/L significantly affected time of first spawn and productivity; the resulting acute-chronic ratio was 3.1.

Concentrations of mercury that affected growth and photosynthetic activity of one saltwater diatom and six species of brown algae range from 10 to 160 ug/L. Bioconcentration factors of 10,000 and 40,000 have been obtained for mercuric chloride and methylmercury with an oyster.

NATIONAL CRITERIA:

Derivation of a water quality criterion for mercury is more complex than for most metals because of methylation of mercury in sediment, in fish, and in the food chain of fish. Apparently almost all mercury currently being discharged is mercury(II). Thus mercury(II) should be the only important possible cause of acute toxicity and the Criterion Maximum Concentrations can be based on the acute values for mercury(II).

The best available data concerning long-term exposure of fish to mercury(II) indicates that concentrations above 0.23 ug/L caused statistically significant effects on the fathead minnow and caused the concentration of total mercury in the whole body

to exceed 1.0 mg/kg. Although it is not known what percent of the mercury in the fish was methylmercury, it is also not known whether uptake from food would increase the concentration in the fish in natural situations. Species such as rainbow trout, coho salmon, and especially the bluegill, might suffer chronic effects and accumulate high residues of mercury about the same as the fathead minnow.

With regard to long-term exposure to methylmercury, McKim et al. (1976) found that brook trout can exceed the FDA action level without suffering statistically significant adverse effects on survival, growth, or reproduction. Thus for methylmercury the Final Residue Value would be substantially lower than the Final Chronic Value.

Basing a freshwater criterion on the Final Residue Value of 0.012 ug/L derived from the bioconcentration factor of 81,700 for methylmercury with the fathead minnow (Olson et al. 1975) essentially assumes that all discharged mercury is methylmercury. On the other hand, there is the possibility that in field situations uptake from food might add to the uptake from water. Similar considerations apply to the derivation of the saltwater criterion of 0.025 ug/L using the BCF of 40,000 obtained for methylmercury with the Eastern oyster (Kopfler, 1974). Because the Final Residue Values for methylmercury are substantially below the Final Chronic Values for mercury(II), it is probably not too important that many fishes, including the rainbow trout, coho salmon, bluegill, and haddock might not be adequately protected by the freshwater and saltwater Final Chronic Values for mercury(II).

In contrast to all the complexities of deriving numerical criteria for mercury, monitoring for unacceptable environmental effects should be relatively straightforward. The most sensitive adverse effect will probably be exceedence of the FDA action level. Therefore, existing discharges should be acceptable if the concentration of methylmercury in the edible portion of exposed consumed species does not exceed the FDA action level.

The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration of mercury does not exceed 0.012 ug/L more than once every 3 years on the average and if the 1-hour average concentration does not exceed 2.4 ug/L more than once every 3 years on the average. If the 4-day average concentration exceeds 0.012 ug/L more than once in a 3-year period, the edible portion of consumed species should be analyzed to determine whether the concentration of methylmercury exceeds the FDA action level.

The procedures described in the Guidelines indicate that, except possibly where a localy important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration of mercury does not exceed 0.025 ug/L more than once every 3 years on the average and if the 1-hour average concentration does not exceed 2.1 ug/L more than once every 3 years on the average. If the 4-day average concentration exceeds 0.025 ug/L more than once

in a 3-year period, the edible protion of consumed species should be analyzed to determine whether the concentration of mathylmercury exceeds the FDA action level.

EPA believes that a measurement such as "acid-soluble" would provide a more scientifically correct basis upon which to establish criteria for metals. The criteria were developed on this basis. However, at this time, no EPA approved-methods for such a measurement are available to implement the criteria through the regulatory programs of the Agency and the States. The Agency is considering development and approval of methods for a measurement such as acid-soluble. Until available, however, EPA recommends applying the criteria using the total recoverable method. This has two impacts: (1) certain species of some metals cannot be analyzed directly because the total recoverable method does not distinguish between individual oxidation states, and (2) these criteria may be overly protective when based on the total recoverable method.

The recommended exceedence frequency of 3 years is the Agency's best scientific judgment of the average amount of time it will take an unstressed system to recover from a pollution event in which exposure to mercury exceeds the criterion. A stressed system, for example, one in which several outfalls occur in a limited area, would be expected to require more time for recovery. The resilience of ecosystems and their ability to recover differ greatly, however, and site-specific criteria may be established if adequate justification is provided.

The use of criteria in designing waste treatment facilities requires the selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria. Limited data or other factors may make their use impractical, in which case one should rely on a steady-state model. The Agency recommends the interim use of 1Q5 or 1Q10 for Criterion Maximum Concentration design flow and 7Q5 or 7Q10 for the Criterion Continuous Concentration design flow in steady-state models for unstressed and stressed systems respectively. These matters are discussed in more detail in the Technical Support Document for Water Quality-Based Toxics Control (U.S EPA, 1985).

HUMAN HEALTH CRITERIA

For the protection of human health from the toxic properties of mercury ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 144 ng/L.

For the protection of human health from the toxic properties of mercury ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 146 ng/L.

NOTE: These values include the consumption of freshwater, estuarine, and marine species.

(45 F.R. 79318 Nov. 28,1980) (50 F.R. 30784, July 29, 1985)
SEE APPENDIX A FOR METHODOLOGY

METHOXYCHLOR

CRITERIA:

100 ug/L for domestic water supply (health);

0.03 ug/L for freshwater and marine aquatic life.

RATIONALE :

The highest level of methoxychlor found to have minimal or no long-term effects in man is 2.0 mg/kg of body weight/day (Lehman, 1965). Where adequate human data are available for corroboration of the animal results, the total "safe" drinking water intake level is assumed to be 1/100 of the no-effect or minimal effect level reported for the most sensitive animal tested, in this case, man.

Applying the available data and based upon the assumptions that 20 percent of the total intake of methoxychlor is from drinking water, and that the average person weighs 70 kg and consumes 2 liters of water per day, the formula for calculating a criterion is 2.0 mg/kg x 0.2 x 70 kg x 1/100 x 1/2 = 0.14 mg/L. A criterion level for domestic water supply of 100 ug/L is recommended.

Few data: are available on acute and chronic effects of methoxychlor on freshwater fish. Merna and Eisele (1973) observed reduced hatchability of fathead minnow (Pimephales promelas) embryos at 0.125 ug/L and lack of spawning at 2.0 ug/L. Yellow perch, Perca ~~flave~~scens, exposed to 0.6 ug/L for 8 months exhibited reduced growth. The 36-hour LC50 concentration was 7.5 and 22 ug/L for the fathead minnow and yellow perch, respectively. Korn and Earnest (1374) obtained a 96-hour LC50

of 3.3 ug/L with juvenile stripped bass, Morone saxatilis, exposed to methoxychlor in a flowing-water bioassay.

Sanders (1972) determined a 96-hour LC50 value of 0.5 ug/L for the crayfish, Orconectes nais. Merna and Eisele (1973) obtained a 96-hour LC50 value of 0.61 ug/L for the scud, Gammarus pseudolimnaeus and 96-hour LC50's ranging from 1.59 to 7.05 ug/L for the crayfish, Orconectes nais, and three aquatic insect larvae. In 28-day exposures, reduction in emergence of mayflies, Stenonema sp., and in pupation of caddisflies, Cheumatospsyche sp., were observed at 0.5 and 0.25 ug/L concentrations, respectively. They also found methoxychlor to be degraded in a few weeks or less in natural waters.

Eisele (1974) conducted a study in which a section of a natural stream was dosed at 0.2 ug/L methoxychlor for 1 year. The near extinction of one species of scud, Hyalella azteca, and reductions in populations of other sensitive species, as well as biomass, were observed. Residue accumulation of up to 1,000 times the level in the stream was observed in first-year crayfish, Orconectes nais. Metcalf et al. (1971) traced the rapid conversion of methoxychlor to water soluble compounds and elimination from the tissues of snails, mosquito larvae and mosquitofish. Thus, methoxychlor appears to be considerably less bioaccumlative in aquatic organisms than some of the other chlorinated pesticides.

Methoxyhlor has a very low accumulation rate in birds and mammals (Stickel, 1973), and relatively low avian (Heath et al. 1972) and mammalian (Hodge et al. 1950) toxicities. No administrative guidelines for acceptable levels in edible fish

tissues have been established by the U.S. Food and Drug Administration.

The above data indicate that 0.1 ug/L methoxychlor would be just below chronic effect level for the fathead minnow and one-fifth the acute toxicity level in a crayfish species. Therefore, a criterion level of 0.03 ug/L is recommended. This criterion should protect fish as sensitive as striped bass and is 10 times lower than the level causing effects on some invertebrate populations in a 1-year dosing of a natural stream.

Bahner and Nimmo (1974) found the 96-hour LC50 of methoxychlor for the pink shrimp, Penaeus duorarum, to be 3.5 ug/L and the 30-day LC50 to be 1.3 ug/L. Using an application factor of 0.01 with the pink shrimp's acute toxicity of 3.5 ug/L, the recommended criterion for the marine environment is 0.03 ug/L.

Butler (1971) found accumulation factors of 470 and 1,500 for the molluscs, Mercenaria mercenaria and Mya arenaria, respectively, when exposed to 1 ug/L methoxychlor for 5 days. Using the 1,500 accumulation factor as a basis, a water concentration of 0.2 ug/L would be required to meet the U.S. Food and Drug Administration's guideline for methoxychlor in meat products. Thus, the recommended marine criterion of 0.03 ug/L is an order of magnitude lower than this concentration.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

MIREX

CRITERION:

0.001 ug/L for freshwater and marine aquatic life.

RATIONAL??.:

Mirex is used to control the imported fire ant Solenopsis saevissima richteri in the southeastern United States. Its use is essentially limited to the control of this insect and it is always presented in bait. In the most common formulation, technical grade mirex is dissolved in soybean oil and sprayed on corncob grits. The bait produced in this manner consists of 0.3 percent mirex, 14.1 percent soybean oil and 85 percent corncob grits. The mirex bait often is applied at a rate of 1.4 kg/ha, equivalent to 4.2 grams of toxicant per hectare.

Relatively few studies have been made of the effects of mirex on freshwater invertebrates og yhrdr, only Dudke et al. (1971) report chemical analyses of mirex in the water. Their study reported effects on two crayfish species exposed to mirex by three techniques. First, field-collected crayfish were exposed to several sublethal concentrations of technical grade mirex solutions €or various periods of time; second, crayfish were exposed to mirex leached from bait (0.3 percent active ingredient); and third, the crayfish were fed mirex bait.

Procambarus blandingi juveniles were exposed to 1 or 5 ug/L for 6 to 144 hours, transferred to clean water and observed for 10 days. After 5 days in clean water, 95 percent of the animals exposed to 1 ug/L for 14 hours were dead. Exposure to 5 ug/L for 6, 24, and 58 hours resulted in 26, 50, and 98 percent mortality 10 days after transfer to clean water. Crayfish, Procambarus

bayi, were exposed to 0.1 and 0.5 ug/L for 48 hours. Four days after transfer to clean water, 65 percent of the animals exposed to 0.1 ug/L were dead. At the 0.5 ug/L concentration, 71 percent of the animals were dead after 4 days in clean water. Tissue residue accumulations (wet weight basis) ranged from 940- to 27,210-fold above water concentrations. In leached bait experiments, 10 bait particles were placed in 2 liters of water but isolated from 20 juvenile crayfish. Thirty percent of the crayfish were dead in 4 days and 95 percent were dead in 7 days. Water analysis indicated mirex concentrations of 0.86 ug/L. In feeding experiments, 108 crayfish each were fed one bait particle. Mortality was noticed on the first day after feeding, and by the sixth day 77 percent were dead. In another experiment, all crayfish were dead 4 days after having been fed 2 bait particles each. From this report it is obvious that mirex is extremely toxic to these species of crayfish. Mortality and accumulation increases with time of exposure to the insecticide. Concentrations as low as 0.1 ug/L or the ingestion of one particle resulted in death.

Research to determine effects of mirex on fish has been concentrated on species which have economic and sport fishery importance. Hyde et al. (1974) applied mirex bait (0.3 percent mirex) at the standard rate (1.4 kg/ha) in four ponds containing channel catfish, Ictalurus punctatus. Three applications were made over an 8-month period with the first application 8 days after fingerling (average weight 18.4 g) catfish were placed in the ponds. Fish were collected at each subsequent application

(approximately 4-month intervals). Two and one half months after the final application, the ponds were drained, all fish were measured and weighed, and the percent survival was calculated. Mirex residues in the fish at termination of the experiment ranged from 0.015 ug/g (ppm) in the fillet to 0.255 ug/g in the fat.

In another study, Van Valin et al. (1988) exposed bluegills, Lepomis macrochirus, and the goldfish, Carassius auratus, to mirex by feeding a mirex-treated diet (1, 3, and 5 mg mirex per kg body weight) or by treating holding ponds with mirex bait (1.3, 100, and 1000 ug/L computed water concentration). They reported no mortality or tissue pathology for the bluegills; however, after 58 days of exposure, gill breakdown in goldfish was found in the 100 and 1000 ug/L contact exposure ponds, and kidney breakdown was occurring in the 1000 ug/L ponds. Mortality in the feeding experiments was not related to the level of exposure, although growth of the bluegills fed 5 ug/L mirex was reduced.

In laboratory and field test systems, reported concentrations of mirex usually are between 0.5 and 1.0 ug/L (Van Valin et al. 1968: Ludke et al. 1971). Although mirex seldom is found above 1 ug/L in the aquatic environment, several field studies have shown that the insecticide is accumulated through the food chain. Borthwick et al. (1973) reported the accumulation of mirex in South Carolina estuaries. Their data revealed that mirex was transported from treated land and marsh to the estuary animals and that accumulation, especially in predators, occurred. In the test area, water supplies consistently were less than 0.01 ug/L.

Residues in fish varied from non-detectable to 0.8 ug/g with 15 percent of the samples containing residues. The amount of mirex and the percent of samples containing mirex increased at higher trophic levels. Fifty-four percent of the raccoons sampled contained mirex residues up to 4.4 ug/g and 78 percent of the birds contained residues up to 17 ug/g. Navgi and de la Cruz (1973) reported average residues for molluscs (0.15 ug/g), fish (0.26 ug/g), insects (0.29 ug/g), crustaceans (0.44 ug/g) and annelids (0.63 ug/g. They also reported that mirex was found in areas not treated with mirex which suggests movement of the pesticide in the environment. Wolfe and Norment (1973) sampled an area for one year following an aerial application of mirex bait (2.1 g mirex/ha). Crayfish residues ranged from 0.04 to 0.16 ug/g. Fish residues were about 2 to 20 times greater than the controls and averaged from 0.01 to 0.78 ug/g. Kaiser (1974), reported the presence of rnirex in fish from the Bay of Quinte, Lake Ontario, Canada. Concentrations range from 0.02 ug/g in the gonads of the northern long nose gar, Lepistosteus osseus, to 0.05 ug/g in the areal fin of the northern pike, Esox lucius. Mirex has never been registered for use in Canada.

Mirex does not appear to be greatly toxic to birds, with LC50's for the young of four species ranging from 547 to greater than 1667 ug/g (Heath et al. 1972). Long-term dietary dosages caused no adverse effect at 3 ug/g with mallards and 13 ug/g with pheasants (Heath and Spann, 1973). However, it has been reported (Stoke et al. 1978) that the persistence of mirex in bird tissue exceeds that of all organochlorine compounds tested except for

DDE. Delayed mortality occurred among birds subjected to doses above expected environmental concentration.

A summary examination of the data available at this time shows a mosaic of effects. Crayfish and channel catfish survival is affected by mirex in the water or by ingestion of the bait particles. Bioaccumulation is well established for a wide variety of organisms but the effect of this bioaccumulation on the aquatic ecosystem is unknown. There is evidence that mirex is very persistent in bird tissue. Considering the extreme toxicity and potential for bioaccumulation, every effort should be made to keep mirex bait particles out of water containing aquatic organisms and water concentrations should not exceed 0.001 ug/L mirex. This value is based upon an application factor of 0.01 applied to the lowest levels at which effects on crayfish have been observed.

Data upon which to base a marine criterion involve several estuarine and marine crustaceans. A concentration of 0.1 ug/L technical grade mirex in flowing seawater was lethal to juvenile pink shrimp, Penaeus durorarum, in a 3-week exposure (Lowe et al. 1971). In static tests with larval stages (megalopal) of the mud crab, Rhithropanopeus harrisii, reduced survival was observed in 0.1 ug/L mirex (Bookhout et al. 1972). In three of four 28-day seasonal flow-through experiments, Tagatz et al. (1975) found reduced survival of Callinectes sapidus, Penaeus durorarum, and grass shrimp, Palaemonetes ~US&, at levels of 0.12 ug/L in summer, 0.06 ug/L in fall and 0.09 ug/L in winter.

Since two reports, Lowe et al. (1971) and Bookhout et al. (1972), stated that effects of mirex on estuarine and marine

crustaceans were observed only after considerable time had elapsed, it seems reasonable that length of exposure is an important consideration €or this chemical. This may not be the case in fresh water since the crayfish were affected within 48 hours. Therefore, a 3- to 4-week exposure might be considered "acute" and by applying an application factor of 0.01 to a reasonable average of toxic-effect levels as summarized above, a recommended marine criterion of 0.001 ug/L results.

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SEE APPENDIX C FOR METHODOLOGY

NAPHTHALENE

CRITERIA:

Aquatic Life

The available data for naphthalene indicate that acute and chronic toxicity to freshwater aquatic life occurs at concentrations as low as 2,300 and 620 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for naphthalene indicate that acute toxicity to saltwater aquatic 1ife occurs at concentrations as low as 2,350 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of naphthalene to sensitive saltwater aquatic life.

Human Health

Using the present guidelines, a satisfactory criterion cannot be derived at this time because of insufficient available data for naphthalene.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

NICKEL

CRITERIA:

Aquatic Life

For total recoverable nickel the criterion (in ug/L) to protect freshwater aquatic life as derived using the Guidelines is the numerical value given by $e^{(0.76[\ln(\text{hardness})]+1.06)}$ as a 24-hour average, and the concentration (in ug/L) should not exceed the numerical value given by $e^{(0.76[\ln(\text{hardness})]+4.02)}$ at any time. For example, at hardnesses of 50, 100, and 200 mg/L as $CaCO_3$ the criteria are 56, 96, and 160 ug/L, respectively, as 24-hour averages, and the concentrations should not exceed 1,100, 1,800, and 3,100 ug/L, respectively, at any time.

For total recoverable nickel the criterion to protect saltwater aquatic life as derived using the Guidelines is 7.1 ug/L as a 24-hour average, and the concentration should not exceed 140 ug/L at any time.

Human Health

For the protection of human health from the toxic properties of nickel ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 632 **ug/L.**

For the protection of human health from the toxic properties of nickel ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 4.77 **mg/L.**

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

NITRATES/NITRITES

CRITERION:

10 mg/L nitrate nitrogen (N) for domestic water supply (health).

INTRODUCTION:

Two gases (molecular nitrogen and nitrous oxide) and five forms of nongaseous, combined nitrogen (amino and amide groups, ammonium, nitrite, and nitrate) are important in the nitrogen cycle. The amino and amide groups are found in soil organic matter and as constituents of plant and animal protein. The ammonium ion either is released from proteinaceous organic matter and urea, or is synthesized in industrial processes involving atmospheric nitrogen fixation. The nitrite ion is formed from the nitrate or the ammonium ions by certain microorganisms found in soil, water, sewage, and the digestive tract. The nitrate ion is formed by the complete oxidation of ammonium ions by soil or water microorganisms; nitrite is an intermediate product of this nitrification process. In oxygenated natural water systems nitrite is rapidly oxidized to nitrate. Growing plants assimilate nitrate or ammonium ions and convert them to protein. A process known as denitrification takes place when nitrate-containing soils become anaerobic and the conversion to nitrite, molecular nitrogen, or nitrous oxide occurs. Ammonium ions may also be produced in some circumstances.

Among the major point sources of nitrogen entry into water bodies are municipal and industrial wastewaters, septic tanks, and feed lot discharges. Diffuse sources of nitrogen include farm-site fertilizer and animal wastes, lawn fertilizer, leachate

from waste disposal in dumps or sanitary landfills, atmospheric fallout, nitric oxide and nitrite discharges from automobile exhausts and other combustion processes, and losses from natural sources such as mineralization of soil organic matter **(NAS, 1972)**. Water reuse systems in some fish hatcheries employ a nitrification process for ammonia reduction; this may result in exposure of the hatchery fish to elevated levels of nitrite (Russo et al. **1974)**.

RATIONALE:

In quantities normally found in food or feed, nitrates become toxic only under conditions in which they are, or may be, reduced to nitrites. Otherwise, at "reasonable" concentration nitrates are rapidly excreted in the urine. High intake of nitrates constitutes a hazard primarily to warmblooded animals under conditions that are favorable to reduction to nitrite. Under certain circumstances, nitrate can be reduced to nitrite in the gastrointestinal tract which then reaches the bloodstream and reacts directly with hemoglobin to produce methemoglobin, consequently impairing transport.

The reaction of nitrite with hemoglobin can be hazardous in infants under 3 months of age. Serious and occasionally fatal poisonings in infants have occurred following ingestion of untreated well waters shown to contain nitrate at concentrations greater than 10 mg/L nitrate nitrogen **(N) (NAS, 1974)**. High nitrate concentrations frequently are found in shallow farm and rural community wells, often as the result of inadequate protection from barnyard drainage or from septic tanks (USPHS,

1961; Stewart et al. 1967). Increased concentrations of nitrates also have been found in streams from farm tile drainage in areas of intense fertilization and farm crop production (Harmeson et al. 1971). Approximately 2,000 cases of infant methemoglobinemia have been reported in Europe and North America since 1945; 7 to 8 percent of the affected infants died (Walton, 1951; Sattelmacher, 1962). Many infants have drunk water in which the nitrate nitrogen content was greater than 10 mg/L without developing methemoglobinemia. Many public water supplies in the United States contain levels that routinely exceed this amount, but only one U.S. case of infant methemoglobinemia associated with a public water supply has ever been reported (Virgil et al. 1965). The differences in susceptibility to methemoglobinemia are not yet understood but appear to be related to a combination of factors including nitrate concentration, enteric bacteria, and the lower acidity characteristic of the digestive systems of baby mammals. Methemoglobinemia systems and other toxic effects were observed when high nitrate well waters containing pathogenic bacteria were fed to laboratory mammals (Wolff et al. 1972). Conventional water treatment has no significant effect on nitrate removal from water (NAS, 1974).

Because of the potential risk of methemoglobinemia to bottle-fed infants, and in view of the absence of substantiated physiological effects at nitrate concentrations below 10 mg/L nitrate nitrogen, this level is the criterion for domestic water supplies. Waters with nitrite nitrogen concentrations over 1

mg/L should not be used for infant feeding. Waters with a significant nitrite concentration usually would be heavily polluted and probably bacteriologically unacceptable.

Westin (1974) determined that the respective 96-hour and 7-day LC50 values for chinook salmon, Oncorhynchus tshawytscha, were 1,310 and 1,080 mg/L nitrate nitrogen in fresh water and 990 and 900 mg/L nitrate nitrogen in 15 o/oo saline water. For fingerling rainbow trout, Salmo gairdneri, the respective 96-hour and 7-day LC50 values were 1,360 and 1,060 mg/L nitrate nitrogen in fresh water, and 1,050 and 900 mg/L nitrate nitrogen in 15 o/oo saline water. Trama (1954) reported that the 96-hour LC50 for bluegills, Lepomis macrochirus, at 20°C was 2,000 mg/L nitrate nitrogen (sodium nitrate) and 420 mg/L nitrate nitrogen (potassium nitrate). Knepp and Arkin (1973) observed that largemouth bass, Micropterus salmoides, and channel catfish, Ictalurus punctatus, could be maintained at concentrations up to 400 mg/L nitrate (90 mg/L nitrate nitrogen) without significant effect upon their growth and feeding activities.

The 96-hour and 7-day LC50 values for chinook salmon, Oncorhynchus tshawytscha, were found to be 0.9 and 0.7 mg/L nitrite nitrogen in fresh water (Westin, 1974). Smith and Williams (1974) tested the effects of nitrite nitrogen and observed that yearling rainbow trout, Salmo gairdneri, suffered a 55 percent mortality after 24 hours at 0.55 mg/L; fingerling rainbow trout suffered a 50 percent mortality after 24 hours of exposure at 1.6 mg/L; and chinook salmon, Oncorhynchus tshawytscha, suffered a 40 percent mortality within 24 hours at

0.5 mg/L. There were no mortalities among rainbow trout exposed to 0.15 mg/L nitrite nitrogen for 48 hours. These data indicate that salmonids are more sensitive to nitrite toxicity than are other fish species, e.g., minnows, Phoxinus laevis, that suffered a 50 percent mortality within 1.5 hours of exposure to 2,030 mg/L nitrite nitrogen, but required 14 days of exposure for mortality to occur at 10 mg/L (Klingler, 1957), and carp, Cyprinus carpio, when raised in a water reuse system, tolerated up to 1.8 mg/L nitrite nitrogen (Saeki, 1965).

Gilbette, et al. (1952) observed that the critical range for creek chub, Semotilus atromaculatus, was 80 to 400 mg/L nitrite nitrogen. Wallen et al. (1957) reported a 24-hour LC50 of 1.6 mg/L nitrite nitrogen, and 48- and 96-hour LC50 values of 1.5 mg/L nitrite nitrogen for mosquitofish, Gambusia affinis. McCoy (1972) tested the nitrite susceptibility of 13 fish species and found that logperch, Percina caprodes, were the most sensitive species tested (mortality at 5 mg/L nitrite nitrogen in less than 3 hours of exposure) whereas carp, Cyprinus carpio, and black bullheads, Ictalurus melas, survived 40 mg/L nitrite nitrogen for a 48-hour exposure period; the common white sucker, Catostomus commersoni, and the quillback, Carpiodes cyprinus, survived 100 mg/L for 48 and 36 hours, respectively.

Russo et al. (1974) performed flow-through nitrite bioassays in hard water (hardness = 199 mg/L $CaCO_3$; alkalinity = 176 mg/L $CaCO_3$; pH = 7.9) on rainbow trout, Salmo gairdneri, of four different sizes, and obtained 96-hour LC50 values ranging from 0.19 to 0.39 mg/L nitrite nitrogen. Duplicate bioassays on 12-gram rainbow trout were continued long enough for their toxicity

curves to level off, and asymptotic LC50 concentrations of 0.14 and 0.15 mg/L were reached in 8 days; on day 19, additional mortalities occurred. For 2-gram rainbow trout, the minimum tested level of nitrite nitrogen at which no mortalities were observed after 10 days was 0.14 mg/L; for the 235-gram trout, the minimum level with no mortality after 10 days was 0.06 mg/L.

It is concluded that (1) levels of nitrate nitrogen at or below 90 mg/L would have no adverse effects on warmwater fish (Knepp and Arkin, 1973); (2) nitrite nitrogen at or below 5 mg/L should be protective of most warmwater fish (McCoy, 1972); and (3) nitrite nitrogen at or below 0.06 mg/L should be protective of salmonid fishes (Russo et al. 1974; Russo and Thurston, 1975). These levels either are not known to occur or would be unlikely to occur in natural surface waters.

Recognizing that concentrations of nitrate or nitrite that would exhibit toxic effects on warm- or coldwater fish could rarely occur in nature, restrictive criteria are not recommended.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

NITROBENZENE

CRITERIA:

Aquatic Life

The available data for nitrobenzene indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 27,000 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No definitive data are available concerning the chronic toxicity of nitrobenzene to sensitive freshwater aquatic life.

The available data for nitrobenzene indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 6,680 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No definitive data are available concerning the chronic toxicity of nitrobenzene to sensitive saltwater aquatic life.

Human Health

For comparison purposes, two approaches were used to derive criterion levels for nitrobenzene. Based on available toxicity data, to protect public health the derived level is 19.8 mg/L. Using available organoleptic data, to control undesirable taste and odor qualities of ambient water the estimated level is 30 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have no demonstrated relationship to potential adverse human health effects.

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SEE APPENDIX B FOR METHODOLOGY

NOTE: The U.S. EPA is currently developing Acceptable Daily Intake (ADI) or Verified Reference Dose (RfD) values for Agency-wide use for this chemical. The new value should

be substituted when it becomes available. The January, 1986, draft Verified Reference Dose document cites an RfD of .0005 mg/kg/day for nitrobenzene.

NITROPHENOLS

CRITERIA:

Aquatic Life

The available data for nitrophenols indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 230 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of nitrophenols to sensitive freshwater aquatic life but toxicity to one species of algae occurs at concentrations as low as 150 ug/L.

The available data for nitrophenols indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 4,850 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of nitrophenols to sensitive saltwater aquatic life.

Human Health

Because of insufficient available data for mono- and trinitrophenols, satisfactory criteria cannot be derived at this time, using the present guidelines.

For the protection of human health from the toxic properties of dinitrophenols and 2,4-dinitro-o-cresol ingested through water and contaminated aquatic organisms, the ambient water criteria are determined to be 70 ug/L and 13.4 ug/L, respectively.

For the protection of human health from the toxic properties of dinitrophenols and 2,4-dinitro-o-cresol ingested through contaminated aquatic organisms alone, the ambient water criteria

are determined to be 14.3 mg/L and 765 ug/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

NITROSAMINES

CRITERIA:

Aquatic Life

The available data for nitrosamines indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 5,850 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of nitrosamines to sensitive freshwater aquatic life.

The available data for nitrosamines indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 3,300,000 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of nitrosamines to sensitive saltwater aquatic life.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to N-nitrosodiethylamine and all other nitrosamines except those listed below, through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the non threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 8.0 ng/L, 0.8 ng/L, and 0.08 ng/L, respectively. If these estimates are made for

consumption of aquatic organisms only, excluding consumption of water, the levels are **12,400** ng/L, **1,240** ng/L, and **124** ng/L, respectively.

For the maximum protection of human health from the potential carcinogenic effects of exposure to N-nitrosodimethylamine through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10-6, and 10^{-7} The corresponding recommended criteria are **14** ng/L, **14** ng/L, and **0.14** ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 160,000 ng/L, 16,000 ng/L, and **1,600** ng/L, respectively.

For the maximum protection of human health from the potential carcinogenic effects of exposure to N-nitrosodibutylamine through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10-6, and 10^{-7}. The corresponding recommended criteria are **64** ng/L, **6.4** ng/L, and **0.64** ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are ***5,868*** ng/L, ***587***

ng/L, and 58.7 ng/L, respectively.

For the maximum protection of human health from the potential carcinogenic effects of exposure to N-nitrosopyrrolidine through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 160 ng/L, 16 ng/L, and 1.6 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 919,000 ng/L, 91,900 ng/L, and 9,190 ng/L, respectively.

For the maximum protection of human health from the potential carcinogenic effects of exposure to N-nitrosodiphenylamine through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 49,000 ng/L, 4,900 ng/L, and 490 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 161,000 ng/L, 16,100 ng/L, and 1,610 ng/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

OIL AND GREASE

CRITERIA:

For domestic water supply: Virtually free from oil and grease, particularly from the tastes and odors that emanate from petroleum products.

For aquatic life:

(1) 0.01 of the lowest continuous flow 96-hour LC50 to several important freshwater and marine species, each having a demonstrated high susceptibility to oils and petrochemicals.

(2) Levels of oils or petrochemicals in the sediment which cause deleterious effects to the biota should not be allowed.

(3) Surface waters shall be virtually free from floating nonpetroleum oils of vegetable or animal origin, as well as petroleum-derived oils.

INTRODUCTION:

It has been estimated that between 5 and 10 million metric tons of oil enter the marine environment annually (Blumer, 1970). A major difficulty encountered in the setting of criteria for oil and grease is that these are not definitive chemical categories, but include thousands of organic compounds with varying physical, chemical, and toxicological properties. They may be volatile or nonvolatile, soluble or insoluble, persistent or easily degraded.

RATIONALE:

Field and laboratory evidence have demonstrated both acute lethal toxicity and long-term sublethal toxicity of oils to aquatic organisms. Events such as the Tampico Maru wreck of 1957 in Baja, California, (Diaz-Piferrer, 1962), and the No. 2 fuel oil spill in West Falmouth, Massachusetts, in 1969

(Hampson and Sanders, 1969), both of which caused immediate death to a wide variety of organisms, are illustrative of the lethal toxicity that may be attributed to oil pollution. Similarly, a gasoline spill in South Dakota in November 1969 (Bugbee and Walter, 1973) was reported to have caused immediate death to the majority of freshwater invertebrates and 2,500 fish, 30 percent of which were native species of trout. Because of the wide range of compounds included in the category of oil, it is impossible to establish meaningful 96-hour LC50 values for oil and grease without specifying the product involved. However, as the data in Table 6 show, the most susceptible category of organisms, the marine larvae, appear to be intolerant of petroleum pollutants, particularly the water soluble compounds, at concentrations as low as 0.1 mg/L.

The long-term sublethal effects of oil pollution refer to interferences with cellular and physiological processes such as feeding and reproduction and do not lead to immediate death of the organism. Disruption of such behavior apparently can result from petroleum product concentrations as low as 10 to 100 ug/L (see Table 7).

Table 7 summarizes some of the sublethal toxicities for various petroleum pollutants and aquatic species. In addition to sublethal effects reported at the 10 to 100 ug/L level, it has been shown that petroleum products can harm aquatic life at concentrations as low as 1 ug/L (Jacobson and Boylan, 1973).

Bioaccumulation of petroleum products presents two especially important public health problems: (1) the tainting of edible,

aquatic species, and (2) the possibility of edible marine organisms incorporating the high boiling, carcinogenic polycyclic aromatics in their tissues. Nelson-Smith (1971) reported that 0.01 mg/L of crude oil caused tainting in oysters. Moore et al. (1973) reported that concentrations as low as 1 to 10 ug/L could lead to tainting within very short periods of time. It has been shown that chemicals responsible for cancer in animals and man (such as 3,4-benzopyrene) occur in crude oil (Blumer, 1970). It also has been shown that marine organisms are capable of incorporating potentially carcinogenic compounds into their body fat where the compounds remain unchanged (Blumer, 1970).

Oil pollutants may also be incorporated into sediments. There is evidence that once this occurs in the sediments below the aerobic surface layer, petroleum oil can remain unchanged and toxic for long periods, since its rate of bacterial degradation is slow. For example, Blumer (1970) reported that No. 2 fuel oil incorporated into the sediments after the West Falmouth spill persisted for over a year, and even began spreading in the form of oil-laden sediments to more distant areas that had remained unpolluted immediately after the spill. The persistence of unweathered oil within the sediment could have a long-term effect on the structure of the benthic community or cause the demise of specific sensitive important species. Moore et al. (1973) reported concentrations of 5 mg/L for the carcinogen 3, 4-benzopyrene in marine sediments.

Mironov (1967) reported that 0.01 mg/L oil produced deformed and inactive flatfish larvae. Mironov (1970) also reported inhibition or delay of cellular division in algae by oil

concentrations of 10^{-4} to 10^{-1} mg/L. Jacobson and Boylan (1973) reported a reduction in the chemotactic perception of food by the snail, Nassarius obsoletus, at kerosene concentrations of 0.001 to 0.004 mg/L. Bellen et al. (1972) reported decreased survival and fecundity in worms at concentrations of 0.01 to 10 mg/L of detergent.

Because of the great variability in the toxic properties of oil, it is difficult to establish a numerical criterion which would be applicable to all types of oil. Thus, an application factor of 0.01 of the 96-hour LC50 as determined by using continuous flow with a sensitive resident species should be employed for individual petrochemical components.

There is a paucity of toxicological data on the ingestion of the components of refinery wastewaters by humans or by test animals. It is apparent that any tolerable health concentrations for petroleum-derived substances far exceed the limits of taste and odor. Since petroleum derivatives become organoleptically objectionable at concentrations far below the human chronic toxicity, it appears that hazards to humans will not arise from drinking oil-polluted waters (Johns Hopkins Univ., 1956; Mckee and Wolf, 1963). Oils of animal or vegetable origin generally are nontoxic to humans and aquatic life.

In view of the problem of petroleum oil incorporation in sediments, its persistence and chronic toxic potential, and the present lack of sufficient toxicity data to support specific criteria, concentrations of oils in sediments should not approach levels that cause deleterious effects to important species or the

bottom community as a whole.

Petroleum and nonpetroleum oils share some similar physical and chemical properties. Because they share common properties, they may cause similar harmful effects in the aquatic environment by forming a sheen, film, or discoloration on the surface of the water. Like petroleum oils, nonpetroleum oils may occur at four levels of the aquatic environment: (a) floating on the surface, (b) emulsified in the water column, (c) solubilized, and (d) settled on the bottom as a sludge. Analogous to the grease balls from vegetable oil and animal fats are the tar balls of petroleum origin which have been found in the marine environment or washed ashore on beaches.

Oils of any kind can cause (a) drowning of waterfowl because of loss of buoyancy, exposure because of loss of insulating capacity of feathers, and starvation and vulnerability to predators because of lack of mobility; (b) lethal effects on fish by coating epithelial surfaces of gills, thus preventing respiration; (c) potential fishkills resulting from biochemical oxygen demand: (d) asphyxiation of benthic life forms when floating masses become engaged with surface debris and settle on the bottom: and (e) adverse aesthetic effects of fouled shorelines and beaches. These and other effects have been documented in the U.S. Department of Health, Education and Welfare report on Oil Spills Affecting the Minnesota and Mississippi Rivers and the 1975 Proceedings of the Joint Conference on Prevention and Control of Oil Spills.

Oils of animal or vegetable origin generally are chemically nontoxic to humans or aquatic life; however, floating sheens of

such oils result in deleterious environmental effects described in this criterion. Thus, it is recommended that surface waters shall be virtually free from floating nonpetroleum oils of vegetable or animal origin. This same recommendation applies to floating oils of petroleum origin since they too may produce similar effects.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

DISSOLVED OXYGEN

NATIONAL CRITERIA:

The national criteria for ambient dissolved oxygen concentrations for the protection of freshwater aquatic life are presented in Table 1. The criteria are derived from the production impairment estimates which are based primarily upon growth data and information on temperature, disease, and pollutant stresses. The average dissolved oxygen concentrations selected are values 0.5 mg/L above the slight production impairment values and represent values between no production impairment and slight production impairment. Each criterion may thus be viewed as an estimate of the threshold concentration below which detrimental effects are expected.

Criteria for coldwater fish are intended to apply to waters containing a population of one or more species in the family Salmonidae {Bailey et al., 1970) or to waters containing other coldwater or coolwater fish deemed by the user to be closer to salmonids in sensitivity than to most warmwater species. Although the acute lethal limit €or salmonids is at or below 3 mg/L, the coldwater minimum has been established at 4 mg/L because a significant proportion of the insect species common to salmonid habitats are less tolerant of acute exposures to low dissolved oxygen than are salmonids. Some coolwater species may require more protection than that afforded by the other life stage criteria for warmwater fish and it may be desirable to protect sensitive coolwater species with the coldwater criteria. Many states have more stringent dissolved oxygen standards for cooler waters, waters that contain either

salmonids, nonsalmonid coolwater fish, or the sensitive centrachid, the smallmouth bas5 The warmwater criteria are necessary to protect early life stages of warmwater fish as sensitive as as channel catfish and to protect other life stages of fish as sensitive as largemouth bass. Criteria for early life stages are intended to apply only where and when these stages occur. These criteria represent dissolved oxygen concentrations which EPA believes provide a reasonable and adequate degree of protection for freshwater aquatic life.

The criteria do not represent assured no-effect levels. However, because the criteria represent worst case conditions (i.e. for wasteload allocation and waste treatment plant design), conditions will be better than the criteria nearly all of the time at most sites. In situations where criteria conditions are just maintained for considerable periods the proposed criteria represent some risk of production impairment. This impairment would depend on innumerable other factors. If slight production impairment or a small but undefinable risk of moderate impairment is unacceptable, than one should use the "no production impairment" values given in the document as means and the "slight production impairment" values as minima. The table which presents these concentrations is reproduced here as table 2.

The criteria do represent dissolved oxygen concentrations believed to protect the more sensitive populations of organisms against potentially damaging production impairment. The dissolved oxygen concentrations in the criteria are intended to be protective at typically high seasonal environmental temperatures for the appropriate taxonomic and life stage classi-

Table 1. Water quality criteria for ambient dissolved oxygen concentration.

	Coldwater Criteria		Warmwater Criteria	
	Early Life Stages[1,2]	Other Life Stages	Early Life Stages[2]	Other Life Stages
30 Day Mean	NA[3]	6.5	NA	5.5
7 Day Mean	9.5 (6.5)	NA	6.0	NA
7 Day Mean Minimum	NA	5.0	NA	4.0
1 Day Minimum[4,5]	8.0 (5.0)	4.0	5.0	3.0

These are water column concentrations recommended to achieve the required intergravel dissolved oxygen concentrations shown in parentheses. The 3 mg/L differential is discussed in the criteria document. For species that have early life stages exposed directly to the water column, the figures in parentheses apply.

Includes all embryonic and larval stages and all juvenile forms to 30-days following hatching.

3 NA (not applicable).

For highly manipulatable discharges, further restrictions apply (see page 37)

All minima should be considered as instantaneous concentrations to be achieved at all times.

fications, temperatures which are often higher than those used in the research from which the criteria were generated, especially for other than early life stages.

Where natural conditions alone create dissolved oxygen concentrations less than 110 percent of the applicable criteria means or minima or both, the minimum acceptable concentration is

90 percent of the natural concentration. These values are similar to those presented graphically by Doudoroff and Shumway (1970) and those calculated from Water Quality Criteria 1972 (NAS/NAE, 1973). Absolutely no anthropogenic dissolved oxygen depression in the potentially lethal area below the 1-day minima should be allowed unless special care is taken to ascertain the tolerance of resident species to low dissolved oxygen.

If daily cycles of dissolved oxygen are essentially sinusoidal, a reasonable daily average is calculated from the day's high and low dissolved oxygen values. A time-weighted average may be required if the dissolved oxygen cycles are decidedly non-sinusoidal. Determining the magnitude of daily dissolved oxygen cycles requires at least two appropriately timed measurements daily, and characterizing the shape of the cycle requires several more appropriately spaced measurements.

Once a series of daily mean dissolved oxygen concentrations are calculated, an average of these daily means can be calculated (Table 3). For embryonic, larval, and early life stages, the averaging period should not exceed 7 days. This short time is needed to adequately protect these often short duration, most sensitive life stages. Other life stages can probably be adequately protected by 30-day averages. Regardless of the averaging period, the average should be considered a moving average rather than a calendar-week or calendar-month average.

Table 2. Dissolved Oxygen Concentrations (mg/L) Versus Quantitative Level of Effect.

1. Salmonid Waters

a. Embryo and Larval Stages

No Production Impairment	=	11* (8)
Slight Production Impairment	=	9* (6)
Moderate Production Impairment	=	8* (5)
Severe Production Impairment	=	7* (4)
Limit to Avoid Acute Mortality	=	6* (3)

(* Note: These are water column concentrations recommended to achieve the required intergravel dissolved oxygen concentrations shown in parentheses. The 3 mg/L difference is discussed in the criteria document.)

b. Other Life Stages

No Production Impairment	=	8
light Production Impairment	=	6
Moderate Production Impairment	=	5
Severe Production Impairment	=	4
Limit to Avoid Acute Mortality	=	3

2. Nonsalmonid Waters

a. Early Life Stages

No Production Impairment	=	6.5
Slight Production Impairment	=	5.5
Moderate Production Impairment	=	5
Severe Production Impairment	=	4.5
Limit to Avoid Acute Mortality	=	4

b. Other Life Stages

No Production Impairment	=	6
Slight Production Impairment	=	5
Moderate Production Impairment	=	4
Severe Production Impairment	=	3.5
Limit to Avoid Acute Mortality	=	3

3. Invertebrates

No Production Impairment	=	8
Some Production Impairment	=	5
Acute Mortality Limit	=	4

Table 3. Sample calculations for determining daily means and 7-day mean dissolved oxygen concentrations (30-day averages are calculated in a similar fashion using 30 days data).

Dissolved Oxygen (mg/L)

Day	Daily Max.	Daily Min.	Daily Mean
1	9.0	7.0	8.0
2	10.0	7.0	8.5
3	11.0	8.0	9.5[b]
4	12.0[a]	8.0	9.5
5	10.0	8.0	9.0
6	11.0	9 0	10.0
7	12.0[a]	10.0	10.5[c]
		57.0	65.0
1-day Minimum		7.0	
7-day Mean Minimum		8.1	
7-day Mean			9.3

a Above air saturation concentration (assumed to be 11.0 mg/L for this example).

b (11.0 + 8.0)2.

c (11 0 +10.0)2.

The criteria have been established on the basis that the maximum dissolved oxygen value actually used in calculating any daily mean should not exceed the air saturation value. This consideration is based primarily on analysis of studies of cycling dissolved oxygen and the growth of largemouth bass (Stewart et al., 1967), which indicated that high dissolved oxygen levels (> 6 mg/L) had no beneficial effect on growth.

During periodic cycles of dissolved oxygen concentrations, minima lower than acceptable constant exposure levels are tolerable so long as:

1. the average concentration attained meets or exceeds the criterion;

2. the average dissolved oxygen concentration is calculated as recommended in Table 3; and

3. the minima are not unduly stressful and clearly are not lethal.

A daily minimum has been included to make certain that no acute mortality of sensitive species occurs as a result of lack of oxygen. Because repeated exposure to dissolved oxygen concentrations at or near the acute lethal threshold will be stressful and because stress can indirectly produce mortality or other adverse effects (e.g., through disease), the criteria are designed to prevent significant episodes of continuous or regularly recurring exposures to dissolved oxygen concentrations at or near the lethal threshold. This protection has been achieved by setting the daily minimum for early life stages at the subacute lethality threshold, by the use of a 7-day averaging period for early life stages, by stipulating a 7-day mean minimum value for other life stages, and by recommending additional limits for manipulatable discharges.

The previous EPA criterion for dissolved oxygen published in Quality Criteria for Water (USEPA, 1976) was a minimum of 5 mg/L (usually applied as a 7Q10) which is similar to the current criterion minimum except for other life stages of warmwater fish which now allows a 7-day mean minimum of 4 mg/L. The new criteria are similar to those contained in the 1968 "Green Book" of the Federal Water Pollution Control Federation (FWPCA, 1968).

A. The Criteria and Monitoring and Design Conditions

The acceptable mean concentrations should be attained most of the time, but some deviation below these values would probably not cause significant harm. Deviations below the mean will probably be serially correlated and hence apt to occur on consecutive days. The significance of deviations below the mean will depend on whether they occur continuously or in daily cycles, the former being more adverse than the latter. Current knowledge regarding such deviations is limited primarily to laboratory growth experiments and by extrapolation to other activity-related phenomena.

Under conditions where large daily cycles of dissolved oxygen occur, it is possible to meet the criteria mean values and consistently violate the mean minimum criteria. Under these conditions the mean minimum criteria will clearly be the limiting regulation unless alternatives such as nutrient control can dampen the daily cycles.

The significance of conditions which fail to meet the recommended dissolved oxygen criteria depend largely upon five factors: (1) the duration of the event; (2) the magnitude of the dissolved oxygen depression; (3) the frequency of recurrence; (4) the proportional area of the site failing to meet the criteria, and (5) the biological significance of the site where the event occurs. Evaluation of an event's significance must be largely case- and site-specific. Common sense would dictate that the magnitude of the depression would be the single most important factor in general, especially if the acute value is violated. A

logical extension of these considerations is that the event must be considered in the context of the level of resolution of the monitoring or modeling effort. Evaluating the extent, duration, and magnitude of an event must be a function of the spatial and temporal frequency of the data. Thus, a single deviation below the criterion takes on considerably less significance where continuous monitoring occurs than where sampling is comprised of once-a-week grab samples. This is so because based on continuous monitoring the event is provably small, but with the much less frequent sampling the event is not provably small and can be considerably worse than indicated by the sample. The frequency of recurrence is of considerable interest to those modeling dissolved oxygen concentrations because the return period, or period between recurrences, is a primary modeling consideration contingent upon probabilities of receiving water volumes, waste loads, temperatures, etc. It should be apparent that return period cannot be isolated from the other four factors discussed above. Ultimately, the question of return period may be decided on a site-specific basis taking into account the other factors (duration, magnitude, areal extent, and biological significance) mentioned above. Future studies of temporal patterns of dissolved oxygen concentrations, both within and between years, must be conducted to provide a better basis for selection of the appropriate return period.

In conducting wasteload allocation and treatment plant design computations, the choice of temperature in the models will be important. Probably the best option would be to use temperatures consistent with those expected in the receiving water over the

critical dissolved oxygen period for the biota.

B. The Criteria and Manipulatable Discharges

If daily minimum DOs are perfectly serially correlated, i.e, if the annual lowest daily minimum dissolved oxygen concentration is adjacent in time to the next lower daily minimum dissolved oxygen concentration and one of these two minima is adjacent to the third lowest daily minimum dissolved oxygen concentration, etc., then in order to meet the 7-day mean minimum criterion it is unlikely that there will be more than three or four consecutive daily minimum values below the acceptable 7-day mean minimum. Unless the dissolved oxygen pattern is extremely erratic, it is also unlikely that the lowest dissolved oxygen concentration will be appreciably below the acceptable 7-day mean minimum or that daily minimum values below the 7-day mean minimum will occur in more than one or two weeks each year. For some discharges, the distribution of dissolved oxygen concentrations can be manipulated to varying degrees. Applying the daily minimum to manipulatable discharges would allow repeated weekly cycles of minimum acutely acceptable dissolved oxygen values, a condition of unacceptable stress and possible adverse biological effect. For this reason, the application of the one day minimum criterion to manipulatable discharges must limit either the frequency of occurrence of values below the acceptable 7-day mean minimum or must impose further limits on the extent of excursions below the 7-day mean minimum. For such controlled discharges, it is recommended that the occurrence of daily minima below the acceptable 7-day mean

minimum be limited to 3 weeks per year or that the acceptable one-day minimum be increased to 4.5 mg/L for coldwater fish and 3.5 mg/L for warmwater fish, Such decisions could be site-specific based upon the extent of control and serial correlation.

PARATHION

CRITERION:

0.04 ug/L for freshwater and marine aquatic life.

RATIONALE:

Acute static LC50 values of the organophosphorus pesticide, parathion, for freshwater fish have ranged generally from about 50 ug/L for more sensiitive species such as bluegills, *Lepomis macrochirus*, to about 2.5 mg/L for the more resistant species such as minnows (U.S. Environ. Prot. Agency, 1975). In flowing water exposures, Spacie (1975) obtained 96-hour LC50 values of 0.5 mg/L, 1.6 mg/L, and 1.76 mg/L for bluegills, *Lepomis macrochirus*, fathead minnows, *Pimephales promelas*, and brook trout, *Salvelinus fontinalis*, respectively. Korn and Earnest (1974) found a 96-hour LC50 of 18 ug/L for juvenile freshwater and estuarine striped bass, *Morone saxatilis*, in a flowing water system,

Few chronic exposure data are available for aquatic organisms. Brown bullheads, *fctalurus nebulosus*, exposed to 30 ug/L parathion for 30 days exhibited tremors; at 60 ug/L they convulsed and were found to have developed a deformed vertebral column (Mount and Boyle, 1969). In a 23-month exposure of bluegills, Spacie (1975) observed deformities (scoliosis and a characteristic protrusion in the throat region) at 0.34 ug/L, but not at 0.16 ug/L. Tremors, convulsions, hypersensitivity, and hemorrhages also were evident at higher concentrations.

Reproductive impairment and deformities were observed in fathead minnows exposed to 4.0 ug/L for 8 1/2 months.

Development of brook trout, S. fontinalis embryos exposed to 32 ug/L was abnormal and mortalities associated with premature hatching were observed. Embryos at 10 ug/L appeared normal. No adverse effects on juveniles and adults was evident during 9 months' exposure to 7 ug/L.

Inhibition of cholinesterase enzymes is the well-established mode of physiological action of parathion and other organic phosphorus pesticides (Weiss, 1958). The degree of inhibition of brain acetylcholinesterase (AChE) activity has been the most frequently used measure of effect of these pesticides. Various studies (Weiss, 1958, 1959, 1961; Murphy et al., 1968; Gibson et al. 1969) have shown the degree of inhibition to be dependent upon toxicant concentration, length of exposure, and species sensitivity. The results of these studies have also indicated that death results from AChE inhibition ranging from 25 to 90 percent of normal. Weiss (1959) also showed that susceptibility depended upon the extent of recovery of AChE activity following prior exposure and that the recovery period for fish exposed to parathion was relatively long. In bluegills, AChE activity was only 50 percent recovered 30 days after exposure to 1 mg/L for 6 to 7 hours (Weiss, 1961).

Some of the other physiological effects observed to result from exposure of fish to parathion have been inhibition of spermatogenesis in guppies, Poecilia reticulata, at 10 ug/L (Billard and deKinkelin, 1970), alternation of oxygen consumption rate in bluegills, Lepomis macrochirus, at 100 ug/L (Dowden, 1966), and liver enlargement associated with increased pesticide-hydrolizing capability in mosquitofish, Gambusia

affinis (Ludke, 1970).

Parathion has been found acutely toxic to aquatic invertebrates at under 1 ug/L e.g., a 50-hour LC50 of 0.8 ug/L for Daphnia magna; 48-hour LC50 of 0.6 ug/L for Daphnia pulex; 48-hour LC50 of 0.37 for Simocephalus serrulatus (a daphnid) (Sanders and Cope, 1966); a 5-day LC50 of 0.93 ug/L for the larval stonefly, Acroneuria pacifica (Jensen and Gaufin, 1964); and a 96-hour LC50 of 0.43 ug/L for the larval caddisfly Hydropsyche californica (Gaufin et al. 1965). Mulla and Khasawinah (1969) obtained a 24-hour LC50 of 0.5 ug/L for 4th instar larvae of the midge Tanypus grodhausi. Spacie (1975) obtained 96-hour LC50's in flow-through bioassays of 0.62 ug/L for Daphnia magna, 0.40 ug/L for the scud, Gammarus fasciatus, and 31.0 ug/L for 4th instar of Chironomous tentans, a midge. Other invertebrates have been found acutely sensitive to parathion in concentrations of from 1 to 30 ug/L in water (U.S. Environ. Prot. Agency, 1975).

Few longer exposures have been conducted. Jensen and Gaufin (1964) obtained 30-day LC50's for Pteronarcys californica and Acroneuria pacifica of 2.2 and 0.44 ug/L, respectively. Spacie (1975) found the 3-week LC50 for Daphnia magna to be 0.14 ug/L. Statistically significant reproductive impairment occurred at concentrations above 0.08 ug/L. A 43-day LC50 of 0.07 ug/L was reported for Gammarus fasciatus and a concentration of 0.04 ug/L produced significantly greater mortality than among controls.

Limited information is available on persistence of parathion in water. Eichelberger and Lichtenberg (1971) determined the

half-life in river water (pH 7.3 - 8.0) to be 1 week. Using AChE inhibitory capacity as the indicator, Weiss and Gakstatter (1964) found the half-life of parathion or its active breakdown products to be 40, 35, and 20 days in "natural" waters having a pH of 5.1. 7.0, and 8.4, respectively. The possibility of breakdown resulting in compounds more toxic than parathion was suggested by Burke and Ferguson (1969) who determined that the toxicity of this pesticide to mosquitofish, Gambusia affinis, was greater in static than in flowing water test systems. Sanders (1972), in 96-hour bioassays with the scud, Gammarus fasciatus, and glass shrimp, Palaemonetes kadiakensis, also observed greater toxicity under static than in flow-through conditions.

Tissue accumulations of parathion by exposed aquatic organisms are not great and do not appear to be very persistent. Mount and Boyle (1969) observed concentrations in the blood of bullhead, Ictalurus melas, up to about 50 times water concentrations. Spacie (1975) found muscle concentrations in chronically exposed brook trout, S. fontinalis, to be several hundred times water concentrations; bluegills, Lepomis macrochirus, had about 25 times water concentrations in their bodies. Leland (1968) demonstrated a biological half-life of parathion in rainbow trout, Salmo gairdneri, exposed and then placed in fresh water to be only 30 to 40 hours. It is not expected that parathion residues in aquatic organisms exposed to the recommended criterion concentrations will be a hazard to consumer organisms.

Weiss and Gakstatter (1964) have shown that 15-day continuous exposure to parathion (1.0 ug/L) can produce progressively greater (i.e., cumulative) brain AChE inhibition in a fish species. After substantial inhibition by parathion exposure, it takes several weeks for brain AChE of exposed fishes to return to normal even though exposure is discontinued (Weiss, 1959, 1961). Inhibition of brain AChE of fishes by 46 percent or more has been associated with harmful effects in exposures to organophosphate pesticides for one life cycle (Eaton, 1970) and for short periods (Carter, 1971; Coppage and Duke, 1971; Coppage, 1972; Coppage and Matthews, 1974; Post and Leasure, 1974; Coppage et al. 1975). It has been shown that a concentration of 10 ug parathion/L of flowing seawater kills 40 to 60 percent of the marine fishes *Lagodon rhomboides* (pinfish) and *Leostomus xanthurus* (spot) in 24 hours and causes about 87 to 92 percent brain AChE inhibition (Coppage and Matthews, 1974.) Similar inhibition of AChE and mortality were caused in sheepshead minnows, *Cyprinodon variegatus*, in 2, 24, 48, and 72 hours at concentrations of 5,000, 2,000, 100, and 10 ug/L, respectively in static tests (Coppage, 1972). These data indicate that reductions of brain AChE activity of marine fishes by 70 to 80 percent or more in short-term exposures to parathion may be associated with some deaths.

Other estimates of parathion toxicity to marine organisms follow. The 48-hour EC50 for parathion to Penaeus duorarum was found to be 0.2 ug/L (Lowe et al. 1970). Lahav and Sarig (1969) reported the 96-hour LC50 for mullet, Mugil cephalus to be 125 ug/L. The shell growth of the oyster, Crassostrea virginica, was found by Lowe et al. (1970) to be decreased by 22 percent after 96 hours in 1.0 mg/L.

An application factor of 0.1 is applied to the 96-hour LC50 data for invertebrates which range upward from 0.4 ug/L. A criteria of 0.04 ug/L is recommended for marine and freshwater aquatic life.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

PENTACHLOROPHENOL

CRITERIA:

Aquatic Life

The available data for pentachlorophenol indicate that acute and chronic toxicity to freshwater aquatic life occurs at concentrations as low as 55 and 3.2 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for pentachlorophenol indicate that acute and chronic toxicity to saltwater aquatic life occur at concentrations as low as 53 and 34 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

Human Health

For comparison purposes, two approaches were used to derive criterion levels for pentachlorophenol. Based on available toxicity data, to protect public health the derived level is 1.01 mg/L. Using available organoleptic data, to control undesirable taste and odor qualities of ambient water the estimated level is 30 ug/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have no demonstrated relationship to potential adverse human health effects.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

pH

CRITERIA:

Range	
5 - 9	Domestic water supplies (welfare)
6.5 - 9.0	Freshwater aquatic life
6.5 - 8.5	Marine aquatic life (but not more than 0.2 units outside of normallyoccurring range.)

INTRODUCTION:

"pH" is a measure of the hydrogen ion activity in a water sample. It is mathematically related to hydrogen ion activity according to the expression: $pH = -\log 10 (H^+)$, where (H^+) is the hydrogen ion activity.

The pH of natural waters is a measure of acid-base equilibrium achieved by the various dissolved compounds, salts, and gases. The principal system regulating pH in natural waters is the carbonate system which is composed of carbon dioxide (CO_2), carbonic acid, (H_2CO_3), bicarbonate ion (HCO_3) and carbonate ions (CO_3). The interactions and kinetics of this system have been described by Stumm and Morgan (1970).

pH is an important factor in the chemical and biological systems of natural waters. The degree of dissociation of weak acids or bases is affected by changes in pH. This effect is important because the toxicity of many compounds is affected by the degree of dissociation. One such example is hydrogen cyanide (HCN). Cyanide toxicity to fish increases as the pH is lowered because the chemical equlibrium is shifted toward an increased concentration of HCN. Similar results have been shown for hydrogen sulfide (H_2S) (Jones, 1964).

The solubility of metal compounds contained in bottom sediments or as suspended material, also is affected by pH. For example, laboratory equilibrium studies under anaerobic conditions indicated that pH was an important parameter involved in releasing manganese from bottom sediments (Delfino and Lee, 1971).

The pH of a water does not indicate ability to neutralize additions of acids or bases without appreciable change. This characteristic, termed "buffering capacity," is controlled by the amounts of alkalinity and acidity present.

RATIONALE:

Knowledge of pH in the raw water used €or public water supplies is important because without adjustment to a suitable level, such waters may be corrosive and adversely affect treatment processes including coagulation and chlorination.

Coagulation for removal of colloidal color by use of aluminum or iron salts generally has an optimum pH range of 5.0 to 6.5 (Sawyer, 1960). Such optima are predicated upon the availability of sufficient alkalinity to complete the chemical reactions.

The effect of pH on chlorine in water principally is on the equilibrium between hypochlorous acid (HOCl) and the hypochlorite ion (OCl^-) according to the reaction:

$$HOCl = H^+ + OCl^-$$

Butterfield (1984) has shown that chlorine disinfection is more effective at values less than pH 7. Another study (Reid and Carlson, 1974) has indicated, however, that in natural waters no significant difference in the kill rate for Escherichia coli was

observed between pH 6 and pH 8.

corrosion of plant equipment and piping in the distribution system can lead to expensive replacement as well as the introduction of metal ions such as copper, lead, zinc, and cadmium. Langelier (1936) developed a method to calculate and control water corrosive activity that employs calcium carbonate saturation theory and predicts whether the water would tend to dissolve or deposit calcium carbonate. By maintaining the pH at the proper level, the distribution system can be provided with a protective calcium carbonate lining which prevents metal pipe corrosion. Generally, this level is above pH 7 and frequently approaches pH 8.3, the point of maximum bicarbonate/carbonate buffering.

Since pH is relatively easily adjusted prior to and during water treatment, a rather wide range is acceptable for waters serving as a source of public water supply. A range of pH from 5.0 to 9.0 would provide a water treatable by typical (coagulation, sedimentation, filtration, and chlorination) treatment plant processes. As the range is extended, the cost of neutralizing chemicals increases.

A review of the effects of pH on fresh water fish has been published by the European Inland Fisheries Advisory Commission (1969). The commission concluded:

> There is no definite pH range within which a fishery is unharmed and outside which it is damaged, but rather, there is a gradual deterioration as the pH values are further removed from the normal range. The pH range which is not directly lethal to fish is 5 - 9; however, the toxicity of several common pollutants is markedly affected by pH changes within this range, and increasing acidity or alkalinity may make these poisons more toxic. Also, an acid discharge may liberate sufficient CO_2 from bicarbonate in the water either

to be directly toxic, or to cause the pH range 5 - 6 to become lethal.

Mount (1973) performed bioassays on the fathead minnow, Pimephales promelas, for a 13-month, one generation time period to determine chronic pH effects. Tests were run at pH levels of 4.5, 5.2.

pH Range	Effect on Fish*
5.0 - 6.0	Unlikely to be harmful to any species unless either the concentration of free CO_2 is greater than 20 ppm, or the water contains iron salts which are precipitated as ferric hydroxide, the toxicity of which is not known.
6.0 - 6.5	Unlikely to be harmful to fish unless free carbon dioxide is present in excess of 100 ppm.
6.5 - 9.0	Harmless to fish, although the toxicity of other poisons may be affected by changes within this range.

EIFAC, 1969

5.9, 6.6, and a control of 7.5. At the two lowest pH values (4.5 and 5.2) behavior was abnormal and the fish were deformed. At pH values less than 6.6, egg production and hatchability were reduced when compared with the control. It was concluded that a pH of 6.6 was marginal for vital life functions.

Bell (1971) performed bioassays with nymphs of caddisflies (two species) stoneflies (four species), dragonflies (two species), and mayflies (one species). All are important fish food organisms. The 30-day TL50 values ranged from 2.45 to 5.38 with the caddisflies being the most tolerant and the mayflies being the least tolerant. The pH values at which 50 percent of the organisms emerged ranged from 4.0 to 6.6 with increasing percentage emergence occurring with the increasing pH values.

Based on present evidence, a pH range of 6.5 to 9.0 appears to provide adequate protection for the life of freshwater fish and bottom dwelling invertebrates fish food organisms. Outside of this range, fish suffer adverse physiological effects increasing in severity as the degree of deviation increases until lethal levels are reached.

Conversely, rapid increases in pH can cause increased NH_3 concentrations that are also toxic. Ammonia has been shown to be 10 times as toxic at pH 8.0 as at pH 7.0 (EIFAC, 1969).

The chemistry of marine waters differs from that of fresh water because of the large concentration of salts present. In addition to alkalinity based on the carbonate system, there is also alkalinity from other weak acid salts such as borate. Because of the buffering system present in seawater, the

naturally occurring variability of pH is less than in fresh water. Some marine communities are more sensitive to pH change than others (NAS, 1974). Normal pH values in seawater are 8.0 to 8.2 at the surface, decreasing to 7.7 to 7.8 with increasing depth (Capurro, 1970). The NAS Committee's review (NAS, 1974) indicated that plankton and benthic invertebrates are probably more sensitive than fish to changes in pH and that mature forms and larvae of oysters are adversely affected at the extremes of the pH range of 6.5 to 9.0. However, in the shallow, biologically active waters in tropical or subtropical areas, large diurnal pH changes occur naturally because of photosynthesis. pH values may range from 9.5 in the daytime to 7.3 in the early morning before dawn. Apparently, these communities are adapted to such variations or intolerant species are able to avoid extremes by moving out of the area.

For open ocean waters where the depth is substantially greater than the euphotic zone, the pH should not be changed more than 0.2 units outside of the naturally occurring variation or in any case outside the range of 6.5 to 8.5. For shallow, highly productive coastal and estuarine areas where naturally occurring variations approach the lethal limits for some species, changes in pH should be avoided, but in any case not exceed the limits established for fresh water, i.e., pH of 6.5 to 9.0. As with freshwater criteria, rapid pH fluctuations that are caused by waste discharges should be avoided. Additional support for these limits is provided by Zirino and Yamamoto (1972). These investigators developed a model which illustrates the effects of variable pH on copper, zinc, cadmium, and lead; small changes in

pH cause large shifts in these metallic complexes. Such changes may affect toxicity of these metals.

For the industrial classifications considered, the NAS report (NAS, 1974) tabulated the range of pH values used by industry for various process and cooling purposes. In general, process waters used varied from pH 3.0 to 11.7, while cooling waters used varied from 5.0 to 8.9. Desirable pH values are undoubtedly closer to neutral to avoid corrosion and other deleterious chemical reactions. Waters with pH values outside these ranges are considered unusable for industrial purposes.

The pH of water applied for irrigation purposes is not normally a critical parameter. Compared with the large buffering capacity of the soil matrix, the pH of applied water is rapidly changed to approximately that of the soil. The greatest danger in acid soils is that metallic ions such as iron, manganese, or aluminum may be dissolved in concentrations which are subsequently directly toxic to plants. Under alkaline conditions, the danger to plants is the toxicity of sodium carbonates and bicarbonates either directly or indirectly (NAS, 1974).

To avoid undesirable effects in irrigation waters, the pH should not exceed a range of 4.5 to 9.0.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

PHENOL

CRITERIA:

Aquatic Life

The available data for phenol indicate that acute and chronic toxicity to freshwater aquatic life occurs at concentrations as low as 10,200 and 2,560 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for phenol indicate that toxicity to saltwater aquatic life occurs at concentrations as low as 5,800 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of phenol to sensitive saltwater aquatic life.

Human Health

For comparison purposes, two approaches were used to derive criterion levels for phenol. Based on available toxicity data, to protect public health the derived level is 3.5 mg/L.

Using available organoleptic data, to control undesirable taste and odor qualities of ambient water the estimated level is 0.3 mg/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criterion, and have no demonstrated relationship to potential adverse human health effects.

NOTE: The U.S. EPA is currently developing Acceptable Daily Intake (ADI) or Verified Reference Dose (RfD) values for Agency-wide use for this chemical. The new value should be substituted when it becomes available. The January, 1986, draft Verified Reference Dose document cites an RfD of 0.1 mg/kg/day for phenol.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

PHOSPHORUS

CRITERION::

0.10 ug/L yellow (elemental) phosphorus for marine or estuarine water.

INTRODUCTION:

Phosphorus in the elemental form is particularly toxic and is subject to bioaccumulation in much the same way as mercury. Phosphorus as phosphate is one of the major nutrients required for plant nutrition and is essential for life. In excess of a critical concentration, phosphates stimulate plant growths. During the past 30 years, a formidable case has developed for the belief that increasing standing crops of aquatic plants, which often interfere with water uses and are nuisances to man, frequently are caused by increasing supplies of phosphorus. Such phenomena are associated with a condition of accelerated eutrophication or aging of waters. Generally, it is recognized that phosphorus is not the sole cause of eutrophication but there is substantiating evidence that frequently it is the key element of all of the elements required by freshwater plants, and generally, it is present in the least amount relative to need. Therefore, an increase in phosphorus allows use of other already present nutrients for plant growth. Further, of all of the elements required for plant growth in the water environment, phosphorus is the most easily controlled by man.

Large deposits of phosphate rock are found near the western shore of Central Florida, as well as in a number of other States. Deposits in Florida are found in the form of pebbles which vary

in size from fine sand to about the size of a human foot. These pebbles are embedded in a matrix of clay and sand. The phosphate rock beds lie within a few feet of the surface and mining is accomplished by using hydraulic water jets and a washing operation that separates the phosphates from waste materials. The process is similar to that of strip-mining. Florida, Idaho, Montana, North Carolina, South Carolina, Tennessee, Utah, Virginia, and Wyoming share phosphate mining activities.

Phosphates enter waterways from several different sources. The human body excretes about one pound per year of phosphorus expressed as "P". The use of phosphate detergents and other domestic phosphates increases the per capita contribution to about 3.5 pounds per year of phosphorus as P. Some industries, such as potato processing, have wastewaters high in phosphates. Crop, forest, idle, and urban land contribute varying amounts of phosphorus-diffused sources in drainage to watercourses. This drainage may be surface runoff of rainfall, effluent from tile lines, or return flow from irrigation. Cattle feedlots, concentrations of domestic duck or wild duck populations, tree leaves, and fallout from the atmosphere all are contributing sources.

Evidence indicates that: (1) high phosphorus concentrations are associated with accelerated eutrophication of waters, when other growth-promoting factors are present: (2) aquatic plant problems develop in reservoirs and other standing waters at phosphorus values lower than those critical in flowing streams: (3) reservoirs and lakes collect phosphates from influent streams

and store a portion of them within consolidated sediments, thus serving as a phosphate sink; and (4) phosphorus concentrations critical to noxious plant growth vary and nuisance growths may result from a particular concentration of phosphate in one geographical area but not in another. The amount or percentage of inflowing nutrients that may be retained by a lake or reservoir is variable and will depend upon: (1) the nutrient loading to the lake or resevoir; (2) the volume of the euphotic zone; (3) the extent of biological activities; (4) the detention time within a lake basin or the time available for biological activities; and (5) the level of discharge from the lake or of the penstock from the reservoir.

Once nutrients are combined within the aquatic ecosystem, their removal is tedious and expensive. Phosphates are used by algae and higher aquatic plants and may be stored in excess of use within the plant cell. With decomposition of the plant cell, some phosphorus may be released immediately through bacterial action €or recycling within the biotic community, while the remainder may be deposited with sediments. Much of the material that combines with the consolidated sediments within the lake bottom is bound permanently and will not be recycled into the system.

RATIONALE :

Elemental Phosphorus

Isom (1960) reported an **LC50** of 0.105 mg/L at **48** hours and 0.025 mg/L at 160 hours for bluegill sunfish, *Lepomis macrochirus*, exposed to yellow phosphorus in distilled water at

26 °C and pH 7. The 125-and 195-hour LC50's of yellow phosphorus to Atlantic cod, Gadus morhua, and Atlantic salmon, Salmo salar, smolts in continuous-exposure experiments were 1.89 and 0.79 ug/L, respectively (Fletcher and Hoyle, 1972). No evidence of an incipient lethal level was observed since the lowest concentration of P_4 tested was 0.79 ug/L. Salmon that were exposed to elemental phosphorus concentrations of 40 ug/L or less developed a distinct external red color and showed signs of extensive hemolysis. The predominant features of P_4 poisoning in salmon were external redness, hemolysis, and reduced hematocrits.

Following the opening of an elemental phosphorus production plant in Long Harbour, Placentia Bay, Newfoundland, divers observed dead fish upon the bottom throughout the Harbour (Peer, 1972). Mortalities were confined to a water depth of less than 18 meters. There was visual evidence of selective mortality among benthos. Live mussels were found within 300 meters of the effluent pipe, while all scallops within this area were dead.

Fish will concentrate elemental phosphorus from water containing as little as 1 ug/L (Idler, 1969). In one set of experiments, a cod swimming in water containing 1 ug/L elemental phosphorus for 18 hours concentrated phosphorus to 50 ug/kg in muscle, 150 ug/kg in fatty tissue, and 25,000 ug/kg in the liver (Idler, 1969; Jangaard, 1970). The experimental findings showed that phosphorus is quite stable in the fish tissues.

The criterion of 0.10 ug/L elemental phosphorus for marine or estuarine waters is .1 of demonstrated lethal levels to important marine organisms and of levels that have been found to result in significant bioaccumulation.

Phosphate Phosphorus

Although a total phosphorus criterion to control nuisance aquatic growths is not presented, it is believed that the following rationale to support such a criterion, which currently is evolving, should be considered.

Total phosphate phosphorus concentrations in excess of 100 ug/L P may interfere with coagulation in water treatment plants. When such concentrations exceed 25 ug/L at the time of the spring turnover on a volume-weighted basis in lakes or reservoirs, they may occasionally stimulate excessive or nuisance growths of algae and other aquatic plants. Algal growths inpart undesirable tastes and odors to water, interfere with water treatment, become aesthetically unpleasant, and alter the chemistry of the water supply. They contribute to the phenomenon of cultural eutrophication.

To prevent the development of biological nuisances and to control accelerated or cultural eutrophication, total phosphates as phosphorus (P) should not exceed 50 ug/L in any stream at the point where it enters any lake or reservoir, nor 25 ug/L within the lake or reservoir. A desired goal for the prevention of plant nuisances in streams or other flowing waters not discharging directly to lakes or impoundments is 100 ug/L total P (Mackenthun, 1973). Most relatively uncontaminated lake districts axe known to have surface waters that contain from 10 to 30 ug/L total phosphorus as P (Hutchinson, 1957).

The majority of the Nation's eutrophication problems are associated with lakes or reservoirs and currently there are more

data to support the establishment of a limiting phosphorus level in those waters than in streams or rivers that do not directly impact such water. There are natural conditions, also, that would dictate the consideration of either a more or less stringent phosphorus level. Eutrophication problems may occur in waters where the phosphorus concentration is less than that indicated above and, obviously, such waters would need more stringent nutrient limits. Likewise, there are those waters within the Nation where phosphorus is not now a limiting nutrient and where the need for phosphorus limits is substantially diminished. Such conditions are described in the last paragraph of this rationale.

There are two basic needs in establishing a phosphorus criterion for flowing waters: one is to control the development of plant nuisances within the flowing water and, in turn, to control and prevent animal pests that may become associated with such plants; the other is to protect the downstream receiving waterway, regardless of its proximity in linear distance. It is evident that a portion of that phosphorus that enters a stream or other flowing waterway eventually will reach a receiving lake or estuary either as a component of the fluid mass, as bed load sediments that are carried downstream, or as floating organic materials that may drift just above the stream's bed or float on its water's surface. Superimposed on the loading from the inflowing waterway, a lake or estuary may receive additional phosphorus as fallout from the air shed or as a direct introduction from shoreline areas.

Another method to control the inflow of nutrients, particularly phosphates, into a lake is that of prescribing an annual loading to the receiving water. Vollenweider (1973) suggests total phosphorus (P) loadings in grams per square meter of surface area per year that will be a critical level for eutrophic conditions within the receiving waterway for a particular water volume where the mean depth of the lake in meters is divided by the hydraulic detention time in years. Vollenweider's data suggest a range of loading values that should result in oligotrophic lake water quality.

Mean Depth/Hydraulic Detention Time	Oligotrophic or Permissible Loading	Eutrophic or Critical Loading
(meters/year)	(grams/meter^2/year)	(grams/meter^2/year)
0.5	0.07	0.14
1.0	0.10	0.20
2.5	0.16	0.32
5.0	0.22	0.45
7.5	0.27	0.55
10.0	0.32	0.63
25.0	0.50	1.00
50.0	0.71	1.41
75.0	0.87	1.73
100.0	1.00	2.00

There may be waterways wherein higher concentrations or loadings of total phosphorus do not produce eutrophy, as well as those waterways wherein lower concentrations or loadings of total

phosphorus may be associated with populations of nuisance organisms. Waters now containing less than the specified amounts of phosphorus should not be degraded by the introduction of additional phosphates.

It should be recognized that a number of specific exceptions can occur to reduce the threat of phosphorus as a contributor to lake eutrophy: 1. Naturally occurring phenomena may limit the development of plant nuisances. 2. Technological or cost-effective limitations may help control introduced pollutants. 3. Waters may be highly laden with natural silts or colors which reduce the penetration of sunlight needed €or plant photosynthesis. 4. Some waters morphometric features of steep banks, great depth, and substantial flows contribute to a history of no plant problems. 5. Waters may be managed primarily for waterfowl or other wildlife. 7. In some waters nutrient other than phosphorus is limiting to plant growth: the level and nature of such limiting nutrient would. not be expected to increase to an extent that would influence eutrophication. 6. In some waters phosphorus control cannot be sufficiently effective under present technology to make phosphorus the limiting nutrient.

No national criterion is presented for phosphate phosphorus for the control of eutrophication.

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SEE APPENDIX C FOR METHODOLOGY

PHTHALATE ESTERS

CRITERIA:

Aquatic Life

The available data for phthalate esters indicate that acute and chronic toxicity to freshwater aquatic life occurs at concentrations as low as 940 and 3 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for phthalate esters indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 2,944 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of phthalate esters to sensitive saltwater aquatic life but toxicity to one species of algae occurs at concentrations as low as 3.4 ug/L.

Human Health

For the protection of human health from the toxic properties of dimethyl phthalate ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 313 mg/L.

For the protection of human health from the toxic properties of dimethyl phthalate ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 2.9 g/l.

For the protection of human health from the toxic properties of diethyl phthalate ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 350 mg/L.

For the protection of human health from the toxic properties of diethyl phthalate ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 1.8 g/l.

For the protection of human health from the toxic properties of dibutyl phthalate ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 34 mg/L.

For the protection of human health from the toxic properties of dibutyl phthalate ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 154 mg/L.

For the protection of human health from the toxic properties of di-2-ethylhexyl phthalate ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 15 mg/L.

For the protection of human health from the toxic properties of di-2-ethylhexyl phthalate ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 50 mg/L.

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SEE APPENDIX B FOR METHODOLOGY

POLYCHLORINATED BIPHENYLS

CRITERIA:

Aquatic Life

For polychlorinated biphenyls the criterion to protect freshwater aquatic life as derived using the Guidelines is 0.014 ug/L as a 24-hour average. The concentration of 0.014 ug/L is probably too high because it is based on bioconcentration factors measured in laboratory studies, but field studies apparently produce factors at least 10 times higher for fishes. The available data indicate that acute toxicity to freshwater aquatic life probably will occur only at concentrations above 2.0 ug/L and that the 24-hour average should provide adequate protection against acute toxicity.

For polychlorinated biphenyls the criterion to protect saltwater aquatic life as derived using the Guidelines is 0.030 ug/L as a 24-hour average. The concentration of 0.030 ug/L is probably too high because it is based on bioconcentration factors measured in laboratory studies, but field studies apparently produce factors at least 10 times higher for fishes. The available data indicate that acute toxicity to saltwater aquatic life probably will only occur at concentrations above 10 ug/L and that the 24-hour average criterion should provide adequate protection against acute toxicity.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to polychlorinated biphenyls through ingestion of contaminated water and contaminated aquatic

organisms, the ambient water concentration should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 0.79 ng/L, 0.079 ng/L, and 0.0079 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 0.79 ng/L, 0.079 ng/L, and 0.0079 ng/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

POLYNUCLEAR AROMATIC HYDROCARBONS

CRITERIA :

Aquatic Life

The limited freshwater data base available for polynuclear aromatic hydrocarbons, mostly from short-term bioconcentration studies with two compounds, does not permit a statement concerning acute or chronic toxicity.

The available data for polynuclear aromatic hydrocarbons indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 300 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of polynuclear aromatic hydrocarbons to sensitive saltwater aquatic life.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to polynuclear aromatic hydrocarbons through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 28.0 ng/L, 2.8 ng/L, and 0.28 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 311.0 ng/L,

31.1 ng/L, and 3.11 ng/L, respectively.

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SEE APPENDIX B FOR METHODOLOGY

SELENIUM

CRITERIA:

Aquatic Life

For total recoverable inorganic selenite the criterion to protect freshwater aquatic life as derived using the Guidelines is 35 ug/L as a 24-hour average, and the concentration should not exceed 260 ug/L at any time.

For total recoverable inorganic selenite the criterion to protect saltwater aquatic life as derived using the Guidelines is 54 ug/L as a 24-hour average, and the concentration should not exceed 410 ug/L at any time.

The available data for inorganic selenate indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 760 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of inorganic selenate to sensitive freshwater aquatic life.

No data are available concerning the toxicity of inorganic selenate to saltwater aquatic life.

Human Health

The ambient water quality criterion for selenium is recommended to be identical to the existing water standard which is 10 ug/L. Analysis of the toxic effects data resulted in a calculated level which is protective of human health against the ingestion of contaminated water and contaminated aquatic organisms. The calculated value is comparable to the present

standard. For this reason a selective criterion based on exposure solely from consumption of 6.5 grams of aquatic organisms was not derived.

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SEE APPENDIX B FOR METHODOLOGY

SILVER

CRITERIA:

Aquatic Life

For freshwater aquatic life the concentration (in ug/L) of total recoverable silver should not exceed the numerical value given by $e^{(1.72[\ln(\text{hardness})]-6.52)}$ at any time. For example, at hardnesses of 50, 100, and 200 mg/L as $CaCO_3$, the concentration of total recoverable silver should not exceed 1.2, 4.1, and 13 ug/L, respectively, at any time. The available data indicate that chronic toxicity to freshwater aquatic life may occur at concentrations as low as 0.12 ug/L.

For saltwater aquatic life the concentration of total recoverable silver should not exceed 2.3 ug/L at any time. No data are available concerning the chronic toxicity of silver to sensitive saltwater aquatic life.

Human Health

The ambient water quality criterion for silver is recommended to be identical to the existing water standard, which is 50 ug/L. Analysis of the toxic effects data resulted in a calculated level which is protective of human health against the ingestion of contaminated water and contaminated aquatic organisms. The calculated value is comparable to the present standard. For this reason a selective criterion based on exposure solely from consumption of 6.5 grams of aquatic organisms was not derived.

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SEE APPENDIX B FOR METHODOLOGY

SOLIDS (DISSOLVED) AND SALINITY

CRITERION:

250 mg/L for chlorides and sulfates
in domestic water supplies (welfare).

INTRODUCTION:

Dissolved solids and total dissolved solids are terms generally associated with freshwater systems and consist of inorganic salts, small amounts of organic matter, and dissolved materials (Sawyer, 1960). The equivalent terminology in Standard Methods is filtrable residue (Standard Methods, 1971). Salinity is an oceanographic term, and although not precisely equivalent to the total dissolved salt content it is related to it (Capurro, 1970). For most purposes, the terms total dissolved salt content and salinity are equivalent. The principal inorganic anions dissolved in water include the carbonates, chlorides, sulfates, and nitrates (principally in ground waters); the principal cations are sodium, potassium, calcium, and magnesium.

RATIONALE:

Excess dissolved solids are objectionable in drinking water because of possible physiological effects, unpalatable mineral tastes, and higher costs because of corrosion or the necessity for additional treatment.

The physiological effects directly related to dissolved solids include laxative effects principally from sodium sulfate and magnesium sulfate and the adverse effect of sodium on certain patients afflicted with cardiac disease and women with toxemia associated with pregnancy. One study was made using data

collected from wells in North Dakota. Results from a questionnaire showed that with wells in which sulfates ranged from 1,000 to 1,500 mg/L, 62 percent of the respondents indicated laxative effects associated with consumption of the water. However, nearly one-quarter of the respondents to the questionnaire reported difficulties when concentrations ranged from 200 to 500 mg/L (Moore, 1952). To protect transients to an area, a sulfate level of 250 mg/L should afford reasonable protection from laxative effects.

As indicated, sodium frequently is the principal component of dissolved solids. Persons on restricted sodium diets may have an intake restricted from 500 to 1,000 mg/day (Nat. Res. Coun., 1954). That portion ingested in water must be compensated by reduced levels in food ingested so that the total does not exceed the allowable intake. Using certain assumptions of water intake (e.g., 2 liters of water consumed per day) and sodium content of food, it has been calculated that for very restricted sodium diets, 20 mg/L in water would be the maximum, while for moderately restricted diets, 270 mg/L would be maximum. Specific sodium levels for entire water supplies have not been recommended but various restricted sodium intakes are recommended because: (1) the general population is not adversely affected by sodium, but various restricted sodium intakes are recommended by physicians for a significant portion of the population, and (2) 270 mg/L of sodium is representative of mineralized waters that may be aesthetically unacceptable, but many domestic water supplies exceed this level. Treatment for removal of sodium in

water supplies is costly (NAS, 1974).

A study based on consumer surveys in 29 California water systems was made to measure the taste threshold of dissolved salts in water (Bruvold et al., 1969). Systems were selected to eliminate possible interferences from other taste-causing substances than dissolved salts. The study revealed that consumers rated waters with 319 to 397 mg/L dissolved solids as "excellent" while those with 1,283 to 1,333 mg/L dissolved solids were "unacceptable" depending on the rating system used. A "good" rating was registered for dissolved solids less than 658 to 755 mg/L. The 1962 PHS Drinking Water Standards recommended a maximum dissolved solids concentration of 500 mg/L unless more suitable supplies were unavailable.

Specific constituents included in the dissolved solids in water may cause mineral tastes at lower concentrations than other constituents. Chloride ions have frequently been cited as having a low taste threshold in water. Data from Ricter and MacLean (1939) on a taste panel of 53 adults indicated that 61 mg/L NaCl was the median level for detecting a difference from distilled water. At a median concentration of 395 mg/L chloride a salty taste was distinguishable, although the range was from 120 to 1,215 mg/L. Lockhart, et al. 1955) evaluated the effect of chlorides on water used for brewing coffee indicated threshold concentrations for chloride ranging from 210 mg/L to 310 mg/L depending on the associated cation. These data indicate that a level of 250 mg/L chlorides is a reasonable maximum level to protect consumers of drinking water.

The causation of corrosion and encrustation of metallic surfaces by water containing dissolved solids is well known. In water distribution systems corrosion is controlled by insulating dissimilar metal connections by nonmetallic materials, using pH control and corrosion inhibitors, or some form of galvanic or impressed electrical current systems (Lehmann, 1964). In household systems water piping, wastewater piping, water heaters, faucets, toilet flushing mechanisms, garbage grinders and both clothes and dishwashing machines incure damage.

By using water with 1,750 mg/L dissolved solids as compared with 250 mg/L, service life was reduced from 70 percent for toilet flushing mechanisms to 30 percent for washing equipment. Such increased corrosion was calculated in 1968 to cost the consumer an additional $0.50 per 1,000 gallons used.

All species of fish and other aquatic life must tolerate a range of dissolved solids concentrations in order to survive under natural conditions. Based on studies in Saskatchewan it has been indicated that several common freshwater species survived 10,000 mg/L dissolved solids, that whitefish and pike-perch survived 15,000 mg/L, but only the stickleback survived 20,000 mg/L dissolved solids. It was concluded that lakes with dissolved solids in excess of 15,000 mg/L were unsuitable for most freshwater fishes (Rawson and Moore, 1944). The 1968 NTAC Report also recommended maintaining osmotic pressure levels of less than that caused by a 15,000 mg/L solution of sodium chloride.

Marine fishes also exhibit variance in ability to tolerate salinity changes. However, fishkills in Laguna Madre off the Texas coast have occurred with salinities in the range of 75 to 100 o/oo. Such concentrated seawater is caused by evaporation and lack of exchange with the Gulf of Mexico (Rounsafell and Everhart, 1953).

Estuarine species of fish are tolerant of salinity changes ranging from fresh to brackish to seawater. Anadromous species likewise are tolerant although evidence indicates that the young cannot tolerate the change until the normal time of migration (Rounsefell and Everhart, 1953). Other aquatic species are more dependent on salinity for protection from predators or require certain minimal salinities for successful hatching of eggs. The oyster drill cannot tolerate salinities less than 12.5 o/oo. Therefore, estuarine segments containing salinities below about 12.5 o/oo produce most of the seed oysters for planting (Rounsefell and Everhart, 1953). Based on similar examples, the 1968 NTAC Report recommended that to protect fish and other marine animals no changes in hydrography or stream flow should be allowed that permanently change isohaline patterns in the estuary by more than 10 percent from natural variation.

Many of the recommended game bird levels for dissolved solids concentrations in drinking water have been extrapolated from data collected on domestic species such as chickens. However, young ducklings were reported poisoned in Suisan Marsh by salt when maximum summer salinities varied from 0.55 to 1.74 o/oo with means as high as 1.26 o/oo (Griffith, 1963).

Indirect effects of excess dissolved solids are primarily the elimination of desirable food plants and other habitat-forming plants. Rapid salinity changes cause plasmolysis of tender leaves and stems because of changes in osmotic pressure. The 1968 NTAC Report recommended the following limits in salinity variation from natural to protect wildlife habitats:

Natural Salinity (o/oo)	Variation Permitted (o/oo)
0 to 3.5	1
3.5 to 13.5	2
13.5 to 35	4

Agricultural uses of water are also limited by excessive dissolved solids concentrations. Studies have indicated that chickens, swine, cattle, and sheep can survive on saline waters up to 15,000 mg/L of salts of sodium and calcium combined with bicarbonates, chlorides, and sulfates but only 10,000 mg/L of corresponding salts of potassium and magnesium. The approximate limit for highly alkaline waters containing sodium and calcium carbonates is 5,000 mg/L (NTAC, 1968).

Irrigation use of water depends not only upon the osmotic effect of dissolved solids, but also on the ratio of the various cations present. In arid and semiarid areas general classification of salinity hazards has been prepared (NTAC, 1968) (see Table 9).

Table 9.-Dissolved Solids Hazard for Irrigation Water (mg/L).

water from which no detrimental effects will usually be noticed----------	500

water which can have detrimental effects on sensitive crops----------	500-1,000
water that may have adverse effects on many crops and requires careful management Practices----------	1,000-2,000
water that can be used for tolerant plants on permeable soils with careful management practices----------	2,000-5,000

The amount of sodium and the percentage of sodium in relation to other cations are often important. In addition to contributing to osmotic pressure, sodium is toxic to certain plants, especially fruits, and frequently causes problems in soil structure, infiltration, and permeability rates (Agriculture Handbook #60, 1954). A high percentage of exchangeable sodium in soils containing clays that swell when wet can cause a soil condition adverse to water movement and plant growth. The exchangeable-sodium percentage (ESP)* is an index of the sodium status of soils. An ESP of 10 to 15 percent is considered excessive if a high percentage of swelling clay minerals is present (Agricultural Handbook #60, 1954).

For sensitive fruits, the tolerance for sodium for irrigation water is for a sodium adsorption ratio (SAR)** of about 4, whereas for general crops and forages a range of 8 to 18 is generally considered usable (NTAC, 1968). It is emphasized that application of these factors must be interpreted in relation to specific soil conditions existing in a given locale and therefore frequently requires field investigation.

Industrial requirements regarding the dissolved solids content of raw waters is quite variable. Table 10 indicates

Table 10.-Total Dissolved Solids Concentrations of Surface Waters That Have Been Used as Sources for Industrial Water Supplies

Industry/Use	Maximum Concentration (mg/L)
Textile	150
Pulp and Paper	1,080
Chemical	2,500
Petroleum	3,500
Primary Metals	1,500
Boiler Make-up	35,000

maximum values accepted by various industries for process requirements (NAS, 1974). Since water of almost any dissolved solids concentration can be de-ionized to meet the most stringent requirements, the economics of such treatment are the limiting factor for industry.

$$*ESP = \frac{100\,[a + b(SAR)]}{1 \quad [a + b(SAR)]}$$

where: a = intercept respresenting experimental error (ranges from -0.06 to 0.01)

b = slope of regression line (ranges from 0.014 to 0.016)

$$**SAR = \text{sodium adsorption ratio} = \frac{Na}{[0.5(Ca + Mg)]^{0.5}}$$

SAR is expressed as milliequivalents

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

SOLIDS (SUSPENDED, SETTLEABLE) AND TURBIDITY

CRITERIA

Freshwater fish and other aquatic life:

Settleable and suspended solids should not reduce the depth of the compensation point for photosynthetic activity by more than 10 percent from the seasonally established norm for aquatic life.

INTRODUCTION:

The term "suspended and settleable solids" is descriptive of the organic and inorganic particulate matter in water. The equivalent terminology used for solids in Standard Methods (APHA, 1971) is total suspended matter for suspended solids, settleable matter for settleable solids, volatile suspended matter for volatile solids and fixed suspended matter for fixed suspended solids. The term "solids" is used in this discussion because of its more common use in the water pollution control literature.

RATIONALE :

Suspended solids and turbidity are important parameters in both municipal and industrial water supply practices. Finished drinking waters have a maximum limit of 1 turbidity unit where the water enters the distribution system. This limit is based on health considerations as it relates to effective chlorine disinfection. Suspended matter provides areas where microorganisms do not come into contact with the chlorine disinfectant (NAS, 1974). The ability of common water treatment processes (i.e., coagulation, sedimentation, filtration, and chlorination) to remove suspended matter to achieve acceptable final turbidities is a function of the composition of the material as well as its concentration. Because of the variability

of such removal efficiency, it is not possible to delineate a general raw water criterion for these uses.

Turbid water interferes with recreational use and aesthetic enjoyment of water. Turbid waters can be dangerous for swimming, especially if diving facilities are provided, because of the possibility of unseen submerged hazards and the difficulty in locating swimmers in danger of drowning (NAS, 1974). The less turbid the water the more desirable it becomes for swimming and other water contact sports. Other recreational pursuits such as boating and fishing will be adequately protected by suspended solids criteria developed for protection of fish and other aquatic life.

Fish and other aquatic life requirements concerning suspended solids can be divided into those whose effect occurs in the water column and those whose effect occurs following sedimentation to the bottom of the water body. Noted effects are similar for both fresh and marine waters.

The effects of suspended solids on fish have been reviewed by the European Inland Fisheries Advisory Commission (EIFAC, 1965). This review in 1965 identified four effects on the fish and fish food populations, namely:

(1) by acting directly on the fish swimming in water in which solids are suspended, and either killing them or reducing their growth rate, resistance to disease, etc.;

(2) by preventing the successful development of fish eggs and larvae;

(3) by modifying natural movements and migrations of fish;

(4) by reducing the abundance of food available to the fish;. ..

Settleable materials which blanket the bottom of water bodies damage the invertebrate populations, block gravel spawning beds, and if organic, remove dissolved oxygen from overlying waters (ElFAC, 1965; Edberg and Hofsten, 1973). In a study downstream from the discharge of a rock quarry where inert suspended solids were increased to 80 mg/L, the density of macroinvertebrates decreased by 60 percent while in areas of sediment accumulation benthic invertebrate populations also decreased by 60 percent regardless of the suspended solid concantrations (Gammon, 1970). similar effects have been reported downstream from an area which was intensively logged. Major increases in stream suspended solids (25 ppm turbidity upstream versus 390 ppm downstream) caused smothering of bottom invertebrates, reducing organism density to only 7.3 per square foot versus 25.5 per square foot upstraam (Tebo, 1955).

When settleable solids block gravel spawning beds which contain eggs, high mortalities result although there is evidence that some species of salmonids will not spawn in such areas (EIFAC, 1965).

It has been postulated that silt attached to the eggs prevents sufficient exchange of oxygen and carbon dioxide between the egg and the overlying water. The important variables are particle size, stream velocity, and degree of turbulence (EIFAC, 1965).

Deposition of organic materials to the bottom sediments can cause imbalances in stream biota by increasing bottom animal density principally worm populations, and diversity is reduced as pollution-sensitive forms disappear (Mackenthun, 1973). Algae likewise flourish in such nutrient-rich areas although forms may become less desirable (Tarzwell and Gaufin, 1953).

Plankton and inorganic suspended materials reduce light penetration into the water body, reducing the depth of thephotic zone. This reduces primary production and decreases fish food. The NAS commitee in 1974 recommended that the depth of light penetration not be reduced by more than 10 percent (NAS, 1974). Additionally, the near surface waters are heated because of the greater heat absorbency of the particulate material which tends to stabilize the water column and prevents vertical mixing (NAS, 1974). Such mixing reductions decrease the dispersion of dissolved oxygen and nutrients to lower portions of the water body.

One partially offsetting benefit of suspended inorganic material in water is the sorption of organic materials such as pesticides. Following this sorption process subsequent sedimentation may remove these materials from the water column into the sediments (NAS, 1974).

Identifiable effects of suspended solids on irrigation use of water include the formation of crusts on top of the soil which inhibits water infiltration and plant emergence, and impedes soil aeration; the formation of films on plant leaves which blocks sunlight and impedes photosynthesis and which may reduce the

marketability of some leafy crops like lettuce, and finally the adverse effect on irrigation reservoir capacity, delivery canals, and other distribution equipment (NAS, **1974).**

The criterion for freshwater fish and other aquatic lifeare essentially that proposed by the National Academy of Sciences and the Great Lakes Water Quality Board.

(QUALITY CRITERIA FOR WATER, JULY **1976)** **PB-263943**
SEE APPENDIX **C** FOR METHODOLOGY

SULFIDE - HYDROGEN SULFIDE

CRITERION:

2 ug/L undissociated H_2S for fish and other aquatic life, fresh and marine water.

INTRODUCTION:

Hydrogen sulfide is a soluble, highly poisonous, gaseous compound having the characteristic odor of rotten eggs. It is detectable in air by humans at a dilution of 0.002 ppm. It will dissolve in water at 4,000 mg/L at 20° C and one atmosphere of pressure. Hydrogen sulfide biologically is an active compound that is found primarily as an anaerobic degradation product of both organic sulfur compounds and inorganic sulfates. Sulfides are constituents of many industrial wastes such as those from tanneries, paper mills, chemical plants, and gas works. The anaerobic decomposition of sewage, sludge beds, algae, and other naturally deposited organic material is a major source of hydrogen sulfide.

When soluble sulfides are added to water they react with hydrogen ions to form HS or H_2S, the proportion of each depending on the pH. The toxicity of sulfides derives primarily from H_2S rather than from the hydrosulfide (HS^-) or sulfide ($S^=$) ions. When hydrogen sulfide dissolves in water it dissociates according to the reactions:

$$H_2S \quad HS_- + H_+ \quad \text{and} \quad HS_- \quad S_= + H_+$$

At pH 9 about 99 percent of the sulfide is in the form of HS_-, at pH 7 the sulfide is equally divided between HS_- and H_2S; and at pH 5 about 99 percent of the sulfide is present as H_2S (NAS

1974). The fact that H_2S is oxidized in well-aerated water by natural biological systems to sulfates or is biologically oxidized to elemental sulfur has caused investigators to minimize the toxic effects of H_2S on fish and other aquatic life.

RATIONALE:

The degree of hazard exhibited by sulfide to aquatic animal life is dependent on the temperature, pH, and dissolved oxygen. At lower pH values a greater proportion is in the form of the toxic undissociated H_2S. In winter when the pH is neutral or below or when dissolved oxygen levels are low but not lethal to fish, the hazard from sulfides is exacerbated. Fish exhibit a strong avoidance reaction to sulfide. Based on data from experiments with the stickleback, Jones (1964) hypothesized that if fish encounter a lethal concentration of sulfide there is a reasonable chance they will be repelled by it before they are harmed. This, of course, assumes that an escape route is open.

Many past data on the toxicity of hydrogen sulfide to fish and other aquatic life have been based on extremely short exposure periods. Consequently, these early data have indicated that concentrations between 0.3 and 0.4 mg/L permit fish to survive (Van Horn 1958, Boon and Follis 1967, Theede et al., 1969). Recent long-term data, both in field situations and under controlled laboratory conditions, demonstrate hydrogen sulfide toxicity at lower concentrations.

Colby and Smiti-i (1967) found that concentrations as high as 0.7 mg/L have been found within 20 mm of the bottom of sludge beds, and the levels of 0.1 to 0.02 mg/L were common within the

first 20 mm of water above this layer. Walleye (Stizostedion vitreum) eggs held in trays in this zone did not hatch. Adelman and Smith (1970) reported that the hatchability of northern pike (Esox lucius) eggs was substantially reduced at 25 ug/L H_2S; at 41 ug/L mortality was almost complete. Northern pike fry had 96-hour LC50 values that varied from 17 to 32 ug/L at normal oxygen levels of 6.0 mg/L. The highest concentration of hydrogen sulfide that had no observable effect on eggs and fry was 14 and 4 ug/L, respectively. Smith and Oseid (1972), working on eggs, fry and juveniles of walleyes and white suckers (Catostomus commersoni) and Smith (1971), Safe levels in working on walleyes and fathead minnows, Pimephales promelas, were found to vary from 2.9 ug/L to 12 ug/L with eggs being the least sensitive and juveniles being the most sensitive in short-term tests. In 96-hour bioassays, fathead minnows and goldfish, Carassius auratus, varied greatly in tolerance to hydrogen sulfide with changes in temperature. They were more tolerant at low temperatures (6 to 10$_o$ C). Holland, et al. (1960) reported that 1.0 mg/L sulfide caused 100 percent mortality in 72 hours with Pacific salmon.

On the basis of chronic tests evaluating growth and survival, the safe H_2S level for bluegill (Lepomis macrochirus) juveniles and adults was 2 ug/L. Egg deposition in bluegills was reduced after 46 days in 1.4 ug/L H_2S (Smith and Oseid, 1974). White sucker eggs were hatched at 15 ug/L, but juveniles showed growth reductions at 1 ug/L. Safe level for fathead minnows were between 2 and 3 ug/L. Studies showed that safe levels for Gammarus Pseudolimnaeus and Hexagenia limbata were 2 and 15 ug/L, respectively (Oseid and Smith, 1974a, 1974b). Some species

typical of normally stressed habitats, Asellus spp., were much more resistant (Oseid and Smith, 1974c).

Sulfide criteria for domestic or livestock use have not been established because the unpleasant odor and taste would preclude such use at hazardous concentrations.

It is recognized that the hazard from hydrogen sulfide to aquatic life is often localized and transient. Available data indicate that water containing concentrations of 2.0 ug/L undissociated H_2S would not be hazardous to most fish and other aquatic wildlife, but concentrations in excess of 2.0 ug/L would constitute a long-term hazard.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

TAINTING SUBSTANCES

CRITERION:

Materials should not be present in concentrations that individually or in combination produce undesirable flavors which are detectable by organoleptic tests performed on the edible portions of aquatic organisms.

RATIONALE:

Fish or shellfish with abnormal flavors, colors, tastes or odors are either not marketable or will result in consumer complaints and possible rejection of the food source even though subsequent lots of organisms may be acceptable. Poor product quality can and has seriously affected or eliminated the commercial fishing industry in some areas. Recreational fishing also can be affected adversely by off-flavored fish. For the majority of sport fishermen, the consumption of their catch is part of their recreation and off-flavored catches can result in diversion of the sportsmen to other water bodies. This can have serious economic impact on the established recreation industries such as tackle and bait sales and boat and cottage rental.

Water Quality Criteria, 1972 (NAS, 1974) lists a number of wastewaters and chemical compounds that have been found to lower the palatability of fish flesh. Implicated wastewaters included those from 2,4-D manufacturing plants, kraft and neutral sulfite pulping processes, municipal wastewater treatment plants, oily wastes, refinery wastes, phenolic wastes, and wastes from slaughterhouses. The list of implicated chemical compounds is long: it includes cresol and phenol compounds, kerosene, naphthol, styrene, toluene, and exhaust outboard motor fuel. As little as 0.1 ug/L o-chlorophenol was reported to cause tainting

of fish flesh.

Shumway and Palensky 1973) determined estimated threshold concentrations for 22 organic compounds. The values ranged from 0.4 ug/L (2,4-dichlorophenol) to 95,000 ug/L (formaldehyde). An additional 12 compounds were tested, 7 of which were not found to impair flavor at or near lethal levels.

Thomas (1973) reviewed the literature review on tainting substances revealed serious problems that have occurred. Detailed studies and methodology used to evaluate the palatability of fishes in the Ohio River as affected by various waste discharges showed that the susceptibility of fishes to the accumulation of tainting substances is variable and dependent upon the species, length of exposure, and the pollutant. As little as 5 ug/L of gasoline can impart off-flavors to fish (Boyle, 1967).

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SEE APPENDIX *c* FOR METHODOLOGY

TEMPERATURE

CRITERIA:

Freshwater Aquatic Life

For any time of year, there are two upper limiting temperatures for a location (based on the important sensitive species found there at that time):

1. One limit consists of a maximum temperature for short exposures that is time dependent and is given by the species-specific equation:

$$\text{Temperature}_{(C_o)} = (1/b)(\log_{10}[\text{time}_{(min)}] - a) - 2_o\ C$$

where: $\log_{10}$ = logarithm to base 10 (common logarithm)

a = intercept on the "y" or logarithmic axis of the line fitted to experimental data and which is available for some species from Appendix 11-C, National Academy of Sciences 1974 document.

b = slope of the line fitted to experimental data and available for some species from Appendix II-C, of the National Academy of Sciences document.

and

2. The second value is a limit on the weekly average temperature that:

a. In the cooler months (mid-October to mid-April in the north and December to February in the south) will protect against mortality of importr to mid-April in the north and December to February in the south) will protect against mortality of important species if the elevated plume temperature is suddenly dropped to the ambient temperature, with the limit being the

acclimation temperature minus 2Pto C when the lower lethal threshold temperature equals the ambient water temperature (in some regions this limitation may also be applicable in summer).

or

b. In the warmer months (April through October in the north and March through November in the south) is determined by adding to the physiological optimum temperature (usually for growth) a factor calculated as one-third of the difference between the ultimate upper incipient lethal temperature and the optimum temperature €or the most sensitive important species (and appropriate life state) that normally is found at that location and time.

or

c. During reproductive seasons (generally April through June and September through October in the north and March through May and October through November in the south) the limit is that temperature that meets site-specific requirements for successful migration, spawning, egg incubation, fry rearing, and other reproductive functions of important species. These local requirements should supersede all other requirements when they are applicable.

or

d. There is a site-specific limit that is found necessary to preserve normal species diversity or prevent appearance of nuisance organisms.

Marine Aquatic Life

In order to assure protection of the characteristic indigenous marine community of a water body segment from adverse thermal effects:

a. the maximum acceptable increase in the weekly average temperature resulting from artificial sources is 1° C (1.8 F) during all seasonsofthe year, providing the summer maxima are not exceeded; and

b. daily temperature cycles characteristic of the water body segment should not be altered in either amplitude or frequency.

Summer thermal maxima, which define the upper thermal limits for the communities of the discharge area, should be established on a site-specific basis. Existing studies suggest the following regional limits:

	Short-term Maximum	Maximum True Daily Mean*
Sub tropical regions (south of Cape Canaveral and Tampa Bay, Florida, and Hawaii	32.2° C (90° F)	29.4° C (85° F)
Cape Hatteras, N.C., to Cape Canaveral, Fla.	32.2° C (90° F)	29.4° C (85° F)
Long Island (south shore) to Cape Hatteras, N.C.	30.6° C (87° F)	27.8° C (82° F)

(* True Daily Mean = average of 24 hourly temperature readings.)

Baseline thermal conditions should be measured at a site where there is no unnatural thermal addition from any source, which is in reasonable proximity to the thermal discharge (within 5 miles) and which has similar hydrography to that of .the receiving waters at the discharge.

INTRODUCTION:

The uses of water by man in and out of its natural situs in the environment are affected by its temperature. Offstream domestic uses and instream recreation are both partially temperature dependent. Likewise, the life associated with the aquatic environment in any location has its species composition and activity regulated by water temperature. Since essentially all of these organisms are so-called "cold blooded" or poikilotherms, the temperature of the water regulates their metabolism and ability to survive and reproduce effectively. Industrial uses for process water and for coolingare likewise regulated by the water's temperature. Temperature, therefore, is an important physical parameter which to some extent regulates many of the beneficial uses of water. The Federal Water Pollution Control Administration in 1967 called temperature a

catalyst, a depressant, an activator, a restrictor, a stimulator, a controller, a killer, one of the most important and most influential water quality characteristics to life in water."

RATIONALE:

The suitability of water for total body immersion is greatly affected by temperature. In temperate climates, dangers from exposure to low temperatures is more prevalent than exposure to elevated water temperatures. Depending on the amount of activity by the swimmer, comfortable temperatures range from 20° C to 30° C. Short durations of lower and higher temperatures can be tolerated by most individuals. For example, for a 30-minute period, temperatures of 10° C or 35° C can be tolerated without harm by most individuals (NAS, 1974).

Temperature also affects the self-purification phenomenon in water bodies and therefore the aesthetic and sanitary qualities that exist. Increased temperatures accelerate the biodegradation of organic material both in the overlying water and in bottom deposits which makes increased demands on the dissolved oxygen resources of a given system. The typical situation is exacerbated by the fact that oxygen becomes less soluble as water temperature increases. Thus, greater demands are exerted on an increasingly scarce resource which may lead to total oxygen depletion and obnoxious septic conditions. These effects have been described by Phelps (1944), Carp (1963), and Velz (1970).

Indicator enteric bacteria, and presumably enteric pathogens, are likewise affected by temperature. It has been shown that both total and fecal coliform bacteria die away more rapidly in the environment with increasing temperatures (Ballentine and

Kittrell, 1968).

Temperature effects have been shown for water treatment processes. Lower temperatures reduce the effectiveness of coagulation with alum and subsequent rapid sand filtration. In one study, difficulty was especially pronounced below 5° C (Hannah, et al., 1967). Decreased temperature also decreases the effectiveness of chlorination. Based on studies relating chlorine dosage to temperature, and with a 30-minute contact time, dosages required for equivalent disinfective effect increased by as much as a factor of 3 when temperatures were decreased from 20° C to 10° C (Reid and Carlson, 1974). Increased temperature may increase the odor of water because of the increased volatility of odor-causing compounds (Bumson, 1938). Odor problems associated with plankton may also be aggravated.

The effects of temperature on aquatic organisms have been the subject of comprehensive literature reviews (Brett, 1956; Fry, 1967; FWPCA, 1967; Kine, 1970) and annual literature reviews published by the Water Pollution Control Federaticn (Coutant, 1968, 1969, 1970, 1971; Coutant and Goodyear, 1972; Coutant and Pfuderer, 1973, 1974). Only highlights from the thermal effects on aquatic life are presented here.

Temperature changes in water bodies can alter the existing aquatic community. The dominance of various phytoplankton groups in specific temperature ranges has been shown. For example, from 20° C to 25° C, diatoms predominated; green algae predominated from 30° C: to 35° C and blue-greens predominated above 35° C

(Cairns, 1956). Likewise, changes from a coldwater fishery to a warm-water fishery can occur because temperature may be directly lethal to adults or fry cause a reduction of activity or limit reproduction (Brett, 1960).

Upper and lower limits for temperature have been established for many aquatic organisms. Considerably more data exist for upper as opposed to lower limits. Tabulations of lethal temperatures for fish and other organisms are available (Jones, 1964: FWPCA, 1967 NAS, 1974). Factors such as diet, activity, age, general health, osmotic stress, and even weather contribute to the lethality of temperature. The aquatic species, thermal accumulation state and exposure time are considered the critical factors (Parker and Xrenkel, 1969).

The effects of sublethal temperatures on metabolism, respiration, behavior, distribution and migration, feeding rate, growth, and reproduction have been summarized by Be Sylva (1969). Another study has illustrated that inside the tolerance zone there is encompassed a more restrictive temperature range in which normal activity and growth occur and yet an even more restrictive zone inside that in which normal reproduction will occur (Brett, 1960).

De Sylva (1969) has summarized available data on the combined effects of increased temperature and toxic materials on fish indicate that toxicity generally increases with increased temperature and that organisms subjected to stress from toxic materials are less tolerant of temperature extremes.

The tolerance of organisms to extremes of temperature is a function of their genetic ability to adapt to thermal changes

within their characteristic temperature range, the acclimation temperature prior to exposure, and the time of exposure to the elevated temperature (Coutant, 1972). The upper incipient lethal temperature or the highest temperature that 50 percent of a sample of organisms can survive is determined on the organism at the highest sustainable acclimation temperature. The lowest temperature that 50 percent of the warm acclimated organisms can survive in is the ultimate lower incipient lethal temperature. True acclimation to changing temperatures requires several days (Brett, 1941). The lower end of the temperature accommodation range for aquatic life is 0° C in fresh water and somewhat less for saline waters. However, organisms acclimated to relatively warm water, when subjected to reduced temperatures that under other conditions of acclimation would not be detrimental, may suffer a significant mortality caused by thermal shock (Coutant, 1972).

Through the natural changes in climatic conditions, the temperatures of water bodies fluctuate daily, as well as seasonally. These changes do not eliminate indigenous aquatic populations, but affect the existing community structure and the geographic distribution of species. Such temperature changes are necessary to induce the reproductive cycles of aquatic organisms and to regulate other life factors (Mount, 1969).

Artificially induced changes such as the return of cooling water or the release of cool hypolimnetic waters from impoundments may alter indigenous aquatic ecosystems (Coutant, 1972). Entrained organisms may be damaged by temperature

increases across cooling water condensers if the increase is sufficiently great or the exposure period sufficiently long. Impingement upon condenser screens, chlorination for slime control, or other physical insults damage aquatic life (Raney, 1969: Patrick, 1969 (b)). However, Patrick (1969(a)) has shown that algae passing through condensers are not injured if the temperature of the outflowing water does not exceed 345° C.

In open waters elevated temperatures nay affect periphyton, benthic invertebrates, and fish, in addition to causing shifts in algal dominance. Trembley (1960) studies of the Delaware River downstream from a power plant concluded that the periphyton population was considerably altered by the discharge.

The number and distribution of bottom organisms decrease as water temperatures increase. The upper tolerance limit for a balanced benthic population structure is approximately 32° C. A large number of these invertebrate species are able to tolerate higher temperatures than those required for reproduction (FWPCA, 1967).

In order to define criteria for fresh waters, Coutant (1972) cited the following was cited as currently definable requirements:

1. Maximum sustained temperatures that are consistent with maintaining desirable levels of productivity,

2. maximum levels of metabolic acclimation to warm temperatures that will permit return to ambient winter temperatures should artificial sources of heat cease,

3. Time-dependent temperature 1imitations for survival of brief exposures to temperature extremes, both upper and lower,

4. Restricted temperature ranges for various states of reproduction, including (for fish) gametogenesis, spawning migration, release of gametes, development of the embryo, commencement of independent feeding (and other activities) by juveni1es, and temperatures required for metamorphosis, emergence, or other activities of lower forms,

5. Thermal limits for diverse species compositions of aquatic communities, particularly where reduction in diversity creates nuisance growths of certain organisms, or where important food sources (food chains) are altered,

6. Thermal requirements of downstream aquatic life (in rivers) where upstream diminution of a coldwater resource will adversely affect downstream temperature requirements.

The major portion of such information that is available, however, is for freshwater fish species rather than lower forms of marine aquatic life.

The temperature-time duration for short-term exposures such that 50 percent of a given population will survive an extreme temperature frequently is expressed mathematically by fitting experimental data with a staright line on a semi-logarithmic plot with time on the logarithmic scale and temperature on the linear scale (see fig. 1). In equation form this 50 percent mortality relationship is:

$$\log^{10} (\text{time}^{(\text{minutes})}) = a + b\ (\text{Temperature}\ (^{\circ}C))$$

where: $\log^{10}$ = logarithm to base 10 (common logarithm)

a = intercept on the "y" or logarithmic axis of the line fitted to experimental data and which is available for some species from Appendix II-C, of the National Academy of Sciences document.

b = slope of the line fitted to experimental data and which is available for some species from Appendix II-C, of the National Academy of Sciences document.

To provide a safety factor so that none or only a few organisms will perish, it has been found experimentally that a

criterion of 2° C below maximum temperature is usually sufficient (Black, 1953). To provide safety for all the organisms, the temperature causing a median mortality for 50 percent of the population would be calculated and reduced by 2° C in the case of an elevated temperature. Available scientific information includes upper and lower incipient lethal temperatures, coefficients "a" and "b" for the thermal resistance equation, and information of size, life stage, and geographic source of the particular test species (Appendix II-C, NAS, 1974).

Maximum temperatures for an extensive exposure (e.g., more than 1 week) must be divided into those for warmer periods and winter. Other than for reproduction, the most temperature-sensitive life function appears to be growth (Coutant, 1972). Coutant (1972) has suggested that a satisfactory estimate of a limiting maximum weekly mean temperature may be an average of the optimum temperature for growth and the temperature for zero net growth.

Because of the difficulty in determining the temperature of zero net growth, essentially the same temperature can be derived by adding to the optimum essentially to temperature (for growth or other physiological functions) a factor calculated as one-third of the difference between the ultimate upper incipient lethal temperature and the optimum temperature (NAS, 1974). In equation form:

$$\text{Maximum weekly average temperature} = \text{optimum temperature} + 1/3\,(\text{ultimate upper incipient lethal temperature} - \text{optimum temperature})$$

Since temperature tolerance varies with various states of development of a particular species, the criterion for a

particular location would be calculated for the most important life form likely to be present during a particular month. One caveat in using the maximum weekly mean temperature is that the limit for short-term exposure must not be exceeded. Example calculations for predicting the summer maximum temperatures for short-term survival and for extensive exposure for various fish species are presented in Table 11. These calculations use the above equations and data from EPA's Environmental Research Laboxatory in Duluth.

The winter maximum temperature must not exceed the ambient water temperature by more than the amount of change a specimen acclimated to the plume temperature can tolerate. Such a change could occur by a cessation of the source of heat or by the specimen being driven from an area by addition of biocides or other factors. However, there are inadequate data to estimate a safety factor for the "no stress" level from cold shocks (NAS, 1974). Figure 2 was developed from available data in the literature (ERL-Duluth, 1976) and can be used for estimating allowable winter temperature increases.

Coutant (1972) has reviewed the effects of temperature on aquatic life reproduction and development. Reproductive events are noted as perhaps the most thermally restricted of all life phases assuming other factors are at or near optimum levels. Natural short-term temperature fluctuations appear to cause reduced reproduction of fish and invertebrates.

TABLE 11.-Example Calculated Values for Maximum Weekly Average Temperatures for Growth and Short-Term Maxima for Survival for Juveniles and Adults During the Summer (Centigrade and Fahrenheit).

Species	Growth[a]		Maxima[b]	
Atlantic salmon	20	(68)	23	(73)
Bigmouth buffalo				
Black crappie	27	(81)		
Bluegill	32	(90)	35	(95)
Brook trout	19	(66)	24	(75)
Carp				
Channel catfish	32	(90)	35	(95)
Coho salmon	18	(64)	24	(75)
Emerald shiner	30	(86)		
Freshwater drum				
Lake herring (Cisco)	17	(63)	25	(77)
Largemouth bass	32	(90)	34	(93)
Northern pike	28	(82)	30	(86)
Rainbow trout	19	(66)	24	(75)
Sauger	25	(77)		
Smallmouth bass	29	(84)		
Smallmouth buffalo				
Sockeye salmon	18	(64)	22	(72)
Striped bass				
Threadfin shad				
White bass				
White crappie	28	(82)		
White sucker	28	(82)		
Yellow perch	29	(84)		

a - Calculated according to the equation (using optimum temperature for growth)

maximum weekly average temperature for growth = optimum temperature + 1/3 (ultimate incipient lethal temperature-optimum temperature.

b - Based on temperature (^{o}C) = 1/b ($\log^{10}$ time$_{(min.)}$ -a)

2° C, acclimation at the maximum weekly average temperature €or summer growth, and data in Appendix II-C of Water Quality Criteria, published by National Academy of Sciences.

c - Based on data for larvae (ERL-Duluth, 1976).

There are indadequate data available quantitating the most temperature-sensitive life stages among various aquatic species. Uniform elevation of temperature a few degrees but still within the spawning range may lead to advanced spawning for spring spawning species and delays for fall spawners. Such changes may not be detrimental unless asynchrony occurs between newly hatched juveniles and their normal food source. Such asynchrony may be most pronounced among anadromous species or other migrants who pass from the warmed area to a normally chilled, unproductive area. Reported temperature data on maximum temperatures for spawning and embryo survival have been summarized in Table 12 (from ERL-Duluth 1976).

Although the limiting effects of thermal addition to estuarine and marine waters are not as conspicuous in the fall, winter, and spring as during the summer season of maximum heat stress, nonetheless crucial thermal limitations do exist. Hence, it is important that the thermal additions to the receiving waters be minimized during all seasons of the year. Size of harvestable stocks of commercial fish and shellfish, particularly near geographic limits of the fishery, appear to be markedly influenced by slight changes in the long-term temperature regime (Dow, 1973).

Jefferies and Johnson (1974) studied the relationship between temperature and annual variation in 7-year catch data for winter flounder, *Pseudopleuronectes americanus*, in Narragansett Bay, Rhode Island, revealed that a 78 percent decrease in annual catch correlated closely with a 0.5°C increase in the average

temperature over the 30-month period between spawning and recruitment into the fishery. Sissenwine's 1974 model predicts a 68 percent reduction of recruitment in yellowtail flounder, *Limanda ferruginea*, with a 1°C long-term elevation in southern New England waters.

TABLE 12.

Summary of Reported Values for
Maximum Weekly Average Temperature for Spawning and Short-Term
Maxima for Embryo Survival During the Spawning Season
(Centigrade and Fahrenheit)

Species	Spawning,[a]		Embryo Survival[b]	
Atlantic Salmon	5	(41)	7	(45)
Bigmouth Buffalo	17	(63)	27	(81)[c]
Black Crappie				
Bluegill	25	77)	34	(93)
Brook Trout	9	48)	13	(55)
carp	21	70)	33	(91)
Channel Catfish	27	81)	29	(84)
Coho Salmon	10	50)	13	(55)
Emerald Shiner	24	75)	28	(82)[c]
Freshwater Drum	21	70)	26	(79)
Lake Herring (Cisco)	3	37)	8	(46)
Largemouth Bass	21	70)	27	(81)
Northern Pike	11	52)	19	(66)
Rainbow Trout	9	48)	13	(55)
Sauger	10	50)	21	(70)
Smallmouth Bass	17	63)		
Smallmouth Buffalo	17	63)	21	(70)
Sockeye Salmon	10	50)	13	(55)
Striped Bass	18	64)	24	(75)
Threadfin Shad	18	64)	34	(93)
White Bass	17	63)	26	(79)
White Crappie	18	64)	23	(73)
White Sucker	10	50)	20	(68)
Yellow Perch	12	54)	20	(68)

a - the optimum or mean of the range of spawning temperatures reported for the species (ERL-Duluth, 1976).

b - the upper temperature for successful incubation and hatching reported for the species (ERL-Duluth, 1976).

c - upper temperature for spawning.

Community balance can be influenced strongly by such temperature-dependent factors as rates of reproduction, recruitment, and growth of each component population. A few degrees elevation in average monthly temperature can appreciably alter a community through changes in interspecies relationships. A 50 percent reduction in the softshell clam fishery in Maine by the green crab, Carcinus maenus, illustrates how an increase in winter temperatures can establish new predator-prey relationships. Over a period of 4 years, there was a natural amelioration of temperature and the monthly mean for the coldest month of each year did not fall below 2°C. This apparently precluded appreciable ice formation and winter cold kill of the green crab and permitted a major expansion of its population, with increased predation of the softshell clam resulting (Glude, 1954: Welch, 1968).

Temperature is a primary factor controlling reproduction and can influence many events of the reproductive cycle from gametogenesis to spawning. Among marine invertebrates, initiation of reproduction (gametogenesis) is often triggered during late winter by attainment of a minimum environmental threshold temperature. In some species, availability of adequate food is also a requisite (Pearse, 1970; Sastry, 1975: deVlaming, 1971). Elevated temperature can limit gametogenesis by preventing accumulation of nutrients in the gonads. This problem could be acute during the winter if food availability and feeding activity is reduced. Most marine organisms spawn during the spring and summer; gametogenesis is usually initiated during the

previous fall. It should also be noted that some species spawn only during the fall (herrinhg), while others during the winter and very early spring. At the higher latitudes, winter breeders include such estuarine community dominants as acorn barnacles, Balanus balanus and B. balanoides, the edible blue mussel Mytilus edulis, sea urchin, Strongylocentrotus drobachiensis, sculpin, and the winter flounder, Pseudopleuronectes americanus. The two boreal barnacles require temperatures below 10°C before egg production will be initiated (Crisp, 1957). It is clear that adaptations for reproduction exist which are dependent on temperature conditions close to the natural cycle.

Juvenile and adult fish usually thermoregulate behaviorally by moving to water having temperatures closest to their thermal preference. This provides a thermal environment which approximates the optimal temperature for many physiological functions, including growth (Neill and Magnuson. 1974). As a consequence, fishes usually are attracted to heated water during the fall, winter, and spring. Avoidance will occur as warmer temperature exceeds the preferendum by 1 to 3°C (Coutant, 1975). This response precludes problems of heat stress for juvenile and adult fishes during the summer, but several potential problems exist during the other seasons. The possibility of cold shock and death of plume-entrained fish resulting from winter plant shutdown is well recognized. Also, increased incidence of disease and a deterioration of physiological condition has been observed among plume-entrained fishes, perhaps because of insufficient food (Massengill, 1973). A weight loss of approximately 10 percent for each 1° C rise in water temperature

has been observed in fish when food is absent. (Phillips et al., 1960) There may also be indirect adverse effects on the indigenous community because of increased predation pressure if thermal addition leads to a concentration of fish which are dependent on this community for their food.

Fish migration is often linked to natural environmental temperature cycles. In early spring, fish employ temperature as their environmental cue to migrate northward (e.g., menhaden, bluefish) or to move inshore (winter flounder). Likewise, water temperature strongly influences timing of spawning runs of anadromous fish into rivers (Leggett and Whitney, 1972). In the autumn, a number of juvenile marine fishes and shrimp are dependent on a drop in temperature to trigger their migration from estuarine nursery grounds for oceanic dispersal or southward migration (Lund and Maltezos, 1970; Talbot, 1966).

Thermal discharges should not alter diurnal and tidal temperature variations normally experienced by marine communities. Laboratory studies show thermal tolerance to be enhanced when animals are maintained under a diurnally fluctuating temperature regime rather than at a constant temperature (Costlow and Bookhout, 1971; Furch, 1972; Hoss, et al.,). A daily cyclic regime can be protective additionally as it reduces duration of exposure to extreme temperatures (Pearce, 1969; Gonzalez, 1972).

Summer thermal maxima should be established to protect the various marine communities within each biogeographic region. During the summer, naturally elevated temperatures may be of

sufficent magnitude to cause death or emigration (Glynn, 1968; Vaughn, 1961). This more commonly occurs in tropical and warm temperate zone waters, but has been reported for enclosed bays and shallow waters in other regions as well (Nichols, 1918). Summer heat stress also can contribute to increased incidence of disease or parasitism (Sinderman, 1965); reduce or block sexual maturation (Thorhaug, et al., 1971: deVlaming, 1972); inhibit or block embryonic cleavage of larval development (Calabrese, 1969); reduce feeding and growth of juveniles and adults (Olla and Studholme, 1971); result in increased predation (Gonzalez, 1972); and reduce productivity of macroalgae and seagrasses (South and Hill, 1970; Zieman, 1970). The general ceilings set forth here are derived from studies delineating limiting temperatures for the more thermally sensitive species or communities of a biogeographic region.

Thermal effects data are presently insufficient to set general temperature limits for all coastal biogeographic regions. The data enumerated in the Appendix, plus any additional data subsequently generated, should be used to develop thermal limits which specifically consider communities relevant to given water bodies.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

CRITERIA:

Aquatic Life

Not enough data are available concerning the effects of 2,3,7,8-TCDD on aquatic life and its uses to allow derivation of national criteria. The available information indicates that acute values for some freshwater animal species are >1.0 ug/L; some chronic values are <0.01 ug/L; and the chronic value for rainbow trout is <0.001 ug/L. Because exposures of some species of fishes to 0.01 ug/L for <6 days resulted in substantial mortality several weeks later, derivation of aquatic life criteria for 2,3,7,8-TCDD may require special consideration. Predicted bioconcentration factors (BCFs) for 2,3,7,8-TCDD range from 3,000 to 900,000, but the available measured BCFs range from 390 to 13,000. If the BCF is 5,000, concentrations >0.00001 ug/L should result in concentrations in edible freshwater and saltwater fish and shellfish that exceed levels identified in a U.S. FDA health advisory. If the BCF is >5,000 or if uptake in a field situation is greater than that in laboratory tests, the value of 0.00001 ug/L will be too high.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of 2,3,7,8-TCDD exposure through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero. This criterion is

based on the nonthreshold assumption for 2,3,7,8-TCDD. However, zero may not be an attainable level at this time.

(49 F.R. 5831, February 15, 1984)
SEE APPENDIX B FOR METHODOLOGY

TETRACHLOROETHYLENE

CRITERIA:

Aquatic Life

The available data for tetrachloroethylene indicate that acute and chronic toxicity to freshwater aquatic life occurs at concentrations as low as **5,280** and 840 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for tetrachloroethylene indicate that acute and chronic toxicity to saltwater aquatic life occurs at concentrations as low as **10,200** and 450 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to tetrachloroethylene through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 8.0 ug/L, 0.80 ug/L, and 0.08 ug/L, respectively. If these estimates are made for

consumption of aquatic organisms only, excluding consumption of water, the levels are 88.5 ug/L, 8.85 ug/L, and 0.88 ug/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

THALLIUM

CRITERIA:

Aquatic Life

The available data for thallium indicate that acute and chronic toxicity to freshwater aquatic life occurs at concentrations as low as 1,400 and 40 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested. Toxicity to one species of fish occurs at concentrations as low as 20 ug/L after 2,600 hours of exposure.

The available data for thallium indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 2,130 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of thallium to sensitive saltwater aquatic life.

Human Health

For the protection of human health from the toxic properties of thallium ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 13 ug/L.

For the protection of human health from the toxic properties of thallium ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 48 ug/L.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

TOLUENE

CRITERIA:

Aquatic Life

The available data for toluene indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 17,500 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of toluene to sensitive freshwater aquatic life.

The available data for toluene indicate that acute and chronic toxicity to saltwater aquatic life occurs at concentrations as low as 6,300 and 5,000 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

Human Health

For the protection of human health from the toxic properties of toluene ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 14.3 mg/L.

For the protection of human health from the toxic properties of toluene ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 424 mg/L.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

NOTE: The U.S. EPA is currently developing Acceptable Daily Intake (ADI) or Verified Reference Dose (RfD) values for Agency-wide use for this chemical. The new value should be substituted when it becomes available. The January, 1986, draft Verified Reference Dose document cites an RfD of 0.3 mg/kg/day for toluene.

TOXAPHENE

CRITERIA:

Aquatic Life

For toxaphene the criterion to protect freshwater aquatic life as derived using the Guidelines is 0.013 ug/L as a 24-hour average, and the concentration should not exceed 1.6 ug/L at any time.

For saltwater aquatic life the concentration of toxaphene should not exceed 0.070 ug/L at any time. No data are available concerning the chronic toxicity of toxaphene to sensitive saltwater aquatic life.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to toxaphene through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero, based on the non threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 7.1 ng/L, 0.71 ng/L, and 0.07 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 7.3 ng/L, 0.73 ng/L, and 0.01 ng/L, respectively.

(45 F.R. 79318, November 28, 1980)

SEE APPENDIX B FOR METHODOLOGY

TRICHLOROETHYLENE

CRITERIA:

Aquatic Life

The available data for trichloroethylene indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 45,000 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of trichloroethylene to sensitive freshwater aquatic life but the behavior of one species is adversely affected at concentrations as low as 21,900 ug/L.

The available data for trichloroethylene indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 2,000 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of trichloroethylene to sensitive saltwater aquatic life.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to trichloroethylene through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 27 ug/L, 2.7 ug/L, and 0.27 ug/L, respectively. If these estimates are made for

consumption of aquatic organisms only, excluding consumption of water, the levels are 807 ug/L, 80.7 ug/L, and 8.07 ug/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

VINYL CHLORIDE

CRITERIA:

Aquatic Life

No freshwater organisms have been tested with vinyl chloride and no statement can be made concerning acute or chronic toxicity.

No saltwater organisms have been tested with vinyl chloride and no statement can be made concerning acute or chronic toxicity.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to vinyl chloride through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 20 ug/L, 2.0 ug/L, and 0.2 ug/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 5,246 ug/L, 525 ug/L, and 52.5 ug/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

ZINC

CRITERIA:

Aquatic Life

For total recoverable zinc the crit rion to protect fre hwater aquatic life as derived using the Guidelines is 47 ug/L as a 24-hour average and the concentration (in ug/L) should not exceed the numerica 1 value given by $e^{(0.83[\ln(hardness)]+1.95)}$ at any time. For example, at hardnesses of 50, 100, and 200 mg/L as $CaCO_3$ the concentration of total recoverable zinc should not exceed 180, 320, and 570 ug/L at any time.

For total recoverable zinc the criterion to protect saltwater aquatic life as derived using the Guidelines is 58 ug/L as a 24-hour average and the concentration should not exceed 170 ug/L at any time.

Human Health

Sufficient data are not available for zinc to derive a level which would protect against the potential toxicity of this compound. Using available organoleptic data, to control undesirable taste and odor quality of ambient water the estimated level is 5 mg/L. It should be recognized that organoleptic data have limitations as a basis for establishing a water quality criteria, and have no demonstrated relationship to potential adverse human health effects.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

APPENDIX A

DERIVATION OF THE 1985 CRITERION

Derivation of numerical national water quality criteria for the protection of aquatic organisms and their uses is a complex process that uses information from many areas of aquatic toxicology. After a decision is made that a national criterion is needed for a particular material, all available information concerning toxicity to, and bioaccumulation by, aquatic organisms is collected, reviewed for acceptability, and sorted. If enough acceptable data on acute toxicity to aquatic animals are available, they are used to estimate the highest 1-hour average concentration that should not result in unacceptable effects on aquatic organisms and their uses. If justified, this concentration is made a function of a water quality characteristic such as pH, salinity, or hardness. Similarly, data on the chronic toxicity of the material to aquatic animals are used to estimate the highest 4-day average concentration that should not cause unacceptable toxicity during a long-term exposure. If appropriate, this concentration is also related to a water quality characteristic.

Data on toxicity to aquatic plants are examined to determine whether plants are likely to be unacceptably affected by concentrations that should not cause unacceptable effects on animals. Data on bioaccumulation by aquatic organisms are used to determine if residues might subject edible species to restrictions by the U.S. Food and Drug Administration or if such residues might harm some wildlife consumers of aquatic life. All other available data are examined for adverse effects that might

be biologically important.

If a thorough review of the pertinent information indicates that enough acceptable data are available, numerical national water quality criteria are derived for fresh water or saltwater or both to protect aquatic organisms and their uses from unacceptable effects due to exposures to high concentrations for short periods of time, lower concentrations for longer periods of time, and combinations of the two.

I. Collection of Data

A. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants, (b) FDA action levels [12], and (c) chronic feeding studies and long-term field studies with wildlife species that regularly consume aquatic organisms.

B. All data that are used should be available in typed, dated, and signed hard copy (publication, manuscript, letter, memorandum, etc.) with enough supporting information to indicate that acceptable test procedures were used and that the results are probably reliable. In some cases it may be appropriate to obtain additional written information from the investigator, if possible. Information that is confidential or privileged or otherwise not available for distribution should not be used.

C. Questionable data, whether published or

unpublished, should not be used. For example, data should usually be rejected if they are from tests that did not contain a control treatment, tests in which too many organisms in the control treatment died or showed signs of stress or disease, and tests in which distilled or deionized water was used as the dilution water without addition of appropriate salts.

D. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the material of concern should not be used.

E. For some highly volatile, hydrolyzable, or degradable materials it is probably appropriate to use only results of flow-through tests in which the concentrations of test material in the test solutions were measured often enough using acceptable analytical methods.

F. Data should be rejected if they were obtained using:

1. Brine shrimp, because they usually occur naturally only in water with salinity greater than 35 g/kg.
2. Species that do not have reproducing wild populations in North America (See Appendix 1).
3. Organisms that were previously exposed to substantial concentrations of the test material or other contaminants.

G. Questionable data, data on formulated mixtures and emulsifiable concentrates, and data obtained with nonresident species or previously exposed organisms may be used to provide auxiliary information but should not be used in the derivation of criteria.

11. Required Data

A. Certain data should be available to help ensure that each of the four major kinds of possible adverse effects receives adequate consideration. Results of acute and chronic toxicity tests with representative species of aquatic animals are necessary so that data available for tested species can be considered a useful indication of the sensitivities of appropriate untested species. Fewer data concerning toxicity to aquatic plants are required because procedures for conducting tests with plants and interpreting the results of such tests are not as well developed. Data concerning bioaccumulation by aquatic organisms are required only if relevant data are available concerning the significance of residues in aquatic organisms.

B. To derive a criterion for freshwater aquatic organisms and their uses, the following should be available:

1. Results of acceptable acute tests (see Section

IV) with at least one species of freshwater animal in at least eight different families such that all of the following are included:

a. the family Salmonidae in the class Osteichthyes

b. a second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)

c. a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)

d. a planktonic crustacean (e.g., cladoceran, copepod, etc.)

e. a benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)

f. an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)

g. a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)

h. a family in any order of insect or any phylum not already represented.

2. Acute-chronic ratios (see Section VI) with species of aquatic animals in at least three different families provided that of the three species:

a. at least one is a fish

b. at least one is an invertebrate

c. at least one is an acutely sensitive freshwater species (the other two may be saltwater species).

3. Results of at least one acceptable test with a freshwater alga or vascular plant (see Section VIII). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should also be available.

4. At least one acceptable bioconcentration factor determined with an appropriate freshwater species, if a maximum permissible tissue concentration is available (see Section IX).

C. To derive a criterion for saltwater aquatic organisms and their uses, the following should be available:

1. Results of acceptable acute tests (see Section IV) with at least one species of saltwater animal in at least eight different families such that all of the following are included:

a. two families in the phylum Chordata

b. a family in a phylum other than Arthropoda or Chordata

c. either the Mysidae or Penaeidae family

d. three other families not in the phylum Chordata (may include Mysidae or Penaeidae, whichever was not used above)

e. any other family.

2. Acute-chronic ratios (see section VI) with species of aquatic animals in at least three different families provided that of the three species:

   a. at least one is a fish

   b. at least one is an invertebrate

   c. at least one is an acutely sensitive saltwater species (the other one may be a freshwater species).

3. Results of at least one acceptable test with a saltwater alga or vascular plant (see Section VIII. If plants are among the aquatic organisms most sensitive to the material, results of a test with a plant in another phylum (division) should also be available.

4. At least one acceptable bioconcentration factor determined with an appropriate saltwater species, if a maximum permissible tissue concentration is available (see Section IX).

D. If all the required data are available, a numerical criterion can usually be derived, except in special cases. For example, derivation of a criterion

might not be possible if the available acute-chronic ratios vary by more than a factor of 10 with no apparent pattern. Also, if a criterion is to be related to a water quality characteristic T (see Sections V and VII), more data will be necessary.

Similarly, if all required data are not available, a numerical criterion should not be derived except in special cases. For example, even if not enough acute and chronic data are available, it might be possible to derive a criterion if the available data clearly indicate that the Final Residue Value should be much lower than either the Final Chronic Value or the Final Plant Value.

E. Confidence in a criterion usually increases as the amount of available pertinent data increases. Thus, additional data are usually desirable.

III. Final Acute Value

A. Appropriate measures of the acute (short-term) toxicity of the material to a variety of species of aquatic animals are used to calculate the Final Acute Value. The Final Acute Value is an estimate of the concentration of the material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acceptable acute tests have been conducted on the material. However, in some cases, if the Species

Mean Acute Value of a commercially or recreationally important species is lower than the calculated Final Acute Value, then that Species Mean Acute Value replaces the calculated Final Acute Value in order to provide protection for that important species.

B. Acute toxicity tests should have been conducted using acceptable procedures [13].

C. Except for tests with saltwater annelids and mysids, results of acute tests during which the test organisms were fed should not be used, unless data indicate that the food did not affect the toxicity of the test material.

D. Results of acute tests conducted in unusual dilution water, e.g., dilution water in which total organic carbon or particulate matter exceeded 5 mg/L, should not be used, unless a relationship is developed between acute toxicity and organic carbon or particulate matter or unless data show that organic carbon, particulate matter, etc., do not affect toxicity.

E. Acute values should be based on endpoints which reflect the total severe acute adverse impact of the test material on the organisms used in the test. Therefore, only the following kinds of data on acute toxicity to aquatic animals should be used:

1. Tests with daphnids and other cladocerans should be started with organisms less than 24 hours old and tests with midges should be stressed with second- or third-instar larvae. The result should be the 48-hr **EC50** based on percentage of organisms immobilized plus percentage of organisms killed. If such an **EC50** is not available from a test, the 48-hr **LC50** should be used in place of the desired 48-hr **EC50.** An **EC50** or **LC50** of longer than 48 hours can be used as long as the animals were not fed and the control animals were acceptable at the end of the test.

2. The result of a test with embryos and larvae of barnacles, bivalve molluscs (clams, mussels, oysters, and scallops), sea urchins, lobsters, crabs, shrimp, and abalones should be the 96-hr **EC50** based on the percentage of organisms with incompletely developed shells plus the percentage of organisms killed. If such an **EC50** is not available from a test, the lower of the 96-hr **EC50** based on the percentage of organisms with incompletely developed shells and the 96-hr **LC50** should be used in place of the desired 96-hr **EC50.** If the duration of the test was between 48 and 96 hours, the **EC50** or **LC50** at the end of the test should be used.

3. The acute values from tests with all other freshwater and saltwater animal species and older life stages of barnacles, bivalve molluscs, sea urchins, lobsters, crabs, shrimps, and abalones should be the 96-hr EC50 based on the percentage of organisms exhibiting loss of equilibrium plus the percentage of organisms immobilized plus the percentage of organisms killed. If such an EC50 is not available from a test, the 96-hr LC50 should be used in place of the desired 96-hr EC50.

4. Tests with single-celled organisms are not considered acute testa, even if the duration was 96 hours or less.

5. If the tests were conducted properly, acute values reported as "greater than" values and those which are above the solubility of the test material should be used, because rejection of such acute values would unnecessarily lower the Final Acute Value by eliminating acute values for resistant species.

F. If the acute toxicity of the material to aquatic animals apparently has been shown to be related to a water quality characteristic such as hardness or particulate matter for freshwater animals or

salinity or particulate matter for saltwater animals, a Final Acute Equation should be derived based on that water quality characteristic. Go to Section V.

G. If the available data indicate that one or more life stages are at least a factor of 2 more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the Species Mean Acute Value (SMAV) because a species can only be considered protected from acute toxicity if all life stages are protected.

H. The agreement of the data within and between species should be considered. Acute values that appear to be questionable in comparison with other acute and chronic data for the same species and for other species in the same genus probably should not be used in calculation of a Species Mean Acute Value. For example, if the acute values available For a species or genus differ by more than a factor of 10, some or all of the values probably should not be used in calculations.

I. For each species for which at least one acute value is available, the Species Mean Acute Value should be calculated as the geometric mean of the results of all flow-through tests in which the concentrations of test material were measured. For a species for which no such result is available,

the Species Mean Acute Value should be calculated as the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial concentrations of test material (nominal concentrations are acceptable for most test materials if measured concentrations are not available).

NOTE: Data reported by original investigators should not be rounded off. Results of all intermediate calculations should be rounded [14] to four significant digits.

NOTE: The geometric mean of N numbers is the Nth root of the product of the N numbers. Alternatively, the geometric mean can be calculated by adding the logarithms of the N numbers, dividing the sum by N, and taking the antilog of the quotient. The geometric mean of two numbers is the square root of the product of the two numbers, and the geometric mean of one number is that number. Either natural (base 0) or common (base 10) logarithms can be used to calculate geometric means as long as they are used consistently within each set of data, i.e., the antilog used must match the logarithm Used.

NOTE: Geometric means, rather than arithmetic means, are used here because the distributions of sensitivities of individual organisms in toxicity tests on most materials and the distributions of sensitivities of species within a genus are more likely to be lognormal than normal. Similarly, geometric means are used for acute-chronic ratios and bioconcentration factors because quotients are likely to be closer to lognormal than normal distributions. In addition, division of the geometric mean of a set of numerators by the geometric mean of the set of corresponding denominators will result in the geometric mean of the set of corresponding quotients.

J. For each genus €or which one or more Species Mean Acute Values are available, the Genus Mean Acute Value should be calculated as the geometric mean of the Species Mean Acute Values available for the genus.

K. Order the Genus Mean Acute Value from high to low.

L. Assign ranks, R, to the Genus Mean Acute Value from "1" for the lowest to "N" €or the highest. If two or more Genus Mean Acute Values are identical, arbitrarily assign them successive ranks.

M. Calculate the cumulative probability, P, €or each Genus Mean Acute Value as R/(N+1).

N. Select the four Genus Mean Acute Value which have cumulative probabilities closest to 0.05 (if there are less than 59 Genus Mean Acute Value, these will always be the four lowest Genus Mean Acute Values).

0. Using the selected Genus Mean Acute Values and Fs, calculate:

$$S_2 = \frac{E(\ln GMAV)_2) - ((E\ln GMAV))_2/4)}{(P) - ((E\ /^p))_2/4)}$$

$$L = (E(\ln GMAV) - S(E(/^p)))/4$$

$$A = S(/^0.05) + L$$

$$FAV = e^A$$

(See [11] for development of the calculation procedure and Appendix 2 for example calculation and computer program.)

NOTE: Natural logarithms (logarithms to base e, denoted as ln) are used herein merely because they are easier to use on some hand calculators and computers than common (base 10) logarithms. Consistent use of either will produce the same result.

P. If for a commercially or recreationally important species the geometric mean of the acute values from flow-through tests in which the concentrations of test material were measured is lower than the calculated Final Acute Value, then that geometric mean should be used as the Final Acute Value instead of the calculated Final Acute

Value.

Q. Go to section VI.

IV. Final Acute Equation

A. When enough data are available to show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be taken into account as described in Sections B-G below or using analysis of covariance [15,16]. The two methods are equivalent and produce identical results. The manual method described below provides an understanding of this application of covariance analysis, but computerized versions of covariance analysis are much more convenient for analyzing large data tests. If two or more factors affect toxicity, multiple regression analysis should be used.

B. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of the acute toxicity values on the corresponding values of the water quality characteristic to obtain the slope and its 95 percent confidence limits for each species.

NOTE: Because the best documented relationship fitting these data is that between hardness and acute toxicity of metals in fresh water and a log-log

relationship, geometric means and natural logarithms of both toxicity and water quality are used in the rest of this section. For relationships based on other water quality characteristics such as pH, temperature, or salinity, no transformation or a different transformation might fit the data better, and appropriate changes will be necessary throughout this section.

c. Decide whether the data for eachspecies are useful, taking into account the range and number of the tested values of the water quality characteristic and the degree of agreement within and between species. For example, a slope based on six data points might be of limited value if it is based only on data for a very narrow range of values of the water quality characteristic. A slope based on only two data points, however, might be useful if it is consistent with other information and if the two points cover a broad enough range of the water quality characteristic. In addition, acute values that appear to be questionable in comparison with other acute and chronic data available for the same species and for other species in the same genus probably should not be used. For example, if after adjustment for the water quality characteristic, the acute values available for a species or genus

differ by more than a factor of 10, probably some or all of the values should be rejected. If useful slopes are not available for at least one fish and one invertebrate or if the available slopes are too dissimilar or if too few data are available to adequately define the relationship between acute toxicity and the water quality characteristic, return to Section IV.G, using the results of tests conducted under conditions and in waters similar to those commonly used for toxicity tests with the species.

D. Individually for each species calculate the geometric mean of the available acute values and then divide each of the acute values for species by the mean for the species. This normalizes the values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0.

E. Similarly normalize the values of the water quality characteristic for each species individually.

F. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in Section B.

NOW, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

G. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope, V, and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

H. For each species calculate the geometric mean, W, of the acute toxicity values and the geometric mean, X, of the values of the water quality characteristic. (These were calculated in steps D and E.)

I. For each species calculate the logarithm, Y, of the Species Mean Acute Value at a selected value, Z, of the water quality characteristic using the equation:

$$Y = \ln W - V(\ln X - \ln Z).$$

J. For each species calculate the SMAV at Z using the equation: $SMAV = e^{Y}$.

NOTE: Alternatively, the Species Mean Acute Values at Z can be obtained by skipping step H using the

equations in steps I and J to adjust each acute value individually to Z, and then calculating the geometric mean of the adjusted values €or each species individually. This alternative procedure allows an examination of the range of the adjusted acute values for each species.

K. Obtain the Final Acute Value at Z by using the procedure described in Section 1V.J-0.

L. If the Species Mean Acute Value at Z of a commercially or recreationally important species is lower than the calculated Final Acute Value at Z, then that Species Mean Acute Value should be used as the Final Acute Value at Z instead of the calculated Final Acute Value.

M. The Final Acute Equation is written as: Final Acute Value = $e^{(V[\ln(\text{water quality characteristic})] + \ln A - V[\ln Z])}$, where V = pooled acute slope and A = Final Acute Value at Z. Because V, A, and Z are known, the Final Acute Value can be calculated for any selected value of the water quality characteristic.

V. Final Chronic Value

A. Depending on the data that are available concerning chronic toxicity to aquatic animals, the Final Chronic Value might be calculated in the same manner as the Final Acute Value or by dividing the Final Acute Value by the Final Acute-

Chronic Ratio. In some cases it may not be possible to calculate a Final Chronic Value.

NOTE: As the name implies, the acute-chronic ratio is a way of relating acute and chronic toxicities. The acute-chronic ratio is basically the inverse of the application factor, but this new name is better because it is more descriptive and should help prevent confusion between 'application factors" and "safety factors." Acute-chronic ratios and application factors are ways of relating the acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond the known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it will usually be greater than 1; this should avoid the confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

B. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which the concentrations of test material in the test solutions were properly measured at appropriate times during the test.

C. Results of chronic tests in which survival,

growth, or reproduction in the control treatment was unacceptably low should not be used. The limits of acceptability will depend on the species.

D. Results of chronic tests conducted in unusual dilution water, e.g., dilution water in which total organic carbon or particulate matter exceeded 5 mg/L, should not be used, unless a relationship is developed between chronic toxicity and organic carbon or particulate matter or unless data show that organic carbon, particulate matter, etc., do not affect toxicity.

E. Chronic values should be based on endpoints and lengths of exposure appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:

1. Life-cycle toxicity tests consisting of exposures of each of two or more groups of individuals of a species to a different concentration of the test material throughout a life cycle. To ensure that all life stages and life processes are exposed, tests with fish should begin with embryos or newly hatched young less than 48 hours old, continue through maturation and reproduction, and should end not less than 24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin

with young less than 24 hours old and last for not less than 21 days. Tests with mysids should begin with young less than 24 hours old and continue until 7 days past the median time of first brood release in the controls. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female. For mysids, data should be obtained and analyzed on survival, growth, and young per female.

2. Partial life-cycle toxicity tests consisting of exposures of each of two or more groups of individuals of a species of fish to a concentration of the test material through most portions of a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. Exposure to the test material should begin with immature juveniles at least 2 months prior to active gonad development, continue through maturation and reproduction,

and end not less than 24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.

3. Early life-stage toxicity tests consisting of 28- to 32-day (60 days. post hatch for salmonids) exposures of the early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained and analyzed on survival and growth.

NOTE: Results of an early life-stage test are used as predictions of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test should not be used because results of such tests are possibly not good predictions of the results of comparable life-cycle or partial 1ife-cycle tests.

F. A chronic value may be obtained by calculating the geometric mean of the lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of adverse effect on any of the specified biological measurements, and (c) below which no tested concentration caused an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of adverse effect on one or more of the specified biological measurements, and (c) above which all tested concentrations also caused such an effect.

NOTE: Because various authors have used a variety of terms and definitions to interpret and report results of chronic tests, reported results should be reviewed carefully. The amount of effect that is considered unacceptable is often based on a statistical hypothesis test, but might also be defined in terms of a specified percent reduction from the controls. A small percent reduction (e.g., 3 percent) might be considered acceptable even if it is statistically significantly different from the control, whereas a large

percent reduction (e.g., 30 percent) might be considered unacceptable even if it is not statistically significant.

G. If the chronic toxicity of the material to aquatic animals apparently has been shown to be related to a water quality characteristic such as hardness or particulate matter for freshwater animals or salinity or particulate matter for saltwater animals, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section VII.

H. If chronic values are available for species in eight families as described in Sections III.B.1 or III.C.1, a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values available for the species, and appropriate Genus Mean Chronic Values should be calculated. The Final Chronic Value should then be obtained using the procedure described in Section 1V.J-0. Then go to Section VI.M.

I. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of the results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in

the same dilution water and in which the concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. The acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water, but in a different study, may be used. If no such acute tests are available, results of acute tests conducted in the same dilution water in a different laboratory may be used. If no such acute tests are available, an acute-chronic ratio should not be calculated.

J. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios available for that species.

K. For some materials the acute-chronic ratio seems to be the same for all species, but for other materials the ratio seems to increase or decrease as the Species Mean Acute Value (SMAV) increases. Thus the Final Acute-Chronic Ratio can be obtained in four ways, depending on the data available:

1. If the Species Mean Acute-Chronic ratio Seems to increase or decrease as the Species Mean Acute Value increases, the Final Acute-Chronic Ratio should be calculated as the geometric mean of the acute-chronic ratios for species

whose Species Mean Acute Values are close to the Final Acute Value.

2. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of 10, the Final Acute-Chronic Ratio should be calculated as the geometric mean of all the Species Mean Acute-Chronic Ratios available for both freshwater and saltwater species.

3. For acute tests conducted on metals and possibly other substances with embryos and larvae of barnacles, bivalve molluscs, sea urchins, lobsters, crabs, shrimp, and abalones (see Section IV.E.2), it is probably appropriate to assume that the acute-chronic ratio is 2. Chronic tests are very difficult to conduct with most such species, but it is likely that the sensitivities of embryos and larvae would determine the results of life-cycle tests. Thus, if the lowest available Species Mean Acute Values were determined with embryos and larvae of such species, the Final Acute-Chronic Ratio should probably be assumed to be 2, so that the Final Chronic Value is equal to the Criterion Maximum Concentration (see Section XI.B)

4. If the most appropriate Species Mean Acute-Chronic Ratios are less than 2.0, and especially if they are less than 1.0, acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be assumed to be 2, so that the Final Chronic Value is equal to the Criterion Maximum Concentration (see Section XI.B).

   If the available Species Mean Acute-Chronic Ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and a Final Chronic Value probably cannot be calculated.

L. Calculate the Final Chronic Value by dividing the Final Acute Value by the Final Acute-Chronic Ratio. If there was a Final Acute Equation rather than a Final Acute Value, see also Section VII.A.

M. If the Species Mean Chronic Value of a commercially or recreationally important species is lower than the calculated Final Chronic Value, then that species Mean Chronic Value should be used as the Final Chronic Value instead of the calculated Final Chronic Value.

N. Go to Section VIII.

VI. Final Chronic Equation

A. A Final Chronic Equation can be derived in two ways. The procedure described here in Section A will result in the chronic slope being the same as the acute slope. The procedure described in Sections B-N usually will result in the chronic slope being different from the acute slope.

1. If acute-chronic ratios are available for enough species at enough values of the water quality characteristic to indicate that the acute-chronic ratio is probably the same for all species and is probably independent of the water quality characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the available Species Mean Acute-Chronic Ratios.

2. Calculate the Final Chronic Value at the selected value Z of the water quality characteristic by dividing the Final Acute Value at Z (see Section V.M) by the Final Acute-Chronic Ratio.

3. Use V = pooled acute slope (see section V.M) as L = pooled chronic slope.

4. Go to Section VII.M.

B. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the

relationship should be taken into account as described in Sections B-G or using analysis of covariance [15,16]. The two methods are equivalent and produce identical results. The manual method described below provides an understanding of this application of covariance analysis, but computerized versions of covariance analysis are much more convenient for analyzing large data sets. If two or more factors affect toxicity, multiple regression analysis should be used.

For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of the chronic toxicity values on the corresponding values of the water quality characteristic to obtain the slope and its 95 percent confidence limits for each species.

NOTE: Because the best documented relationship fitting these data is that between hardness and acute toxicity of metals in freshwater and a log-log relationship, geometric means and natural logarithms of both toxicity and water quality are used in the rest of this section. For relationships based on other water quality characteristics such as pH, temperature, or

salinity, no transformation or a different transformation might fit the data better, and appropriate changes will be necessary throughout this section. It is probably preferable, but not necessary, to use the same transformation that was usedwiththeacutevalues in Section V.

D. Decide whether the data for each speciesare useful, taking into account the range and number of the tested values of the water quality characteristic and the degree of agreement within and between species. For example, a slope based on six datapointsmightbeof limited value if it is based only on data €or a very narrow range of values of the water quality characteristic, A slope based on only two data points, however, might be useful if it is consistent with other information and if the two points cover a broad enough range of the water quality characteristic. In addition, chronic values that appear to be questionable in comparison with other acute and chronic data available €or the same species and for other species in the same genus probably should not be used. For example, if after adjustment for the water quality characteristic, the chronic values available for a species or genus differ by more than a factor of 10, probably some or all of the values should be rejected. If

a useful chronic slope is not available for at least one species or if the available slopes are too dissimilar or if too few data are available to adequately define the relationship between chronic toxicity and the water quality characteristic, it might be appropriate to assume that the chronic slope is the same as the acute slope, which is equivalent to assuming that the acute-chronic ratio is independent of the water quality characteristic. Alternatively, return to Section VI.H, using the results of tests conducted under conditions and in waters similar to those commonly used for toxicity tests with the species.

E. Individually for each species calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species. This normalizes the chronic values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0.

F. Similarly normalize the values of the water quality characteristic for each species individually.

G. Individually for each species perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and the 95 percent confidence

limits will be identical to those obtained in Section B. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

H. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope, L, and its 95 percent confidence limits, If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

I. For each species calculate the geometric mean, M, of the toxicity values and the geometric mean, P, of the values of the water quality characteristic. (These were calculated in steps E and F.)

J. For each species calculate the logarithm, Q, of the Species Mean Chronic Value at a selected value, Z, **of** the water quality characteristic using the equation: $Q = \ln M - L(\ln P - \ln Z)$.

NOTE: Although **it is** not necessary, it will usually be best to use the same value of the water quality characteristic here as was used in section V.I.

K. For each species calculate a Species Mean Chronic Value at Z using the equation: $SMCV = e^{Q}$.

NOTE: Alternatively, the Species Mean Chronic Values at **Z** can be obtained by skipping step **J**, using the equations in steps **J** and **K** to adjust each acute value individually to Z, and then calculating the geometric means **of** the adjusted values for each species individually. This alternative procedure allows an examination of the range of the adjusted chronic values **for** each species.

L. Obtain the Final Chronic Value at Z by using the procedure described in Section IV.J-O.

M. If the Species Mean Chronic Value at Z of a commercially or recreationally important species is lower than the calculated Final Chronic Value at Z, then that Species Mean Chronic Value should be used as the Final Chronic Value at Z instead of the calculated Final Chronic Value.

N. The Final Chronic Equation is written as: Final Chronic Value = e(L[ln(water quality characteristic)] + ln S - L[ln Z]), where L = pooled chronic slope and S = Final Chronic Value at Z. Because L, S and Z are known, the Final Chronic Value can be calculated for any selected value of the water quality characteristic.

VII. Final Plant Value

A. Appropriate measures of the toxicity of the material to aquatic plants are used to compare the relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting the results of toxicity tests with plants are not well developed, results of tests with plants usually indicate that criteria which adequately protect aquatic animals and their uses will probably also protect aquatic plants and their uses.

B. A plant value is the result of a 96-hr test conducted with an alga or a chronic test conducted with an aquatic vascular plant.

NOTE: A test of the toxicity of a metal to a plant usually should not be used if the medium contained an excessive amount of a complexing agent, such as EDTA, that might affect the toxicity of the metal. Concentrations of EDTA above about 200 ug/L should probably be considered excessive.

C. The Final Plant Value should be obtained by selecting the lowest result from a test with an important aquatic plant species in which the concentrations of test material were measured and the endpoint was biologically important.

VIII. Final Residue Value

A. The Final Residue Value is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from affecting marketability because of exceedence of applicable FDA action levels and (b) protect wildlife, including fishes and birds, that consume aquatic organisms from demonstrated unacceptable effects. The Final Residue Value is the lowest of the residue values that are obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration or bioaccumulation factors. A maximum permissible tissue concentration is either (a) an FDA action level [12] for fish oil or for the edible portion of fish or shellfish, or (b) a maximum acceptable dietary intake based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no maximum permissible tissue concentration is available, go to Section X because no Final Residue Value can be derived.

B. ~ioconcentrationFactors (BCFs) and bioaccumulation factors (BAFs) are quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution in which the organism had been living. A BCF is intended to account only for net uptake directly from water, and thus almosthastobemeasured in a laboratory test. Some uptake during the bioconcentration test might not bedirectly fromwater if the food sorbs some of the test material before it is eaten by the test organisms. A BAF is intended to account for net uptake from both food and water in a real-world situation. A BAF almost has to be measured in a field situation in which predators accumulate the material directly from water and by consuming prey that itself could have accumulated the material from both food and water. The BCF and BAF are probably similar for a material with a low BCF, but the BAF is probably higher than the BCF for materials with high BCFs. Although BCFs are not too difficult to determine, very few BAFs have been measured acceptably because it is necessary to make enough measurements of the concentration of the material in water to show that it was reasonably constant for a long enough period of time over the range of territory inhabited by the

organisms. Because so few acceptable BAFs are available, only BCFs will be discussed further. However, if an acceptable BAF is available for a material, it should be used instead of any available BCFs.

C. If a maximum permissible tissue concentration is available for a substance (e.g., parent material, parent material plus metabolites, etc.), the tissue concentration used in the calculation of the BCF should be for the same substance. otherwise the tissue concentration used in the calculation of the BCF should be' that of the material and its metabolites which are structurally similar and are not much more soluble in water than the parent material.

D. 1. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of the test material in tissue and in the test solution, and the exposure continued at least until either apparent steady-state or 28 days was reached. Steady-state is reached when the BCF does not change significantly over a period of time, such a 2 days or 16 percent of the length of the exposure, whichever is longer. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached, (b) the highest BCF

obtained, if apparent steady-state was not reached, and (c) the projected steady-state BCF, if calculated.

2. Whenever a BCF is determined for a lipophilic material, the percent lipids should also be determined in the tissue(s) for which the BCF was calculated.

3. A BCF obtained from an exposure that adversely affected the test organisms may be used only if it is similar to a BCF obtained with unaffected organisms of the same species at lower concentrations that did not cause adverse effects.

4. Because maximum permissible tissue concentrations are almost never based on dry weights, a BCF calculated using dry tissue weights must be converted to a wet tissue weight basis. If no conversion factor is reported with the BCF, multiply the dry weight BCF by 0.1 for plankton and by 0.2 for individual species of fishes and invertebrates [17].

5. If more than one acceptable BCF is available for a species, the geometric mean of the available values should be used, except that if the BCFs are from different lengths of exposure and the BCF increases with length of

exposure, the BCF for the longest exposure should be used.

E. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:

1. For each available maximum acceptable dietary intake derived from a chronic feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute' or represent a major portion of the diet of the tested wildlife species.

2. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion (muscle for decapods, muscle with or without skin for fishes, adductor muscle for scallops, and total soft tissue for other bivalve molluscs) of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.

F. For lipophilic materials, it might be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material Seems to be proportional to percent lipids from one tissue to another and from one species to another

[18-20], extrapolations can be made from tested tissues or species to untested tissues or species an the basis of percent lipids.

1. For each BCF for which the percent lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a 1 percent lipid basis by dividing the BCF by the percent lipids. This adjustment to a 1 percent lipid basis is intended to make all the measured BCFs far a material comparable regardless of the species or tissue'withwhich the BCF was measured.

2. calculate the geometric mean normalized BCF. Data for both saltwater and freshwater species should be used to determine the mean normalized BCF, unless the data show that the normalized BCFs are probably not similar.

3. Calculate all possible residue values by dividing the available maximum permissible tissue concentrations by the mean normalized BCF and by the percent lipids values appropriate to the maximum permissible tissue concentrations, i.e.,

$$\text{Residue value} = \frac{\text{(maximum permissible tissue concentration)}}{\text{(mean normalized BCF)(appropriate percent lipids)}}$$

tissue concentration) Residue value = (mean normalized BCF) (appropriate percent lipids)

a. For an FDA action level for fish oil, the

appropriate percent lipids value is 100.

b. For an FDA action level for fish, the appropriate percent lipids value is 11 for freshwater criteria and 10 for saltwater criteria because FDA action levels are applied on a species-by-species basis to commonly consumed species. The highest lipid contents in the edible portions of important consumed species are about 11 percent for both the freshwater chinook salmon and lake trout and about 10 percent for the saltwater Atlantic herring [21].

c. For a maximum acceptable dietary intake derived from a chronic feeding study or a long-term field study with wildlife, the appropriate percent lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.

G. The Final Residue Value is obtained by selecting the lowest of the available residue values.

NOTE: In some cases the Final Residue Value will not be low enough. For example, a residue value calculated from an FDA action level will probably result in an average concentration in the edible portion of a fatty species that is at the action level. Some individual organisms, and possibly some species, will have residue concentrations higher than the mean value but no mechanism has been devised to provide appropriate additional protection. Also, some chronic feeding studies and long-term field studies with wildlife identify concentrations that cause adverse effects but do not identify concentrations which do not cause adverse effects; again, no mechanism has been devised to provide appropriate additional protection. These are some of the species and uses that are not protected at all times in all places.

X. other Data

Pertinent information that could not be used in earlier sections might be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other adverse effect that has been shown to be biologically important. Especially important are data for species for which no

other data are available. Data from behavioral, biochemical, physiological, microcosm, and field studies might also be available. Data might be available from tests conducted in unusual dilution water (see IV.D and VI.D), from chronic tests in which the concentrations were not measured (see VI.B), from tests with previously exposed organisms (see II.F), and from tests on formulated mixtures or emulsifiable concentrates (see II.D). Such data might affect a criterion if the data were obtained with an important species, the test concentrations were measured, and the endpoint was biologically important.

XI. Criterion

A. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.

B. The Criterion Maximum Concentration (CMC) is equal to one-half the Final Acute Value.

C. The Criterion Continuous Concentration (CCC) is equal to the lowest of the Final Chronic Value, the Final Plant Value, and the Final Residue Value, unless other data (see Section X) show that a lower value should be used. If toxicity is related to a water quality characteristic, the Criterion continuous concentration is obtained from the Final Chronic Equation, the Final Plant Value, and the Final Residue Value by selecting

the one, or the combination, that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section X) show that a lower value should be used.

D. Round [14] both the Criterion Maximum Concentration and the Criterion Continuous Concentration to two significant digits.

E. The criterion is stated as:

The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that, except possibly where a locally important species is very sensitive, (1) aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration of (2) does not exceed (3) ug/L more than once every 3 years on the average and if the 1-hour average concentration does not exceed (4) ug/L more than once every 3 years on the average.

where (1) = insert "freshwater" or "saltwater"

(2) = insert name of material

(3) = insert the Criterion Continuous Concentration

(4) = insert the Criterion Maximum Concentration.

XII. Final Review

A. The derivation of the criterion should be carefully reviewed by rechecking each step of the Guidelines. Items that should be especially checked are:

1. If unpublished data are used, are they well documented?
2. Are all required data available?
3. Is the range of acute values for any species greater than a factor of lo?
4. Is the range of Species Mean Acute Values for any genus greater than a factor of 10?
5. Is there more than a factor of 10 difference between the 'four lowest Genus Mean Acute Values?
6. Are any of the four lowest Genus Mean Acute Values questionable?
7. Is the Final Acute Value reasonable in comparison with the Species Mean Acute Values and Genus Mean Acute Values?
8. For any commercially or recreationally important species, is the geometric mean of the acute values from flow-through tests in which the concentrations of test material were measured lower than the Final Acute Value?
9. Are any of the chronic values questionable?

10. Are chronic values available for acutely sensitive species?

11. Is the range of acute-chronic ratios greater than a factor of 10?

12. Is the Final Chronic Value reasonable in comparison with the available acute and chronic data?

13. Is the measured or predicted chronic value for any commercially or recreationally important species below the Final Chronic Value?

14. Are any of the other data important?

15. Do any data look like they might be outliers?

16. Are there any deviations from the Guidelines? Are they acceptable?

B. On the basis of all available pertinent laboratory and field information, determine if the criterion is consistent with sound scientific evidence. If it is not, another criterion, either higher or lower, should be derived using appropriate modifications of these Guidelines.

APPENDIX B

SUMMARY OF THE 1980 AQUATIC LIFE GUIDELINES

The Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life and its Uses were developed to describe an objective, internally consistent, and appropriate way of ensuring that water quality criteria for aquatic life would provide, on the average, a reasonable amount of protection. The resulting criteria are not intended to provide 100 percent protection of all species and all uses of aquatic life all of the time, but they are intended to protect most species in a balanced, healthy aquatic community.

Minimum data requirements are identified in four areas; acute toxicity to animals (eight data points), chronic toxicity to animals (three data points), toxicity to plants, and residues. Data on acute toxicity are needed for a variety of fish and invertebrate species and are used to derive a Final Acute Value. By taking into account the number and relative sensitivities of the tested species, the Final Acute Value is designed to protect most, but not necessarily all, of the tested and untested species.

Data on chronic toxicity to animals can be used to derive a Final Chronic Value by two different means. If chronic values are available for a specified number and array of species, a Final Chronic Value can be calculated directly. If not, an acute-chronic ratio is derived and then used with the Final Acute Value to obtain the Final Chronic Value.

The Final Plant Value is obtained by selecting the lowest plant toxicity value based on measured concentrations.

The Final Residue Value is intended to protect wildlife which consume aquatic organisms and the marketability of aquatic organisms. Protection of the marketability of aquatic organisms is, in actuality, protection of a use of that water body (commercial fishery). Two kinds of data are necessary to calculate the Final Residue Value: a bioconcentration factor (BCF) and a maximum permissible tissue concentration, which can be an FDA action level or can be the result of a chronic wildlife feeding study. For lipid-soluble pollutants, the BCF is normalized for percent lipids and then the Final Residue Value is calculated by dividing the maximum permissible tissue concentration by the normalized BCF and by an appropriate percent lipid value. BCFs are normalized for percent lipids since the BCF measured for any individual aquatic species is generally proportional to the percent lipids in that species.

If sufficient data are available to demonstrate that one or more of the final values should be related to a water quality characteristic, such as salinity, hardness, or suspended solids, the final value(s) are expressed as a function of that characteristic.

After the four final values (Final Acute Value, Final Chronic Value, Final Plant Value, and Final Residue Value) have been obtained, the criterion is established with the Final Acute value becoming the maximum value and the lowest of the other three values becoming the 24-hour average value. All of the data used to calculate the four final values and any additional pertinent information are then reviewed to determine if the criterion is reasonable. If sound scientific evidence indicates that the

criterion should be raised or lowered, appropriate changes are made as necessary.

The November 28, 1980, Guidelines have been revised from the earlier published versions (43 FR 21506, May 18, 1978; 43 FR 29028, July 5, 1978; 44 FR 15926, March 15, 1979). Details have been added in many places and the concept of a minimum data base has been incorporated. In addition, three adjustment factors and the species sensitivity factor have been deleted. These modifications were the result of the Agency's analysis of public comments and comments received from the Science Advisory Board on earlier versions of the Guidelines. These comments and the Resultant modifications are addressed fully in Appendix D to this notice.

Criteria for the Protection of Human Health

Interpretation of the Human Health Criteria

The human health criteria issued today are summarized in Appendix A of this Federal Register notice. Criteria for the protection of human health are based on their carcinogenic, toxic, or organoleptic (taste and odor) properties. The meanings and practical uses of the criteria values are distinctly different depending on the properties on which they are based.

The objective of the health assessment portions of the criteria documents is to estimate ambient water concentrations which, in the case of noncarcinogens, prevent adverse health effects in humans, and in the case of suspect or proven carcinogens, represent various levels of incremental cancer risk.

Health assessments typically contain discussions of four elements: exposure, pharmacokinetics, toxic effects, and criterion formulation.

The exposure section summarizes information on exposure routes: ingestion directly from water, indirectly from consumption of aquatic organisms found in ambient water, other dietary sources, inhalation, and dermal contact. Exposure assumptions are used to derive human health criteria. Most criteria are based solely on exposure from consumption of water containing a specified concentration of a toxic pollutant and through consumption of aquatic organisms which are assumed to have bioconcentrated pollutants from the water in which they live. Other multimedia routes of exposure such as air, nonaquatic diet, or dermal are not factored into the criterion formulation for the vast majority of pollutants because of lack of data. The criteria are calculated using the combined aquatic exposure pathway and also using the aquatic organism ingestion exposure route alone. In criteria reflecting both the water consumption and aquatic organism ingestion routes of exposure, the relative exposure contribution varies with the propensity of a pollutant to bioconcentrate, with the consumption of aquatic organisms becoming more important as the bioconcentration factor (BCF) increases. As additional information on total exposure is assembled for pollutants for which criteria reflect only the two specified aquatic exposure routes, adjustments in water concentration values may be made. The demonstration of significantly different exposure patterns will become an element

of a process to adapt/modify human health-based criteria to local conditions, somewhat analogous to the aquatic life criteria modification process discussed previously. It is anticipated that States at their discretion will be able to set appropriate human health criteria based on this process.

Specific health-based criteria are developed only if a weight of evidence supports the occurrence of the toxic effect and if dose/response data exist from which criteria can be estimated. The pharmacokinteics section reviews data on absorption, distribution, metabolism, and excretion to assess the biochemical fate of the compounds in the human and animal system. The toxic effects section reviews data on acute, subacute, and chronic toxicity, synergistic and antagonistic effects, and specific information on mutagenicity, teratogenicity, and carcinogenicity. From this review, the toxic effect to be protected against is identified taking into account the quality, quantity, and weight of evidence characteristic of the data. The criterion formulation section reviews the highlights of the text and specifies a rationale for criterion development and the mathematical derivation of the criterion number.

Within the limitations of time and resources, current published information of significance was incorporated into the human health assessments. Review articles nad reports were used for data evaluation and synthesis. Scientific judgment was exercised in reviewing and evaluating the data in each criteria document and in identifying the adverse effects for which protective criteria were published.

Criteria for suspect or proven carcinogens are presented as concentrations in water associated with a range of incremental cancer risks to man. Criteria for noncarcinogens represent levels at which exposure to a single chemical is not anticipated to produce adverse effects in man. In a few cases, organoleptic (taste and odor) data form the basis for the criterion. While this type of criterion does not represent a value which directly affects human health, it is presented as an estimate of the level of a pollutant that will not produce unpleasant taste or odor either directly from water consumption or indirectly by consumption of aquatic organisms found in ambient waters. A criterion developed in this manner is judged to be as useful as other types of criteria in protecting designated water uses. In addition, where data are available, toxicity-based criteria are also presented for pollutants with derived organoleptic criteria. The choice of criteria used in water quality standards for these pcllutants will depend upon the designated use to be protected. In the case of a multiple use water body, the criterion protecting the most sensitive use will be applied. Finally, for several pollutants no criteria are recommended because insufficient information is available for quantitative criterion formulation.

Risk Extrapolation

Because methods do not exist to establish the presence of a threshold for carcinogenic effects, EPA's policy is that there is no scientific basis for estimating "safe" levels for carcinogens. The criteria for carcinogens, therefore, state that the

recommended concentration for maximum protection of human health is zero. In addition, the Agency has presented a range of concentrations corresponding to incremental cancer risks of to 10_{-5} (one additional case of cancer in populations ranging from 10 million to 100,000, respectively). Other concentrations representing different risk levels may be calculated by use of the Guidelines. The risk estimate range is presented for information purposes and does not represent an Agency judgment on a "acceptable" risk level.

Summary of the Human Health Guidelines

The health assessments and corresponding criteria were derived based on Guidelines and Methodology Used in the Preparation of Health Effect Assessment Chapters of the Consent Decree Water Criteria Documents (the Guidelines) developed by EPA'S Office of Research and Development. The estimation of health risk associated with human exposure to environmental pollutants requires predicting the effect of low doses for up to a lifetime in duration. A combination of epidemiological and animal dose/response data is considered the preferred basis for quantitative criterion derivation.

No-effect (noncarcinogen) or specified risk (carcinogen) concentrations were estimated by extrapolation from animal toxicity or human epidemiology studies using the following basic exposure assumptions: a 70-kilogram male person (Report of the Task Group on Reference Man, International Commission for Radiation Protection, November 23, 1957) as the exposed individual; the average daily consumption of freshwater and

estuarine fish and shellfish products equal to 6.5 grams/day; and the average ingestion of 2 liters/day of water (Drinking Water and Health, National Academy of Sciences, National Research Council, 1977). Criteria based on these assumptions are estimated to be protective of an adult male who experiences average exposure conditions.

Two basic methods were used to formulate health criteria, depending on whether the prominent adverse effect was cancer or other toxic manifestations. The following sections detail these methods.

Carcinogens

Extrapolation of cancer responses from high to low doses and subsequent risk estimation from animal data are performed using a linearized multi-stage model. This procedure is flexible enough to fit all monotonically-increasing dose response data, since it incorporates several adjustable parameters. The multi-stage model is a linear nonthreshold model as was the "one-hit" model originally used in the proposed criteria documents. The linear nonthreshold concept has been endorsed by the four agencies in the Interagency Regulatory Liaison Group and is less likely to underestimate risk at the low doses typical of environmental exposure than other models that could be used. Because of the uncertainties associated with dose response, animal-to-human extrapolation, and other unknown factors; because of the use of average consumptions; and because of the serious public health consequences that could result if risks were underestimated, EPA believes that it is prudent to use conservative methods to

estimate risk in the water quality criteria program. The linearized multistage model is more systematic and invokes fewer arbitrary assumptions than the "one-hit" procedure previously used.

It should be noted that extrapolation models provide estimates of risk since a variety of assumptions are built into any model. Models using widely different assumptions may produce estimates ranging over several orders of magnitude. Since there is at present no way to demonstrate the scientific validity of any model, the use of risk extrapolation models is a subject of debate in the scientific community. However, risk extrapolation is generally recognized as the only tool available at this time for estimating the magnitude of health hazards associated with nonthreshold toxicants and has been endorsed by numerous Federal agencies and scientific organizations, including EPA's Carcinogen Assessment Group, the National Academy of Sciences, and the Interagency Regulatory Liaison Group, as a useful means of assessing the risks of exposure to various carcinogenic pollutants.

Noncarcinogens

Health criteria based on toxic effects of pollutants other than carcinogenicity are estimates of concentrations which are not expected to produce adverse effects in humans. They are based upon Acceptable Daily Intake (ADI) levels and are generally derived using no-observed-adverse-effect-level data from animal studies although human data are used wherever available. The ADI is calculated using safety factors to account for uncertainties

inherent in extrapolation from animal to man. In accordance with the National Research Council recommendations (Drinking Water and Health, National Academy of Sciences, National Research Council, 1977), safety factors of 10, 100, or 1,000 are used, depending on the quality and quantity of data. In some instances extrapolations are made from inhalation studies or limits to approximate a human response from ingestion using the Stokinger-Woodward model (Journal of American Water Works Association, 1958). Calculations of criteria from ADIs are made using the standard exposure assumptions (2 liters of water, 6.5 grams of edible aquatic products, and an average body weight of 70 kg).

APPENDIX C

THE PHILOSOPHY OF THE 1976 WATER QUALITY CRITERIA

Water quality criteria specify concentrations of water constituents which, if not exceeded, are expected to support an organic ecosystem suitable for the higher uses of water. Such criteria are derived from scientific facts obtained from experimental or *in situ* observations that depict organic responses to a defined stimulus or material under identifiable or regulated environmental conditions for a specified time period.

Water quality criteria are not intended to offer the same degree of strategy for survival and propagation at all times to all organisms within a given ecosystem. They are intended not only to protect essential and significant life in water and the direct users of water, but also to protect life that is dependent on life in water for its existence, or that may consume intentionally or unintentionally any edible portion of such life.

The criteria levels for domestic water supply incorporate available data for human health protection. Such values are different from the criteria levels necessary for protection of aquatic life. The Agency's interim primary drinking water regulations (40 Federal Register 59566 December 24, 1975), as required by the Safe Drinking Water Act (42 U.S.C. 300f, et seq.), incorporate applicable domestic water supply criteria. Where pollutants are identified in both the quality criteria for domestic water supply and the Drinking Water Standards, the concentration levels are identical. Water treatment may not significantly affect the removal of certain pollutants.

What is essential and significant life in water? Do Daphnia or stonefly nymphs qualify as such life? Why does 1/100th of a concentration that is lethal to 50 percent of the test organisms (LC50) constitute a criterion in some instances, whereas 1/2 or 1/10th of some effect levels constitutes a criterion in other instances? These are questions that often are asked of those who undertake the task of criteria formulation.

The universe of organisms composing life in water is great in both kinds and numbers. As in the human population, physiological variability exists among individuals of the same species in response to a given stimulus. A much greater response variation exists among species of aquatic organisms. Thus, aquatic organisms do not exhibit the same degree of harm, individually or by species, from a given concentration of a toxicant or potential toxicant within the environment. In establishing a level or concentration of a quality constituent as a criterion it is necessary to ensure a reasonable degree of safety for those more sensitive species that are important to the functioning of the aquatic ecosystem even though data on the response of such species to the quality constituent under consideration may not be available. The aquatic food web is an intricate relationship of predator and prey organisms. A water constituent that may in some way destroy or eliminate an important segment of that food web would, in all likelihood, destroy or seriously impair other organisms associated with it.

Although experimentation relating to the effects of particular substances under controlled conditions began in the

early 1900's, the effects of any substance on more than a few of the vast number of aquatic organisms have not been investigated. Certain test animals have been selected by investigators for intensive investigation because of their importance to man, their availability to the researcher, and their physiological responses to the laboratory environment. As general indicators of organism responses such test organisms are representative of the expected results for other associated organisms. In this context Daphnia or stoneflies or other associated organisms indicate the general levels of toxicity to be expected among untested species. In addition, test organisms are themselves vital 1inks within the food web that results in the fish population in a particular waterway.

The ideal data base for criteria development would consist of information on a large percentage of aquatic species and would show the community response to a range of concentrations for a tested constituent during a long time period. This information is not available but investigators are beginning to derive such information for a few water constituents. Where only 96-hour bioassay data are available, judgmental prudence dictates that a substantial safety factor be employed to protect all life stages of the test organism in waters of varying quality, as well as associated organisms within the aquatic environment that have not been tested and that may be more sensitive to the test constituent. Application factors have been used to provide the degree of protection required. Safe levels for certain chlorinated hydrocarbons and certain heavy metals were estimated by applying an 0.01 application factor to the 96-hour LC50 value

for sensitive aquatic organisms. Flow-through bioassays have been conducted for some test indicator organisms over a substantial period of their life history. In a few other cases, information is available for the organism's natural life or for more than one generation of the species. Such data may indicate a minimal effect level, as well as a no-effect level.

The word "criterion" should not be used interchangeably with or as a synonym for the word '*standard.**The word "criterion" represents a constituent concentration or level associated with a degree of environmental effect upon which scientific judgment may be based. As it is currently associated with the water environment it has come to mean a designated concentration of a constituent that, when not exceeded, will protect an organism, an organism community, or a prescribed water use or quality with an adequate degree of safety. A criterion, in some cases, may be a narrative statement instead of a constituent concentration. On the other hand, a standard connotes a legal entity for a particular reach of waterway or €or an effluent. A water quality standard may use a water quality criterion as a basis for regulation or enforcement, but the standard may differ from a criterion because of prevailing local natural conditions, such as naturally occurring organic acids, or because of the importance of a particular waterway, economic considerations, or the degree of safety to a particular ecosystem that may be desired.

Toxicity to aquatic life generally is expressed in terms of acute (short term) or chronic (long-term) effects. Acute toxicity refers to effects occurring in a short time period:

often death is the end point. Acute toxicity can be expressed as the lethal concentration for a stated percentage of organisms tested, or the reciprocal, which is the tolerance limit of a percentage of surviving organisms, Acute toxicity for aquatic organisms generally has been expressed for 24 to 96-hour exposures.

chronic toxicity refers to effects through an extended time period. Chronic toxicity may be expressed in terms of an observation period equal to the lifetime of an organism or to the time span of more than one generation. Some chronic effects may be reversible, but most are not.

Chronic effects often occur in the species population rather than in the individual. If eggs fail to develop or the sperm does not remain viable, the species would be eliminated from an ecosystem because of reproductive failure. Physiological stress may make a species less competitive with others and may result in a gradual population decline or absence from an area. The elimination of a microcrustacean that serves as a vital food during the larval period of a fish's life could result ultimately in the elimination of the fish from an area. The phenomenon of bioaccumulation of certain materials may result in chronic toxicity to the ultimate consumer in a food chain. Thus, fish may mobilize lethal toxicants from their fatty tissues during periods of physiological stress. Egg shells of predatory birds may be weakened to a point of destruction in the nest. Bird chick embryos may have increased mortality rates. There may be a hazard to the health of man if aquatic organisms with toxic residues are consumed.

The fact that living systems, i.e., individuals, populations, species, and ecosystems, can take up, accumulate, and bioconcentrate manmade and natural toxicants is well documented. In aquatic systems biota are exposed directly to pollutant toxicants through submersion in a relatively efficient solvent (water) and are exposed indirectly through food webs and other biological, chemical, and physical interactions. Initial toxicant levels, if not immediately toxic and damaging, may accumulate in the biota or sediment over time and increase to levels that are lethal or sublethally damaging to aquatic organisms or to consumers of these organisms. Water quality criteria reflect a knowledge of the capacity for environmental accumulation, persistence, and effects of specific toxicants in specific aquatic systems.

Ions of toxic materials frequently cause adverse effects because they pass through the semipermeable membranes of an organism. Molecular diffusion through membranes may occur for some compounds such as pesticides, polychlorinated biphenyls, and other toxicants. Some materials may not pass through membranes in their natural or waste-discharged state, but in water they may be converted to states that have increased ability to affect organisms. For example, certain microorganisms can methylate mercury, thus producing a material that more readily enters physiological systems. Some materials may have multiple effects: for example, an iron salt may not be toxic; an iron floc or gel may be an irritant or clog fish gills to effect asphyxiation; iron at low concentrations can be a trace nutrient

but at high concentrations it can be a toxicant. Materials also can affect organisms if their metabolic byproducts cannot be excreted. Unless otherwise stated, criteria are based on the total concentration of the substance because an ecosystem can produce chemical, physical, and biological changes that may be detrimental to organisms living in or using the water.

In prescribing water quality criteria, certain fundamental principles dominate the reasoning process. In establishing a level or concentration as a criterion for a given constituent it was assumed that other factors within the aquatic environment are acceptable to maintain the integrity of the water. Interrelationships and interactions among organisms and their environment, as well as the interrelationships of sediments and the constituents they contain to the water above, are recognized as fact.

Antagonistic and synergistic reactions among many quality constituents in water also are recognized as fact. The precise definition of such reactions and their relative effects on particular segments of aquatic life have not been identified with scientific precision. Historically much of the data to support criteria development was of an ambient concentration-organism response nature. Recently, data are becoming available on long-term chronic effects on particular species. Studies now determine carcinogenic, teratogenic, and other insidious effects of toxic materials.

Some unpolluted waters in the Nation may exceed designated criteria for particular constituents. There is variability in the natural quality of water and certain organisms become adapted

to that quality, which may be considered extreme in other areas. Likewise, it is recognized that a single criterion cannot identify minimal quality for the protection of the integrity of water for every aquatic ecosystem in the Nation. To provide an adequate degree of safety to protect against long-term effects may result in a criterion that cannot be detected with present analytical tools. In some cases, a mass balance calculation can provide a means of assurance that the integrity of the waterway is not being degraded.

Water quality criteria do not have direct regulatory impact, but they form the basis for judgment in several Environmental Protection Agency programs that are derived from water quality considerations. For example, water quality standards developed by the States under section 303 of the Act and approved by EPA are to be based on the water quality criteria, appropriately modified to take account of local conditions. The local conditions to be considered include actual and projected uses of the water, natural background levels of particular constituents, the presence or absence of sensitive important species, characteristics of the local biological community, temperature and weather, flow characteristics, and synergistic or antagonistic effects of combinations of pollutants.

Similarly, by providing a judgment on desirable levels of ambient water quality, water quality criteria are the starting point in deriving toxic pollutant effluent standards pursuant to section 307(a) of the Act. Other EPA programs that use water quality criteria involve drinking water standards, the ocean

dumping program, designation of hazardous substances, dredge spoil criteria development, removal of in-place toxic materials, thermal pollution, and pesticide registration.

To provide the water resource protection for which they are designed, quality criteria should apply to virtually all of the Nation's navigable waters with modifications for local conditions as needed. To violate quality criteria for any substantial length of time or in any substantial portion of a waterway may result in an adverse affect on aquatic life and perhaps a hazard to man or other consumers of aquatic life.

Quality criteria have been designed to provide long-term protection. Thus, they may provide a basis for effluent standards, but it is not intended that criteria values become effluent standards. It is recognized that certain substances may be applied to the aquatic environment with the concurrence of a governmental agency for the precise purpose of controlling or managing a portion of the aquatic ecosystem: aquatic herbicides and piscicides are examples of such substances. For such occurrences, criteria obviously do not apply. It is recognized further that pesticides applied according to official label instructions to agricultural and forest lands may be washed to a receiving waterway by a torrential rainstorm. Under such conditions it is believed that such diffuse source inflows should receive consideration similar to that of a discrete effluent discharge and that in such instances the criteria should be applied to the principal portion of the waterway rather than to that peripheral portion receiving the diffuse inflow.

The format for presenting water quality criteria includes a concise statement of the dominant criterion or criteria for a particular constituent followed by a narrative introduction, a rationale that includes justification for the designated criterion or criteria, and a listing of the references cited within the rationale. An effort has been made to restrict supporting data to those which either have been published or are in press awaiting publication. A particular constituent may have more than one criterion to ensure more than one water use or condition, i.e., hard or soft water where applicable, suitability as a drinking water supply source, protection of human health when edible portions of selected biota are consumed, provision for recreational bathing or waterskiing, and permitting an appropriate factor of safety to ensure protection for essential warm-or coldwater associated biota.

Criteria are presented for those substances that may occur in water where data indicate the potential for harm to aquatic life, or to water users, or to the consumers of the water or aquatic life. Presented criteria do not represent an all-inclusive list of constituent contaminants. omissions from criteria should not be construed to mean that an omitted quality constituent is either unimportant or non-hazardous.

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QUALITY CRITERIA FOR WATER 1986

UPDATE #1

*ALDRIN-DIELDRIN

CRITERIA:

Aquatic Life

Dieldrin

For dieldrin the criterion to protect freshwater aquatic life as derived using the Guidelines is 0.0019 ug/L as a 24-hour average, and the concentration should not exceed 2.5 ug/L at any time.

For dieldrin the criterion to protect saltwater aquatic life as derived using the Guidelines is 0.0019 ug/L as a 24-hour average, and the concentration should not exceed 0.71 ug/L at any time.

Aldrin

For freshwater aquatic life the concentration of aldrin should not exceed 3.0 ug/L at any time. No data are available concerning the chronic toxicity of aldrin to sensitive freshwater aquatic life.

For saltwater aquatic life the concentration of aldrin should not exceed 1.3 ug/L at any time. No data are available concerning the chronic toxicity of aldrin to sensitive saltwater aquatic life.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to aldrin through ingestion of contaminated water and contaminated aquatic organisms, the

*Indicates suspended, canceled or restricted by U.S. EPA office of Pesticides and Toxic Substances

ambient water concentration should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase'of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6} and 10^{-7} The corresponding recommended criteria are **0.74** ng/L, **0.074** ng/L, and **0.0074** ng/L, respectively. **If** these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are **0.79** ng/L, **0.079** ng/L, and **0.0079** ng/L, respectively.

For the maximum protection of human health from the potential carcinogenic effects of exposure to dieldrin through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6} and 10^{-7}. The corresponding recommended criteria are **0.71** ng/L, 0.071 ng/L, and 0.0071 ng/L, respectively. If these above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are **0.76** ng/L, **0.076** ng/L, and **0.0076** ng/L, respectively.

(45 F.R. 79318, November **28, 1980)**
SEE APPENDIX **B** FOR METHODOLOGY

AMMONIA

SUMMARY:

All concentrations used herein are expressed as un-ionized ammonia (NH_3), because NH_3, not the ammonium ion (NH_4^+) has been demonstrated to be the principal toxic form of ammonia. The data used in deriving criteria are predominantly from flow through tests in which ammonia concentrations were measured. Ammonia was reported to be acutely toxic to freshwater organisms at concentrations (uncorrected for pH) ranging from **0.53** to **22.8** mg/L NH_3 for **19** invertebrate species representing **14** families and **16** genera and from **0.083** to **4.60** mg/L NH_3 for **29** fish species from **9** families and **18** genera. Among fish species, reported **96**-hour LC50 ranged from **0.083** to **1.09** mg/L for salmonids and from **0.14** to **4.60** mg/L NH_3 for nonsalmonids. Reported data from chronic tests on ammonia with two freshwater invertebrate species, both daphnids, showed effects at concentrations (uncorrected for pH) ranging from **0.304** to 1.2 mg/L NH_3, and with nine freshwater fish species, from five families and seven genera, ranging from **0.0017** to **0.612** mg/L NH_3.

Concentrations of ammonia acutely toxic to fishes may cause loss of equilibrium, hyperexcitability, increased breathing, cardiac output and oxygen uptake, and, in extreme cases, convulsions, coma, and death. At lower concentrations ammonia has many effects on fishes, including a reduction in hatching success, reduction in growth rate and morphological development, and pathologic changes in tissues of gills, livers, and kidneys.

Several factors have been shown to modify acute NH_3 toxicity in fresh water. Some factors alter the concentration of un-ionized ammonia in the water by affecting the aqueous ammonia equilibrium, and some factors affect the toxicity of un-ionized ammonia itself, either ameliorating or exacerbating the effects of ammonia. Factors that have been shown to affect ammonia toxicity include dissolved oxygen concentration, temperature, pH, previous acclimation to ammonia, fluctuating or intermittent exposures, carbon dioxide concentration, salinity, and the presence of other toxicants.

The most well-studied of these is pH; the acute toxicity of NH_3 has been shown to increase as pH decreases. Sufficient data exist from toxicity tests conducted at different pH values to formulate a mathematical expression to describe pH-dependent acute NH_3 toxicity. The very limited amount of data regarding effects of pH on chronic NH_3 toxicity also indicates increasing NH_3 toxicity with decreasing pH, but the data are insufficient to derive a broadly applicable toxicity/pH relationship. Data on temperature effects on acute NH_3 toxicity are limited and somewhat variable, but indications are that NH_3 toxicity to fish is greater as temperature decreases. There is no information available regarding temperature effects on chronic NH_3 toxicity.

Examination of pH and temperature-corrected acute NH_3 toxicity values among species and genera of freshwater organisms showed that invertebrates are generally more tolerant than fishes, a notable exception being the fingernail clam. There is no clear trend among groups of fish; the several most sensitive

tested species and genera include representatives from diverse families (Salmonidae, Cyprinidae, Percidae, and Centrarchidae). Available chronic toxicity data for freshwater organisms also indicate invertebrates (cladocerans, one insect species) to be more tolerant than fishes, again with the exception of the fingernail clam. When corrected for the presumed effects of temperature and pH, there is also no clear trend among groups of fish for chronic toxicity values, the most sensitive species including representatives from five families (Salmonidae, Cyprinidae, Ictaluridae, Centrarchidae, and Catostomidae) and having chronic values ranging by not much more than a factor or two. The range of acute-chronic ratios for 10 species from 6 families was 3 to 43, and acute-chronic ratios were higher for the species having chronic tolerance below the median. Available data indicate that differences in sensitivities between warm and coldwater families of aquatic organisms are inadequate to warrant discrimination in the national ammonia criterion between bodies of water with "warm" and "coldwater" fishes: rather, effects of organism sensitivities on the criterion are most appropriately handled by site-specific criteria derivation procedures.

Data for concentrations of NH_3 toxic to freshwater phytoplankton and vascular plants, although limited, indicate that freshwater plant species are appreciably more tolerant to NH_3 than are invertebrates or fishes. The ammonia criterion appropriate for the protection of aquatic animals will therefore in all likelihood be sufficiently protective of plant life.

Available acute and chronic data for ammonia with saltwater organisms are very limited, and insufficient to derive a saltwater criterion. A few saltwater invertebrate species have been tested, and the prawn Macrobrachiurn rosenbergii was the most sensitive. The few saltwater fishes tested suggest greater sensitivity than freshwater fishes. Acute toxicity of NH_3 appears to be greater at low pH values, similar to findings in freshwater. Data for saltwater plant species are limited to diatoms, which appear to be more sensitive than the saltwater invertebrates for which data are available.

More quantitative information needs to be published on the toxicity of ammonia to aquatic life. Several key research needs must be addressed to provide a more complete assessment of ammonia toxicity. These are: (1) acute tests with additional saltwater fish species and saltwater invertebrate species; (2) life-cycle and early life-stage tests with representative freshwater and saltwater organisms from different families, with particular attention to trends of acute-chronic ratios; (3) fluctuating and intermittent exposure tests with a variety of species and exposure patterns; (4) more complete tests of the individual and combined effects of pH and temperature, especially for chronic toxicity; (5) more histopathological an6 histochenical research with fishes, which would provide a rapid means of identifying and quantifying sublethal ammonia effects; and (6) studies on effects of dissolved and suspended solids on acute and chronic toxicity.

NATIONAL CRITERIA:

The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if:

(1) the 1-hour* average concentration of un-ionized ammonia (in mg/L NH_3) does not exceed, more often than once every 3 years on the average, the numerical value given by 0.52/FT/FPH/2,

where:

$$FT = 10^{0.03\ 20-TCAP)}; \quad TCAP \leq T \leq 30$$

$$10^{0.03\ 20-T)}; \quad 0 \leq T \leq TCAP$$

$$FPH = 1 \quad ; \quad 8 < pH < 9$$

$$\frac{1+10^{7.4-pH}}{1.25} \quad ; \quad 6.5 \leq pH \leq 7.7$$

TCAP = 20 C; Salmonids or other sensitive coldwater species present

= 25 C; Salmonids and other sensitive coldwater species absent

(*An averaging period of 1 hour may not be appropriate if excursions of concentrations to greater than 1.5 times the average occur during the hour; in such cases, a shorter averaging period may be needed.)

(2) the 4-day average concentration of un-ionized ammonia (in mg/L NH_3) does not exceed, more often than once every 3 years on the average, the average* numerical value given by 0.80/FT/FPH/RATIO, where FT and FPH are as above and:

$$\text{RATIO} = 16 \qquad ; 7.7 \leq pH \leq 9$$

$$= 24 \; \frac{10^{7.7-pH}}{1+10^{7.4pH}} \qquad ; 6.5 \leq pH \leq 7.7$$

TCAP = 15 C; Salmonids or other sensitive coldwater species present

= 20 C; Salmonids and other sensitive coldwater species absent

(*Because these formulas are nonlinear in pH and temperature, the criterion should be the average of separate evaluations of the formulas reflective of the fluctuations of flow, pH, and temperature within the averaging period; it is not appropriate in general to simply apply the formula to average pH, temperature, and flow.)

The extremes for temperature (0, 30) and pH (6.5, 9) given in the above formulas are absolute. It is not permissible with current data to conduct any extrapolations beyond these limits. In particular, there is reason to believe that appropriate criteria at pH > 9 will be lower than the plateau between pH 8 and 9 given above.

Criteria concentrations for the pH range 6.5 to 9.0 and the temperature range 0 C to 30 C are provided in the following tables. Total ammonia concentrations equivalent to each un-ionized ammonia concentration are also provided in these tables. There are limited data on the effect of temperature on chronic toxicity. EPA will be conducting additional research on the effects of temperature on ammonia toxicity in order to fill perceived data gaps. Because of this uncertainty, additional site-specific information should be developed before these

criteria are used in wasteload allocation modeling. For example, the chronic criteria tabulated for sites lacking salmonids are less certain at temperatures much below 20 C than those tabulated at temperatures near 20 C. Where the treatment levels needed to meet these criteria below 20 C may be substantial, use of site-specific criteria is strongly suggested. Development of such criteria should be based upon site-specific toxicity tests.

Data available for saltwater species are insufficient to derive a criterion for saltwater.

The recommended exceedence frequency of 3 years is the Agency's best scientific judgment of the average amount of time it will take an unstressed system to recover from a pollution event in which exposure to ammonia exceeds the criterion. A stressed system, for example, one in which several outfalls occur in a limited area, would be expected to require more time for recovery. The resilience of ecosystems and their ability to recover differ greatly, however, and site-specific criteria may be established if adequate justification is provided.

The use of criteria in designing waste treatment facilities requires the selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria. Limited data or other factors may make their use impractical, in which case one should rely on a steady-state model. The Agency recommends the interim use of 1Q5 or 1Q10 for Criterion Maximum Concentration design flow and 7Q5 or 7Q10 for the Criterion Continuous Concentration design flow in steady-state models for unstressed and stressed systems respectively.

12) 4-day average concentrations for ammonia.*

pH	0 C	5 c	10 C	15 C	20 C	25 C	30 C
A. Salmonids or Other Sensitive Coldwater Species Present							
Un-ionized Ammonia (mg/liter NH_3)							
6.50	0.0007	0.0009	0.0013	0.0019	0.0019	0.0019	0.0019
6.75	0.0012	0.0017	0.0023	0.0033	0.0033	0.0033	0.0033
7.00	0.0021	0.0029	0.0042	0.0059	0.0059	0.0059	0.0059
7.25	0.0037	0.0052	0.0074	0.0105	0.0105	0.0105	0.0105
7.50	0.0066	0.0093	0.0132	0.0186	0.0186	0.0186	0.0186
7.75	0.0105	0.01 53	0.022	0.03 1	0.03 I	0.031	0.031
8.00	0.0126	0.0177	0.025	0.035	0.035	0.035	0.035
8.25	0.0126	0.0177	0.025	0.035	0.035	0.035	0.035
8.50	0.0126	0.0177	0.025	0.035	0.035	0.035	0.035
8.75	0.0126	0.0177	0.025	0.035	0.035	0.035	0.035
9.00	0.0126	0.0177	0.025	0.035	0.035	0.035	0.035
Total Ammonia (mg/liter NH_3)							
6.50	2.5	2.4	2.2	2.2	1.49	I.04	0.73
6.75	2.5	2.4	2.2	2.2	1.49	1.04	0.73
7.00	2.5	2.4	2.2	2.2	149	1.04	Q.74
7.25	2.5	2.4	2.2	2.2	I.50	1.04	0.74
7.50	2.5	2.4	2.2	2.2	I.%	1.05	0.74
7.75	2.3	2.2	2.1	2.0	1.40	0.99	0.71
8.00	1.53	I.44	1.37	1.33	0.93	0.66	0.47
8.25	0.87	0.82	0.70	0.76	054	0.39	0.28
8.50	0.49	0.47	0.45	0.44	0.32	0.23	0.17
8.75	0.28	0.27	0.26	0.27	0.19	0.15	0.11
9.00	0.16	0.16	0.16	0.16	0.13	0.10	0.0C
B. Salmonids and Other Sensitive Coldwater Species Absent†							
Un-ionized Ammonia (mg/liter NH_3)							
6.50	0.0007	0.0009	0.0013	0.0019	0.0026	0.0026	0.0026
6.75	0.0012	0.0017	0.0023	0.0033	0.0047	0.0047	0.0047
7.00	0.0021	0.0029	0.0042	0.0059	0.0083	0.0083	0.0083
7.25	0.0037	0.0052	0.0074	0.0105	0.0148	0.0148	0.0148
7.50	0.0066	0.0093	0.0132	0.0186	0.026	0.026	0.026
7.75	0.0109	0.0153	0.022	0.03 1	0.043	0.043	0.043
8.00	0.0126	0.0177	0.025	0.035	0.050	0.050	0.050
8.25	0.0126	0.0177	0.025	0.035	0.050	0.050	0.050
8.50	0.0126	0.0177	0.025	0.035	0.050	0.050	0.050
8.75	0.0120	0.0177	0.025	0.035	0.050	0.050	0.050
9.00	0.0126	0.0177	0.025	0.035	0.050	0.050	0.050
Total Ammonia (mg/liter NH_3)							
6.50	2.5	2.4	2.2	2.2	21	1.46	I.03
6.75	2.5	2.4	2.2	2.2	2 1	I.47	1-04
7.00	2.5	2.4	2.2	2.2	2.1	I.47	1.04
7.25	2.5	2.4	2.2	2.2	2 1	1.48	1.05
7.50	2.5	2.4	2.2	2.2	21	1.49	1.06
7.75	2.3	2.2	2.1	2.0	I.98	1.39	I.00
8.00	1.53	1.44	1.37	1.33	1.31	0.93	0.67
8.25	0.87	0.82	0.78	0.76	0.76	0.54	0.40
8.50	0.49	0.47	0.45	0.44	0.45	0.33	0.25
8.75	0.28	0.27	0.26	0.27	0.27	0.21	0.16
9.00	0.16	0.16	0.16	0.16	0.17	0.14	0.11

* To convert these values to mg/liter N, multiply by 0.822.

† Site-specific criteria development Is strongly suggested at temperatures above 20 C because of the limited data available to generate tho criteria recommendation, and at temperatures below 20 C because of the limited data and because small changes In the criteria may have significant impact on the level of treatment required In meeting tho recommended criteria.

(1) One-hour average concentrations for ammonia.*

pH	0 C	5 c	10 C	15 C	20 C	25 C	30 C
A. Salmonids or Other Sensitive Coldwater Species Present							
Un-ionized Ammonia (mg/liter NH_3)							
6.50	0.0091	0.0129	0.0182	0.026	0.036	0.036	0.036
6.75	0.0149	0.021	0.030	0.042	0.059	0.059	0.059
7.00	0.023	0.033	0.046	0.066	0.093	0.093	0.093
7.25	0.034	0.048	0.068	0.095	0.135	0.135	0.135
7.50	0.045	0.064	0.091	0.128	0.181	0.181	0.181
7.75	0.056	0.080	0.113	0.159	0.22	0.22	0.22
8.00	0.065	0.092	0.130	0.184	0.26	0.26	0 3 6
8.25	0.065	0.092	0.130	0.184	0.26	0.26	0.26
8.50	0.065	0.092	0.130	0.184	0.26	0.26	0.26
8.75	0.065	0.092	0.130	0.184	0.26	0.26	0.26
9.00	0.065	0.092	0.130	0.184	0 3 6	0.26	0.26
Total Ammonia (mg/liter NH_3)							
6.50	35	33	31	30	29	20	14.3
6.75	32	30	28	27	27	18.6	13.2
7.00	28	26	25	24	23	16.4	11.6
7.25	23	22	20	19.7	19.2	13.4	9.5
7.50	17.4	16.3	15.5	14.9	14.6	10.2	7.3
7.75	12.2	11.4	10.9	10.5	10.3	7.2	5.2
8.00	8.0	7.5	7.1	6.9	6.8	4.8	3.5
8.25	4.5	4.2	4.1	4.0	3.9	2.8	2.1
8.50	26	2.4	2.3	2.3	2.3	1.71	1.28
8.75	1.47	1.40	1.37	1.38	1.42	1.07	0.83
9.00	0.86	0.83	0.83	0.86	0.91	0.72	0.58
B. Salmonids and Other Sensitive Coldwater Species Absent							
Un-ionized Ammonia (mg/liter NH_3)							
6.50	0.009 1	0.0129	0.0182	0.026	0.036	0.051	0.051
6.75	0.0149	0.021	0.030	0.042	0.059	0.084	0.084
7.00	0.023	0.033	0.046	0.066	0.093	0.131	0.131
7.25	0.034	0.046	0.068	0.095	0.135	0.190	0.190
7.50	0.045	0.064	0.091	0.128	0.181	0.26	0.26
7.75	0.056	0.080	0.113	0.159	0.22	0.32	0.32
8.00	0.065	0.092	0.130	0.184	0.26	0.37	0.37
8.25	0.065	0.092	0.130	0.184	0.26	0.37	0.37
8.50	0.065	0.092	0.130	0.184	0.26	0.37	0.37
8.75	0.065	0.092	0.130	0.184	0.26	0.37	0.37
9.00	0.065	0.092	0.130	0.184	0.26	0.37	0.37
Total Ammonia (mg/liter NH_3)							
6.50	35	33	31	30	29	29	20
6.75	32	30	28	27	27	26	18.6
7.00	28	26	25	24	23	23	16.4
7.25	23	22	20	19.7	19.2	19.0	13.5
7.50	17.4	16.3	15.5	14.9	14.6	14.5	10.3
7.75	12.2	11.4	10.9	10.5	10.3	10.2	7.3
8.00	8.0	7.5	7.1	6.9	6.8	6.8	4.9
0.25	4.5	4.2	4.1	4.0	3.9	4.0	2.9
8.50	2.6	2.4	23	2.3	2.3	2.4	1.81
8.75	1.47	1.40	1.37	1.38	1.42	1.52	1.18
9.00	0.86	0.83	0.83	0.86	0.91	1.01	0.82

* To convert these values to mg/liter N, multiply by 0.822.

The Agency acknowledges that the Criterion Continuous Concentration stream flow averaging period used for steady-state wasteload allocation modeling may be as long as 30 days in situations involving POTWs designed to remove ammonia where limited variability of effluent pollutant concentration and resultant concentrations in receiving waters can be demonstrated. In cases where low variability can be demonstrated, longer averaging periods for the ammonia Criterion Continuous Concentration (e.g., 30-day averaging periods) would be acceptable because the magnitude and duration of exceedences above the Criterion Continuous Concentration would be sufficiently limited. These matters are discussed in more detail in the Technical Support Document for Water Quality-Based Toxics Control (U.S. EPA, 1985a).

(50 F.R. 30784, July 29, 1985)
SEE APPENDIX A FOR METHODOLOGY

BERYLLIUM

CRITERIA:

Aquatic Life

The available data for beryllium indicate that acute and chronic toxicity to freshwater aquatic life occur at concentrations as low as 130 and 5.3 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested. Hardness has a substantial effect on acute toxicity.

The limited saltwater data base available for beryllium does not permit any statement concerning acute or chronic toxicity.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to beryllium through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero, based on the non threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 68 ng/L, 6.8 ng/L, and 0.68 ng/L, respectively. If these estimates are made for

consumption of aquatic organisms only, excluding consumption of water, the levels are 1170 ng/L, 117.0 ng/L, and 11.71 ng/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

BORON

CRITERION:

750 ug/L for long-term irrigation on sensitive crops.

INTRODUCTION:

Boron is not found in its elemental form in nature: it is usually found as a sodium or calcium borate salt. Boron salts are used in fire retardants, the production of glass, leather tanning and finishing industries, cosmetics, photographic materials, metallurgy and for high energy rocket fuels. Elemental boron also can be used in nuclear reactors for neutron absorption. Borates are used as "burnable" poisons.

RATIONALE:

Boron is an essential element for growth of plants but there is no evidence that it is required by animals. The maximum concentration found in 1,546 samples of river and lake waters from various parts of the United States was 5.0 mg/L; the mean value was 0.1 mg/L (Kopp and Kroner, 1967). Ground waters could contain substantially higher concentrations at certain places. The concentration in seawater is reported as 4.5 mg/L in the form of borate (NAS, 1974). Naturally occurring concentrations of boron should have no effects on aquatic life.

The minimum lethal dose for minnows exposed to boric acid at 20 °C for 6 hours was reported to be 18,000 to 19,000 mg/L in distilled water and 19,000 to 19,500 mg/L in hard water (Le Clerc and Devlaminck, 1955: Le Clerc, 1960).

In the dairy cow, 16 to 20 g/day of boric acid for 40 days

produced no ill effects (McKee and Wolf, 1963).

Sensitive crops have shown toxic effects at 1000 ug/L or less of boron (Richards, 1954). Bradford (1966), in a review of boron deficiencies and toxicities, stated that when the boron concentration in irrigation waters was greater than 0.75 ug/L, some sensitive plants such as citrus began to show injury. Biggar and Fireman (1960) showed that with neutral and alkaline soils of high absorption capacities, water containing 2 ug/L boron might be used for some time without injury to sensitive plants. The criterion of 750 ug/L is thought to protect sensitive crops during long-term irrigation.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOLOGY

CHLORINATED BENZENES

CRITERIA

Aquatic Life

The available data for chlorinated benzenes indicate that acute toxicity to fresh water aquatic life at concentrations as low as 250 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of the more toxic of the chlorinated benzenes to sensitive freshwater aquatic life but toxicity occurs at concentrations as low as 50 ug/L for a fish species exposed for 7.5 days.

The available data for chlorinated benzenes indicate that acute and chronic toxicity to saltwater aquatic life occur at concentrations as low as 160 and 129 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

Human Health

For comparison purposes, two approaches were used to derive criterion levels for monochlorobenzene. Based on available toxicity data, for the protection of public health, the derived level is 488 ug/L. Using available organoleptic data, for controlling undesirable taste and odor quality of ambient water, the estimated level is 20 ug/L. It should be recognized that organoleptic data as a basis for establishing a water quality criteria have limitations and have no demonstrated relationship to potential adverse human health effects.

Trichlorobenzenes

Due to the insufficiency in the available information for the trichlorobenzenes, a criterion cannot be derived at this time using the present guidelines.

1,2,4,5-Tetrachlorobenzene

For the protection of human health from the toxic properties of 1,2,4,5-tetrachlorobenzene ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 38 ug/L.

For the protection of human health from the toxic properties of 1,2,4,5-tetrachlorobenzene ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 48 ug/L.

Pentachlorobenzene

For the protection of human health from the toxic properties of pentachlorobenzene ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 74 ug/L.

For the protection of human health from the toxic properties of pentachlorobenzene ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 85 ug/L.

Hexachlorobenzene

For the maximum protection of human health from the potential carcinogenic effects due to exposure of hexachlorobenzene through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero based on the non-threshold assumption for this chemical. However, zero

level may not be attainable at the present time. Therefor, the levels which may result in incramental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 7.2 ng/L, 0.72 ng/L, and 0.072 ng/L, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 7.4 ng/L, 0.74 ng/L and 0.074. ng/L respectively.

(45 F.R. 79316, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

DICHLOROPROPANES/DICHLOROPROPENES

CRITERIA:

Aquatic Life

The available data for dichloropropanes indicate that acute and chronic toxicity to freshwater aquatic life occurs at concentrations as low as 23,000 and 5,700 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for dichloropropene indicate that acute and chronic toxicity to freshwater aquatic life occurs at concentrations as low as 6,060 and 244 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for dichloropropane indicate that acute and chronic toxicity to saltwater aquatic life occur at concentrations as low as 10,300 and 3,040 ug/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for dichloropropene indicate that acute toxicity to saltwater aquatic 1ife occurs at concentrations as low as 790 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of dichloropropene to sensitive saltwater aquatic life.

Human Health

Using the present guidelines, a satisfactory criterion cannot be derived at this time because of insufficient avaiiable data for dichloropropanes.

For the protection of human health from the toxic properties of dichloropropenes ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 87 ug/L.

For the protection of human health from the toxic properties of dichloropropenes ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 14.1 mg/L.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B €OR METHODOLOGY

*ENDRIN

CRITERIA:

Aquatic Life

For endrin the crit rion to protect fr shwater aquatic li e as derived using the Guidelines is 0.0023 ug/L as a 24-hour average, and the concentration should not exceed 0.18 ug/L at any time.

For endrin the criterion to protect saltwater aquatic life as derived using the Guidelines is 0.0023 ug/L as a 24-hour average, and the concentration should not exceed 0.037 ug/L at any time.

Human Health

The ambient water quality criterion for endrin is recommended to be identical to the existing water standard which is 1.0 ug/L. Analysis of the toxic effects data resulted in a calculated level which is protective of human health against the ingestion of contaminated water and contaminated aquatic organisms. The calculated value is comparable to the present standard. For this reason a selective criterion based on exposure solely from consumption of 6.5 g of aquatic organisms was not derived.

*Indicates suspended, canceled or restricted by U.S. EPA Office of Pesticides and Toxic Substances

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

HEPTACHLOR

CRITERIA:

Aquatic Life

For heptachlor the criterion to protect freshwater aquatic life as derived using the Guidelines is 0.0038 ug/L as a 24-hour average, and the concentration should not exceed 0.52 ug/L at any time.

For heptachlor the criterion to protect saltwater aquatic life as derived using the Guidelines is 0.0036 ug/L as a 24-hour average, and the concentration should not exceed 0.053 ug/L at any time.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to heptachlor through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero, based on the non threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 2.78 ng/L, 0.28 ng/L, and 0.026 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 2.85 ng/L, 0.29 ng/L, and 0.029 ng/L, respectively.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

HEXACHLOROCYCLOHEXANE

CRITERIA:

Aquatic Life

Lindane

For lindane the criterion to protect freshwater aquatic life as derived using the Guidelines is 0.080 ug/L **as a** 24-hour average and the concentration should not exceed ***2.0*** ug/L at any time.

For saltwater aquatic life the concentration of lindane should not exceed 0.16 ug/L at any time. No data are available concerning the chronic toxicity of lindane to sensitive saltwater aquatic life.

BHC

The available data for a mixture of isomers of **BHC** indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 100 ug/L and would occur at lower concentrations anong species that are more sensitive than those tested. No data are available concerning the chronic toxicity of a mixture of isomers of **BHC** to sensitive freshwater aquatic life.

The available data for a mixture of isomers of BHC indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 0.34 ug/L and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of a mixture of isomers of **BHC** to sensitive saltwater aquatic life.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to alpha-hexachlorocyclohexane through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10-6 and 10^{-7}. The corresponding recommended criteria are 92 ng/L, 9.2 ng/L, and .92 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 310 ng/L, 31.0 ng/L, and 3.10 ng/L, respectively.

For the maximum protection of human health from the potential carcinogenic effects of exposure to beta-hexachlorocyclohexane through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 163 ng/L, 16.3 ng/L, and 1.63 ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 547 ng/L, 54.7 ng/L, and 5.47 ng/L, respectively.

For the maximum protection of human health from the potential carcinogenic effects due to exposure of gama-hexachlorocyclohexane through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chenical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are **186** ng/L, **18.6** ng/L, and **1.86** ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are **625** ng/L, **62.5** ng/L, and **6.25** ng/L, respectively.

For the maximum protection of human health from the potential carcinogenic effects of exposure to technical-hexachlorocyclohexane through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero, based on the nonthreshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are **123** ng/L, **12.3** ng/L, and **1.23** ng/L, respectively. If these estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are **414** ng/L, **41.4** ng/L, and **4.14** ng/L, respectively.

Using the present guidelines, satisfactory criteria cannot be derived at this tine for delta and epsilon hexachlorocyclohexane because of insufficient available data.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

MIREX

CRITERION:

0.001 ug/L for freshwater and marine aquatic life.

RATIONALE:

Mirex is used to control the imported fire ant Solenopsis saevissima richteri in the southeastern United States. Its use is essentially limited to the control of this insect and it is always presented in bait. In the most common formulation, technical grade mirex is dissolved in soybean oil and sprayed on corncob grits. The bait produced in this manner consists of 0.3 percent mirex, 14.7 percent soybean oil and 85 percent corncob grits. The mirex bait often is applied at a rate of 1.4 kg/ha, equivalent to 4.2 grams of toxicant per hectare.

Relatively few studies have been made of the effects of mirex on freshwater invertebrates of 'these, only Ludke et al. (1971) report chemical analyses of mirex in the water. Their study reported effects on two crayfish species exposed to mirex by three techniques. First, field-collected crayfish were exposed to several sublethal concentrations of technical grade mirex solutions for various periods of time: second, crayfish were exposed to mirex leached from bait (0.3 percent active ingredient); and third, the crayfish were fed mirex bait.

Procambarus blandingi juveniles were exposed to 1 or 5 ug/L for 6 to 144 hours, transferred to clean water and observed for 10 days. After 5 days in clean water, 95 percent of the animals exposed to 1 ug/L for 144 hours were dead. Exposure to 5 ug/L for 6, 24, and 58 hours resulted in 26, 50, and 98 percent mortality 10 days after transfer to clean water. Crayfish,

Procambarus hayi, were exposed to 0.1 and 0.5 ug/L for 48 hours. Four days after transfer to clean water, 65 percent of the animals exposed to 0.1 ug/L were dead. At the 0.5 ug/L concentration, 71 percent of the animals were dead after 4 days in clean water. Tissue residue accumulations (wet weight basis) ranged from 940- to 27,210-fold above water concentrations. In leached bait experiments, 10 bait particles were placed in 2 liters of water but isolated from 20 juvenile crayfish. Thirty percent of the crayfish were dead in 4 days and 95 percent were dead in 7 days. Water analysis indicated mirex concentrations of 0.86 ug/L. In feeding experiments, 108 crayfish each were fed one bait particle. Mortality was noticed on the first day after feeding, and by the sixth day 77 percent were dead. In another experiment, all crayfish were dead 4 days after having been fed 2 bait particles each. From this report it is obvious that mirex is extremely toxic to these species of crayfish. Mortality and accumulation increase with time of exposure to the insecticide. Concentrations as low as 0.1 ug/L or the ingestion of one particle resulted in death.

Research to determine effects of mirex on fish has been concentrated on species which have economic and sport fishery importance. Hyde et al. (1974) applied mirex bait (0.3 percent mirex) at the standard rate (1.4 kg/ha) in four ponds containing channel catfish, *Ictalurus punctatus*. Three applications were made over an 8-month period with the first application 8 days after fingerling (average weight 18.4 g) catfish were placed in the ponds. Fish were collected at each subsequent application

(approximately 4-month intervals). Two and one half months after the final application, the ponds were drained, all fish were measured and weighed, and the percent survival was calculated. Mirex residues in the fish at termination of the experiment ranged from 0.015 ug/g (ppm) in the fillet to 0.255 ug/g in the fat.

In another study, Van Valin et al. (1968) exposed bluegills, Lepomis macrochirus, and the goldfish, Carassius auratus, to mirex by feeding a mirex-treated diet (1, 3, and 5 mg mirex per kg body weight) or by treating holding ponds with mirex bait (1.3, 100, and 1000 ug/L computed water concentration). They reported no mortality or tissue pathology for the bluegills: however, after 56 days of exposure, gill breakdown in goldfish was found in the 100 and 1000 ug/L contact exposure ponds, and kidney breakdown was occurring in the 1000 ug/L ponds. Mortality in the feeding experiments was not related to the level of exposure, although growth of the bluegills fed 5 ug/L mirex was reduced.

In laboratory and field test systems, reported concentrations of mirex usually are between 0.5 and 1.0 ug/L (Van Valin et al. 1968: Ludke et al. 1971). Although mirex seldom is found above 1 ug/L in the aquatic environment, several field studies have shown that the insecticide is accumulated through the food chain. Borthwick et al. (1973) reported the accumulation of mirex in South Carolina estuaries. Their data revealed that mirex was transported from treated land and marsh to the estuary animals and that accumulation, especially in predators, occurred. In the test area, water samples consistently were less than 0.01 ug/L.

Residues in fish varied from non-detectable to 0.8 ug/g with 15 percent of the samples containing residues. The amount of mirex and the percent of samples containing mirex increased at higher trophic levels. Fifty-four percent of the raccoons sampled contained mirex residues up to 4.4 ug/g and 78 percent of the birds contained residues up to 17 ug/g. Nagvi and de la Cruz (1973) reported average residues for molluscs (0.15 ug/g), fish (0.26 ug/g), insects (0.29 ug/g), crustaceans (0.44 ug/g) and annelids (0.63 ug/g). They also reported that mirex was found in areas not treated with mirex which suggests movement of the pesticide in the environment. Wolfe and Norment (1973) sampled an area for one year following an aerial application of mirex bait (2.1 g mirex/ha). Crayfish residues ranged from 0.04 to 0.16 ug/g. Fish residues were about 2 to 20 times greater than the controls and averaged from 0.01 to 0.76 ug/g. Kaiser (1974), reported the presence of mirex in fish from the Bay of Quinte, Lake Ontario, Canada. Concentrations range from 0.02 ug/g in the gonads of the northern long nose gar, Lepistosteus osseus, to 0.05 ug/g in the post-anal fin of the northern pike, Esox lucius. Mirex has never been registered for use in Canada.

Mirex does not appear to be greatly toxic to birds, with LC50's for the young of four species ranging from 547 to greater than 1667 ug/g (Heath et al. 1972). Long-term dietary dosages caused no adverseeffect at 3 ug/g with mallards and 13 ug/g with pheasants (Heath and Spann, 1973). However, it has been reported (Stickel et al. 1973) that the persistence of mirex in bird tissue exceeds that of all organochlorine compounds tested except

for DDE. Delayed mortality occurred among birds subjected to doses above expected environmental concentration.

A summary examination of the data available at this time shows a mosaic of effects. Crayfish and channel catfish survival is affected by mirex in the water or by ingestion of the bait particles. Bioaccumulation is well established for a wide variety of organisms but the effect of this bioaccumulation on the aquatic ecosystem is unknown, There is evidence that mirex is very persistent in bird tissue. Considering the extreme toxicity and potential for bioaccumulation, every effort should be made to keep mirex bait particles out of water containing aquatic organisms and water concentrations should not exceed 0.001 ug/L mirex. This value is based upon an application factor of 0.01 applied to the lowest levels at which effects on crayfish have been observed.

Data upon which to base a marine criterion involve several estuarine and marine crustaceans. A concentration of 0.1 ug/L technical grade mirfx in flowing seawater was lethal to juvenile pink shrimp, Penaeus durorarum, in a 3-week exposure (Lowe et al. 1971). In static tests with larval stages (megalopal) of the mud crab, Rhithropanopeus harrisii, reduced survival was observed in 0.1 ug/L mirex (Bookhout et al. 1972). In three of four 28-day seasonal flow-through experiments, Tagatz et al. (1975) found reduced survival of Callinectes sapidus, Penaeus durorarum, and grass shrimp, Palaemonetes pugio, at levels of 0.12 ug/L in summer, 0.06 ug/L in fall and 0.09 ug/L in winter.

Since two reports, Lowe et al. (1971) and Bookhout et al. (1972), stated that effects of mirex on estuarine and marine

crustaceans were observed only after considerable time had elapsed, it seems reasonable that length of exposure is an important consideration for this chemical. This may not be the case in fresh water since the crayfish were affected within 48 hours. Therefore, a 3- to 4-week exposure night be considered "acute" and by applying an application factor of 0.01 to a reasonable average of toxic-effect levels as summarized above, a recommended marine criterion of 0.001 ug/L results.

(QUALITY CRITERIA FOR WATER, JULY 1976) PB-263943
SEE APPENDIX C FOR METHODOMGY

NICKEL

CRITERIA:

Aquatic Life

For total recoverable nickel the criterion (in ug/L) to protect freshwater aquatic life as derived using the Guidelines is the numerical value given by $e^{(0.76[\ln(\text{hardness})]+1.06)}$ as a 24-hour average, and the concentration (in ug/L) should not exceed the numerical value given by $e^{(0.76[\ln(\text{hardness})]+4.02)}$ at any time. For example, at hardnesses of 50, 100, and 200 mg/L as $CaCO^3$ the criteria are 56, 96, and 160 ug/L, respectively, as 24-hour averages, and the concentrations should not exceed 1,100, 1,800, and 3,100 ug/L, respectively, at any time.

For total recoverable nickel the criterion to protect saltwater aquatic life as derived using the Guidelines is 7.1 ug/L as a 24-hour average, and'the concentration should not exceed 140 ug/L at any time.

Human Health

For the protection of human health from the toxic properties of nickel ingested through water and contaminated aquatic organisms, the ambient water criterion is determined to be 13.4 ug/L.

For the protection of human health from the toxic properties of nickel ingested through contaminated aquatic organisms alone, the ambient water criterion is determined to be 100. ug/L.

(45 F.R. 79318, November 28, 1980)
SEE APPENDIX B FOR METHODOLOGY

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

CRITERIA:

Aquatic Life

Not enough data are available concerning the effects of 2,3,7,8-TCDD on aquatic life and its uses to allow derivation of national criteria. The available information indicates that acute values for some freshwater animal species are >1.0 ug/L; some chronic values are <0.01 ug/L; and the chronic value for rainbow trout is <0.001 ug/L. Because exposures of some species of fishes to 0.01 ug/L for <6 days resulted in substantial mortality several weeks later, derivation of aquatic life criteria for 2,3,7,8-TCDD may require special consideration. Predicted bioconcentration factors (BCFs) for 2,3,7,8-TCDD range from 3,000 to 900,000, but the available measured BCFs range from 390 to 13,000. If the BCF is 5,000, concentrations >0.00001 ug/L should result in concentrations in edible freshwater and saltwater fish and shellfish that exceed levels identified in a U.S. FDA health advisory. If the BCF is >5,000 or if uptake in a field situation is greater than that in laboratory tests, the value of 0.00001 ug/L will be too high.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of 2,3,7,8-TCDD exposure through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero. This criterion is

based on the nonthreshold assumption for 2,3,7,8-TCDD. However, zero may not be an attainable level at this time. Therefore, the levels that may result in an increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 1.3×10^{-7}, 1.3×10^{-8} and 1.3×10^{-9} ug/L, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 1.4×10^{-7}, 1.4×10^{-8} and 1.4×10^{-9} ug/L, respectively. If these estimates are made for comsumption of water only, the levels are 2.2×10^{-6}, 2.2×10^{-7} and 2.2×10^{-8} ug/L, respectively.

(49 **F.R.** 5831, February 15, 1984)
SEE **APPENDIX** B €OR METHODOLOGY

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WATER QUALITY CRITERIA SUMMARY

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF WATER REGULATIONS AND STANDARDS
STANDARDS BRANCH (WH-585)
401 M STREET S.W.
WASHINGTON, DC 20460

update 1.0

September 2. 1986

	PRIORITY	TYPE	CONCENTRATIONS IN ug/L: FRESH ACUTE CRITERIA	FRESH CHRONIC CRITERIA	MARINE ACUTE CRITERIA	MARINE CHRONIC CRITERIA	UNITS PER LITER: WATER AND FISH INGESTION	FISH CONSUMPTION ONLY	DRINKING WATER M.C.L.	DATE/REFERENCE	# OF STATES WITH AQUATIC LIFE STANDARD
ACENAPTHENE	Y	N	*1,700.	*520.	*970.	*710.				1980 FR	1
ACROLEIN	Y	N	*68.	*21.	*55.		320.ug	780.ug		1980 FR	1
ACRYLONITRILE	Y	Y	*7,550.	*2,600.			0.058ug**	0.65ug**		1980 FR	1
ALDRIN	Y	Y	3.0		1.3		0.074ng**	0.079ng**		1980 FR	16
ALKALINITY	N	N		20,000.						1976 RB	
AMMONIA	N	N	CRITERIA ARE pH AND TEMPERATURE DEPENDENT - SEE DOCUMENT							1985 FR	24
ANTIMONY	Y	N	*9,000.	*1,600.			146.ug	45,000.ug		1980 FR	1
ARSENIC	Y	Y					2.2ng**	17.5ng**	0.05mg	1980 FR	21
ARSENIC(PENT)	Y	Y	*850.	*48.	*2,319.	*13.				1985 FR	21
ARSENIC(TRI)	Y	Y	360.	190.	69.	36.				1985 FR	21
ASBESTOS	Y	Y					30M f/L**			1980 FR	
BACTERIA	N	N	FOR PRIMARY RECREATION AND SHELLFISH USES - SEE DOCUMENT						<1/100ml	1986 FR	56
BARIUM	N	N					1.mg		1.0mg	1976 RB	8
BENZENE	Y	Y	*5,300.		*5,100.	*700.	0.66ug**	40.ug**		1980 FR	1
BENZIDINE	Y	Y	*2,500.				0.12ng**	0.53ng**		1980 FR	6
BERYLLIUM	Y	Y	*130.	*5.3			6.8ng**	117.ng**		1980 FR	8
BHC	Y	N	*100.		*0.34					1980 FR	
CADMIUM	Y	N	3.9*	1.1*	43.	9.3	10.ug		0.010mg	1985 FR	21
CARBON TETRACHLORIDE	Y	Y	*35,200.		*50,000.		0.4ug**	6.94ug**		1980 FR	1
CHLORDANE	Y	Y	2.4	0.0043	0.09	0.004	0.46ng**	0.48ng**		1980 FR	12
CHLORINATED BENZENES	Y	Y	*250.	*50.	*160.	*129.	488.ug			1980 FR	1
CHLORINATED NAPHTHALENES	Y	N	*1,600.		*7.5					1980 FR	1
CHLORINE	N	N	19.	11.	13.	7.5				1985 FR	21
CHLOROALKYL ETHERS	Y	N	*238,000.							1980 FR	1
CHLOROETHYL BIS-2	Y	Y					0.03ug**	1.36ug**		1980 FR	
CHLOROFORM	Y	Y	*28,900.	*1,240.			0.19ug**	15.7ug**		1980 FR	1
CHLOROISOPROPYL (BIS-2)	Y	N					34.7ug	4.36mg		1980 FR	
CHLOROMETHYL ETHER (BIS)	Y	N					0.00000376ng**	0.00184ug**		1980 FR	
CHLOROPHENOL 2	Y	N	*4,380.	*2,000.						1980 FR	1
CHLOROPHENOL 4	N	N			*29,700.					1980 FR	1
CHLOROPHENOXY HERBICIDES (2,4,5,-TP)	N	N					10.ug			1980 FR	
CHLOROPHENOXY HERBICIDES (2,4-D)	N	N					100.ug			1976 RB	7
CHLORO-4 METHYL-3 PHENOL	N	N	*30.							1980 FR	
CHROMIUM (HEX)	Y	N	16.	11.	1,100.	50.	50.ug		0.05mg	1985 FR	24
CHROMIUM (TRI)	N	N	1,700.*	210.*	*10,300.		170.mg	3,433.mg	0.05mg	1985 FR	24
COLOR	N	N	NARRATIVE STATEMENT - SEE DOCUMENT							1976 RB	
COPPER	Y	N	18.*	12.*	2.9	2.9				1985 FR	20
CYANIDE	Y	N	22.	5.2	1.	1.	200.ug			1985 FR	23
DDT	Y	Y	1.1	0.001	0.13	0.001	0.024ng**	0.024ng**		1980 FR	16
DDT METABOLITE (DDE)	Y	Y	*1,050.		*14.					1980 FR	
DDT METABOLITE (TDE)	Y	Y	*0.06		*3.6					1980 FR	
DEMITON	Y	N		0.1		0.1				1976 RB	
DIBUTYL PHTHALATE	Y	N					35.mg	154.mg		1980 FR	
DICHLOROBENZENES	Y	N	*1,120.	*763.	*1,970.		400.ug	2.6mg		1980 FR	1
DICHLOROBENZIDINE	Y	Y					0.01ug**	0.020ug**		1980 FR	1
DICHLOROETHANE 1,2	Y	Y	*118,000.	*20,000.	*113,000.		0.94ug**	243.ug**		1980 FR	1
DICHLOROETHYLENES	Y	Y	*11,600.		*224,000.		0.033ug**	1.85ug**		1980 FR	1
DICHLOROPHENOL 2,4	N	N	*2,020.	*365.			3.09mg			1980 FR	1
DICHLOROPROPANE	Y	N	*23,000.	*5,700.	*10,300.	*3,040.				1980 FR	1
DICHLOROPROPENE	Y	N	*6,060.	*244.	*790.		87.ug	14.1mg		1980 FR	1
DIELDRIN	Y	Y	2.5	0.0019	0.71	0.0019	0.071ng**	0.076ng**		1980 FR	16
DIETHYL PHTHALATE	Y	N					350.mg	1.8g		1980 FR	
DIMETHYL PHENOL 2,4	Y	N	*2,120.							1980 FR	
DIMETHYL PHTHALATE	Y	N					313.mg	2.9g		1980 FR	
DINITROTOLUENE 2,4	N	N					0.11ug**	9.1ug**		1980 FR	
DINITROPHENOLS	Y	N					70.ug	14.3mg		1980 FR	
DINITROTOLUENE	N	Y	*330.	*230.	*590.	*370.				1980 FR	1
DINITRO-O-CRESOL 2,4,	Y	N					13.4ug	765.mg		1980 FR	
DIOXIN (2,3,7,8 - TCDD)	Y	Y	*<0.01	*<0.00001			0.000013ng**	0.000014ng**		1984 FR	1
DIPHENYLHYDRAZINE	Y	N					42.ng**	0.56ug**		1980 FR	1
DIPHENYLHYDRAZINE 1,2	Y	N	*270.							1980 FR	
DI-2-ETHYLHEXYL PHTHALATE	Y	N					15.mg	50.mg		1980 FR	
ENDOSULFAN	Y	N	0.22	0.056	0.034	0.0087	74.ug	159.ug		1980 FR	10
ENDRIN	Y	N	0.18	0.0023	0.037	0.0023	1.ug		0.0002mg	1980 FR	18
ETHYLBENZENE	Y	N	*32,000.		*430.		1.4mg	3.28mg		1980 FR	
FLUORANTHENE	Y	N	*3,980.		*40.	*16.	42.ug	54.ug		1980 FR	1
GASES, TOTAL DISSOLVED	N	N	NARRATIVE STATEMENT - SEE DOCUMENT							1976 RB	
GUTHION	N	N		0.01		0.01				1976 RB	8

HALOETHERS	Y	N	*360.	*122.						1980	FR	
HALOMETHANES	Y	Y	*11,000.		*12,000.	*6,400.	0.19ug**	15.7ug**		1980	FR	
HEPTACHLOR	Y	Y	0.52	0.0038	0.053	0.0036	0.28ng**	0.29ng**		1980	FR	12
HEXACHLOROETHANE	N	Y	*980.	*540.	*940.		1.9ug	8.74ug		1980	FR	1
HEXACHLOROBENZENE	Y	N					0.72ng**	0.74ng**		1980	FR	
HEXACHLOROBUTADIENE	Y	Y	*90.	*9.3	*32.		0.45ug**	50.ug**		1980	FR	2
HEXACHLOROCYCLOHEXANE (LINDANE)	Y	Y	2.0	0.08	0.16				0.004mg	1980	FR	12
HEXACHLOROCYCLOHEXANE - ALPHA	Y	Y					9.2ng**	31.ng**		1980	FR	
HEXACHLOROCYCLOHEXANE - BETA	Y	Y					16.3ng**	54.7ng**		1980	FR	
HEXACHLOROCYCLOHEXANE - GAMA	Y	Y					18.6ng**	62.5ng**		1980	FR	
HEXACHLOROCYCLOHEXANE - TECHNICAL	Y	Y					12.3ng**	41.4ng**		1980	FR	
HEXACHLOROCYCLOPENTADIENE	Y	N	*7.	*5.2	*7.		206.ug			1980	FR	3
IRON	W	W		1,000.			0.3mg			1976	RB	15
ISOPHORONE	Y	N	*117,000.		*12,900.		5.2mg	520.mg		1980	FR	
LEAD	Y	N	82.+	3.2+	140.	5.6	50.ug		0.05mg	1985	FR	20
MALATHION	N	N		0.01		0.01				1976	RB	7
MANGANESE	N	N					50.ug	100.ug		1976	RB	7
MERCURY	Y	N	2.4	0.012	2.1	0.025	144.ng	146.ng	0.002mg	1985	FR	17
METHOXYCHLOR	N	N		0.03		0.03	100.ug		0.1mg	1976	RB	12
MIREX	N	N		0.001		0.001				1976	RB	7
MONOCHLOROBENZENE	Y	N					488.ug			1980	FR	
NAPHTHALENE	Y	N	*2,300.	*620.	*2,350.					1980	FR	1
NICKEL	Y	N	1,800.+	96.+	140.	7.1	13.4ug	100.ug		1980	FR	10
NITRATES	N	N					10.mg		10.mg	1976	RB	5
NITROBENZENE	Y	N	*27,000.		*6,690.		19.8mg			1980	FR	1
NITROPHENOLS	Y	N	*230.	*150.	*4,850.					1980	FR	1
NITROSAMINES	Y	Y	*5,850.		*3,300,000		0.8ng**	1240.ng**		1980	FR	
NITROSODIBUTYLAMINE N	Y	Y					6.4ng**	587.ng**		1980	FR	
NITROSODIETHYLAMINE N	Y	Y					0.8ng**	1,786.ng**		1980	FR	
NITROSODIMETHYLAMINE N	Y	Y					1.4ng**	16,000.ng**		1980	FR	
NITROSODIPHENYLAMINE N	Y	Y					4,900.ng**	16,100.ng**		1980	FR	
NITROSOPYRROLIDINE N	Y	Y					16.ng**	91,900.ng**		1980	FR	
OIL AND GREASE	N	N	NARRATIVE STATEMENT - SEE DOCUMENT							1976	RB	58
	N	N	WARMWATER AND COLDWATER CRITERIA MATRIX - SEE DOCUMENT							1986	FR	56
	N	N		0.04		0.04				1976	RB	8
	Y	Y	2.0	0.014	10.	0.03	0.079ng**	0.079ng**		1980	FR	16
	Y	N	*7,240.	*1,100.	*390.	*281.				1980	FR	1
	Y	N					74.ug	85.ug		1980	FR	
	Y	N	*55.	*3.2	*53.	*34.	1.01mg			1980	FR	2
PH	N	N		6.5-9		6.5-8.5				1976	RB	56
PHENOL	Y	N	*10,200.	*2,560.	*5,800.		3.5mg			1980	FR	23
PHOSPHORUS ELEMENTAL	N	N				0.1				1976	RB	
PHTHALATE ESTERS	Y	N	*940.	*3.	*2,944.	*3.4				1980	FR	6
POLYNUCLEAR AROMATIC HYDROCARBONS	Y	Y			*300.		2.8ng**	31.1ng**		1980	FR	1
SELENIUM	Y	N	260.	35.	410.	54.	10.ug		0.01mg	1980	FR	15
SILVER	Y	N	4.3+	0.12	2.3		50.ug		0.05mg	1980	FR	14
SOLIDS DISSOLVED AND SALINITY	N	N					250.mg			1976	RB	56
SOLIDS SUSPENDED AND TURBIDITY	N	N	NARRATIVE STATEMENT - SEE DOCUMENT							1976	RB	44
SULFIDE-HYDROGEN SULFIDE	N	N		2.		2.				1976	RB	
TEMPERATURE	N	N	SPECIES DEPENDENT CRITERIA - SEE DOCUMENT							1976	RB	56
TETRACHLORINATED ETHANES	Y	N	*9,320.							1980	FR	
TETRACHLOROBENZENE 1,2,4,5	Y	N					38.ug	48.ug		1980	FR	
TETRACHLOROETHANE 1,1,2,2	Y	Y		*2,400.	*9,620.		0.17ug**	10.7ug**		1980	FR	1
TETRACHLOROETHANES	Y	N	*9,320.							1980	FR	1
TETRACHLOROETHYLENE	Y	Y	*5,280.	*840.	*10,200.	*450.	0.8ug**	8.85ug**		1980	FR	1
TETRACHLOROPHENOL 2,3,5,6	Y	N				*440.				1980	FR	
THALLIUM	Y	N	*1,400.	*40.	*2,130.		13.ug	48.ug		1980	FR	2
TOLUENE	Y	N	*17,500.		*6,300.	*5,000.	14.3mg	424.mg		1980	FR	1
TOXAPHENE	Y	Y	1.6	0.013	0.07		0.71ng**	0.73ng**	0.005mg	1980	FR	17
TRICHLORINATED ETHANES	Y	Y	*18,000.							1980	FR	
TRICHLOROETHANE 1,1,1	Y	N			*31,200.		18.4mg	1.03g		1980	FR	1
TRICHLOROETHANE 1,1,2	Y	Y		*9,400.			0.6ug**	41.8ug**		1980	FR	1
TRICHLOROETHYLENE	Y	Y	*45,000.	*21,900.	*2,000.		2.7ug**	80.7ug**		1980	FR	1
TRICHLOROPHENOL 2,4,5	Y	N					2,600.ug			1980	FR	
TRICHLOROPHENOL 2,4,6	N	Y		*970.			1.2ug**	3.6ug**		1980	FR	
VINYL CHLORIDE	Y	Y					2.ug**	525.ug**		1980	FR	
[illegible]	Y	N	320.+	43.	170.	58.				1980	FR	19

g = grams
mg = milligrams
ug = micrograms
ng = nanograms
f = fibers

Y=YES
N=NO

+ = HARDNESS DEPENDENT CRITERIA (100mg/L used)
* = INSUFFICIENT DATA TO DEVELOP CRITERIA. VALUE PRESENTED IS THE L.O.E.L. - LOWEST OBSERVED EFFECT LEVEL.
** = HUMAN HEALTH CRITERIA FOR CARCINOGENS REPORTED FOR THREE RISK LEVELS. VALUE PRESENTED IS THE 10-6 RISK LEVEL.

FR = FEDERAL REGISTER
RB = QUALITY CRITERIA FOR WATER, 1976 (REDBOOK)

M.C.L. = MAXIMUM CONTAMINANT LEVEL

Note: This chart is for general information; please use criteria documents or detailed summaries in "Quality Criteria for Water 1986" for regulatory purposes.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF WATER REGULATIONS AND STANDARDS

CRITERIA AND STANDARDS DIVISION

UPDATE #2

to

"QUALITY CRITERIA FOR WATER 1986"

May 1, 1987

This is the second update to the EPA document "Quality Criteria for Water 1986". Included in this package are criteria summaries for contaminants that were recently revised as well as a criteria summary for a new contaminant. Several hand corrections are also included.

Revised	New	Hand corrections
INDEX	CHLORPYRIFOS	AMMONIA
SUMMARY CHART		CYANJDE
NICKEL		CHLORINATED ETHANES
PARATHION		
PENTACHLOROPHENOL		
TOXAPHENE		
ZINC		

Directions:

a. Replace sections that have been revised with new sections.
b. Insert new section alphabetically.
c. Make the identified hand corrections.

For additional information contact:
EPA's Criteria and Standards Division
at (202) 475-7315.

HAND CORRECTIONS

Ammonia - page 5, center page, third line of equation should be changed from

"FPH = 1 ; 8 < pH < 9"

to

"FPH = 1 ; 8 ≤ pH ≤ 9"

Cyanide - last page, include the following after the last line

"(45 F.R. 79318, Nov. 28, 1980) (50 F.R. 30784, July 29, 1985)"
"SEE APPENDIX A FOR METHODOLOGY"

Chlorinated Ethanes - last page, change

"1.03 ug/l"

to

"1.03 g/L"

Water Quality Criteria Summary

Note: This chart is for general information; please use criteria documents or detailed summaries in "Quality Criteria for Water 1986" for regulatory purposes.

			CONCENTRATIONS IN µg/L				UNITS PER LITER				
	Priority Pollutant	Carcinogen	Fresh Acute Criteria	Fresh Chronic Criteria	Marine Acute Criteria	Marine Chronic Criteria	Water and Fish Ingestion	Fish Consumption Only	Drinking Water M.C.L.	Date Reference	# of States With Aquatic Life Standard
ACENAPTHENE	Y	N	*1,700.	*520.	*970.	*710.				1980 FR	1
ACROLEIN	Y	N	*68.	*21.	*55.		320.µg	780.µg		1980 FR	1
ACRYLONITRILE	Y	Y	*7,550.	*2,600.			0.058µg**	0.65µg**		1980 FR	
ALDRIN	Y	Y	3.0		1.3		0.074ng**	0.079ng**		1980 FR	16
ALKALINITY	N	N		20,000.						1976 RB	
AMMONIA	N	N	CRITERIA ARE pH AND TEMPERATURE DEPENDENT—SEE DOCUMENT							1985 FR	24
ANTIMONY	Y	N	*9,000.	*1,600.			146.µg	45,000.µg		1980 FR	1
ARSENIC	Y	Y					2.2ng**	17.5ng**	0.05mg	1980 FR	21
ARSENIC (PENT)	Y	Y	*850.	*48	*2,319	*13.				1985 FR	21
ARSENIC (TRI)	Y	Y	360.	190.	69.	36.				1985 FR	21
ASBESTOS	Y	Y					30x1/L**			1980 FR	
BACTERIA	N	N	FOR PRIMARY RECREATION AND SHELLFISH USES—SEE DOCUMENT						<1/100ml	1986 FR	56
BARIUM	N	N					1.mg		1.0mg	1976 RB	8
BENZENE	Y	Y	*5,300.		*5,100.	*700.	0.66µg**	40.µg**		1980 FR	1
BENZIDINE	Y	Y	*2,500				0.12ng**	0.53ng**		1980 FR	8
BERYLLIUM	Y	Y	*130.	*5.3			6.8ng**	117.ng**		1980 FR	8
BHC	Y	N	*100.		*0.34					1980 FR	
CADMIUM	Y	N	3.9 +	1.1 +	43.	9.3	10.µg		0.010mg	1985 FR	21
CARBON TETRACHLORIDE	Y	Y	*35,200.		*50,000		0.4µg**	6.94µg**		1980 FR	1
CHLORDANE	Y	Y	2.4	0.0043	0.09	0.004	0.46ng**	0.48ng**		1980 FR	12
CHLORINATED BENZENES	Y	Y	*250	*50	*160	*129				1980 FR	1
CHLORINATED NAPHTHALENES	Y	N	*1,600.		*7.5					1980 FR	1
CHLORINE	N	N	19.	11.	13.	7.5				1985 FR	21
CHLOROALKYL ETHERS	Y	N	*238,000.							1980 FR	1
CHLOROETHYL ETHER (BIS-2)	Y	Y					0.03µg**	1.36µg**		1980 FR	
CHLOROFORM	Y	Y	*28,900.	*1,240.			0.19µg**	15.7µg**		1980 FR	1
CHLOROISOPROPYL ETH ER (BIS-2)	Y	N					34.7µg	4.36mg		1980 FR	
CHLOROMETHYL ETHER (BIS)	N	Y					0.00000376ng**	0.00184µg**	+	1980 FR	
CHLOROPHENOL 2	Y	N	*4,380.	*2,000.						1980 FR	1
CHLOROPHENOL 4	N	N			*29,700.					1980 FR	1
CHLOROPHENOXY HERBICIDES (2,4,5,-TP)	N	N					10.µg			1980 FR	
CHLOROPHENOXY HERBICIDES (2,4-D)	N	N					100.µg			1976 RB	7
CHLORPYRIFOS	N	N	0.083	0.041	0.011	0.0056				1986 FR	
CHLORO-4 METHYL-3 PHENOL	N	N	*30.							1980 FR	
CHROMIUM (HEX)	Y	N	16.	11.	1,100.	50.	50.µg		0.05mg	1985 FR	24
CHROMIUM (TRI)	N	N	1,700. +	210. +	*10,300.		170.mg	3,433.mg	0.05mg	1985 FR	24
COLOR	N	N	NARRATIVE STATEMENT—SEE DOCUMENT							1976 RB	
COPPER	Y	N	18. +	12. +	2.9	2.9				1985 FR	20
CYANIDE	Y	N	22.	5.2	1.	1.	200.µg			1985 FR	23
DDT	Y	Y	1.1	0.001	0.13	0.001	0.024ng**	0.024ng**		1980 FR	16
DDT METABOLITE (DDE)	Y	Y	*1,050.		*14.					1980 FR	
DDT METABOLITE (TDE)	Y	Y	*0.06		*3.6					1980 FR	
DEMETON	Y	N		0.1		0.1				1976 RB	
DIBUTYL PHTHALATE	Y	N					35.mg	154.mg		1980 FR	
DICHLOROBENZENES	Y	N	*1,120.	*763.	*1,970.		400.µg	2.6mg		1980 FR	1
DICHLOROBENZIDINE	Y	Y					0.01µg**	0.020µg**		1980 FR	1
DICHLOROETHANE 1,2	Y	Y	*118,000.	*20,000.	*113,000.		0.94µg**	243.µg**		1980 FR	1
DICHLOROETHYLENES	Y	Y	*11,600.		*224,000.		0.033µg**	1.85µg**		1980 FR	1
DICHLOROPHENOL 2,4	N	N	*2,020.	*365.			3.09mg			1980 FR	1
DICHLOROPROPANE	Y	N	*23,000.	*5,700.	*10,300.	*3,040.				1980 FR	1
DICHLOROPROPENE	Y	N	*6,060.	*244.	*790.		87.µg	14.1mg		1980 FR	1
DIELDRIN	Y	Y	2.5	0.0019	0.71	.0019	0.071ng**	0.076ng**		1980 FR	16
DIETHYL PHTHALATE	Y	N					350.mg	1.8g		1980 FR	
DIMETHYL PHENOL 2,4	Y	N	*2,120.							1980 FR	
DIMETHYL PHTHALATE	Y	N					313.mg	2.9g		1980 FR	
DINITROTOLUENE 2,4	N	Y					0.11µg**	9.1µg**		1980 FR	
DINITROTOLUENE	Y	N					70.µg	14.3mg		1980 FR	
DINITROTOLUENE	N	Y	*330.	*230.	*590.	*370.				1980 FR	1
DINITRO-O-CRESOL 2,4	Y	N					13.4µg	765.µg		1980 FR	
DIOXIN (2,3,7,8-TCDD)	Y	Y	*0.01	*0.00001			0.000013ng**	0.000014ng**		1984 FR	1
DIPHENYLHYDRAZINE	Y	N					42.ng**	0.56µg**		1980 FR	1
DIPHENYLHYDRAZINE 1,2	Y	N	*270							1980 FR	
DI-2-ETHYL HEXYL PHTHALATE	Y	N					15.mg	50.mg		1980 FR	

ENDOSULFAN	Y	N	0.22	0.056	0.034	0.0087	74.µg	159.µg		1980 FR	10
ENDRIN	Y	N	0.18	0.0023	0.037	0.0023	1.µg		0.0002mg	1980 FR	18
ETHYLBENZENE	Y	N	*32,000.		*430.		1.4mg	3.28mg		1980 FR	
FLUORANTHENE	Y	N	*3,980.		*40.	*16.	42.µg	54.µg		1980 FR	1
GASSES, TOTAL DISSOLVED	N	N	NARRATIVE STATEMENT—SEE DOCUMENT							1976 RB	
GUTHION	N	N		0.01		0.01				1976 RB	8
HALOETHERS	Y	N	*360.	*122.						1980 FR	
HALOMETHANES	Y	Y	*11,000.		*12,000	*6,400.	0.19µg**	15.7µg**		1980 FR	
HEPTACHLOR	Y	Y	0.52	0.0036	0.053	0.0036	0.28ng**	0.29ng**		1980 FR	12
HEXACHLOROETHANE	N	Y	*980.	*540.	*940.		1.9µg	8.74µg		1980 FR	1
HEXACHLOROBENZENE	Y	N					0.72ng**	0.74ng**		1980 FR	
HEXACHLOROBUTADIENE	Y	Y	*90.	*9.3	*32.		0.45µg**	50.µg**		1980 FR	2
HEXACHLOROCYCLOHEXANE (LINDANE)	Y	Y	2.0	0.08	0.16				0.004mg	1980 FR	12
HEXACHLOROCYCLOHEXANE–ALPHA	Y	Y					9.2ng**	31.ng**		1980 FR	
HEXACHLOROCYCLOHEXANE–BETA	Y	Y					16.3ng**	54.7ng**		1980 FR	
HEXACHLOROCYCLOHEXANE–GAMA	Y	Y					18.6ng**	62.5ng**		1980 FR	
HEXACHLOROCYCLOHEXANE–TECHNICAL	Y	Y					12.3ng**	41.4ng**		1980 FR	
HEXACHLOROCYCLOPENTADIENE	Y	N	*7.	*5.2	*7.		206.µg			1980 FR	3
IRON	N	N		1,000.			0.3mg			1976 RB	15
ISOPHORONE	Y	N	*117,000.		*12,900.		5.2mg	520.mg		1980 FR	
LEAD	Y	N	82. +	3.2 +	140.	5.6	50.µg		0.05mg	1985 FR	20
MALATHION	N	N		0.1		0.1				1976 RB	7
MANGANESE	N	N					50.µg	100.µg		1976 RB	7
MERCURY	Y	N	2.4	0.012	2.1	0.025	144.ng	146.ng	0.002mg	1985 FR	17
METHOXYCHLOR	N	N		0.03		0.03	100.µg		0.1mg	1976 RB	12
MIREX	N	N		0.001		0.001				1976 RB	7
MONOCHLOROBENZENE	Y	N					488.µg			1980 FR	
NAPHTHALENE	Y	N	*2,300.	*620.	*2,350.					1980 FR	1
NICKEL	Y	N	1,400. +	160 +	75	8.3	13.4µg	100.µg		1986 FR	10
NITRATES	N	N					10.mg		10.mg	1976 RB	5
NITROBENZENE	Y	N	*27,000.		*6,680.		19.8mg			1980 FR	1
NITROPHENOLS	Y	N	*230.	*150.	*4,850.					1980 FR	1
NITROSAMINES	Y	Y	*5,850.		*3,300,000					1980 FR	1
NITROSODIBUTYLAMINE N	Y	Y					6.4ng**	587.ng**		1980 FR	
NITROSODIETHYLAMINE N	Y	Y					0.8ng**	1,240.ng**		1980 FR	
NITROSODIMETHYLAMINE N	Y	Y					1.4ng**	16,000.ng**		1980 FR	
NITROSODIPHENYLAMINE N	Y	Y					4,900.ng**	16,100.ng**		1980 FR	
NITROSOPYRROLIDINE N	Y	Y					16.ng**	91,900.ng**		1980 FR	
OIL AND GREASE	N	N	NARRATIVE STATEMENT—SEE DOCUMENT							1976 RB	56
OXYGEN DISSOLVED	N	N	WARMWATER AND COLDWATER CRITERIA MATRIX—SEE DOCUMENT							1986 FR	56
PARATHION	N	N	0.065	0.013						1986 FR	8
PCB's	Y	Y	2.0	0.014	10	0.03	0.079ng**	0.079ng**		1980 FR	16
PENTACHLORINATED ETHANES	N	N	*7,240.	*1,100.	*390.	*281.				1980 FR	1
PENTACHLOROBENZENE	N	N					74.µg	85.µg		1980 FR	
PENTACHLOROPHENOL	Y	N	***20.	***13.	13.	*7.9	1.01mg			1986 FR	2
pH	N	N		6.5-9		6.5-8.5				1976 RB	56
PHENOL	Y	N	*10,200.	*2,560.	*5,800.		3.5mg			1980 FR	23
PHOSPHORUS ELEMENTAL	N	N				0.1				1976 RB	
PHTHALATE ESTERS	Y	N	*940.	*3.	*2,944.	*3.4				1980 FR	6
POLYNUCLEAR AROMATIC HYDROCARBONS	Y	Y			*300.		2.8ng**	31.1ng**		1980 FR	1
SELENIUM	Y	N	260.	35.	410.	54.	10.µg		0.01mg	1980 FR	15
SILVER	Y	N	4.1 +	0.12	2.3		50.µg		0.05mg	1980 FR	14
SOLIDS DISSOLVED AND SALINITY	N	N					250.mg			1976 RB	56
SOLIDS SUSPENDED AND TURBIDITY	N	N	NARRATIVE STATEMENT—SEE DOCUMENT							1976 RB	44
SULFIDE-HYDROGEN SULFIDE	N	N		2.		2.				1976 RB	
TEMPERATURE	N	N	SPECIES DEPENDENT CRITERIA—SEE DOCUMENT							1976 RB	56
TETRACHLORINATED ETHANES	Y	N	*9,320.							1980 FR	
TETRACHLOROBENZENE 1,2,4,5	Y	N					36.µg	48.µg		1980 FR	
TETRACHLOROETHANE 1,1,2,2	Y	Y		*2,400.	*9,020.		0.17µg**	10.7µg**		1980 FR	1
TETRACHLOROETHANES	Y	N	*9,320.							1980 FR	1
TETRACHLOROETHYLENE	Y	Y	*5,280.	*840.	*10,200.	*450.	0.8µg**	8.85µg**		1980 FR	1
TETRACHLOROPHENOL 2,3,6,2	Y	N				*440.				1980 FR	
THALLIUM	Y	N	*1,400.	*40.	*2,130.		13.µg	48.µg		1980 FR	2
TOLUENE	Y	N	*17,500.		*6,300.	*5,000.	14.3mg	424.mg		1980 FR	1
TOXAPHENE	Y	Y	0.73	0.0002	0.21	0.0002	0.71ng**	0.73ng**	0.005mg	1986 FR	17
TRICHLORINATED ETHANES	Y	Y	*18,000.							1980 FR	
TRICHLOROETHANE 1,1,1	Y	N			*31,200.		18.4mg	1.03g		1980 FR	1
TRICHLOROETHANE 1,1,2	Y	Y		*9,400.			0.6µg**	41.8µg**		1980 FR	1
TRICHLOROETHYLENE	Y	Y	*45,000.	*21,900.	*2,000.		2.7µg**	80.7µg**		1980 FR	1
TRICHLOROPHENOL 2,4,5	N	N					2,600.µg			1980 FR	
TRICHLOROPHENOL 2,4,6	Y	Y		*970.			1.2µg**	3.6µg**		1980 FR	
VINYL CHLORIDE	Y	Y					2.µg**	525.µg**		1980 FR	
ZINC	Y	N	120. +	110 +	95	98				1987 FR	19

g = grams
mg = milligrams
µg = micrograms
ng = nanograms
M.L. = liters

Y = YES
N = NO

M.C.L. – MAXIMUM CONTAMINANT LEVEL

+ = Hardness Dependent Criteria (100 mg L used)

* = Insufficient Data to Develop Criteria. Value Presented is the L.O.E.L.—Lowest Observed Effect Level.

** = Human Health Criteria For Carcinogens Reported For Three Risk Levels. Value Presented is the 10-6 Risk Level.

*** = pH Dependent Criteria (7.8 pH used)

FR = FEDERAL REGISTER
RB = QUALITY CRITERIA FOR WATER, 1976 (REDBOOK)

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CHLORPYRIFOS

Summary

The acute values for eighteen freshwater species in fifteen genera range from 0.11 ug/L for an amphipod to greater than 806 ug/L for two fishes and a snail. The bluegill is the most acutely sensitive fish species with an acute value of 10 ug/L, but seven intervetebrate genera are more sensitive. Smaller organisms seem to be more acutely sensitive than larger ones.

Chronic toxicity data are available for one freshwater species, the fathead minnow. Unacceptable effects occurred in second generation larvae at 0.12 ug/L, which was the lowest concentration tested. The resulting acute-chronic ratio was greater than 1,417.

Little information is available on the toxicity of chlorpyrifos to freshwater plants, although algal blooms frequently follow field applications of chlorpyrifos. The only available bioconcentration test on chlorpyrifos with a freshwater species was with the fathead minnow and resulted in a bioconcentration factor of 1,673.

The acute toxicity of chlorpyrifos has been determined for 15 species of saltwater animals in 12 genera with the acute values ranging from 0.01 ug/L for the Korean shrimp, Palaemon macrodactvlus, to 1.911 ug/L for larvae of the eastern oyster, Crassostrea virginica. Arthropods are particularly sensitive to chlorpyrifos. Among the 10 species of fish tested, the 96-hr LC50s range from 0.58 ug/L for striped bass to 520 ug/L for gulf

toadfish. Fish larvae are more sensitive than other life stages. Growth of the mysid, Mysidopsis bahia, was reduced at 0.004 ug/L in a life-cycle test. In early life-stage tests, the California grunion, Leuresthes tenuis, was the most sensitive of the six fishes, with growth being reduced at 0.30 ug/L. Of the seven acute-chronic ratios that have been determined with saltwater species, the five lowest range from 1.374 to 12.50, whereas the highest is 228.5.

Concentrations of chlorpyrifos affecting six species of saltwater phytoplankton range from 138 to 10,000 ug/L. BCFs ranged from 100 to 5,100 when the gulf toadfish was exposed to concentrations increasing from 1.4 to 150 ug/L. Steady-state BCFs averaged from 100 to 757 for five fishes exposed in early life-stage tests.

National Criteria

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate, that except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of chlorpyrifos does not exceed 0.041 ug/L more than once every three years on the average and if the one-hour average concentration does not exceed 0.083 ug/L more than once every three years on the average.

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of

Aquatic Organisms and Their Uses" indicate, that except possibly where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of chlorpyrifos does not exceed 0.0056 ug/L more than once every three years on the average and if the one-hour average concentration does not exceed 0.011 ug/L more than once every three years on the average.

Three years is the Agency's best scientific judgment of the average amount of time aquatic ecosystems should be provided. between excursions. The resiliences of ecosystems and their abilities to recover differ greatly, however, and site-specific allowed excursion frequencies may be established if adequate justification is provided.

Use of criteria for developing water quality-based permit limits and for designing waste treatment facilities requires selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria. Limited data or other consideretions might make their use impractical, in which case one must rely on a steady-state model.

(51 F.R. 43665, December 3, 1986)
SEE APPENDIX A FOR METHODOLOGY

NICKEL

Summary

Acute values with twenty-one freshwater species in 18 genera range from 1,101 ug/L for a cladoceran to 43,240 ug/L for a fish. Fishes and invertebrates are both spread throughout the range of sensitivity. Acute values with four species are significantly correlated with hardness. Data are available concerning the chronic toxicity of nickel to two invertebrates and two fishes in freshwater. Data available for two species indicate that chronic toxicity decreases as hardness increases. The measured chronic values ranged from 14.77 ug/L with Daphnia magna in soft water to 526.7 ug/L with the fathead minnow in hard water. Five acute-chronic ratios are available for two species in soft and hard water and range from 14 to 122.

Nickel appears to be quite toxic to freshwater algae, with concentrations as low as 50 ug/L producing significant inhibition. Bioconcentration factors for nickel range from 0.8 for fish muscle to 193 for a cladoceran.

Acute values for twenty-three saltwater species in twenty genera range from 151.7 ug/L with juveniles of a mysid of to 1,100,000 ug/L with juveniles and adults of a clam. The acute values for the four species of fish range from 7,598 to 350.000 ug/L. The acute toxicity of nickel appears to be related to salinity, but the form of the relationship appears to be species-dependent.

Mysidopsis bahia is the only saltwater species with which an acceptable chronic test has been conducted on nickel. Chronic

exposure to 141 ug/L and greater resulted in reduced survival and reproduction. The measured acute-chronic ratio was 5.478.

Bioconcentration factors in saltwater range from 261.8 with a oyster to 675 with a brown alga.

National Criteria

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate, that except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of nickel (in ug/L) does not exceed the numerical value given by $e^{(0.8460[\ln(\text{hardness})]+1.1645)}$ more than once every three. years on the average and if the one-hour average concentration (in ug/L) does not exceed the numerical value given by $e^{(0.8460[\ln(\text{hardness})]+3.3612)}$ more than once every three years on the average. For example, at hardnesses of 50. 100, and 200 mg/L as $CaCO_3$ the four-day average concentrations of nickel are 88, 160, and 280 ug/L, respectively, and the one-hour average concentrations are 790, 1400. and 2500 ug/L.

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate, that except possibly where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of nickel does not exceed 8.3 ug/L more than once every three years on the average and if the one-hour average concentration does not exceed

75 ug/L more than once every three years on the average.

"Acid-soluble'' is probably the best measurement at present for expressing criteria for metals and the criteria for nickel were developed on this basis. However, at this time, no EPA approved method for such a measurement is available to implement criteria for metals through the regulatory programs of the Agency and the States. The Agency is considering development and approval of a method for a measurement such as "acid-soluble." Until one is approved, however, EPA recommends applying criteria for metals using the total recoverable method. This has two impacts: (1) certain species of some metals cannot be measured because the total recoverable method cannot distinguish between individual oxidation States, and *(2)* in some cases these criteria might be overly protective when based on the total recoverable method.

Three years is the Agency's best scientific judgment of the average amount of time aquatic ecosystems should be provided between excursions. The resiliences of ecosystems and their abilities to recover differ greatly, however, and site-specific allowed excursion frequencies may be established if adequate justification is provided.

Use of criteria for developing water quality-based permit limits and for designing waste treatment facilities requires selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria. Limited data or other considerations might make their use impractical, in which case one must rely on a steady-state model.

(51 F.R. 43665, December 3, 1986)
SEE APPENDIX A FOR METHODOLOGY

PARATHION

Summary

The acute values for thirty-seven freshwater species in thirty-one genera range from 0.04 ug/L for an early instar of a crayfish, Orconectes nais, to 5,230 ug/L for two species of tubificid worms. For Daphnia magna, the chronic value and acute-chronic ratio are 0.0990 ug/L and 10.10 respectively. Chronic toxicity values are available for two freshwater fish species, the bluegill and the fathead minnow, with chronic values of 0.24 ug/L and 6.3 ug/L, and acute-chronic ratios of 2.121 and 79.45. respectively. Two freshwater algae were affected by toxaphene concentrations of 30 and 390 ug/L, respectively. Bioconcentration factors determined with three fish species ranged from 27 to 573.

The acute values that are available for saltwater species are 11.5 and 17.8 ug/L for the Korean shrimp, Palaemon macrodactvlus. and 17.8 ug/L for the striped bass, Morone saxatilis. No data are available concerning the chronic toxicity of parathion to saltwater species, toxicity to saltwater plants, or bioaccumulation by saltwater species. Some data indicate that parathion is acutely lethal to commercially important saltwater shrimp at concentrations as low as 0.24 ug/L. Measurement of acetylcholinesterase (AChE) in fish tissue might be useful for diagnosing fish kills caused by parathion.

National Criteria

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of

Aquatic Organisms and Their Uses" indicate, that except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of parathion does not exceed 0.013 ug/L more than once every three years on the average and if the one-hour average concentration does not exceed 0.065 ug/L more than once every three years on the average.

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" require the availability of specified data for the derivation of a criterion. A saltwater criterion for parathion cannot be derived because very few of the required data are available.

Three years is the Agency's best scientific judgment of the average amount of time aquatic ecosystems should be provided between excursions. The resiliences of ecosystems and 'their abilities to recover differ greatly, however, and site-specific allowed excursion frequencies may be established if adequate justification is provided.

Use of criteria for developing water quality-based permit limits and for designing waste treatment facilities requires selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria. Limited data or other considerations might make their use impractical, in which case one must rely on a steady-state model.

(51 F.R. 43665, December 3, 1986)

SEE APPENDIX A FOR METHODOLOGY

PENTACHLOROPHENOL (PCP)

Summary

The acute and chronic toxicity of PCP to freshwater animals increased as pH and dissolved oxygen concentration of the water decreased. Generally, toxicity also increased with increased temperature. The estimated acute sensitivities of **36** species at pH = **6.5** ranged from **4.355** ug/L for larval common carp to **>43,920** ug/L for a crayfish. At pH **= 6.5,** the lowest and highest estimated chronic values of **<1.835** and **79.66** ug/L, respectively, were obtained with different cladoceran species. Chronic toxicity to fish was affected by the presence of impurities, with industrial-grade PCP being more toxic than purified samples. Mean acute-chronic ratios for six freshwater species ranged from **0.8945** to **>15.79,** but the mean ratios for the four most acutely sensitive species only range **0.8945** to **5.035.** Freshwater algae were affected by concentrations as low as **7.5** ug/L, whereas vascular plants were affected at **189** ug/L and above. Bioconcentration factors ranged from **7.3** to **1,066** for three species of fish.

Acute toxicity values from tests with 18 species of saltwater animals, representing **17** genera, range from **22.63** ug/L for late yolk-sac larvae of the Pacific herring, Clupea narenqus pallasi, to 18,000 ug/L for adult blue mussels, Mvtllus edulis. The embryo and larval stages of invertebrates and the late larval premetamorphosis stage of fish appear to be the most sensitive life stages to PCF. With few exceptions, fish are more sensitive

than invertebrates to PCP. Salinity, temperature, and pH have a slight effect on the toxicity of PCP to some saltwater animals.

Life-cycle toxicity tests have been conducted with the sheepshead minnow, Cyprinodon variegatus. and the polychaete worm, Ophrvotrocha diadema. The chronic value for the minnow is 64.31 ug/L and the acute-chronic ratio is 6.873. Unfortunately, no statistical analysis of the data from the test with the worm is available.

The EC50s for saltwater plants range from 17.40 ug/L for the diatom, Skeletonema costatum, to 3.600 ug/L for the green alga, Dunaliella tertiolecta. Apparent steady-state BCFs are available for the eastern oyster. Crassostrea virginica, and two saltwater fishes and range from 10 to *82.*

National Criteria

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate, that except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration (in ug/L) of pentachlorophenol does not exceed the numerical value given by $e^{[1.005(pH)-5.290]}$ more than once every three years on the average and if the one-hour average concentration (in ug/L) does not exceed the numerical value given by $e^{[1.005(pH)-4.830]}$ more than once every three years on the average. For example, at pH = 6.5. 7.8, and 9.0 the four-day average concentrations of pentachlorophenol are 3.5, 13, and 43 ug/L, respectively, and the

one-hour average concentrations are 5.5, 20, and 68 ug/L. At pH = 6.8, a pentachlorophenol concentration of 1.74 ug/L caused a 50% reduction in the growth of yearling sockeye salmon in a 56-day test.

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate, that except possibly where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of pentachlorophenol does not exceed 7.9 ug/L more than once every three years on the average and if the one-hour average concentration does not exceed 13 ug/L more than once every three years on the average.

Three years is the Agency's best scientific judgment of the average amount of time aquatic ecosystems should be provided between excursions. The resiliences of ecosystems and their abilities to recover differ greatly, however, and site-specific allowed excursion frequencies may be established if adequate justification is provided.

Use of criteria for developing water quality-based permit limits and for designing waste treatment facilities requires selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria. Limited data or other considerations might make their use impractical, in which case one must rely on a steady-state model.

(51 F.R. 43665, December 3, 1986)
SEE APPENDIX A FOR METHODOLOGY

TOXAPHENE

Summary

The acute sensitivities of 36 freshwater species in 28 genera ranged from 0.8 ug/L to 500 ug/L. Such important fish species as the channel catfish, largemouth bass, chinook and coho salmon, brook, brown and rainbow trout, striped bass, and bluegill had acute senitivities ranging from 0.8 ug/L to 10.8 ug/L. Chronic values for four freshwater species range from less than 0.039 ug/L for the brook trout to **0.1964** ug/L for the channel catfish. The growth of algae was affected at 100 to 1,000 ug/L, and bioconcentration factors from laboratory tests ranged from 3.100 to **90,000.** Concentrations in lake trout in the Great Lakes have frequently exceeded the U.S. FDA action level of 5 mg/kg, even though the concentrations in the water seem to be only 1 to **4** ng/L. These concentrations in lake water are thought to have resulted from toxaphene being transported to the Great Lakes from remote sites, the locations of which are not well known.

The acute toxicity of toxaphene to 15 species of saltwater animals ranges from 0.53 for pinfish, Laqodon rhomoides. to **460.000** ug/L for the adults of the clam, Ranqia cuneata. Except for resistant species tested at concentrations greater than toxaphene's water solubility, acute values for most species were within a factor of 10. The toxicity of toxaphene was found to decrease slightly with increasing salinity for adult blue crabs. Callinectes sapidus, whereas no relationship between toxicity and salinity was observed with the three spine stickleback,

Gasterosteus aculeatus. Temperature significantly affected the toxicity of toxaphene to blue crabs.

Early life-stage toxicity tests have been conducted with the sheepshead minnow, Cyprinodon variegatus, and the longnose killifish, Fundulus similis, whereas life-cycle tests have been conducted with the sheepshead minnow and a mysid. For the sheepshead minnow, chronic values of 1.658 ug/L from the early life-stage test and 0.7141 ug/L from the life-cycle toxicity test are similar to the 96-hr LC50 of 1.1 ug/L. Killifish are more chronically sensitive with effects noted at 0.3 ug/L. In the life-cycle test with the mysid, no adverse effects were observed at the highest concentration tested, which was only slightly below the 96-hr LC50, resulting in an acute-chronic ratio of 1.132.

Toxaphene is bioconcentrated by an oyster, Crassostrea virsinica, and two fishes, C. variegatus and F. similis, to concentrations that range from 9,380 to 70.140 times that in the test solution.

National Criteria

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate, that except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of toxaphene does not exceed 0.0002 ug/L more than once every three years on the average and if the one-hour average concentration does not

exceed 0.73 ug/L more than once every three years on the average. If the concentration of toxaphene does exceed 0.0002 ug/L, the edible portions of consumed species should be analyzed to determine whether the concentration of toxaphene exceeds the FDA action level of 5 mg/kg. If the channel catfish is as acutely sensitive as some data indicate it might be, it will not be protected by this criterion.

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate, that except possibly where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of toxaphene does not exceed 0.0002 ug/L more than once every three years on the average and if the one-hour average concentration does not exceed 0.21 ug/L more than once every three years on the average. If the concentration of toxaphene does exceed 0.0002 ug/L, the edible portions of consumed species should be analyzed to determine whether the concentration of toxaphene exceeds the FDA action level of 5 mg/kg.

Three years is the Agency's best scientific judgment of the average amount of time aquatic ecosystems should be provided between excursions. The resiliences of ecosystems and their abilities to recover differ greatly, however, and site-specific allowed excursion frequencies may be established if adequate justification is provided.

Use of criteria for developing water quality-based permit limits and for designing waste treatment facilities requires

selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria. Limited data or other considerations might make their use impractical, in which case one must rely on a steady-state model.

(51 F.R. 43665, December 3, 1986)
SEE APPENDIX A FOR METHODOLOGY

ZINC

Summary

Acute toxicity values are available for 43 species of freshwater animals and data for eight species indicate that acute toxicity decreases as hardness increases. When adjusted to a hardness of 50 mg/L, sensitivities range from 50.70 ug/L for *Ceriodaphnia reticulata* to 88,960 ug/L for a damselfly. Additional data indicate that toxicity increases as temperature increases. Chronic toxicity data are available for nine freshwater species. Chronic values for two invertebrates ranged from 46.73 ug/L for *Daphnia magna* to >5,243 ug/L for the caddisfly, *Clistoronla magnificia*. Chronic values for seven fish species ranged from 36.41 ug/L for the flagfish, *Jordanella floridae*, to 854.7 ug/L for the brook trout, *Salvelinus fontinalis*. Acute-chronic ratios ranged from 0.2614 to 41.20. but the ratios for the sensitive species were all less than 7.3.

The sensitivity range of freshwater plants to zinc is greater than that for animals. Growth of the alga, *Selenastrum capriocornutum*, was inhibited by 30 ug/L. On the other hand, with several other species of green algae, 4-day EC50s exceeded 200,000 ug/L. Zinc was found to bioaccumulate in freshwater animal tissues from 51 to 1,130 times the concentration present in the water.

Acceptable acute toxicity values for zinc are available for 33 species of saltwater animals including 26 invertebrates and 7 fish. LC50s range from 191.5 ug/L for cabezon, *Scorpanichthys*

marmoratus to 320.400 ug/L for adults of another clam, *Macoma balthica*. Early life stages of saltwater invertebrates and fishes are generally more sensitive to zinc than juveniles and adults. Temperature has variable and inconsistent effects on the sensitivity of saltwater invertebrates to zinc. The sensitivity of saltwater vertebrate animals to zinc decreases with increasing salinity, but the magnitude of the effect is species-specific.

A life-cycle test with the mysid, *Mysidopsia bahia*, found unacceptable effects at 120 ug/L, but not at 231 ug/L, and the acute-chronic ratio was 2.997. Seven species of saltwater plants were affected at concentrations ranging from 19 to 10,100 ug/L. Bioaccumulation data for zinc are available for seven species of saltwater algae and five species of saltwater animals. Steady-state zinc bioconcentration factors for the twelve species range from 3.692 to 23.820.

National Criteria

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate, that except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of zinc (in ug/L) does not exceed the numerical value given by $e^{(0.8473[\ln(\text{hardness})]+0.7614)}$ more than once every three years on the average and if the one-hour average concentration (in ug/L) does not exceed the numerical value given by $e^{(0.8473[\ln(\text{hardness})]+0.8604)}$ more than once every three years on the

average. For example, at hardnesses of 50, 100, and 200 mg/L as $CaCO_3$, the four-day average concentrations of zinc are 59, 110 and 190 ug/L, respectively, and the one-hour average concentrations are 65, 120, and 210 ug/L. If the striped bass is as sensitive as some data indicate, it will not be protected by this criterion.

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate, that except possibly where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of zinc does not exceed 86 ug/L more than once every three years on the average and if the one-hour average concentration does not exceed 95 ug/L more than once every three years on the average.

"Acid-soluble'' is probably the best measurement at present for expressing criteria for metals and the criteria for zinc were developed on this basis. However, at this time no EPA approved method for such a measurement is available to implement criteria for metals through the regulatory programs of the Agency and the States. The Agency is considering development and approval of a method for a measurement such as "acid-soluble.'' Until one is approved, however, EPA recommends applying criteria for metals using the total recoverable method. This has two impacts: (1) certain species of some metals cannot be measured because the total recoverable method cannot distinguish between individual oxidation States, and (2) in some cases these criteria might be overly protective when based on the total recoverable method.

Three years is the Agency's best scientific judgment of the average amount of time aquatic ecosystems should be provided between excursions. The resiliences of ecosystems and their abilities to recover differ greatly, however, and site-specific allowed excursion frequencies may be established if adequate justification is provided.

Use of criteria for developing water quality-based permit limits and for designing waste treatment facilities requires selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria. Limited data or other considerations might make their use impractical, in which case one must rely on a steady-state model.

(52 F.R. 6213, March 2. 1987)

SEE APPENDIX A FOR METHODOLOGY

COMMONWEALTH of VIRGINIA

DEPARTMENT OF ENVIRONMENTAL QUALITY

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Memorandum

SUBJECT: Water Guidance Memo No. 02-2002
Fish Kill Guidance Manual Second Edition

TO: Regional Directors (with attachment)

FROM: Larry Lawson, P.E., Director
Division of Water Program Coordination

DATE: March 4, 2002

COPIES: Environmental Field Managers, Regional Biologists, Regional Compliance and Enforcement Managers, Regional Water Permit Managers, and Alan Pollock (without attachment)

Attached is the second edition of the DEQ Fish Kill Investigation Guidance Manual that was developed in coordination with the Department of Game and Inland Fisheries and DEQ Regional and Central Office staffs. This manual supercedes Water Guidance Memo No. 98-2006, Fish Kill Investigation Guidance Manual, dated September 14, 1998. The manual should serve as a guide for fish kill investigations and employs procedures that are consistent with the U.S Fish and Wildlife Service and the American Fisheries Society published guidelines.

The document provides guidance regarding field equipment required for an investigation, field observations and sample collections for locating the source and extent of the kill, fish counting and fish cost assessment guidelines, documentation of expenses for cost recovery, etc.

If you have any questions on the contents of this manual, please feel free to contact Rick Browder with the Water Quality Standards & Biological Programs at 804/698-4134.

Disclaimer

This document provides procedural guidance to staff. This document is guidance only. It does not establish or affect legal rights or obligations. It does not establish a binding norm and is not finally determinative of issues addressed. Agency decisions in any particular case will be made by applying the State Water Control Law and the implementation regulations on the basis of the site specific facts.

LGL/scj

Attachment

FISH KILL INVESTIGATION GUIDANCE MANUAL

SECOND EDITION

WATER QUALITY STANDARDS AND BIOLOGICAL MONITORING PROGRAMS

RICHMOND, VIRGINIA

March 2002

EXECUTIVE SUMMARY

The Department of Environmental Quality (DEQ) Fish Kill Investigation Guidance Manual is designed to guide field personnel through an investigation of a fish kill event. It is intended for use in a first response scenario by trained personnel. It is not designed as a "cookbook" for untrained personnel or to be used as a substitute for formal training. Procedures outlined in this manual are the product of many fish kill investigations undertaken since the creation of the State Water Control Board in 1946, which was a predecessor to the DEQ. These procedures are consistent with agency guidelines published by the U.S. Fish and Wildlife Service and the American Fisheries Society.

This document covers the pertinent functions of a complete investigation and includes: defining the extent of the kill area; locating and stopping the source of the kill; notifying other appropriate parties; collecting the necessary information to substantiate the cause of the kill; determining the number and kind of fish killed; and presenting the data collected for potential enforcement action.

CHRONOLOGY OF THE GUIDANCE MANUAL AND ACKNOWLEDGEMENTS

This guidance manual was prepared by the Department of Environmental Quality (DEQ) Office of Water Quality Standards and Biological Programs (WQS & BP). On October 30, 1997 a draft version of the first version of the guidance was presented to personnel of the Department of Game and Inland Fisheries (DGIF) and to DEQ Central and Regional Office Staff who have fish kill investigation and cost recovery responsibilities at a meeting in Charlottesville, Virginia. On August 1998 the first version was finalized and presented to the DGIF and to the DEQ regional and central offices. On December 7, 2001 (WQS & BP) staff requested comments from the same and additional DGIF & DEQ staff members who participated in the 1997 Charlottesville meeting to update the Manual. Few changes to the manual were requested and in March 2002, the second version of the DEQ Fish Kill Guidance Manual was completed. The final preparation of the first and second versions of this guidance manual is due to the extensive effort by various DGIF and DEQ staff (listed below).

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PURPOSE/POLICY STATEMENT

The purposes of fish kill investigations are: to determine the cause of fish kill events in the waters of Virginia; to assess environmental damage caused by such kills; and to determine the parties responsible for the kill. Evidence gathered during investigations may become part of enforcement actions to seek reimbursement of investigation and fish replacement costs by the responsible party (State Water Control Law §62.1-44.15) and to address violations of Virginia's Water Quality Standards (9 VAC 25-260-20A). Fish kill investigations also serve as the impetus for further studies which may reveal environmental or human health concerns in cases where water quality standards are not being met and/or where the consumption of contaminated or diseased fish is possible.

Reports of fish kills are investigated on the assumption that fish kills represent a potentially serious loss of aquatic resources.

BACKGROUND

Fish kills may be obvious indicators of serious water quality degradation. In addition, the loss of the Commonwealth's natural resources merits investigation and, if possible, compensation for damages sustained. Fish kill investigations have been a necessary function of the State Water Control Board (SWCB) since its inception in 1946. In 1970, the State Water Control Law was modified to make the SWCB responsible for investigating fish kills. The modification also empowered the SWCB to recover the costs of investigations, the replacement costs of dead fish, and to impose penalties for violations (**see Appendix A** of applicable statutory and regulatory requirements). In 1993, the SWCB merged with the State Air Pollution Control Board, the Waste Management Board, and the Council on the Environment to form the Department of Environmental Quality (DEQ). The DEQ regulates water discharges, air emissions, and waste management in Virginia.

Responsibility for handling fish kill events lies in three divisions of the DEQ; Regional Offices (RO), the Central Office of Enforcement (OE), and the Water Quality Standards and Biological Programs unit (WQS&BP).

Investigations of fish kill events are conducted by the RO pollution response teams. Reports are forwarded to the RO enforcement staff and reside in RO files. The Central OE serve in a technical assistance capacity.

Throughout the course of an investigation, coordination among the RO's, OE, and WQS&BP is essential. The immediate staff time requirements of fish kill investigations may place hardships on all offices involved, possibly resulting in the postponement or cancellation of routinely scheduled work assignments. Although personnel are kept on call for such investigations, staff requirements may, at times, be expected to exceed that which is readily available.

This document is based on information contained in the U.S. Fish & Wildlife Service Field Manual for the Investigation of Fish Kills (1990) and the American Fisheries Society (AFS) Investigation and Valuation of Fish Kills (1992). Investigators should refer to those documents for detailed information regarding investigations of fish kills. Investigators are expected to set priorities and exercise varying degrees of technical judgement in carrying out fish kill investigations. This document is not to be viewed as a standard operating procedure.

RESPONSIBILITIES

Responsibility for the fish kill investigation program is shared among the RO, Central OE, and the Office of WQS&BP in the following manner.

Regional Offices (RO)

Investigate fish kills in their region. Log the initial report and assign a pollution incident report number (IR#) to the investigation. Coordinate notifications to other state and local agencies as appropriate.

Conduct fish kill investigations when appropriate and maintain a field log book.

Document all investigative costs. Obtain figures for the replacement costs of fish from the Department of Game and Inland Fisheries (DGIF) and figures for the costs of lab tests from the Division of Consolidated Laboratory Services (DCLS) if the kill is not due to natural causes.

Write the fish kill reports and maintain report files.

The report should be in a finished form so that it may be presented to the party responsible for the kill. Each report should contain at least the information required to complete the "Virginia Department of Environmental Quality Fish Kill Report/Notification", and its appropriate attachments (see Appendix B).

Regional OE evaluate fish kill reports to ensure that appropriate enforcement action and cost recovery may be undertaken when warranted and return written reports to the regional investigator when incomplete.

Request technical review by WQS&BP staff when needed.

Seek recovery of investigative and fish costs when appropriate. All proceedings concerning judicial action to be taken by the DEQ shall be coordinated through the Central OE. In addition, enforcement actions to be taken against major VPDES facilities and actions involving state-wide policy or precedent-setting issues, shall be reviewed with Central OE in advance of final disposition.

Central Office of Enforcement (OE)

Update enforcement procedural manual as necessary.

Provide interpretation and guidance on applicable statutory, regulatory and policy requirements, to the RO, as necessary.

Coordinate judicial action on fish kills with the Office of the Attorney General and the RO.

Review cases involving VPDES majors, state-wide policy and precedent-setting issues.

Assist in obtaining inspection warrants.

Water Quality Standards and Biological Programs (WQS&BP)

Update fish kill investigation procedural manuals and guidelines as necessary.

Provide technical assistance to the RO upon request.

Provide technical review of fish kill reports for RO upon request.

Provide guidance to regional investigators in obtaining the appropriate literature for reference data.

Assist RO with unusual or large fish kill investigations(i.e. fish counts, etc.).

PRELIMINARY TASKS

Receipt of the Fish Kill Report

Upon receipt of a fish kill report, as much information as possible should be obtained from the person reporting the fish kill.

Contact Information

The name, address, telephone number, and any other pertinent information about the caller should be recorded in case there is a need for later contact.

Fish Kill Location

The exact location and description of the kill site should be obtained, not only for the purpose of finding the kill area but also for trip preparation. Precise information on route numbers, intersections, railroad crossings, landmarks, etc. are essential. One should also ask whether the kill site is a lake requiring a large boat, or a small wadeable stream.

Magnitude of the Kill

The approximate size of the kill area in acres or stream miles, the species and sizes of fish, and the approximate number of fish involved should be obtained.

Condition of Fish and Other Observations

It is helpful to the investigator to know if the fish are still dying; such information will enable the investigator to determine how recently the kill occurred and the possibility of the pollutant still being present or released. Also, it is helpful to get the caller's opinion as to the time and cause of the fish kill (e.g., information on recent watershed activities upstream of the fish kill, such as pesticide spraying, fuel or chemical spills, agricultural or construction activity, dumping of fish by commercial fisherman, unusual odors, color of water, foam or oil sheen on the surface of the water. In addition, any obvious external marks or lesions on fish, etc.).

Notifications

The initial fish kill report is usually received by the RO or the Department of Emergency Services (DES) and may be shared via phone or facsimiles. Subsequent notifications within DEQ, and to other state (e.g. the Virginia Department of Health regarding threatened public and/or private water supplies, etc.), federal, local agencies, and private facilities are handled by the RO. It is recommended that the Virginia Department of Game and Inland Fisheries be notified when large or significant fish kills occur e.g., if a fish kill occurs in waters containing threatened or endangered species **(see Appendix C)**. Notifications to the above parties do not necessarily indicate that the RO Office desires assistance with field investigations. The RO will originate any request for assistance. In special cases where technical assistance in the investigation is required, the WQS&BP may be contacted for guidance.

FORMAL FISH KILL INVESTIGATION

A quick response to a fish kill is essential and the incident should be treated as an emergency response situation; however, some office preparation should take place prior to departing for the field in order to conduct an efficient investigation. The investigator should locate the fish kill area on maps. USGS topographical maps and Virginia county maps are ideal for field work and enable the investigator to perceive the terrain and water area involved in order to prepare appropriate equipment for the investigation. The investigator may perform a quick search of the pollution complaint files to determine if the fish kill is a continuing or recurring problem. Permit files and maps may also be searched to identify discharges that are present in the area.

Finally, before departing the office, all field equipment and sample containers should be checked to determine if they are sufficient to conduct the investigation.

The primary purpose of the fish kill investigation is to determine the cause of death (natural or otherwise), the mechanism of death (toxicosis, asphyxia, septicemia, etc.),conditions that lead to death, and the size and number of each species killed (Meyer and Barclay 1990). Field tests, sampling, and other observations should be geared toward answering these basic questions and to identify, if possible, a responsible party to stop or contain the pollution discharged and to prevent future fish kills. Every investigation must be handled on a case-by-case basis and no document can cover all aspects of an investigation. The following sections will aid in the use of specific techniques, either investigative or technical, and may be applied as necessary to individual fish kills.

Location/Confirmation

The first step in the investigation is to locate and confirm the kill, and determine if fish are still dying. Next, attempt to **locate the source** of the pollutant and take measures to **stop and contain** the pollutant with local Fire Department, DES, and/or responsible party assistance. **The DEQ does not expect employees to risk their personal safety in responding to chemical/hazardous spills or any pollution complaint**. In instances where hazardous chemicals, explosives, and flammable materials are involved, (particularly if one is not familiar with the material), **stand clear** and **contact the DES for help**.

Problems of Entry

Investigators on the scene have an obligation and a responsibility to report anything that appears harmful or damaging to the environment. As state employees, investigators should report any problems to the appropriate state agency. If an investigator deems that an inspection of a facility or posted property is appropriate or essential to the investigation, permission to enter the facility or posted property should be requested from a company official or property owner. Normally, immediate access will be granted, however, if an investigator is denied entry for any reason, the investigator should immediately contact RO management. Central OE may also be contacted for assistance in obtaining inspection warrants.

Field Tests

If fish are found dead or dying, the investigator should conduct initial field tests (i.e. pH, dissolved oxygen, temperature, chlorine, conductivity, salinity) in the immediate area where dead fish are found, using properly calibrated instruments. Calibration data should be logged to ensure that any field test measurements can be defended in court. All field test locations, date and time, method, and measurements should be documented in a waterproof field notebook and initialed by the investigator taking the test. Field tests may be used to trace the source of a spill or pollutant discharge and to delineate the area impacted by a spill.

Sampling

Once initial field tests have been made, water column, sediment, and/or fish samples should be collected for lab analyses. Reliable results can be obtained if a few basic principles are followed. For example, ensure that the sample taken is truly representative of the stream, use proper sampling techniques (see pages 8-1, 8-2), preserve and protect the samples until they are analyzed, and use proper sample chain of custody procedures (see Water Quality Assessment Operating Procedures Manual-legal sampling handling procedures and Guidance Memo No 00-2016 Chain of Custody Policy and Procedures in Appendix L). Be sure to collect samples of everything that is pertinent, for example all discharges in the area that may be involved. **REMEMBER: Any sample(s) collected in excess of those needed for validation of the cause or mechanism of the kill can always be discarded later**. Preserved (formalin or frozen) voucher specimens of fish submitted for testing should be kept to later confirm data if required.

General Observations

After appropriate field tests and samples have been taken, general observations and photographs and/or video of the fish kill should be made. As quickly as possible, establish the area of the kill. Estimate the number, size and species of fish involved. If fish are still dying, observe their behavior; whether they are listless, frantic, spiraling, suffering from a loss of equilibrium, etc. Examine the fish externally for gross abnormalities such as open lesions, hemorrhaging, etc. **(see Appendix D)**. Collect a few live fish and keep on ice **(see Pathology 8-6)**. A macroinvertebrate benthic survey should be conducted in nontidal streams or rivers to provide important clues as to the cause of the kill and to document the total impact of the pollution event (see Benthic Survey 8-3). Algal observations should also be made. Dense algal blooms may create a low dissolved oxygen condition in the water column during night time algal respiration and indirectly cause a fish kill. In addition, certain algae produce toxins that can directly cause a fish kill **(see Algae 8-9)**. General observations should be made as quickly as possible. Do not try to get specific details at this time. If additional help is needed for further investigation, request it at this time.

Reference or Control Station

Establishment of a reference station is necessary for comparison of the field test results, field samples, and observations of aquatic life. If the limits of the kill can be found, then a reference or control station should be selected either upstream from the kill or, if the kill is in a tidal estuary, far enough away from the kill area as not to be affected by tidal influence. If the kill has occurred in the headwaters of a stream, then another, comparable stream may be used as a reference. It would be beneficial to have a control or reference sample for each type of field test and/or sample collected.

Continued investigation (e.g. field testing, sampling, etc.) after initial observations and sampling may be necessary. The investigator should use the initial information as a starting point to begin a more detailed investigation/sample plan.

Press Release

Only after the initial investigation has been completed is there enough information to respond to inquiries by the press or other concerned parties. The size and character of some fish kills necessitates a quick response; however, the release of information will be conducted under the direct responsibility of the Regional Director under the same guidelines as other pollution events.

Counting Dead Fish

The DEQ has been given the responsibility for assessing the damage to aquatic life caused by pollution incidents. The State Water Control Law is very specific for fish kills: the SWCB shall have the duty "To investigate any large-scale killing of fish" (§62.1-44.15 (11) **see Appendix A**). Thus, the counting of fish killed is a responsibility of the DEQ staff. Just counting dead fish, unfortunately, is not enough. Since the costs of fish replacement are assigned by DGIF, the count must contain the necessary information for the DGIF to make assessments as well as for DEQ to determine the total natural resource damage. Species level identification should be obtained for all fish killed except for certain members of Cyprinidae (carps and minnows) (i.e. *Notropis* spp.) and Percidae (perch and darters) which are often similar in appearance. Counts of generalized cyprinids and percids should be conducted for replacement cost analysis. In waters containing endangered species, species level identification should be conducted for all species. Numerous publications exist that can help investigators identify fish to species level in the field. Two publications specific to fishes in Virginia are referenced at the end of this manual (Jenkins and Burkhead, 1993, and McIninch and Garman, 1998). In addition, the Department of Game and Inland Fisheries on-line Wildlife Information Database may be accessed at www.dgif.state.va.us/wildlife/index.cfm to quickly generate a list of fish and other aquatic and semi-aquatic animals including species listed as threatened, endangered, and special concern that are expected to occur within a three mile radius of the fish kill area.

The following procedure explains the methods used and the information required to ensure accurate and responsible fish kill counts.

Counts used in fish kill reports can come from several sources. Most counts will be done by the DEQ staff, but count information also has been obtained in the past from the DGIF, the Virginia Institute of Marine Science (VIMS), and the Virginia Marine Resources Commission (VMRC). Every count, no matter who makes it, must be conducted utilizing an acceptable technique (i.e. AFS guidelines). Accepting count information from other sources must be handled carefully as the DEQ will ultimately be responsible for the accuracy of the information reported.

Three primary methods may be employed to conduct fish kill counts: total count, standard procedural count, and estimates.

Total Count

The best method of counting fish is a total count of every fish, starting downstream and working upstream. The total count is the most indisputable method available, since it is an exact count of every fish the investigator saw.
The total count method is preferred and should be done whenever possible. However, in many cases, a kill will be of such magnitude that a total count will be impossible.

Standard Procedural Count

Lacking a total count, a standard procedural count involving the counting of fish within sampling areas and extrapolating the total number of fish killed, is the next best method. The DEQ utilizes the AFS "Investigation and Valuation of Fish Kills, Special Publication No. 24", 1992 and the "Sourcebook for Investigation and Valuation of Fish Kills", Supplement to Special Publication No. 24, 1993) as the official methodology for standard procedural counts **(see Appendix E)**. To conduct a standard procedural count, the furthest upstream and downstream locations that dead fish are found are first determined. Then the investigator delineates sample segments at regular intervals between the upstream and downstream locations on a map.

For most fish kills, a count of dead fish in one 100 yard segment per 1/2 mile of stream is acceptable. This type of count is commonly used by the staff and meets the minimum evidentiary requirements for legal action. However, the larger the percentage of affected stream counted, the more accurate the computed estimate will be. Therefore, the investigator may decide to count more than one 100 yard segment per 1/2 mile. Within each 1/2 mile section, the investigator must choose a representative area to count. Often access is limited but, if possible, randomly select a 100 yard reach of stream that is representative of the characteristics of the stream. The investigator must be careful not to bias the count, particularly if dams or other obstructions which accumulate fish are present. Keep in mind that the segment count is a sample and should be a good representative of the whole, not an exception. For example, if one encounters a dam or obstacle where many fish are trapped, the downstream count will be greatly influenced by the obstruction.

The distance between sample segments may have to be adjusted to accommodate the total length of the kill and the investigative resources available. Ascertaining the length of the kill is important before starting the count so the proper distance between segments can be determined. As a rule of thumb (it will vary depending on stream depth and accessibility), a two person counting team can count about three or four 100 yard segments in one day. Since it is desirable to make the count within a single day to avoid duplicate counts of drifting fish, the investigator must judge his or her segment length and distance carefully. For example, if only two people are available for counting dead fish and the fish kill is determined to be 10 river miles long, it will take the crew five to ten days to count 100 yard segments, spaced 1/2 mile apart as recommended by protocol. In this case, the investigator could call for assistance from other regional personnel; if additional assistance is not available, an alternative would be to make the segments about 2 or 2 1/2 miles apart. For large fish kills, as many as six to twelve persons may be involved in counts if the 1/2 mile segment guideline is followed.

Final fish counts for the stream segments should be recorded on the "Fish Kill Count Form" **(see Appendix F)**.

The number of fish killed for each species size class is calculated using either the mean number of fish counted per segment or an expansion factor for the total number of fish counted in the sample segments.

Example:
In a 2 mile long fish kill, 240 2-inch bluegills were counted in 4 segments of 100 yards, spaced 1/2 mile a part.

(a) Mean number of fish counted per segment:

mean number = 240 fish / 4 segments = 60 fish per segment

In 2 miles, there are 35.2 segments each 100 yards long, so

60 fish per segment x 35.2 segments = 2,112 2-inch bluegills killed;

2100 2-inch bluegills killed; estimate based on appropriate significant digits (AFS, 1992).

(b) Expansion factor:

$$\text{expansion factor} = \frac{(1{,}760 \text{ yards per mile} \times 2.0 \text{ miles})}{(4 \text{ segments} \times 100 \text{ Yards per segment})} = 8.80$$

240 fish x 8.80 = 2,112 Fish:

2100 2-inch bluegills killed; estimate based on appropriate significant digits (AFS, 1992).

Procedural counts have been modified for fish kills in narrow completely and incompletely accessible streams, narrow streams with drifting fish, wide streams, large meandering streams, lakes, and multiple day counts. Detailed procedures and counting examples are presented in **Appendix E**.

Estimate

If neither a total count nor a procedural count can be made, then an estimate should be made. Estimates have mostly been used for large menhaden kills, where a million fish may be a foot deep in a small channel. Estimates provide an idea of the magnitude of a fish kill.

There are several more important points to remember. The investigator should look closely at the fish species involved in the kill even if no replacement cost is to be assigned.

Primarily, one should remember that threatened or endangered species or rare endemic populations may be severely impacted. In addition, other aquatic or semi-aquatic animals may have been killed. Impacts to benthic invertebrates, amphibians, reptiles, birds, and mammals should be documented and the total environmental impact of the pollution incident should be reported.

It should be noted that counting procedures typically underestimate the number of fish killed for a variety of reasons. For example, fish may not be counted that are too deep or too small to be seen, or they may have been scavenged by predators. In addition, time is against investigators of fish kill events. For example, Hayne et. al. reported that estimates for the number of fish killed decrease by approximately 50% after 24 Hours of the fish kill (Nielson and Johnson, 1983). The underestimation may be reduced by more complex and more costly counting techniques, however, most state agencies reason that a rapid defensible estimate better serves the needs of the public than a more complete and costly study (AFS, 1993).

COSTS

Fish Cost Analysis

It is DEQ policy to have the DGIF determine the replacement costs of the fish that are killed. In order for the fish to be assigned a cost by DGIF, the fish must be correctly measured and identified, due to the fact that restocking values differ with the size and species of fish involved. Determining which information to obtain is facilitated by the publication "Investigation and Valuation of Fish Kills" (American Fisheries Society, 1992; **see Appendix E**). This publication represents the results of the AFS Pollution Committee work on the value of fish species and the cost of their replacement. These costs are now accepted by the Southeastern Division of AFS. Familiarization with this document will assist the investigator in determining what information to gather on fish counted in the field. In cases where only weight is acceptable, a representative collection of species sizes must be made. This collection is weighed in the field using accurate scales and the values expanded for the total number of fish killed. Fish that cannot be identified must be collected and identified later. Remember to record the number of the unknown fish at each segment and their size. In order for the DGIF to assign costs, information is recorded on a "Replacement Cost of Fish" form (**see Appendix G**). This form is officially transmitted to DGIF via the Regional Director. The form is dated and the fish kill number (IR #) is listed. To assist in the evaluation of replacement costs, the body of water and location of the kill is also noted. The most important part of the form is the itemized listing of fish killed. Each species is listed separately by size and number counted. Continuation sheets may be used if needed. The form is filled out by the investigator and sent directly to the Director of the Fisheries Division at DGIF via the Regional Director. DGIF assigns the costs, signs and returns the original copy to the regional investigator. The original form becomes part of the case file for each fish kill investigation.

Cost of Investigation

By law the DEQ may recover the costs incurred as a result of a fish kill investigation, as well as any cost incurred by the DGIF in investigating the kill **(Appendix A)**. Hence, investigators should keep records of investigative expenses as a basis for cost recovery. The Pollution Complaint Investigation Total Cost Summary Form **(see Appendix H)** is designed to document investigation costs. The form is used to record the actual costs incurred per individual during the investigation and the costs of any follow-up investigation, report writing, etc.. The form has several major parts. The first part deals with hourly labor costs. The grade is the grade the employee held at the time of the investigation. The grade standard hourly rate is multiplied times the number of hours spent on the investigation to arrive at a total labor cost. The next part of the form deals with vehicle mileage or other transportation costs and is self explanatory. The following part deals with food and lodging. These expenses are reported exactly as claimed for reimbursement on travel vouchers. Lastly, under materials and equipment, items may be included such as costs of ice, tolls, film, sample jars, laboratory costs (obtained from the catalog of laboratory services if conducted by DCLS), etc. The expenses are totaled and the form is signed and dated by the staff involved in the investigation. Disk one at the back of this manual includes an Excel program file (FISHKIL1.XLS) expense form that automatically calculates pay grade rates and totals all expenses. If DGIF personnel assist with an investigation they should also complete an expense form. The original form is submitted to the regional enforcement staff with the final report. The last part of the form is the sum of all costs for each investigator and materials and equipment expenses. If a responsible party is identified, this is the amount that will be requested for reimbursement.

CAUSES OF FISH KILLS

Fish kills may have many different causes which can be grouped under seven general categories:

1. Industrial Operations
2. Municipal Operations (domestic sewage systems)
3. Agriculture and related activities
4. Construction/other causes
5. Transportation operations/storage
6. Natural causes
7. Fish dumping from commercial operations

Industrial Operations

Fish kills can occur as a result of industrial operations. Once the outfall of a suspected industrial waste discharge has been located, an attempt should be made to identify the owner of the facility from which the outfall originates and encourage the owner or operator to halt the suspected toxic discharge. A sample of the discharge should be collected as soon as possible, preferably at the location where the waste leaves the facility property. If an in-plant inspection is warranted, contact the plant manager or person in charge and request a brief tour of the facility. If denied entry, contact Regional Office management or the Central Office of Enforcement. During a tour; the investigator can obtain general information concerning the products manufactured; raw materials used in the manufacturing process; quantities, sources, and characteristics of wastes generated; and waste treatment units if any. The plant manager may be able to supply a flow diagram of plant operations. The investigator should also request specific information concerning facility operations (i.e., accidental spills, etc.) immediately prior to the beginning of the fish kill.

Municipal Operations

Waste discharges from municipal or domestic sewage treatment plants may contain domestic sewage or industrial wastes combined with domestic sewage. These wastes may have been partially treated at the treatment plant or discharged untreated directly into a stream. Since the municipality or owner or operator of the sewage system is generally held responsible for any discharge from such a system, the owner/operator or their representative (i.e. city engineer, public works supervisor, a subdivision developer ,etc.) should be contacted when the samples of the suspect wastewater discharge are collected. The investigator should obtain information about plant operations. If the cause of the fish kill is determined to be the result of industrial waste discharging to a municipal treatment facility and then to a stream, data about the industry and its discharge should be obtained from municipal officials.

Agriculture and Related Activities

Fish kills can occur as a result of pollution from agricultural practices such as crop dusting, fertilizer application, and manure or other organic material discharges to a stream. Fish kills resulting from these agricultural operations are usually associated with runoff due to rainfall. The source or type of pollution may be difficult to identify and may involve a large nonpoint source area. Talking to local residents may help pinpoint the problem area. Runoff from fields, drainage ditches, and small streams leading to the kill area may provide good sampling sites to trace the cause. Noting changes in turbidity in the stream may help to locate possible sources of runoff. Analyses of sediment samples are usually more reliable than water samples where pesticides or herbicides are suspected as a cause of the kill.

Construction/Other Causes

Fish kills may result from mining activities as well as from such temporary or intermittent activities such as; mosquito spraying; construction activities involving chemicals, concrete, and oils; and weed spraying with herbicides or other toxic substances. As with agricultural activities, tracing the cause of these kills is difficult and may require extensive investigation.

Transportation Operations

Fish kills occurring as a result of transportation accidents/incidents are usually readily identified. The investigation should include specifics of the accident such as vehicle license number, vehicle owners, cargo type, DOT placard number, etc..

The **DEQ does not expect employees to risk their personal safety in responding to chemical/hazardous spills or any pollution complaint.** In instances where hazardous chemicals, explosives, and flammable materials are involved, (particularly if one is not familiar with the material), **stand clear** and **contact the DES for help**.

Natural Causes

There are several possible natural causes for fish kills:

1. Oxygen depletion due to ice and snow cover on surface waters.
2. Oxygen depletion at night because of plant respiration or at anytime during the day because of naturally occurring organic compounds in the water.
3. Abrupt temperature changes.
4. Epidemic and endemic diseases, parasites, and other naturally occurring biological causes.
5. Lake water inversion during vernal or autumnal turnover which results in toxic material or anoxic water being brought to the surface.
6. Poor lake management resulting in overcrowding or introduction of the wrong species.
7. Fish spawning stress.
8. *Pfiesteria piscicida* and *Pfiesteria* like microbes often called *Pfiesteria* complex are dinoflagellates that have been implicated in recent fish kills in coastal waters in North Carolina and in the Pocomoke River near the Virginia Maryland border on the Eastern Shore. The DEQ has developed response and safety plans to deal with *Pfiesteria* related fish kills. **See Appendix M** for a copy of the DEQ plans and for information regarding *Pfiesteria.* Up to date information regarding *Pfiesteria* fish kills may be found at the Virginia Institute of Marine Science Web site (www.vims.edu).

There are few truly "natural" kills. Almost all kills, including disease outbreaks, occur due to external stresses. It is just as important, therefore, to identify these environmental stresses as it is to identify the disease causing organism(s).

Fish Dumping from Commercial Operations

This problem occurs predominantly in the piedmont and tidewater regions but can occur anywhere there are commercial fishing operations. Most reported kills result from the emptying of nets or wash-down operations. In addition, commercial nets that break may release large numbers of dead or dying fish. Commercial fisherman in Virginia coastal waters are required to report such incidents to the VMRC.

ADDITIONAL METHODS TO ASSESS ENVIRONMENTAL IMPACTS

The following are additional methods of obtaining information that may assist in further enhancing or defining the cause(s) of fish kills and assessing environmental impacts. Their use is not mandatory, but one or more may be employed at the investigator's discretion.

Water Samples

Substances in solution or suspension largely determine the quality of water. Fish are affected both directly and indirectly by these substances. The addition of dissolved or suspended material to water or the altering of amounts of substances naturally found in the environment can be harmful to fish and thereby cause a fish kill. The analysis of water by collecting water samples is a standard procedure for fish kills. The actual collection is, therefore, of considerable importance. The fish kill investigator should use appropriate containers that have been properly cleaned. Otherwise, the chemical data received will be invalid.

Water samples should be collected in flowing water where the water is well mixed. Place the mouth of the container a few inches below the water surface. This is done to avoid collecting floating material (except where the material is the suspected pollutant). Air should be excluded from the container when samples are to be analyzed for dissolved oxygen, BOD, pH, acidity, alkalinity, chlorine, volatile organics, sulfur dioxide, and hydrogen sulfide. Table 3 in Section 2.0 of the Water Quality Assessment Operating Procedures Manual (the WQAOP manual) lists analytical parameters, recommended containers, preservation and holding times as mandated by EPA (40 CFR Part 136). The volume of sample required by the DCLS to analyze the requested parameters is also listed in Table 3. Specific preservation procedures for the most commonly sampled parameters are described in Section 2.0 of the WQAOP manual.

Also, be sure to identify and handle all samples properly as described in Section 5.0 of the WQAOP manual and DEQ Guidance Memo 00-2016 **(see Appendix L)**. Be certain that all sample tags and lab sheets are filled out completely. DCLS will reject and may discard any samples with improper documentation.

Knowing what parameters to sample for may be the most difficult part of collecting water samples. If the fish kill occurs below a sewage treatment plant, the investigator should collect samples for BOD, COD, nutrients, metals, organic priority pollutants and pesticides analysis if the area is industrialized. If a fish kill occurs below certain industrial discharges the nature of the industrial activity should determine the type of sample collection needed. For example, below a pulp or paper mill, investigators may collect samples for BOD, COD, pH, and phenols for analysis. In agricultural areas the investigator may collect samples for pesticides, metals, and nutrients from fertilizers for analysis.

Sediment Samples

In some cases, the collection of water samples is not enough. The investigator may have arrived after the toxic discharge has stopped, the pollutant may have passed downstream or it may have become diluted beyond recognition in the receiving water. This presents the investigator with a formidable problem. He or she must not only attempt to identify a pollutant no longer in the water but also must find a source which may no longer be discharging. It is at this point one may consider collecting sediment samples.

The sediment sample offers the investigator the opportunity to recover pollutant residues, some of which may have settled out or may have become attached to particles of material in the water column which have settled. Obviously, sediment itself has some different physical and chemical characteristics than the overlying water. Analysis of some parameters is not appropriate due to the differing chemistry or physical properties of sediment versus water.

Sediment samples do offer a good chance of identifying metals, pesticides, and some organic materials that may be the cause of the fish kill. The amount of a minimum sample (one pint) is nearly the same for all uses, but the location of the sediment collection may vary. In the case of fish kills, where very recent contamination is involved, one is interested in only a few top millimeters of sediment. A grab or scoop of sediment may dilute the recent surface contaminate beyond recognition. The type of sediment involved is also important and should be specified on the lab sheet. The absorption of some pollutants in silty mud may differ greatly from that of sand. As always, a good control is essential. Analysis of sediment samples is time- consuming and expensive. Such samples should be collected to identify a specific pollutant and the analysis cancelled if found unnecessary to verify the pollutant type. Detailed procedures for sampling sediments may be found in Section 2.0 of the WQAOP manual. Preserve and protect the samples until they are analyzed, and use proper sample chain of custody procedures (see Water Quality Assessment Operating Procedures Manual-legal sampling handling procedures and Guidance Memo No 00-2016 Chain of Custody Policy and Procedures in Appendix L).

Benthic Macroinvertebrate Surveys

The term "Benthic Macroinvertebrate" refers to invertebrate animals such as larval and adult insects, molluscs, aquatic worms, and crustaceans that live on the bottoms of lakes, streams, estuaries and ocean floors. Although susceptibility to toxic compounds varies among the invertebrate groups, a concentration of a toxic substance that will kill fish will also kill benthic organisms. After a compound toxic to benthic organisms enters the water via a spill or a discharge, the number of benthic organisms living on the bottom downstream from the spill or discharge will be reduced, compared to upstream control areas. The boundary between the affected and unaffected areas is usually distinct.

This difference in the concentrations of bottom dwelling organisms above and below a spill or discharge point makes benthic surveys useful in pinpointing the source of a toxic pollutant when its origin is unknown. After or even during a fish kill, by examining the benthic life as you proceed to the head of a kill area, you may discover the sharp boundary between the affected and unaffected zones. This boundary may point to a particular discharge in an area with many discharges close together. It may also be possible to trace the path of the toxicant into tributaries too small to contain permanent fish life, which might have otherwise been overlooked without a cursory benthic inspection. Even when the source of pollution is known, information on how the benthic life was affected still helps build your case against the polluter. The benthic studies utilize the EPA approved Rapid Bioassessment Protocols for Use in Wadable Streams and Rivers (EPA 841-B-99-002) to assess benthic communities. For the most meaningful results, the benthic macroinvertebrate survey should be completed within three weeks. Benthic organisms themselves are slow to recolonize. The differences between the affected and unaffected areas will remain distinct for several weeks, after which time, recolonization by benthic organisms drifting downstream with the current makes the differences continually less apparent. A benthic survey also helps to determine the severity of a fish kill and the total impact on the stream's biota. Since most benthic animals are a source of fish food, a severe reduction in their numbers over a lengthy stretch of stream would inhibit recolonization of the stream by fishes.

Bioassay Toxicity Testing

Bioassay toxicity tests are not recommended to investigate potential toxicants during fish kill investigations. Rather, if the specific chemical can be identified, the manufacturer or the Material Safety Data Sheet (MSDS) should be consulted to determine the toxicity of the chemical. Many chemicals are required to be tested on aquatic organisms before they are marketed and the information is available from one of these two sources. Another option is to search the EPA on-line AQUIRE Database at www.epa.gov/ecotox. This database provides information regarding the toxicity of chemicals to aquatic organisms.

If a toxicity test is to be conducted it must be performed by a contract laboratory (private or university) since the DEQ no longer has the capability to conduct bioassay toxicity testing.

Arranging a Toxicity Test for Fish Kill Investigations

1. Contact a contract laboratory to determine if a test can be performed and establish a contractual agreement in accordance with DEQ contract procedures, and arrange sample delivery. Due to time constraints, a base contract with a bioassay laboratory should be established before a fish kill event.

2. Collect at least 3 gallons of the material to be tested. Immediately store on ice.

3. Include information as to the composition of the sample and an MSDS if available. (This protects the staff from known hazards).

4. Ship the sample overnight or deliver the sample, on ice, to the laboratory selected for testing.

Chemical Analyses of Fish Tissue

Chemical analysis of fish tissue samples may prove to be of value in fish kill investigations. As with many investigative practices, the dead fish may yield information as to the cause of death. It is important to remember that fish tissue analyses are an indirect method of identifying the pollutant involved.

Affected fish must also be carefully chosen. They must be representative of the kill; they should be alive rather than dead. Fresh dead fish may be acceptable, but during the summer months significant deterioration of organs and muscle tissue may occur within hours. Individual fish make better samples than composites, since composites tend to average concentrations of pollutants and make range determinations difficult.

The use and analysis of fish samples is far too complex to cover fully here. The "DEQ Quality Assurance/Quality Control Project Plan for the Fish Tissue and Sediment Monitoring Program" gives a breakdown of fish handling procedures **(see Appendix I)**. Further handling and analysis is best determined on an individual basis.

Shellfish

Shellfish, when present, offer excellent opportunities for analysis of many metals, pesticides, and organic pollutants. Being filter feeders and unable to move about, oysters, clams, and even freshwater mussels tend to accumulate and concentrate pollutants in their tissues. Sample variation is decreased if animals of the same size are taken from a single location.

Handling of shellfish samples is relatively easy once the animal is obtained from the bottom. Since oysters and clams can live for days out of water, simply keep them cool and bring them in for shipment to the laboratory.

Control or reference samples are often a serious problem. Since collection is normally done in tidal areas, one must collect control samples a considerable distance from the affected area to avoid contamination. Unfortunately, shellfish may be of different sizes or not present at all if salinity is different in the control area.

For specific instructions on the preparation of shellfish samples **see Appendix I**.

Pathology

The causes behind fish disease may be divided into three categories:

1) Pathogenic organisms (bacterial, viral, fungal, or protozoan),

2) Toxins or poisons,

3) Physical stress or changes in water quality

Populations of fish may be affected by one or a combination of the above factors with a resulting fish kill. Investigation of fish kills and the diagnosis of diseases should be a step-by-step procedure. It should be the goal of the investigator to gather as much information as possible at a kill site so that the agent(s) behind the disease may be accurately determined.

Consult the "Fish Kill Pathological Examination Report" form for the water quality data required to perform the pathological examination **(see Appendix J)**.

Notations on conditions at a kill site and the affected species may often be as helpful to the diagnostician as samples sent to the lab. Investigators should pay close attention to the behavior of ailing fish and accurately record any abnormalities. Attention should be given to the species, size, appearance, and distribution of affected fish on-site. Thoughtful estimations of the numbers of sick or dying fish as well as the size of the water body are also helpful. Estimating the size of a kill can often be accomplished by counting the number of dead or dying fish per unit length of shoreline or surface area of water. Finally, measurement of water quality parameters (pH, DO, temperature, conductivity, etc.) may help in diagnosis.

Careful consideration should be given to the selection of diagnostic specimens to be sent in for analyses. Affected fish that are near death or freshly dead should be used whenever possible.

Poor or grossly decomposed samples not only provide little information, they may also act to confuse or mislead the examiner. Any specimens that were dead upon collection should be clearly noted for the diagnostician and they should be packaged separate from those collected alive, to prevent contamination. Samples for analysis should be collected as gently as possible, clearly labeled, and shipped or transferred for examination as soon as possible.

Fish for pathological examination are normally shipped to one of the following laboratories;

1) Leetown West Virginia Science Center, Contact Dr. Vicki Blazer (304) 725-8461 Ext. 434, Biological Resource Division, USGS - Eastern Region , 1700 Leetown Road, Kearneysville, West Virginia 25430.

2) The College of William & Mary, Virginia Institute of Marine Sciences, Contact Dr. Wolfgang Volgelbein, (804) 684-7261, e-mail: wolf@vims.edu, Chesapeake Bay Hall Room N107, N125, Gloucester Point, Va 23062

3) Virginia Tech, Contact Dr. Stephen Smith (540) 231-5131, e-mail: stsmith7@vt.edu, Biomed. Sciences & Pathology Phase II, Room 121, College of Veterinary Medicine, Blacksburg, Va 24061

The WQS&BP office should be contacted prior to shipping to coordinate the shipment of fish collected for pathological examination. Specimens that cannot be examined immediately must be preserved for future investigation. The majority of fish pathogens (especially ectoparasites) will quickly drop off or perish during the decay of host tissues. The investigator who selects diagnostic specimens must first make an assumption of the general cause of the disease in order to use the most suitable method of preservation. Common methods of preservation and probable uses are listed below.

CHILLING is generally used to preserve samples where the freezing or chemical fixation of host tissues would prevent the isolation or identification of disease agents. Whole fish or fish parts may be preserved in this manner if the time until examination is measured in hours. Bacteria, fungi, protozoans, as well as histological and blood samples are generally preserved by this method. Diseases in which the appearance of the fish tissues is important in diagnosis should also be preserved by this process. The best procedure for chilling is to place the specimen in a waterproof container and transfer on wet ice or under refrigeration.

FREEZING is used in those cases where living organisms are thought to be present in the specimen and the destruction of tissue cells is not important. Tissues thought to be harboring larger parasites may be preserved in this manner. Tissue samples to be analyzed for the presence of chemicals or toxins should also be preserved by freezing. Specimens should be frozen as soon as possible to avoid overgrowth of contaminating organisms. The samples should be placed in a waterproof container on dry ice or in a mechanical freezer.

All samples should remain frozen until they are ready for examination.

CHEMICAL preservatives are generally used when the viability of organisms is not necessary and contamination of tissues by fixatives does not hinder examination. Tissue samples and large animal parasites may be preserved in this manner. Specimens selected for chemical preservation should be small enough to allow penetration of the preservative within a short time. Fishes up to five cm can be placed directly into the preservative while larger specimens should be opened along the abdomen before being placed in the chemical. Common chemical preservatives include a 70% isopropyl alcohol or a 10% solution of formalin.

Fish samples are collected for pathological examination whenever the investigator feels that the kill is the result of pathogenic organisms or under conditions where unusual symptoms are noted. For the purposes of pathological analysis, fish kills may be divided into two groups, those kills which are the result of pathogenic organisms (which may be viral, bacterial, fungal, or protozoan) and those that are the result of the introduction of a toxic material or materials.

In the first group, the goal of the investigator is to determine the specific pathogen involved so that a more concise analysis of the cause of the kill is possible and, in some instances, so that appropriate remedial action may be taken. Important field observations would be species involved; size class, behavior, and appearance of affected fish; distribution of affected fish; and the normal water quality parameters of DO, pH, and temperature.

In the second group, fish samples are usually taken with an eye to toxicity testing. For example, a fish kill occurs and a given toxicant is suspected but the literature fails to adequately support this hypothesis. Pathological examinations are performed on the affected fish and abnormalities are noted. Later, a toxicity test is conducted using similar circumstances and the fish from this test also undergo pathological examination. If the abnormalities noted on the kill fish correspond to those noted on the test fish then the investigator has an expanded data base to support the original hypothesis. It is important that control fish also be examined so that any abnormalities noted can be directly attributed to the toxicant and not to factors such as other toxicants, spawning stress, seasonal variation, and the like.

It is important that the investigator understand that fish samples submitted for pathological examination (concerning toxicant caused fish kills) in no way replace fish tissue, sediment or water samples collected for chemical analyses.

Algae

Under certain circumstances and in particular periods of high algal productivity, algae or their toxic products may cause illness or death in man, fish, and other animals. There are two basic groups of algae responsible for fish kills. These are the bluegreen algae and the armored dinoflagellates. Although both groups occur in both fresh and saltwater, the bluegreen algae are known to cause toxicity problems in freshwater. In marine waters the predominant organisms causing toxicity are the dinoflagellates (see *Pfiesteria* page 7-3 and Appendix M) but other groups have also been implicated. Problems with dinoflagellates usually occur south of Virginia.

Instances of acute and often fatal poisoning of aquatic animals, farm animals, and birds are numerous, especially if the animals drink water containing the greatest concentration of plankton during an algal bloom. Most of the outbreaks of algal poisoning have occurred during periods of continuous hot weather, when the water had a high organic content, and when the floating algae were concentrated in the water body by wind or current. Algae samples should be taken if such conditions precede or occur during a fish kill.

Algae samples should be preserved (with copper sulfate and formalin or Lugol's solution) and, if possible, non-preserved samples should be collected in quart containers. The non-preserved samples should be stored and shipped at approximately 4°C. (see DEQ Sampling Techniques for Chlorophyll and Algae, Appendix K). It is acceptable to arrange for other state institutions to perform algal taxonomic identifications. Recently, Dr. Harold Marshall (phone 757 683-4204, e-mail hmarshal@odu.edu) at ODU has performed many algae identifications for the Tidewater and Piedmont regional offices. If an outside source is used, a contract and purchase order may be required.

Special Studies/Surveys

Follow-up surveys can be conducted if needed. Investigators may perform special studies/surveys to determine if the kill area is still receiving pollutants or whether the problem has been corrected. The survey can aid in determining the source of a pollutant and will also provide data on the effectiveness of a facility's waste treatment process. A macroinvertebrate benthic survey can be conducted by the regional biologist on the stream, lake, or estuary involved. The benthic survey can ascertain the effect of the discharge on the body of water which receives the wastes. The survey may also judge the severity of the effect of the discharge and the extent of the area affected. Some kills may involve only a few dead fish because the stream supported only a small number of fish, yet a benthic survey may reveal that the stream has been impacted for miles.

DATA INTERPRETATION AND REPORT PREPARATION

After all the field work and additional research has been completed, the chemical and biological laboratory samples have been analyzed, and the results returned to the investigator, the data are ready to be organized and interpreted into a final written report. The investigator should consider all phases of the investigation when determining the cause of the kill. The data should be arranged in a logical sequence to make interpretation as easy as possible.

The first step is to look at the field test results. These results should be compared to the DEQ Water Quality Standards (9 VAC 25-260-20.A) and any standards violation noted. Literature research is then necessary to determine if the violation is extreme enough to cause a fish kill. The WQS&BP can be contacted for assistance with the literature search. In addition, the internet may also be used to conduct quick information searches.

The investigator should then check the field observations. Does the fish behavior confirm the water quality violation demonstrated by the field tests as the cause of the kill or is another cause suspected? The investigator should check the pathologist's report for the possibility of a disease causing the kill. If this proves to be the case, the cause for the pathogen outbreak should be investigated further to determine the extent of water quality degradation.

The next step is to review the chemical and biological analysis results. Sample results in the affected area are compared to the control stations. Chemical data which appear abnormal are compared to data found in the literature. This is where the investigator may have to rely on toxicity tests for a source of comparison to his or her chemical data. Most literature values are specific to certain aquatic life and water conditions. Therefore, the need may arise for duplication of the specific conditions present at the time of the kill. Standards violations are fairly straightforward and require little additional work. The biological data can augment the chemical data and in some instances indicate the cause of the kill. Macroinvertebrate benthic analyses can be very useful for determining the extent of damage. The algal data are important because if the species present is identified as a producer of toxin, the algae could have directly caused the kill.

All of the above are not necessary to prove the cause of every kill. Important points in substantiating a kill are locating the source, the responsible party, and the causative agent.

As long as these three steps are covered, the report is complete. It should be remembered that any fish kill may require legal action to collect penalties, recover replacement costs of the dead fish and investigation costs, as well as to secure injunctive relief to prevent future kills. Keeping the legal possibilities in mind, the investigation should include as many of the steps above necessary to support a legal case.

Remember: The most serious error an investigator can make is to assume that conditions obvious to him in the field can be easily demonstrated in a final report. Data, sample analyses, photos, detailed notes in a waterproof field log book, other evidence, etc., must be able to convince someone with less knowledge of the subject than the investigator.

REFERENCES

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Meyer, Fred P., and Lee A. Barclay, editors, 1990. Field Manual for the Investigation of Fish Kills. U.S. Fish and Wildlife Serv., Resour. Publ. 177. 120 pp. (Note: copies of this publication may be purchased from the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161, or from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20401, Stock Number 024-010-00685-4).

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APPENDIX A

STATE WATER CONTROL LAW §62.1-44.15

§ 62.1-44.15. Powers and duties.

It shall be the duty of the Board and it shall have the authority:

(1) [Repealed.]

(2) To study and investigate all problems concerned with the quality of state waters and to make reports and recommendations.

(2a) To study and investigate methods, procedures, devices, appliances, and technologies which could assist in water conservation or water consumption reduction.

(2b) To coordinate its efforts toward water conservation with other persons or groups, within or without the Commonwealth.

(2c) To make reports concerning, and formulate recommendations based upon, any such water conservation studies to ensure that present and future water needs of the citizens of the Commonwealth are met.

(3a) To establish such standards of quality and policies for any state waters consistent with the general policy set forth in this chapter, and to modify, amend or cancel any such standards or policies established and to take all appropriate steps to prevent quality alteration contrary to the public interest or to standards or policies thus established, except that a description of provisions of any proposed standard or policy adopted by regulation which are more restrictive than applicable federal requirements, together with the reason why the more restrictive provisions are needed, shall be provided to the standing committee of each house of the General Assembly to which matters relating to the content of the standard or policy are most properly referable. The Board shall, from time to time, but at least once every three years, hold public hearings pursuant to subsection B of § 2.2-4007 but, upon the request of an affected person or upon its own motion, hold hearings pursuant to § 2.2-4009, for the purpose of reviewing the standards of quality, and, as appropriate, adopting, modifying, or canceling such standards. Whenever the Board considers the adoption, modification, amendment or cancellation of any standard, it shall give due consideration to, among other factors, the economic and social costs and benefits which can reasonably be expected to obtain as a consequence of the standards as adopted, modified, amended or cancelled. The Board shall also give due consideration to the public health standards issued by the Virginia Department of Health with respect to issues of public health policy and protection. If the Board does not follow the public health standards of the Virginia Department of Health, the Board's reason for any deviation shall be made in writing and published for any and all concerned parties.

(3b) Except as provided in subdivision (3a), such standards and policies are to be adopted or modified, amended or cancelled in the manner provided by the Administrative Process Act (§ 2.2-4000 et seq.).

(4) To conduct or have conducted scientific experiments, investigations, studies, and research to discover methods for maintaining water quality consistent with the purposes of this chapter. To this end the Board may cooperate with any public or private agency in the conduct of such experiments, investigations and research and may receive in behalf of the Commonwealth any moneys which any such agency may contribute as its share of the cost under any such cooperative agreement. Such moneys shall be used only for the purposes for which they are contributed and any balance remaining after the conclusion of the experiments, investigations, studies, and research, shall be returned to the contributors.

(5) To issue, revoke or amend certificates under prescribed conditions for: (a) the discharge of sewage, industrial wastes and other wastes into or adjacent to state waters; (b) the alteration otherwise of the physical, chemical or biological properties of state waters; (c) excavation in a wetland; or (d) on and after October 1, 2001, the conduct of the following activities in a wetland: (i) new activities to cause draining that significantly alters or degrades existing wetland acreage or functions, (ii) filling or dumping, (iii) permanent flooding or impounding, or (iv) new activities that cause significant alteration or degradation of existing wetland acreage or functions.

(5a) All certificates issued by the Board under this chapter shall have fixed terms. The term of a Virginia Pollution Discharge Elimination System permit shall not exceed five years. The term of a Virginia Water Protection Permit shall be based upon the projected duration of the project, the length of any required monitoring, or other project operations

or permit conditions; however, the term shall not exceed fifteen years. The term of a Virginia Pollution Abatement permit shall not exceed ten years, except that the term of a Virginia Pollution Abatement permit for confined animal feeding operations shall be ten years. The Department of Environmental Quality shall inspect all facilities for which a Virginia Pollution Abatement permit has been issued at least once every five years, except that the Department shall inspect all facilities covered by the Virginia Pollution Abatement permit for confined animal feeding operations annually. Department personnel performing inspections of confined animal feeding operations shall be certified under the voluntary nutrient management training and certification program established in § 10.1-104.2. The term of a certificate issued by the Board shall not be extended by modification beyond the maximum duration and the certificate shall expire at the end of the term unless an application for a new permit has been timely filed as required by the regulations of the Board and the Board is unable, through no fault of the permittee, to issue a new permit before the expiration date of the previous permit.

(5b) Any certificate issued by the Board under this chapter may, after notice and opportunity for a hearing, be amended or revoked on any of the following grounds or for good cause as may be provided by the regulations of the Board:

1. The owner has violated any regulation or order of the Board, any condition of a certificate, any provision of this chapter, or any order of a court, where such violation results in a release of harmful substances into the environment or poses a substantial threat of release of harmful substances into the environment or presents a hazard to human health or the violation is representative of a pattern of serious or repeated violations which, in the opinion of the Board, demonstrates the owner's disregard for or inability to comply with applicable laws, regulations, or requirements;

2. The owner has failed to disclose fully all relevant material facts or has misrepresented a material fact in applying for a certificate, or in any other report or document required under this law or under the regulations of the Board;

3. The activity for which the certificate was issued endangers human health or the environment and can be regulated to acceptable levels by amendment or revocation of the certificate; or

4. There exists a material change in the basis on which the permit was issued that requires either a temporary or a permanent reduction or elimination of any discharge controlled by the certificate necessary to protect human health or the environment.

(6) To make investigations and inspections, to ensure compliance with any certificates, standards, policies, rules, regulations, rulings and special orders which it may adopt, issue or establish and to furnish advice, recommendations, or instructions for the purpose of obtaining such compliance. In recognition of §§ 32.1-164 and 62.1-44.18, the Board and the State Department of Health shall enter into a memorandum of understanding establishing a common format to consolidate and simplify inspections of sewage treatment plants and coordinate the scheduling of the inspections. The new format shall ensure that all sewage treatment plants are inspected at appropriate intervals in order to protect water quality and public health and at the same time avoid any unnecessary administrative burden on those being inspected.

(7) To adopt rules governing the procedure of the Board with respect to: (a) hearings; (b) the filing of reports; (c) the issuance of certificates and special orders; and (d) all other matters relating to procedure; and to amend or cancel any rule adopted. Public notice of every rule adopted under this section shall be by such means as the Board may prescribe.

(8a) To issue special orders to owners (i) who are permitting or causing the pollution, as defined by § 62.1-44.3, of state waters to cease and desist from such pollution, (ii) who have failed to construct facilities in accordance with final approved plans and specifications to construct such facilities in accordance with final approved plans and specifications, (iii) who have violated the terms and provisions of a certificate issued by the Board to comply with such terms and provisions, (iv) who have failed to comply with a directive from the Board to comply with such directive, (v) who have contravened duly adopted and promulgated water quality standards and policies to cease and desist from such contravention and to comply with such water quality standards and policies, (vi) who have violated the terms and provisions of a pretreatment permit issued by the Board or by the owner of a publicly owned treatment works to comply with such terms and provisions or (vii) who have contravened any applicable pretreatment standard

or requirement to comply with such standard or requirement; and also to issue such orders to require any owner to comply with the provisions of this chapter and any decision of the Board.

(8b) Such special orders are to be issued only after a hearing with at least thirty days' notice to the affected owners, of the time, place and purpose thereof, and they shall become effective not less than fifteen days after service as provided in § 62.1-44.12; provided that if the Board finds that any such owner is grossly affecting or presents an imminent and substantial danger to (i) the public health, safety or welfare, or the health of animals, fish or aquatic life; (ii) a public water supply; or (iii) recreational, commercial, industrial, agricultural or other reasonable uses, it may issue, without advance notice or hearing, an emergency special order directing the owner to cease such pollution or discharge immediately, and shall provide an opportunity for a hearing, after reasonable notice as to the time and place thereof to the owner, to affirm, modify, amend or cancel such emergency special order. If an owner who has been issued such a special order or an emergency special order is not complying with the terms thereof, the Board may proceed in accordance with § 62.1-44.23, and where the order is based on a finding of an imminent and substantial danger, the court shall issue an injunction compelling compliance with the emergency special order pending a hearing by the Board. If an emergency special order requires cessation of a discharge, the Board shall provide an opportunity for a hearing within forty-eight hours of the issuance of the injunction.

(8c) The provisions of this section notwithstanding, the Board may proceed directly under § 62.1-44.32 for any past violation or violations of any provision of this chapter or any regulation duly promulgated hereunder.

(8d) With the consent of any owner who has violated or failed, neglected or refused to obey any regulation or order of the Board, any condition of a permit or any provision of this chapter, the Board may provide, in an order issued by the Board against such person, for the payment of civil charges for past violations in specific sums not to exceed the limit specified in § 62.1-44.32 (a). Such civil charges shall be instead of any appropriate civil penalty which could be imposed under § 62.1-44.32 (a) and shall not be subject to the provisions of § 2.2-514. Such civil charges shall be paid into the state treasury and deposited by the State Treasurer into the Virginia Environmental Emergency Response Fund pursuant to Chapter 25 (§ 10.1-2500 et seq.) of Title 10.1, excluding civil charges assessed for violations of Article 9 (§ 62.1-44.34:8 et seq.) or 10 (§ 62.1-44.34:10 et seq.) of Chapter 3.1 of this title, or a regulation, administrative or judicial order, or term or condition of approval relating to or issued under those articles.

The amendments to this section adopted by the 1976 Session of the General Assembly shall not be construed as limiting or expanding any cause of action or any other remedy possessed by the Board prior to the effective date of said amendments.

(9) To make such rulings under §§ 62.1-44.16, 62.1-44.17 and 62.1-44.19 as may be required upon requests or applications to the Board, the owner or owners affected to be notified by certified mail as soon as practicable after the Board makes them and such rulings to become effective upon such notification.

(10) To adopt such regulations as it deems necessary to enforce the general water quality management program of the Board in all or part of the Commonwealth, except that a description of provisions of any proposed regulation which are more restrictive than applicable federal requirements, together with the reason why the more restrictive provisions are needed, shall be provided to the standing committee of each house of the General Assembly to which matters relating to the content of the regulation are most properly referable.

(11) To investigate any large-scale killing of fish.

(a) Whenever the Board shall determine that any owner, whether or not he shall have been issued a certificate for discharge of waste, has discharged sewage, industrial waste, or other waste into state waters in such quantity, concentration or manner that fish are killed as a result thereof, it may effect such settlement with the owner as will cover the costs incurred by the Board and by the Department of Game and Inland Fisheries in investigating such killing of fish, plus the replacement value of the fish destroyed, or as it deems proper, and if no such settlement is reached within a reasonable time, the Board shall authorize its executive secretary to bring a civil action in the name of the Board to recover from the owner such costs and value, plus any court or other legal costs incurred in connection with such action.

(b) If the owner is a political subdivision of the Commonwealth, the action may be brought in any circuit court within

the territory embraced by such political subdivision. If the owner is an establishment, as defined in this chapter, the action shall be brought in the circuit court of the city or the circuit court of the county in which such establishment is located. If the owner is an individual or group of individuals, the action shall be brought in the circuit court of the city or circuit court of the county in which such person or any of them reside.

(c) For the purposes of this subsection the State Water Control Board shall be deemed the owner of the fish killed and the proceedings shall be as though the State Water Control Board were the owner of the fish. The fact that the owner has or held a certificate issued under this chapter shall not be raised as a defense in bar to any such action.

(d) The proceeds of any recovery had under this subsection shall, when received by the Board, be applied, first, to reimburse the Board for any expenses incurred in investigating such killing of fish. The balance shall be paid to the Board of Game and Inland Fisheries to be used for the fisheries' management practices as in its judgment will best restore or replace the fisheries' values lost as a result of such discharge of waste, including, where appropriate, replacement of the fish killed with game fish or other appropriate species. Any such funds received are hereby appropriated for that purpose.

(e) Nothing in this subsection shall be construed in any way to limit or prevent any other action which is now authorized by law by the Board against any owner.

(f) Notwithstanding the foregoing, the provisions of this subsection shall not apply to any owner who adds or applies any chemicals or other substances that are recommended or approved by the State Department of Health to state waters in the course of processing or treating such waters for public water supply purposes, except where negligence is shown.

(12) To administer programs of financial assistance for planning, construction, operation, and maintenance of water quality control facilities for political subdivisions in this Commonwealth.

(13) To establish policies and programs for effective area-wide or basin-wide water quality control and management. The Board may develop comprehensive pollution abatement and water quality control plans on an area-wide or basin-wide basis. In conjunction with this, the Board, when considering proposals for waste treatment facilities, is to consider the feasibility of combined or joint treatment facilities and is to ensure that the approval of waste treatment facilities is in accordance with the water quality management and pollution control plan in the watershed or basin as a whole. In making such determinations, the Board is to seek the advice of local, regional, or state planning authorities.

(14) To establish requirements for the treatment of sewage, industrial wastes and other wastes that are consistent with the purposes of this chapter; however, no treatment shall be less than secondary or its equivalent, unless the owner can demonstrate that a lesser degree of treatment is consistent with the purposes of this chapter.

(15) To promote and establish requirements for the reclamation and reuse of wastewater that are protective of state waters and public health as an alternative to directly discharging pollutants into waters of the state. The requirements shall address various potential categories of reuse and may include general permits and provide for greater flexibility and less stringent requirements commensurate with the quality of the reclaimed water and its intended use. The requirements shall be developed in consultation with the Department of Health and other appropriate state agencies. This authority shall not be construed as conferring upon the Board any power or duty duplicative of those of the State Board of Health.

(16) To establish and implement policies and programs to protect and enhance the Commonwealth's wetland resources. Regulatory programs shall be designed to achieve no net loss of existing wetland acreage and functions. Voluntary and incentive-based programs shall be developed to achieve a net resource gain in acreage and functions of wetlands. The Board shall seek and obtain advice and guidance from the Virginia Institute of Marine Science in implementing these policies and programs.

Code 1950, § 62.1-27; 1968, c. 659; 1970, c. 638; 1972, c. 741; 1975, c. 335; 1976, c. 621; 1977, c. 32; 1978, c 827; 1984, c. 11; 1985, cc. 249, 397; 1988, cc. 167, 328; 1989, c. 389; 1990, c. 717; 1991, cc. 239, 718; 1993, c. 456; 1994, c. 698; 1998, cc. 805, 863; 2000, cc. 972, 1032, 1054.)

APPENDIX B

DEQ FISH KILL REPORT/NOTIFICATION

VIRGINIA DEPARTMENT OF ENVIRONMENTAL QUALITY
FISH KILL REPORT/NOTIFICATION

Incident #_____ Stream_____Basin_____

City/County_____Region_____

Date Investigated_____Investigator_____

Reported by: Name_____

Address_____

Phone (Home)_____(Work)_____

Reported to: Name_____

Office_____Date_____

****

Date Kill Started_____Date Kill Ended_____Total # Killed_____

Fish Killed (common name)_____

_____

Location of Kill_____

Area Involved_____

Cause of Kill_____

Staff Hours_____ Total Cost of Investigation_____

Investigator's Signature_____Date_____

****

Attachments (Check all included forms. As a minimum Attachments A and B should be included for investigated kills.)

____ A - Investigator's Expense
____ C - Summary Memo
____ E - Fish Cost
____ G - Lab Analyses
____ I - Benthic/Algal Results
____ K - Bioassay Costs
____ M - Pathology Costs

____ B - Map
____ D - Fish Count
____ F - Fish Description
____ H - Lab Cost
____ J - Bioassay Results
____ L - Pathology Results
____ N - Total Costs

****

Remarks/Recommendations/Action Taken

Copies: Enforcement (orig.), Regional Office

APPENDIX C

DEPARTMENT OF GAME AND INLAND FISHERIES REGIONAL NOTIFICATION LIST

Dept. of Game & Inland Fisheries, Fisheries Division

P. O. Box 11104
Richmond, Virginia 23230-1104
804/367-1004
Fax: 804/367-2628

FAX TRANSMISSION COVER SHEET

Date	*January 30, 2002*
To:	*Rick Browder*
Fax:	*698-4522*
Re:	*VDGIF, Fisheries Division Biologists List*
Sender:	*Gary F. Martel* GFM

YOU SHOULD RECEIVE 7 PAGE(S), INCLUDING THIS COVER SHEET. IF YOU DO NOT RECEIVE ALL THE PAGES, PLEASE CALL 804/367-0509.

Per our discussion, I am attaching a current list of Fisheries Division biologists. If we can be of further assistance, feel free to call.

LOUDOUN
ARLINGTON
FAIRFAX
PRINCE WILLIAM
FAUQUIER
WARREN
PAGE
SHENANDOAH
FREDERICK
CLARKE
ROCKINGHAM
CULPEPER
MADISON
GREENE
ORANGE
SPOTSYLVANIA
4
5
AUGUSTA
HIGHLAND
BATH
ALLEGHANY
BOTETOURT
ROCKBRIDGE
NELSON
N. BEDFORD
LOUISA
CAROLINE
NORTHUMBERLAND
ACCOMACK
NORTHAMPTON
HANOVER
GOOCHLAND
FLUVANNA
AMHERST
BUCKINGHAM
CUMBERLAND
POWHATAN
ALBEMARLE
CRAIG
APPOMATTOX
AMELIA
CHESTERFIELD
NEW KENT
CHARLES CITY
PRINCE GEORGE
SURRY
BEDFORD
CAMPBELL
PRINCE EDWARD
NOTTOWAY
DINWIDDIE
SUSSEX
SOUTHAMPTON
ISLE OF WIGHT
SUFFOLK
CHESAPEAKE
VIRGINIA BEACH
NORFOLK
NEWPORT NEWS
HAMPTON
YORKTOWN
GLOUCESTER
MATHEWS
MIDDLESEX
LANCASTER
ESSEX
KING AND QUEEN
KING WILLIAM
BUCHANAN
DICKENSON
WISE
RUSSELL
TAZEWELL
BLAND
GILES
MONTGOMERY
PULASKI
WYTHE
SMYTH
GRAYSON
WASHINGTON
SCOTT
LEE
CARROLL
PATRICK
HENRY
FLOYD
FRANKLIN
PITTSYLVANIA
HALIFAX
CHARLOTTE
LUNENBURG
MECKLENBURG
BRUNSWICK
DINWIDDIE
2
1

FISHERIES DIVISION

Richmond Office

NAME	JOB TITLE	ADDRESS	OFFICE PHONE	HOME PHONE
Gary F. Martel	Director	4010 W. Broad St. Richmond, VA 23230	804-367-1004	
Samuel S. West	Engineering Technician	4010 W. Broad Street Richmond, VA 23230	804-367-1644	
Frances H. Anderson	Executive Secretary	4010 W. Broad Street Richmond, VA 23230	804-367-0509	
Fred D. Leckie, Jr.	Assistant Director Operations	4010 W. Broad Street Richmond, VA 23230	804-367-8944	
Christine L. Butor	Secretary	4010 W. Broad Street Richmond, VA 23230	804-367-8629	
Ron Southwick	Assistant Director Hatcheries	4010 W. Broad Street Richmond, VA 23230	804-367-1292	
Dean L Fowler	Outreach Biologist	5806 Mooretown Road Williamsburg, VA 23188	757-253-4180	
Linda S. Stone	Secretary	4010 W. Broad Street Richmond, VA 23230	804-367-1293	
Anne Skalski-Windle	Aquatic Education Coordinator	4010 W. Broad Street Richmond, VA 23230	804-367-6778	
	Sport Fishing Education Specialist	4010 W. Broad Street Richmond, VA 23230	804-367-0141	

Region I

Mitchell D. Norman	Regional Fisheries Manager	500 Hinton Avenue Chesapeake, VA 23323	757-558-4731	

District 1

Work Area: Charles City, Essex, Gloucester, James City, King George, King William, King & Queen, Lancaster, Mathews, Middlesex, New Kent, Northumberland, Richmond, Westmoreland, York, and the cities of Hampton and Newport News.

Robert S. Greenlee	Fisheries Biologist	5806 Mooretown Road Williamsburg, VA 23188	757-253-4170	
Mukhtar A. Farooqi	Fisheries Biologist	5806 Mooretown Road Williamsburg, VA 23188	757-253-4172	
Douglas E. Smith	Fisheries Technician	5806 Mooretown Road Williamsburg, VA 23188	757-253-4172	

District 2

Work Area: Accomack, Greensville, Isle of Wight, Northampton, Prince George, Southampton, Surry, Sussex, and the cities of Chesapeake, Norfolk, Portsmouth, Suffolk, and Virginia Beach.

Christopher C. Long	Fisheries Biologist	500 Hinton Avenue Chesapeake, VA 23323	757/558-4773	

Region II

Arthur L. LaRoche, III	Regional Fisheries Manager	209 E. Cleveland Ave. Vinton, VA 24179	540-857-7704	
Angela Overstreet	Secretary	209 E. Cleveland Ave. Vinton, VA 24179	540-857-7704	

District 1

Work Area: Amelia, Appomattox, Buckingham, Brunswick, Charlotte, Cumberland, Dinwiddie, Halifax, Lunenburg, Mecklenburg, Nottoway, Prince Edward, and the city of Petersburg. (Hopewell)

Daniel P. Michaelson	Fisheries Biologist	H.C. 6, Box 46 Farmville, VA 23901	434-392-9645	
Victor J. DiCenzo	Fisheries Biologist	H.C. 6, Box 46 Farmville, VA 23901	434-392-9645	
Eric M. Brittle	Assistant Fisheries Biologist	H.C. 6, Box 46 Farmville, VA 23901	434-392-9645	

District 2

Work Area: Amherst, Bedford, Botetourt, Campbell, Franklin, Henry, Nelson, Patrick, Pittsylvania, Roanoke, and the cities of Lynchburg and Roanoke.

Daniel M. Wilson	Fisheries Biologist Senior	1132 Thomas Jefferson Road Forest, VA 24551-9223	434-525-7522	
Scott M. Smith	Fisheries Biologist	1132 Thomas Jefferson Road Forest, VA 24551-9223	434-525-7522	

Region III

William B. Kittrell, Jr.	Regional Fisheries Manager	1796 Highway Sixteen Marion, VA 24354	276-783-4860	

District 1

Work Area: Bland, Carroll, Craig, Floyd, Giles, Montgomery, Pulaski, and Wythe.

John C. Copeland	Fisheries Biologist Senior	2206 South Main Street Suite C Blacksburg, VA 24060	540 951-7923	
Joe Williams	Fisheries Biologist	2206 South Main Street Suite C Blacksburg, VA 24060	540-951-7923	

District 2

Work Area: Buchanan, Dickenson, Grayson, Lee, Scott, Russell, Smyth, Tazewell, Washington, and Wise.

Thomas M. Hampton	Fisheries Biologist Senior	1796 Highway Sixteen Marion, VA 24354	276-783-4860	
George C. Palmer	Fisheries Biologist	1796 Highway Sixteen Marion, VA 24354	276-783-4860	
Clifford R. Kirk	Fisheries Technician	1796 Highway Sixteen Marion, VA 24354	276-783-4860	

Region IV

Larry O. Mohn	Regional Fisheries Manager	P. O. Box 996 Verona, VA 24482	540-248-9360	

District 1

Work Area: Alleghany, Augusta, Bath, Highland, Rockbridge, and the cities of Lexington and Waynesboro

Paul E. Bugas, Jr.	Fisheries Biologist Senior	P. O. Box 996 Verona, VA 24482	540-248-9360	
Bradley A. Trumbo	Fisheries Technician	P. O. Box 996 Verona, VA 24482	540-248-9360	

District 2

Work Area: Clarke, Frederick, Page, Rockingham, Shenandoah, and Warren, and the Cities of Harrisonburg and Winchester

Stephen J. Reeser	Fisheries Biologist	P. O. Box 996 Verona, VA 24482	540-248-9360	

Region V

John W. Kauffman	Regional Fisheries Manager	Suite 100 900 Natural Resource Dr. Charlottesville VA 22903	434-296-4731	

District 1

Work Area: Albemarle, Chesterfield, Fluvanna, Goochland, Greene, Hanover, Henrico, Louisa, Madison, Powhatan, Rappahannock, and the cities of Charlottesville and Richmond.

Price P. Smith	Fisheries Biologist Senior	12104 Washington Highway Ashland, Va. 23005	804-752-5503	
Thomas P. Gunter, Jr.	Fisheries Biologist	12104 Washington Highway Ashland, Va. 23005	804-752-5503	
Alan Weaver	Fish Passage Coordinator	12104 Washington Highway Ashland, Va. 23005	804-752-5504	

District 2

Work Area: Arlington, Caroline, Culpeper, Fairfax, Fauquier, Loudon, Orange, Prince William, Spotsylvania, and Stafford.

Stephen J. Owens	Fisheries Biologist Senior	1320 Belman Road Fredericksburg VA 22401	540-899-4179	
John S. Odenkirk	Fisheries Biologist	1320 Belman Road Fredericksburg VA 22401	540-899-4179	
Scott W. Herrmann	fisheries Technician	1320 Belman Road Fredericksburg VA 22401	540-899-4179	

APPENDIX D

CLINICAL SIGNS

CLINICAL SIGNS

BEHAVIOR
- ☐ Gasping
- ☐ Flashing
- ☐ Lethargic
- ☐ Fin Twitching
- ☐ Convulsions
- ☐ In Shallow Water
- ☐ Around Inflow
- ☐ Around Drain
- ☐ Around Aeration
- ☐ Head up - tail down
- ☐ Head-tail whirling
- ☐ Long. axis whirling
- ☐ Pect. fins folded forward
- ☐ Anorexia
- ☐ Belly-up
- ☐ Loss of Balance
- ☐ Other

PHYSICAL – External (Rate)
- ☐ Normal ___/___
- ☐ Emaciated ___/___
- ☐ Depigmented ___/___
- ☐ Hyperpigmented ___/___
- ☐ Exophthalmia ___/___
- ☐ Endophthalmia ___/___
- ☐ Swollen Belly ___/___
- ☐ Scoliosis & Lordosis ___/___

FINS – Eroded
- ☐ Dorsal ___/___
- ☐ Pectoral ___/___
- ☐ Pelvic ___/___
- ☐ Anal ___/___
- ☐ Adipose ___/___
- ☐ Caudal ___/___

LESION – Shape
- ☐ Irregular ___/___
- ☐ Regular ___/___

LESION – Appearance
- ☐ Clean ___/___
- ☐ Dirty ___/___
- ☐ Yellow ___/___
- ☐ Red ___/___
- ☐ White ___/___

LESION – Location
- ☐ Fins ___/___
- ☐ Head ___/___
- ☐ Cranial Foramen ___/___
- ☐ Eyes ___/___
- ☐ Mouth ___/___
- ☐ Peduncle ___/___
- ☐ Ventral ___/___
- ☐ Dorsal ___/___
- ☐ Lateral ___/___

LESION – Size
- ☐ 1-5 mm ___/___
- ☐ 5-10 mm ___/___
- ☐ 1.25 cm ___/___
- ☐ 1-2.5 cm ___/___
- ☐ >2.5 cm ___/___

HEMORRHAGES (Rate)
- ☐ Fins ___/___
- ☐ Head ___/___
- ☐ Mouth ___/___
- ☐ Eyes ___/___
- ☐ Peduncle ___/___
- ☐ Ventral ___/___
- ☐ Dorsal ___/___
- ☐ Lateral ___/___
- ☐ Vent ___/___
- ☐ Cranial Foramen ___/___

HEMORRHAGES – Size
- ☐ Petchiae ___/___
- ☐ Eccymoses ___/___
- ☐ Suffusion ___/___

ULCER – Location
- ☐ Fins ___/___
- ☐ Head ___/___
- ☐ Eyes ___/___
- ☐ Mouth ___/___
- ☐ Peduncle ___/___
- ☐ Ventral ___/___
- ☐ Dorsal ___/___
- ☐ Lateral ___/___

ULCER – Size
- ☐ 1-5 mm ___/___
- ☐ 5-10 mm ___/___
- ☐ 1.25 cm ___/___
- ☐ 1-2.25 cm
- ☐ >2.5 cm ___/___

ULCER – Shape
- ☐ Irregular ___/___
- ☐ Regular ___/___

ULCER – Appearance
- ☐ Clean ___/___
- ☐ Dirty ___/___
- ☐ Yellow ___/___
- ☐ Red ___/___

GILLS
- ☐ Normal ___/___
- ☐ Pale ___/___
- ☐ Brown ___/___
- ☐ Cherry Red ___/___
- ☐ Necrotic ___/___
- ☐ Hyperplasia ___/___
- ☐ Gas Bubbles ___/___
- ☐ Aneurisms ___/___
- ☐ Hyperemia ___/___
- ☐ Cellular Edema ___/___
- ☐ Golden Spherules ___/___
- ☐ Clubbed ___/___
- ☐ Swollen ___/___
- ☐ Puffy ___/___
- ☐ Other ___/___
- ☐ Hamburger Gill ___/___
- ☐ Postmortem Change ___/___

GAS BUBBLES (Rate)
- ☐ Gills ___/___
- ☐ Fins ___/___
- ☐ Skin ___/___

PHYSICAL – Internal
- ☐ Normal ___/___
- ☐ Postmortem Change ___/___

INTESTINE
- ☐ Normal ___/___
- ☐ Hemorrhagic ___/___
- ☐ Flaccid ___/___
- ☐ Gas ___/___
- ☐ Mucus ___/___
- ☐ Feces ___/___
- ☐ Fluid ___/___
- ☐ Intussusception ___/___

STOMACH
- ☐ Normal ___/___
- ☐ Hemorrhagic ___/___
- ☐ Gas ___/___
- ☐ Mucus ___/___
- ☐ Food ___/___
- ☐ Fluid ___/___

KIDNEY
- ☐ Normal ___/___
- ☐ Pale ___/___
- ☐ Hemorrhagic ___/___
- ☐ Swollen ___/___
- ☐ Brown ___/___
- ☐ Soft ___/___

LIVER
- ☐ Normal ___/___
- ☐ Pale ___/___
- ☐ Hemorrhagic ___/___
- ☐ Brown ___/___
- ☐ Black ___/___
- ☐ Mottled ___/___
- ☐ Congested

SPLEEN
- ☐ Congested ___/___
- ☐ Normal ___/___
- ☐ Mottled ___/___
- ☐ Brown ___/___
- ☐ Hemorrhagic ___/___

SWIM BLADDER
- ☐ Normal ___/___
- ☐ Hemorrhagic ___/___

BLOOD
- ☐ Normal ___/___
- ☐ Anemic ___/___
- ☐ Brown ___/___
- ☐ Black ___/___
- ☐ Cherry Red ___/___
- ☐ Methemoglobin ___/___
- ☐ Hct ___

COELOM
- ☐ Ascites ___/___
- ☐ Cloudy ___/___
- ☐ Bloody ___/___
- ☐ Clear ___/___
- ☐ Gas ___/___

Histology Samples Taken ☐ _____

Tissues Taken _____

_____

DIAGNOSIS _____

PARASITES

A ☐ Ichthyoboda
B ☐ Ich
C ☐ Chilodon
D ☐ Trichodina
E ☐ Trichophrya
F ☐ Ambiphrya
G ☐ Epistylis
H ☐ Henneguya
I ☐ Monogenea (Gills)
J ☐ Gyrodactylus
K ☐ Yellow Grub
L ☐ White Grub
M ☐ Black Grub
N ☐ Lernaea
O ☐ Other_____
P ☐ Bodamonas
Q ☐ Apiosoma

BACTERIA

A ☐ A. hydrophila
B ☐ A. sobria
C ☐ Aeromonas sp.
D ☐ Plesiomonas shigelloides
E ☐ E. tarda
F ☐ E. ictaluri
G ☐ Flexibacter external
H ☐ Other myxobacteria
I ☐ Pseudomonas f.
J ☐ Pseudomonas sp.
K ☐ Klebsiella
L ☐ Enterobacter
M ☐ Proteus
N ☐ Unknown
O ☐ Other_____
P ☐ Flexibacter internal

FUNGI

A ☐ External
B ☐ Systemic
C ☐ Branchiomyces
D ☐ Other_____

VIRUSES

A ☐ CCV
B ☐ GSV
C ☐ Lymphocystis
D ☐ Other_____

WATER QUALITY

A ☐ Ammonia
B ☐ Nitrite
C ☐ Gas Bubble
D ☐ DO Depletion
E ☐ Thermal Shock
F ☐ pH
G ☐ Other_____
H ☐ Suspected DO Depletion

NUTRITIONAL

A ☐

TOXICITY

A ☐ Bluegreen algae
B ☐ Overtreatment_____
C ☐ Pesticides
D ☐ Other_____

MISCELLANEOUS

A ☐ Handling
B ☐ Genetic
C ☐ Tumors
D ☐ Crowding
E ☐ Moving
F ☐ Inadequate Sample_____
G ☐ Unknown
H ☐ Inspection
I ☐ Routine Check
J ☐ Other_____
K ☐ Hamburger Gill
L ☐ Anemia
M ☐ Winter Kill

Histology Results_____

_____

_____

Remarks _____

_____

APPENDIX E

AFS MONETARY VALUES OF FRESHWATER FISH AND FISH-KILL COUNTING GUIDELINES

Appendix E-1... American Fisheries Society. 1992. Investigation and valuation of fish kills. American Fisheries Society Publication 24. Bethesda, Maryland.

Appendix E-2... American Fisheries Society. 1993. Sourcebook for investigating and valuation of fish kills. American Fisheries Society Special Publication 24 (supplement). Bethesda, Maryland.

APPENDIX F

DEQ FISH KILL COUNT FORM

VA DEQ FISH KILL COUNT

Incident # _____ Page____of _____

Segment #	Species	Size Total Length (inches)	Number

APPENDIX G

REPLACEMENT COST OF FISH FORM

REPLACEMENT COST OF FISH

Incident #_____

Stream_____ City/County_____

Species (Common Name)	Size (Inches)	Number	Individual Costs	Total Cost

Grand Total_____

Signed_____
(Chief, Fish Div. DGIF)

Date_____

APPENDIX H

DEQ TOTAL COST SUMMARY

Department of Environmental Quality
Fish Kill Investigation Cost Calculation and Payment Instructions

1. Determine total DEQ fish kill investigation costs.

The agency fish kill investigation recovery worksheet file is found in DEQ intranet forms/admin/finance/finance forms. Each fish kill cost recovery case should be saved with the Pollution Incident Report Number (IR#), (formally called pollution complaint number, PC#) as the file name. Completed and signed originals of the form should be maintained with the appropriate cost support in the regional or central office fish kill investigation files.

a. The hourly rate is calculated from the employee's annual salary. Administrative and managerial oversight costs will be calculated using DEQ's established indirect cost rate of 28.5%. A standard benefit rate of 25% will be applied to direct salaries.
b. Standard mileage rates are established for pool cars (19 cents), agency owned (19 cents), and private vehicles (32.5 cents). Vehicle cost recovery is based on number of vehicle miles documented on the vehicle log sheet.
c. Actual costs for employee meals, lodging, and other travel expenses as documented on the employee's travel voucher will be recovered.
d. Actual laboratory sampling costs will be recovered based on the current catalog of sampling costs for the specific type of testing performed.
e. The cost of the use of agency boats will be calculated based on the standard hourly rate established for the boat motor. Costs will be calculated based on hours used to whole hours.
f. Other miscellaneous costs incurred such as ice, film processing, and apparel will be recovered based on the actual costs incurred. These costs are documented on the agency purchase requisition, petty cash voucher, or travel voucher. The costs of inventory or "stock" (ex. Gloves) items should be based on the documented current fair market price.

2. Determine replacement cost of the fish

3. The responsible party should send two separate checks for DEQ cost recovery and DGIF fish replacement. Each check should be made payable to "The Treasurer of Virginia" and MUST include the Pollution Incident Number (formally PC #) and refer to either DEQ cost or DGIF fish replacement costs. Both checks should be sent to the following DEQ lock box address:

Receipts Control
P.O. Box 10150
Richmond, VA 23240

Pollution Complaint Investigation
Time, Travel and Materials Cost Recovery Schedule
Expenses Incurred by the Department of Environmental Quality
for Fish Kill Investigation
Spreadsheet completion instruction form

Hourly rates are established based on the employee's annual salary. Costs for administration and management oversight are included in the 28.5% indirect cost rate. The spreadsheet automatically calculates rates when annual salary and hours are added to spreadsheet.
Boat costs are standardized based on size of boat motor. See below for rates

How to complete the form:

1) Enter the PC incident # in cell D6
2) Enter the Date in cell H6
3) Enter the site in cell D7
4) Enter the Region Code in D8
5) Enter the employee's last name & first initial in B11 for the 1st,B12 for the second employee, etc.
6) Matching the employee's number, enter their respective time codes in cells B21 through B25.
7) Enter the employee's annual salary in cells C21 through C25 respectively.
8) Enter time and units as 3.0; 3.25; etc. in cells D21 through D25
8) Enter any travel dates in cells D31 /E31 through D35/E35.
9) Enter the number of miles traveled in either the Pool, Agency or Private column (cells F31/F35 , G31/G35 or H31/H35.)
10) Enter the total amount of Meals, Overnight Lodging and Other Travel costs in cells F, G, H 41/45 respectively.
11) For Additional Materials and or Equipment purchased/used complete the necessary cells under the appropriate headings in cells C50 through H 55 Laboratory costs are calculated based on the type of sample and sample cost. Refer to the catalog of lab services for individual sample costs. Other costs include ice, film, etc.
12) Print using the Smarticon.
13) Save the File with the PC incident # as the file name in k:\agency\forms\costrecol\ sub-directory.

Standard Boat Usage Rates by Motor Size:

100 hp and greater	$20.00	per hour
Under 100 hp	$15.00	per hour
Electro Fisher	$20.00	per hour

Pollution Incident Investigation
Time, Travel and Materials Cost Recovery Schedule
Expenses incurred by the Department of Environmental Quality
for Fish Kill Investigation

Incident Report (IR)		Date	
IR Site Location			
Region			

	Name	Signature		
Emp #1			Date	
Emp #2			Date	
Emp #3			Date	
Emp #4			Date	
Emp #5			Date	
Supervisor			Date	

Time Spent	Time Code	Salary	Hours	Extended Cost	Benefits @ 25%	Indirect @28.5%	Total Time Costs
Emp #1				0.00	0.00	0.00	0.00
Emp #2				0.00	0.00	0.00	0.00
Emp #3				0.00	0.00	0.00	0.00
Emp #4				0.00	0.00	0.00	0.00
Emp #5				0.00	0.00	0.00	0.00
Sub-Total Employee Time			0	0.00	0.00	0.00	0.00

Travel-Vehicle	Date(s) From	To	No of Miles Pool @ 0.19	Agency @ 0.19	Private 0.325	Cost
Emp #1						0.00
Emp #2						0.00
Emp #3						0.00
Emp #4						0.00
Emp #5						0.00
Sub-Total Employee Travel			0.00	0.00	0.00	0.00

Travel - Meal and Lodging

	Meals	Lodging	Other	Costs
Emp #1				0.00
Emp #2				0.00
Emp #3				0.00
Emp #4				0.00
Emp #5				0.00
Sub-Total Employee Meals and Lodging	0.00	0.00	0.00	0.00

Materials and Equipment Used

Activity	Date	Vendor	Hours/ Units	Rate/ Costs	Costs
Boat Usage					0.00
Laboratory Analysis					0.00
Laboratory Analysis					0.00
Other- Specify					0.00

Other- Specify	0.00
Other- Specify	0.00
Sub-total Equipment and Supplies	0.00
Total Time, Travel and Materials Cost Recovery Schedule	0.00

APPENDIX I

DEQ FISH TISSUE AND SEDIMENT MONITORING PROGRAM (FORMERLY CORE PROGRAM) SAMPLING HANDLING PROCEDURE FROM THE USEPA. 2000. GUIDANCE FOR ASSESSING CHEMICAL CONTAMINANT DATA FOR USE IN FISH ADVISORIES, VOL. I. FISH SAMPLING AND ANALYSIS. WASHINGTON, DC.

Sample Handling

Fish samples should be carefully handled throughout the study to prevent contamination by the sampler's hands or field equipment. The following items are of particular importance:

sample preservation - dry or wet ice in field (sample frozen within 24 hrs.), freeze in lab
holding times - 6 month maximum for initial samples
sample storage - frozen
sample ID- sample number, fish collection data form, laboratory reporting form, bag labels
sample weight - 100 g **minimum** per composite

Fish handling procedure

The fish collected are to be handled with clean hands. Fish are wrapped in aluminum foil (dull side to the fish). If fish are to be analyzed as individual samples, they should be individually wrapped. For normal Core sampling, fish of the same sample may be wrapped in the same piece of foil. The wrapped fish are placed in plastic bags according to species or composite sample grouping and placed on ice. Usually, the three composite samples from each station are placed inside another plastic bag before being iced to decrease the chance of water infiltration. Once back from the field, the samples are immediately removed from ice and stored in the freezer until ready for shipment to DCLS contract laboratory.

Each DCLS lab prefers a 50 gram sample, however, the **minimum** sample weight requirements are listed below:

Metals 15 grams
Pesticides 35 grams
Trace Organics 35 grams

Laboratory reporting forms and sample identification tags for each sample should be completed upon conclusion of sample preparation for each station.

If samples are shipped to the DCLS laboratory, the DEQ laboratory coordinator should be contacted prior to submission of samples.

Laboratory Data Sheet Completion for STORET Data Entry

Correct completion of the laboratory data sheets is important to ensure that the fish tissue contaminant data is efficiently entered into the EPA STORET data base. CORE Procedure Appendix E lists the fish species commonly encountered while sampling in Virginia's waters along with the STORET numerical and alphabetic code. For other fish species STORET codes contact the STORET coordinator (see Section 2). STORET data printouts have a number of mislabeled columns. Appendix K is a copy of a STORET data retrieval. Please note that columns "Time of Day", "Medium" and "SMK or Depth" are incorrectly identified.

Appendix E

Fish Commonly Collected for Core Fish Tissue Program

PREDATOR SPECIES

COMMON NAME	STORET ALPHA CODE	STORET NUMERIC CODE
Bluegill	BGS	08
Bowfin	BF	68
Channel catfish	CHC	16
Flier	FR	412
Green sunfish	GSF	25
Largemouth bass	LMB	31
Longear sunfish	LSF	72
Oyster toadfish	TOAD	528
Pumpkinseed	SF	38
Rainbow trout	RBT	39
Redbreast sunfish	RBS	70
Rock bass	RKB	43
Smallmouth bass	SMB	47
Striped bass	STB	52
Sunfish	CSN	521
Weakfish (Grey trout)	WKF	180
White catfish	WHC	58
White crappie	WHS	59
White perch	WP	60
Yellow perch	YP	63

BOTTOM FEEDER SPECIES

COMMON NAME	STORET ALPHA CODE	STORET NUMERIC CODE
Carp	C	12
Croaker*	CRK	115
Fallfish	FFH	208
Gizzard shad	GSH	21
Menhaden	ATM	165
Northern hogsucker	NHO	94
Oyster	CRV	217
River Chub	RC	73
Shorthead redhorse	SRD	101
Silver redhorse	SRH	170
Snail bullhead	SBH	488
Spot*	SPT	181
Summer Flounder	SSF	183
White sucker	SWU	61
Yellow bullhead	YEB	62

*: also used as a predator for human health risk.

sh_spp

species of interest, dynamics of dispersion of pollutants of interest, or geographical location. Taking a simple random sample of lakes may not achieve sufficient coverage, whereas taking a stratified random sample approach may require more lakes be sampled than can be afforded. A conservative approach may be to look at the "worst case scenario". States may decide to sample the lakes that are believed to have the highest levels of pollutants, based on historical contaminant data, current water and sediment sampling results, or other variables. Another approach would be to select one or two of the factors described above ("representativeness"), stratify the lakes according to these factors, and select a random sample within each stratum. The set of factors for stratification may change every few years or so if it is deemed that some other factors are becoming more indicative of the levels of contamination.

6.2 SAMPLE COLLECTION

Sample collection activities should be initiated in the field only after an approved sampling plan has been developed. This section discusses recommended sampling equipment and its use, considerations for ensuring preservation of sample integrity, and field recordkeeping and chain-of-custody procedures associated with sample processing, preservation, and shipping.

6.2.1 Sampling Equipment and Use

In response to the variations in environmental conditions and target species of interest, fisheries biologists have had to devise sampling methods that are intrinsically selective for certain species and sizes of fish and shellfish (Versar, 1982). Although this selectivity can be a hindrance in an investigation of community structure, it is not a problem where tissue contaminant analysis is of concern because tissue contaminant data can best be compared only if factors such as differences in taxa and size are minimized.

Collection methods can be divided into two major categories, active and passive. Each collection method has advantages and disadvantages. Various types of sampling equipment, their use, and their advantages and disadvantages are summarized in Table 6-4 for fish and in Table 6-5 for shellfish. **Note:** Either active or passive collection methods may be used as long as the methods selected result in collection of a representative fish sample of the type consumed by local sport and subsistence fishers.

A basic checklist of field sampling equipment and supplies is shown in Table 6-6. Safety considerations associated with the use of a boat in sample collection activities are summarized in Table 6-7.

6.2.1.1 Active Collection—

Active collection methods employ a wide variety of sampling techniques and devices. Devices for fish sampling include electroshocking units, seines, trawls,

Table 6-4. Summary of Fish Sampling Equipment

Device	Use	Advantages	Disadvantages
ACTIVE METHODS			
Electrofishing	Shallow rivers, lakes, and streams.	Most efficiency nonselection method. Minimal damage to fish. Adaptable to a number of sampling conditions (e.g., boat, wading, shorelines). Particularly useful at sites where other active methods cannot be used (e.g., around snags and irregular bottom contours).	Nonselective–stuns or kills most fish. Cannot be used in brackish, salt, or extremely soft water. Requires extensive operator training. DANGEROUS when not used properly.
Seines	Shallow rivers, lakes, and streams. Shoreline areas of estuaries.	Relatively inexpensive and easily operated. Mesh size selection available for target species.	Cannot be used in deep water or over substrates with an irregular contour. Not completely efficient as fish can evade the net during seining operation.
Trawls	Various sizes can be used from boats in moderate to deep open bodies of water (10 to >70 m depths).	Effective in deep waters not accessible by other methods. Allows collection of a large number of samples.	Requires boat and trained operators.
Angling	Generally species selective involving use of hook and line.	Most selective method. Does not require use of large number of personnel or expensive equipment.	Inefficient and not dependable.
Purchasing specimens from commercial fishers	Only in areas where target species are commercially harvested.	Most cost-effective and efficient means of obtaining commercially valuable species from harvested waters.	Limited use; commercially harvested areas may not include sampling sites chosen for fish contaminant monitoring. The field collection staff should accompany the commercial fishers and should remove the required samples from the collection device. This will ensure the proper handling of the specimens and accurate recording of the collection time and sampling location.
PASSIVE METHODS			
Gill nets	Lakes, rivers, and estuaries. Where fish movement can be expected or anticipated.	Effective for collecting pelagic fish species. Relatively easy to operate. Requires less fishing effort than active methods. Selectivity can be controlled by varying mesh size.	Not effective for bottom-dwelling fish or populations that do not exhibit movement patterns. Nets prone to tangling or damage by large and sharp spined fish. Gill nets will kill captured specimens, which, when left for extended periods, may undergo physiological changes.
Trammel nets	Lakes, rivers, and estuaries. Where fish movement can be expected or anticipated. Frequently used where fish may be scared into the net.	Slightly more efficient than a straight gill net.	(Same as for gill nets.) Tangling problems may be more severe. Method of scaring fish into net requires more personnel or possibly boats in deep water areas.
Hoop, Fyke and Pound Nets	Shallow rivers, lakes, and estuaries when currents are present or when movements of fish are predictable. Frequently used in commercial operations.	Unattended operation. Very efficient in regard to long-term return and expended effort. Particularly useful in areas where active methods are impractical.	Inefficient for short term. Difficult to set up and maintain.
D-Traps	Used for long-term capture of slow-moving fish, particularly bottom species. Can be used in all environments.	Easy to operate and set. Unattended operation. Particularly useful for capturing bottom-dwelling organisms in deep waters or other types of inaccessible areas. Relatively inexpensive–often can be hand made.	Efficiency is highly variable. Not effective for pelagic fish or fish that are visually oriented. Less efficient for all species when water is clear rather than turbid. Not a good choice for a primary sampling technique, but available as backup for other methods.

Source: Versar, 1982.

Table 6-5. Summary of Shellfish Sampling Equipment

Device	Use	Advantages	Disadvantages
ACTIVE METHODS			
Seines	Shallow shoreline areas of estuaries.	Relatively inexpensive and easily operated. Mesh size selection available for target crustacean species (e.g., shrimp and crabs).	Cannot be used in deep water or over substrates with an irregular contour. Not completely efficient as crustaceans can evade the net during seining operation.
Trawls	Various sizes can be used from boats in moderate to deep open bodies of water (10 to >70 m depths).	Effective in deeper waters not accessible by other methods. Allows collection of a large number of samples.	Requires boat and trained operators.
Mechanical grabs Double-pole-operated grab buckets	Used from boat or pier. Most useful in shallow water areas less than 6 m deep including lakes, rivers, and estuaries.	Very efficiency means of sampling bivalves (e.g., clams and oysters) that are located on or buried in bottom sediments.	At depths greater than 6 m, the pole-operated devices become difficult to operate manually.
Tongs or double-handled grab sampler	Most useful in shallow water, lakes, rivers, and estuaries. Generally used from a boat.	Very efficient means of sampling oysters, clams, and scallops. Collection of surrounding or overlying sediments is not required and the jaws are generally open baskets. This reduces the weight of the device and allows the washing of collected specimens to remove sediments.	At depths greater than 6 m, the pole-operated devices become difficult to operate manually.
Line or cable-operated grab buckets			
Ekman grab	Used from boat or pier to sample soft to semisoft substrates.	Can be used in water of varying depths in lakes, rivers, and estuaries.	Possible incomplete closure of jaws can result in sample loss. Must be repeatedly retrieved and deployed. Grab is small and is not particularly effective in collecting large bivalves (calms and oysters).
Petersen grab	Deep lakes, rivers, and estuaries for sampling most substrates.	Large sample is obtained; grab can penetrate most substrates.	Grab is heavy, may require which for deployment. Possible incomplete closure of jaws can result is sample loss. Must be repeatedly retrieved and deployed.
Ponar grab	Deep lakes, rivers, and estuaries for sampling sand, silt, or clay substrates.	Most universal grab sampler. Adequate on most substrates. Large sample is obtained intact.	Possible incomplete closure of jaws can result in sample loss. Must be repeatedly retrieved and deployed.
Orange peel grab	Deep lakes, rivers, and estuaries for sampling most substrates.	Designed for sampling hard substrates.	Grab is heavy, may require winch for deployment. Possible incomplete closure of jaws can result in sample loss. Must be repeatedly retrieved and deployed. Grab is small and not particularly effective in collecting large bivalves (clams and oysters).
Biological or hydraulic dredges	Dragged along the bottom of deep waterbodies to collect large stationary invertebrates.	Qualitative sampling of large area of bottom substrate and benthic community. Length of tows can be relatively short if high density of shellfish exists in sampling area.	If the length of the tow is long, it is difficult to pinpoint the exact location of the sample collection area. Because of the scouring operation of the dredge, bivalve shells may be damaged. All bivalve specimens should be inspected and individuals with cracked or damaged shells should be discarded.

(continued)

Table 6-5. (continued)

Device	Use	Advantages	Disadvantages
ACTIVE METHODS (continued)			
Scoops, shovels	Used in shallow waters accessible by wading or SCUBA equipment for collection of hard clams *(Mercenaria mercenaria)* or soft-shell clam *(Mya arenaria)*.	Does not require a boat; sampling can be done from shore.	Care must be taken not to damage the shells of bivalves while digging in substrate.
Scrapers	Used in shallow waters accessible by wading or SCUBA equipment for collection of oysters *(Crassostrea virginica)* or mussels *(Mytilus sp)*.	Does not require a boat; sampling can be done from shore.	Care must be taken not to damage shells of bivalves while removing them from hard substrate.
Rakes	Used in shallow waters accessible by wading or can be used from a boat.	Does not require a boat; sampling can be done close to shore. Can be used in soft sediments to collect clams or scallops and can also be used to dislodge oysters or mussels that are attached to submerged objects such as rocks and pier pilings.	Care must be taken not to damage the shells of the bivalves while raking or dislodging them from the substrate.
Purchasing specimens from commercial fishers	Only in areas where target species are commercially harvested.	Most cost-effective and efficient means of obtaining bivalves for pollutant analysis from commercially harvested waters.	Limited use; commercially harvested areas may not include sampling sites chosen for shellfish contaminant monitoring. The field collection staff should accompany the commercial fishers and should remove the required samples from the collection device. This will ensure the proper handling of the specimens and accurate recording of the exact collection time and sampling location.
PASSIVE METHODS			
D-traps	Used for capture of slow-moving crustaceans (crabs and lobsters) that move about on or just above the substrate.	Can be used in a variety of environments. Particularly useful for capturing bottom-dwelling organisms in deep water or other inaccessible areas. Relatively inexpensive, can be hand made.	Catch efficiency is highly variable. Not a good choice for a primary sampling technique, but valuable as a backup for other methods.

Table 6-6. Checklist of Field Sampling Equipment and Supplies for Fish and Shellfish Contaminant Monitoring Programs

- ☐ Boat supplies
  - ☐ Fuel supply (primary and auxiliary supply)
  - ☐ Spare parts repair kit
  - ☐ Life preservers
  - ☐ First aid kit (including emergency phone numbers of local hospitals, family contacts for each member of the sampling team)
  - ☐ Spare oars
  - ☐ Nautical charts of sampling site locations
- ☐ Collection equipment (e.g., nets, traps, electroshocking device)
- ☐ Recordkeeping/documentation supplies
  - ☐ Field logbook
  - ☐ Sample request forms
  - ☐ Specimen identification labels
  - ☐ Chain-of-Custody (COC) Forms and COC tags or labels
  - ☐ Indelible pens
- ☐ Sample processing equipment and supplies
  - ☐ Holding trays
  - ☐ Fish measuring board (metric units)
  - ☐ Calipers (metric units)
  - ☐ Shucking knife
  - ☐ Balance to weigh representative specimens for estimating tissue weight (metric units)
  - ☐ Aluminum foil (extra heavy duty)
  - ☐ Freezer tape
  - ☐ String
  - ☐ Several sizes of plastic bags for holding individual or composite samples
  - ☐ Resealable watertight plastic bags for storage of Field Records, COC Forms, and Sample Request Forms
- ☐ Sample preservation and shipping supplies
  - ☐ Ice (wet ice, blue ice packets, or dry ice)
  - ☐ Ice chests
  - ☐ Filament-reinforced tape to seal ice chests for transport to the central processing laboratory

Table 6-7. Safety Considerations for Field Sampling Using a Boat

- Field collection personnel **should not** be assigned to duty alone in boats.
- Life preservers should be worn at all times by field collection personnel near the water or on board boats.
- If electrofishing is the sampling method used, there must be two shutoff switches--one at the generator and a second on the bow of the boat.
- All deep water sampling should be performed with the aid of an experienced, licensed boat captain.
- All sampling during nondaylight hours, during severe weather conditions, or during periods of high water should be avoided or minimized to ensure the safety of field collection personnel.
- All field collection personnel should be trained in CPR, water safety, boating safety, and first aid procedures for proper response in the event of an accident. Personnel should have local emergency numbers readily available for each sampling trip and know the location of the hospitals or other medical facilities nearest each sampling site.

and angling equipment (hook and line). Rotenone, a chemical piscicide, has been used extensively to stun fish prior to their collection with seines, trawls, or other sampling devices. Rotenone has not been found to interfere with the analysis of the recommended organic target analytes (see Table 4-1) when the recommended analysis procedures are used. See Section 8 for additional information on appropriate analysis methods for the recommended organic target analytes. Devices for shellfish sampling include seines, trawls, mechanical grabs (e.g., pole- or cable-operated grab buckets and tongs), biological and hydraulic dredges, scoops and shovels, rakes, and dip nets. Shellfish can also be collected manually by SCUBA divers. Although active collection requires greater fishing effort, it is usually more efficient than passive collection for covering a large number of sites and catching the relatively small number of individuals needed from each site for tissue analysis (Versar, 1982). Active collection methods are particularly useful in shallow waters (e.g., streams, lake shorelines, and shallow coastal areas of estuaries).

One aspect of sample collection that is of paramount importance is that the sampling team must ensure the collection of live, intact fish and shellfish for use in sample analysis for human risk assessment. It is highly desirable to collect live, intact fish and shellfish that have not been mutilated by the collection gear and that do not have any skin, shell, or carapace lacerations or fin deterioration that would allow body fluids to leak out of the specimen or contaminants to pass into the specimen after collection. For example, some fish collected by electro-shocking methods may have ruptured organs due to the electroshocking procedure. Fish that are found floating dead at a site should not be used for sample analysis for human risk assessments. For these reasons, EPA recommends that any specimens that show any skin, shell, or carapace lacerations or fin deterioration of any kind not used for chemical analysis.

Active collection methods have distinct disadvantages for deep water sampling. They require more field personnel and more expensive equipment than passive collection methods. This disadvantage may be offset by coordinating sampling efforts with commercial fishing efforts. Purchasing fish and shellfish from commercial fishers using active collection devices is acceptable; however, field sampling staff should accompany the commercial fishers during the collection operation to ensure that samples are collected and handled properly and to verify the sampling site location. The field sampling staff then remove the target species directly from the sampling device and ensure that sample collection, processing, and preservation are conducted as prescribed in sample collection protocols, with minimal chance of contamination. This is an excellent method of obtaining specimens of commercially important target species, particularly from the Great Lakes and coastal estuarine areas (Versar, 1982). More detailed descriptions of active sampling devices and their use are provided in Battelle (1975), Bennett, et al., (1970), Gunderson and Ellis (1986), Hayes (1983), Mearns and Allen (1978), Pitt (1981), Puget Sound Estuary Program (1990b), Versar (1982), and Weber (1973).

6.2.1.2 Passive Collection—

Passive collection methods employ a wide array of sampling devices for fish and shellfish, including gill nets, fyke nets, trammel nets, hoop nets, pound nets, and d-traps. Passive collection methods generally require less fishing effort than active methods but are usually less desirable for shallow water sample collection because of the ability of many species to evade these entanglement and entrapment devices. These methods normally yield a much greater catch than would be required for a contaminant monitoring program and are time consuming to deploy. In deep water, however, passive collection methods are generally more efficient than active methods. Crawford and Luoma (1993) caution that passive collection devices (e.g., gill nets) should be checked frequently to ensure that captured fish do not deteriorate prior to removal from the sampling device. Versar (1982, 1984) and Hubert (1983) describe passive sampling devices and their use in more detail. It is highly desirable to collect live, intact fish that have not been mutilated by the collection gear and that do not have any skin lacerations or fin deterioration. For these reasons, EPA recommends that fish captured in passive collection devices not remain in the water for more than 24 hours after the passive collection device is first deployed and that specimens that show any skin or fin deterioration or external lacerations of any kind not used for chemical analysis.

Purchasing fish and shellfish from commercial fishers using passive collection methods is acceptable; however, field sampling staff should accompany the fishers during both the deployment and collection operations to ensure that samples are collected and handled properly and to verify the sampling site location. The field sampling staff can then ensure that sample collection, processing, and preservation are conducted as prescribed in sample collection protocols, with minimal chance of contamination.

6.2.2 Preservation of Sample Integrity

The primary QA consideration in sample collection, processing, preservation, and shipping procedures is the preservation of sample integrity to ensure the accuracy of target analyte analyses. Sample integrity is preserved by prevention of loss of contaminants already present in the tissues and prevention of extraneous tissue contamination (Smith, 1985).

Loss of contaminants already present in fish or shellfish tissues can be prevented in the field by ensuring that the skin on fish specimens has not been lacerated by the sampling gear or that the carapace of crustaceans or shells of bivalves have not been cracked during sample collection resulting in loss of tissues and/or fluids that may contain contaminants. Once the samples have reached the laboratory, further care must be taken during thawing (if specimens are frozen) to ensure that all liquids from the thawed specimens are retained with the tissue sample as appropriate (see Sections 7.2.2, 7.2.3, and 7.2.4).

Sources of extraneous tissue contamination include contamination from sampling gear, grease from ship winches or cables, spilled engine fuel (gasoline or diesel), engine exhaust, dust, ice chests, and ice used for cooling. All potential sources of contamination in the field should be identified and appropriate steps taken to minimize or eliminate them. For example, during sampling, the boat should be positioned so that engine exhausts do not fall on the deck. Ice chests should be scrubbed clean with detergent and rinsed with distilled water after each use to prevent contamination. To avoid contamination from melting ice, samples should be placed in waterproof plastic bags (Stober, 1991). Sampling equipment that has obviously been contaminated by oils, grease, diesel fuel, or gasoline should not be used. All utensils or equipment that will be used directly in handling fish or shellfish (e.g., fish measuring board or calipers) should be cleaned in the laboratory prior to each sampling trip, rinsed in acetone and pesticide-grade hexane, and stored in aluminum foil until use (Versar, 1982). Between sampling sites, the field collection team should clean each measurement device by rinsing it with ambient water and rewrapping it in aluminum foil to prevent contamination.

Note: Ideally, all sample processing (e.g., resections) should be performed at a sample processing facility under cleanroom conditions to reduce the possibility of sample contamination (Schmitt and Finger, 1987; Stober, 1991). However, there may be some situations in which state staff find it necessary to fillet finfish or resect edible turtle or shellfish tissues in the field prior to packaging the samples for shipment to the processing laboratory. This practice should be avoided whenever possible. If states find that filleting fish or resecting other edible tissues must be performed in the field, a clean area should be set up away from sources of diesel exhaust and areas where gasoline, diesel fuel, or grease are used to help reduce the potential for surface and airborne contamination of the samples from PAHs and other contaminants. Use of a mobile laboratory or use of a portable resection table and enclosed hood would provide the best environment for sample processing in the field. General guidance for conducting sample

processing under cleanroom conditions is provided in Section 7.2.1. States should review this guidance to ensure that procedures as similar as possible to those recommended for cleanroom processing are followed. If sample processing is conducted in the field, a notation should be made in the field records and on the sample processing record (see Figure 7-2). Procedures for laboratory processing and resection are described in Section 7.2. Procedures for assessing sources of sample contamination through the analyses of field and processing blanks are described in Section 8.3.3.6.

6.2.3 Field Recordkeeping

Thorough documentation of all field sample collection and processing activities is necessary for proper interpretation of field survey results. For fish and shellfish contaminant studies, it is advisable to use preprinted waterproof data forms, indelible ink, and writing implements that can function when wet (Puget Sound Estuary Program, 1990b). When multicopy forms are required, no-carbon-required (NCR) paper is recommended because it allows information to be forwarded on the desired schedule and retained for the project file at the same time.

Four separate preprinted sample tracking forms should be used for each sampling site to document field activities from the time the sample is collected through processing and preservation until the sample is delivered to the processing laboratory. These are

- Field record form
- Sample identification label
- Chain-of-custody (COC) label or tag
- COC form.

6.2.3.1 Field Record Form—

The following information should be included on the field record for each sampling site in both **Tier 1** screening (Figures 6-3 and 6-4) and **Tier 2** intensive studies as appropriate (Figures 6-5 and 6-6):

- Project number
- Sampling date and time (give date in a Year 2000 compliant format [YYYYMMDD] and specify convention used for time, e.g., 24-h clock)
- Sampling site location (including site name and number, county/parish, latitude/longitude, waterbody name/segment number, waterbody type, and site description)
- Sampling depth (specify units of depth)
- Collection method
- Collectors' names and signatures
- Agency (including telephone number and address)

Field Record for Fish Contaminant Monitoring Program — Screening Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

County/Parish: _____ Lat./Long.: _____

Waterbody Name/Segment Number: _____

Waterbody Type: ☐ RIVER ☐ LAKE ☐ ESTUARY

Site Description: _____

Collection Method: _____

Collector Name: _____
(print and sign)

Agency: _____ Phone: (____) _____

Address: _____

FISH COLLECTED

Bottom Feeder—Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (mm)	Sex	Fish #	Length (mm)	Sex
001	_____	___	006	_____	___
002	_____	___	007	_____	___
003	_____	___	008	_____	___
004	_____	___	009	_____	___
005	_____	___	010	_____	___

$\frac{\text{Minimum size}}{\text{Maximum size}}$ x 100 = _____ >75% Composite mean length _____ mm

Notes (e.g., morphological anomalies): _____

Predator—Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (mm)	Sex	Fish #	Length (mm)	Sex
001	_____	___	006	_____	___
002	_____	___	007	_____	___
003	_____	___	008	_____	___
004	_____	___	009	_____	___
005	_____	___	010	_____	___

$\frac{\text{Minimum size}}{\text{Maximum size}}$ x 100 = _____ ≥ 75% Composite mean length _____ mm

Notes (e.g., morphological anomalies): _____

Figure 6-3. Example of a field record for fish contaminant monitoring program—screening study.

Field Record for Shellfish Contaminant Monitoring Program — Screening Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

County/Parish: _____ Lat./Long.: _____

Waterbody Name/Segment Number: _____

Waterbody Type: ☐ RIVER ☐ LAKE ☐ ESTUARY

Site Description: _____

Collection Method: _____

Collector Name: _____
(print and sign)

Agency: _____ Phone: (____) _____

Address: _____

SHELLFISH COLLECTED

Bivalve Species Name: _____

Composite Sample #: _____ Number of Individuals: _____

Bivalve #	Size (mm)	Bivalve #	Size (mm)	Bivalve #	Size (mm)
001	_____	018	_____	035	_____
002	_____	019	_____	036	_____
003	_____	020	_____	037	_____
004	_____	021	_____	038	_____
005	_____	022	_____	039	_____
006	_____	023	_____	040	_____
007	_____	024	_____	041	_____
008	_____	025	_____	042	_____
009	_____	026	_____	043	_____
010	_____	027	_____	044	_____
011	_____	028	_____	045	_____
012	_____	029	_____	046	_____
013	_____	030	_____	047	_____
014	_____	031	_____	048	_____
015	_____	032	_____	049	_____
016	_____	033	_____	050	_____
017	_____	034	_____		

$\frac{\text{Minimum size}}{\text{Maximum size}} \times 100 =$ _____ ≥ 75% Composite mean size _____ mm

Notes (e.g., morphological anomalies): _____

Figure 6-4. Example of a field record for shellfish contaminant monitoring program—screening study.

Field Record for Fish Contaminant Monitoring Program — Intensive Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

County/Parish: _____ Lat./Long.: _____

Waterbody Name/Segment Number: _____

Waterbody Type: ☐ RIVER ☐ LAKE ☐ ESTUARY

Site Description: _____

Collection Method: _____

Collector Name: _____
(print and sign)

Agency: _____ Phone: (____) _____

Address: _____

FISH COLLECTED

Species Name: _____ **Replicate Number**: _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (mm)	Sex (M, F, or I)	Fish #	Length (mm)	Sex (M, F, or I)
001	_____	___	006	_____	___
002	_____	___	007	_____	___
003	_____	___	008	_____	___
004	_____	___	009	_____	___
005	_____	___	010	_____	___

$\frac{\text{Minimum length}}{\text{Maximum length}}$ x 100 = _____ % Composite mean length _____ mm

Notes (e.g., morphological anomalies): _____

Species Name: _____ **Replicate Number**: _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (mm)	Sex (M, F, or I)	Fish #	Length (mm)	Sex (M, F, or I)
001	_____	___	006	_____	___
002	_____	___	007	_____	___
003	_____	___	008	_____	___
004	_____	___	009	_____	___
005	_____	___	010	_____	___

$\frac{\text{Minimum length}}{\text{Maximum length}}$ x 100 = _____ ≥ 75% Composite mean length _____ mm

Notes (e.g., morphological anomalies): _____

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Figure 6-5. Example of a field record for fish contaminant monitoring program—intensive study.

Field Record for Fish Contaminant Monitoring Program — Intensive Study (con.)

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION:

Site Name/Number: _____

County/Parish: _____ Lat./Long.: _____

FISH COLLECTED

Species Name: _____ **Replicate Number:** _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (mm)	Sex (M, F, or I)	Fish #	Length (mm)	Sex (M, F, or I)
001	_____	___	006	_____	___
002	_____	___	007	_____	___
003	_____	___	008	_____	___
004	_____	___	009	_____	___
005	_____	___	010	_____	___

$\frac{\text{Minimum length}}{\text{Maximum length}}$ x 100 = _____ % Composite mean length _____ mm

Notes (e.g., morphological anomalies): _____

Species Name: _____ **Replicate Number:** _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (mm)	Sex (M, F, or I)	Fish #	Length (mm)	Sex (M, F, or I)
001	_____	___	006	_____	___
002	_____	___	007	_____	___
003	_____	___	008	_____	___
004	_____	___	009	_____	___
005	_____	___	010	_____	___

$\frac{\text{Minimum length}}{\text{Maximum length}}$ x 100 = _____ % Composite mean length _____ mm

Notes (e.g., morphological anomalies): _____

Species Name: _____ **Replicate Number:** _____

Composite Sample #: _____ Number of Individuals: _____

Fish #	Length (mm)	Sex (M, F, or I)	Fish #	Length (mm)	Sex (M, F, or I)
001	_____	___	006	_____	___
002	_____	___	007	_____	___
003	_____	___	008	_____	___
004	_____	___	009	_____	___
005	_____	___	010	_____	___

$\frac{\text{Minimum length}}{\text{Maximum length}}$ x 100 = _____ ≥ 75% Composite mean length _____ mm

Notes (e.g., morphological anomalies): _____

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Figure 6-5. (continued)

Field Record for Shellfish Contaminant Monitoring Program — Intensive Study

Project Number: _____ Sampling Date and Time: _____

SITE LOCATION

Site Name/Number: _____

County/Parish: _____ Lat./Long.: _____

Waterbody Name/Segment Number: _____

Waterbody Type: ☐ RIVER ☐ LAKE ☐ ESTUARY

Site Description: _____

Collection Method: _____

Collector Name: _____
(print and sign)

Agency: _____ Phone: (____) _____

Address: _____

SHELLFISH COLLECTED

Species Name: _____ **Replicate Number:** _____

Composite Sample #: _____ Number of Individuals: _____

Shellfish #	Size (mm)	Sex	Shellfish #	Size (mm)	Sex	Shellfish #	Size (mm)	Sex
001			018			035		
002			019			036		
003			020			037		
004			021			038		
005			022			039		
006			023			040		
007			024			041		
008			025			042		
009			026			043		
010			027			044		
011			028			045		
012			029			046		
013			030			047		
014			031			048		
015			032			049		
016			033			050		
017			034					

$\frac{\text{Minimum size}}{\text{Maximum size}}$ x 100 = _____ ≥ 75% Composite mean size _____ mm

Notes (e.g., morphological anomalies): _____

Figure 6-6. Example of a field record for shellfish contaminant monitoring program—intensive study.

- Species collected (including species common and scientific name, composite sample number, individual specimen number, number of individuals per composite sample, number of replicate samples, total length/size [mm], sex [male, female, indeterminate])

Note: States should specify a unique numbering system to track samples for their own fish and shellfish contaminant monitoring programs.

- Percent difference in size between the smallest and largest specimens to be composited (smallest individual length [or size] divided by the largest individual length [or size] x 100; should be $\geq$75 percent) and mean composite length or size (mm)

- Notes (including visible morphological abnormalities, e.g., fin erosion, skin ulcers, cataracts, skeletal and exoskeletal anomalies, neoplasms, or parasites).

6.2.3.2 Sample Identification Label—

A sample identification label should be completed in indelible ink for each individual fish or shellfish specimen after it is processed to identify each sample uniquely (Figure 6-7). The following information should be included on the sample identification label:

- Species scientific name or code number
- Total length/size of specimen (mm)
- Specimen number
- Sample type: F (fish fillet analysis only)
  S (shellfish edible portion analysis only)
  W (whole fish analysis)
  O (other fish tissue analysis)

<table>
<tr><td colspan="2">Species Name or Code</td><td colspan="2">Sample Type</td></tr>
<tr><td>Total Length or Size (mm)</td><td colspan="3">Sampling Site (name/number)</td></tr>
<tr><td colspan="3" rowspan="2">Specimen Number
☐ ☐ ☐ ☐ ☐ — ☐ ☐ ☐</td><td>Sampling Date (YYYMMDD)</td></tr>
<tr><td>Time (24-h clock)</td></tr>
</table>

Figure 6-7. Example of a sample identification label.

- Sampling site—waterbody name and/or identification number
- Sampling date/time (give date in a Year 2000 compliant format [YYYYMMDD] and specify convention for time, e.g., 24-h clock).

A completed sample identification label should be taped to each aluminum-foil-wrapped specimen and the specimen should be placed in a waterproof plastic bag.

6.2.3.3 Chain-of-Custody Label or Tag—

A COC label or tag should be completed in indelible ink for each individual fish specimen. The information to be completed for each fish is shown in Figure 6-8.

<table>
<tr><td colspan="2">Project Number</td><td colspan="4">Collection Agency (name, address, phone)</td></tr>
<tr><td colspan="3">Sampling Site (name and/or ID number)</td><td colspan="3">Sampler (name and signature)</td></tr>
<tr><td colspan="2">Composition Number/Specimen Number(s)</td><td colspan="2">Chemical Analyses
☐ All target analytes
☐ Others (specify) _____</td><td colspan="2">Study Type</td></tr>
<tr><td colspan="2" rowspan="3">Sampling Date (YYYYMMDD) Time (24-h clock)</td><td colspan="2" rowspan="3">_____
_____</td><td rowspan="3">Screening</td><td>Intensive</td></tr>
<tr><td>Phase I ☐</td></tr>
<tr><td>Phase II ☐</td></tr>
<tr><td colspan="2" rowspan="2">Species Name or Code</td><td colspan="2">Processing</td><td colspan="2">Type of Ice</td></tr>
<tr><td>Whole Body</td><td>Resection</td><td>Wet</td><td>Dry</td></tr>
<tr><td colspan="6">Comments</td></tr>
</table>

Figure 6-8. Example of a chain-of-custody tag or label.

After all information has been completed, the COC label or tag should be taped or attached with string to the outside of the waterproof plastic bag containing the individual fish sample. Information on the COC label/tag should also be recorded on the COC form (Figure 6-9).

Because of the generally smaller size of shellfish, several individual aluminum-foil-wrapped shellfish specimens (within the same composite sample) may be placed in the same waterproof plastic bag. A COC label or tag should be completed in indelible ink for each shellfish composite sample. If more than 10 individual

Chain-of-Custody Record

Project Number	Collecting Agency (name, address, phone)	Sampling Date	Chemical Analyses
Samplers (print and sign)		Container ____ of ____	

Composite Number	Specimen Nos.	Sampling Time	Study Type Scr	Study Type Int	Sampling Site (name/number)	All Target Contaminants	Specific Contaminants Only (Specify)	Comments

Delivery Shipment Record	Deliver/Ship to: (name, address and phone)	Date/Time Shipped:
Delivery Method ☐ Hand carry ☐ Shipped		

Relinquished by: *(signature)*	Date / Time	Received by: *(signature)*	Relinquished by: *(signature)*	Date / Time	Received by: *(signature)*
Relinquished by: *(signature)*	Date / Time	Received for Central Processing Laboratory by: *(signature)*	Date / Time	Remarks:	

Laboratory Custody:

Released Name/Date	Received Name/Date	Purpose	Location

Figure 6-9. Example of a chain-of-custody record form.

shellfish are to be composited, several waterproof plastic bags may have to be used for the same composite. It is important not to place too many individual specimens in the same plastic bag to ensure proper preservation during shipping, particularly during summer months. Information on the COC label/tag should also be recorded on the COC form (Figure 6-9).

6.2.3.4 Chain-of-Custody Form—

A COC form should be completed in indelible ink for each shipping container (e.g., ice chest) used. Information recommended for documentation on the COC form (Figure 6-9) is necessary to track all samples from field collection to receipt at the processing laboratory. In addition, this form can be used for tracking samples through initial laboratory processing (e.g., resection) as described in Section 7.2.

Prior to sealing the ice chest, one copy of the COC form and a copy of the field record sheet should be sealed in a resealable waterproof plastic bag. This plastic bag should be taped to the inside cover of the ice chest so that it is maintained with the samples being tracked. Ice chests should be sealed with reinforced tape for shipment.

6.2.3.5 Field Logbook—

In addition to the four sample tracking forms discussed above, the field collection team should document in a field logbook any additional information on sample collection activities, hydrologic conditions (e.g., tidal stage), weather conditions, boat or equipment operations, or any other unusual activities observed (e.g., dredging) or problems encountered that would be useful to the program manager in evaluating the quality of the fish and shellfish contaminant monitoring data.

6.3 SAMPLE HANDLING

6.3.1 Sample Selection

6.3.1.1 Species Identification—

As soon as fish, shellfish, and turtles are removed from the collection device, they should be identified by species. Nontarget species or specimens of target species that do not meet size requirements (e.g., juveniles) should be returned to the water. Species identification should be conducted only by experienced personnel knowledgeable of the taxonomy of species in the waterbodies included in the contaminant monitoring program. Taxonomic keys, appropriate for the waters being sampled, should be consulted for species identification. Because the objective of both the screening and intensive monitoring studies is to determine the magnitude of contamination in specific fish, shellfish, and turtle species, it is necessary that all individuals used in a composite sample be of a single species. **Note:** Correct species identification is important and different species should never be combined in a single composite sample.

When sufficient numbers of the target species have been identified to make up a composite sample, the species name and all other appropriate information should be recorded on the field record forms (Figures 6-3 through 6-6).

Note: EPA recommends that, when turtles are used as the target species, target analyte concentrations be determined for each turtle rather than for a composite turtle sample.

6.3.1.2 Initial Inspection and Sorting—

Individual fish of the selected target species should be rinsed in ambient water to remove any foreign material from the external surface. Large fish should be stunned by a sharp blow to the base of the skull with a wooden club or metal rod. This club or rod should be used solely for the purpose of stunning fish, and care should be taken to keep it reasonably clean to prevent contamination of the samples (Versar, 1982). Small fish may be placed on ice immediately after capture to stun them, thereby facilitating processing and packaging procedures. Once stunned, individual specimens of the target species should be grouped by species and general size class and placed in clean holding trays to prevent contamination. All fish should be inspected carefully to ensure that their skin and fins have not been damaged by the sampling equipment, and damaged specimens should be discarded (Versar, 1982).

Freshwater turtles should be rinsed in ambient water and their external surface scrubbed if necessary to remove any foreign matter from their carapace and limbs. Each turtle should be inspected carefully to ensure that the carapace and extremities have not been damaged by the sampling equipment, and damaged specimens should be discarded (Versar, 1982). Care should be taken when handling large turtles, particularly snapping turtles; many can deliver severe bites. Particularly during procedures that place fingers or hands within striking range of the sharp jaws, covering the turtle's head, neck, and forelimbs with a cloth towel or sack and taping it in place is often sufficient to prevent injury to the field sampling crew (Frye, 1994).

After inspection, each turtle should be placed individually in a heavy burlap sack or canvas bag tied tightly with a strong cord and then placed in an ice-filled cooler. Placing turtles on ice will slow their metabolic rate, making them easier to handle. **Note:** It is recommended that each turtle be analyzed as an individual sample, especially if the target turtle species is not abundant in the waterbody being sampled or if the collected individuals differ greatly in size or age. Analysis of individual turtles can provide an estimate of the maximum contaminant concentrations to which recreational or substistence fishers are exposed. Target analyte concentrations in composite samples represent averages for a specific target species population. The use of these values in risk assessment is appropriate if the objective is to estimate the average concentration to which consumers of the target species are exposed over a long period of time. The use of long exposure periods (e.g., 70 years) is typical for the assessment of

carcinogenic effects, which may be manifest over an entire lifetime (see Volume II of this guidance series). Noncarcinogenic effects, on the other hand, may cause acute health effects over a relatively short period of time (e.g., hours or days) after consumption. The maximum target analyte contaminant concentration may be more appropriate than the average target analyte concentration for use with noncarginogenic target analytes (U.S. EPA, 1989d). This is especially important for those target analytes for which acute exposures to very high concentrations may be toxic to consumers.

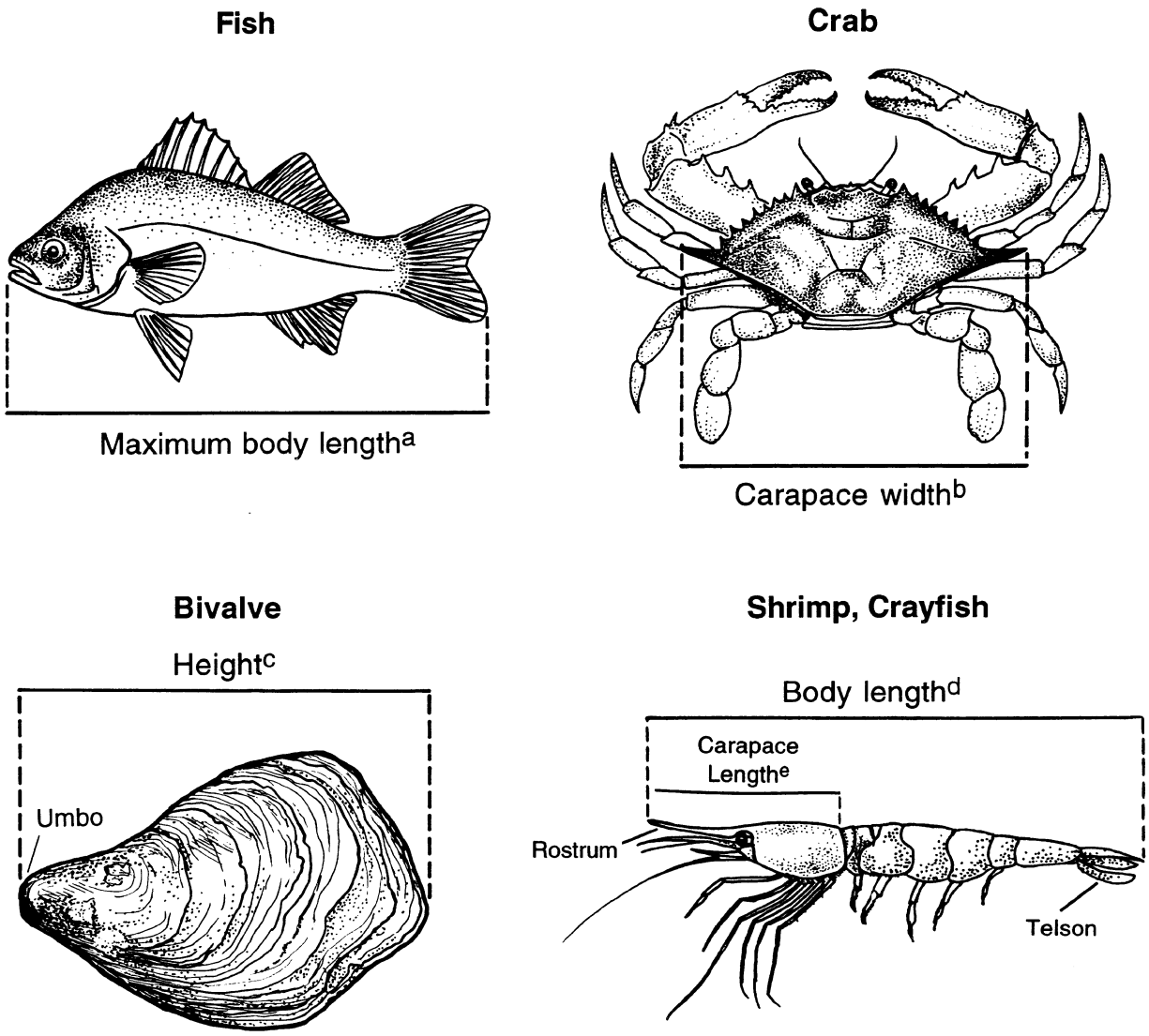
Stone et al. (1980) reported extremely high concentrations of PCBs in various tissues of snapping turtles from a highly contaminated site on the Hudson River. Contaminant analysis of various turtle tissues showed mean PCB levels of 2,991 ppm in fatty tissue, 66 ppm in liver tissue, and 29 ppm in eggs as compared to 4 ppm in skeletal muscle. Clearly, inclusion of the fatty tissue, liver, and eggs with the muscle tissues as part of the edible tissues will increase observed residue concentrations over those detected in muscle tissue only. States interested in using turtles as target species should review Appendix C for additional information on the use of individual samples in contaminant monitoring programs.

Bivalves (oysters, clams, scallops, and mussels) adhering to one another should be separated and scrubbed with a nylon or natural fiber brush to remove any adhering detritus or fouling organisms from the exterior shell surfaces (NOAA, 1987). All bivalves should be inspected carefully to ensure that the shells have not been cracked or damaged by the sampling equipment and damaged specimens should be discarded (Versar, 1982). Crustaceans, including shrimp, crabs, crayfish, and lobsters, should be inspected to ensure that their exoskeletons have not been cracked or damaged during the sampling process, and damaged specimens should be discarded (Versar, 1982). After shellfish have been rinsed, individual specimens should be grouped by target species and placed in clean holding trays to prevent contamination.

A few shellfish specimens may be resected (edible portions removed) to determine wet weight of the edible portions. This will provide an estimate of the number of individuals required to ensure that the recommended sample weight (200 g) is attained. **Note:** Individuals used to determine the wet weight of the edible portion should not be used for target analyte analyses.

6.3.1.3 Length or Size Measurements—

Each fish within the selected target species should be measured to determine total body length (mm). To be consistent with the convention used by most fisheries biologists in the United States, maximum body length should be measured as shown in Figure 6-10. The maximum body length is defined as the length from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are compressed dorsoventrally) (Anderson and Gutreuter, 1983).



[a] Maximum body length is the length from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are compressed dorsoventrally (Anderson and Gutreuter, 1983).

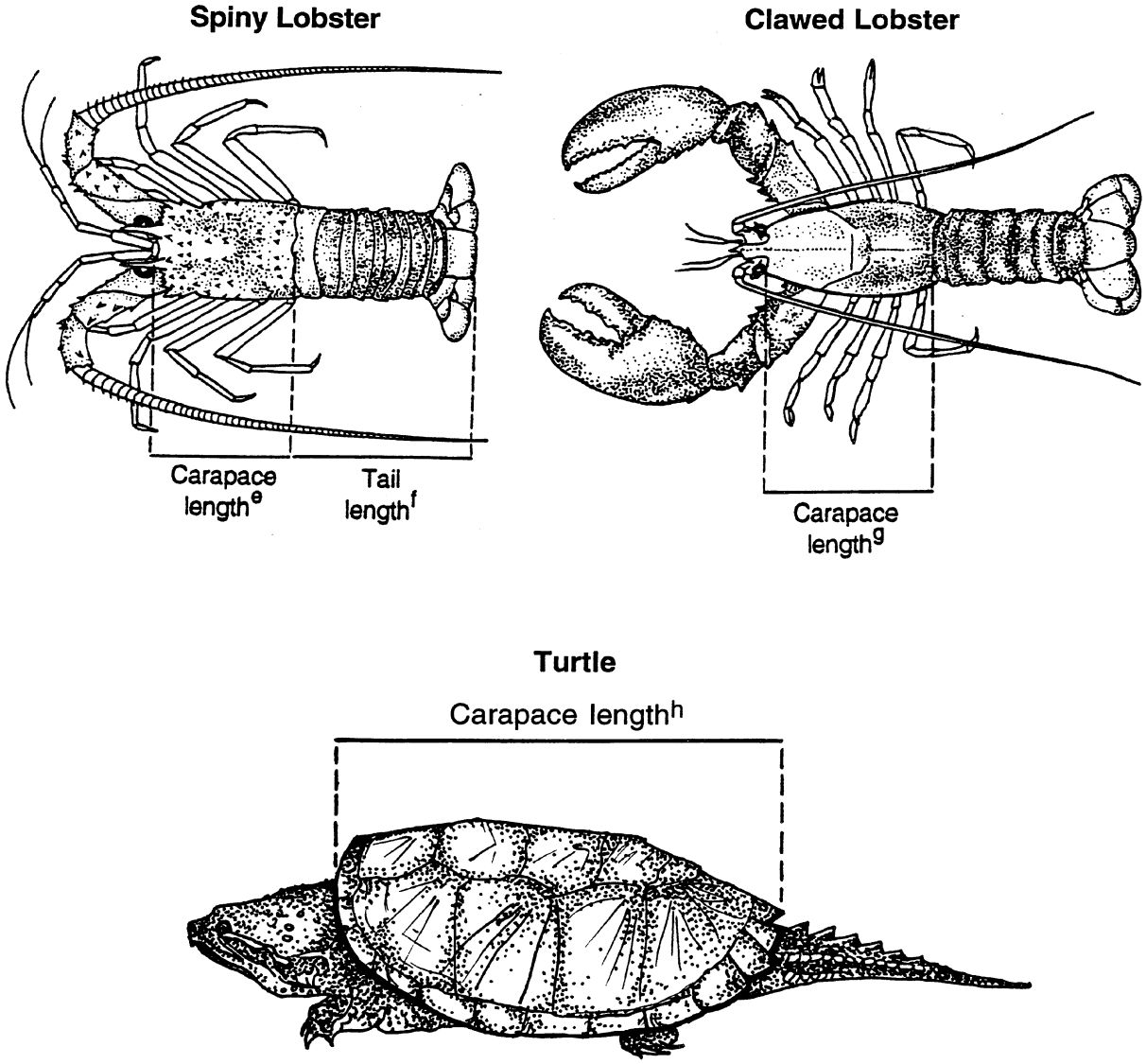
[b] Carapace width is the lateral distance across the carapace (from tip of spine to tip of spine (U.S. EPA, 1990c).

[c] Height is the distance from the umbo to the anterior (ventral) shell margin (Galtsoff, 1964).

[d] Body length is the distance from the tip of the rostrum to the tip of the telson (Texas Water Commission, 1990).

[e] Carapace length is distance from top of rostrum to the posterior margin of the carapace.

Figure 6-10. Recommended measurements of body length and size for fish, shellfish, and turtles.



[e] Carapace length is the distance from the anterior-most edge of the groove between the horns directly above the eyes, to the rear edge of the top part of the carapace as measured along the middorsal line of the back (Laws of Florida Chapter 46-24.003).

[f] Tail length is the distance measured lengthwise along the top middorsal line of the entire tail to rear-most extremity (this measurement shall be conducted with the tail in a flat straight position with the tip of the tail closed) (Laws of Florida Chapter 46-24.003).

[g] Carapace length is the distance from the rear of the eye socket to the posterior margin of the carapace (New York Environmental Conservation Law 13-0329.5.a and Massachusetts General Laws Chapter 130).

[h] Carapace length is the straight-line distance from the anterior margin to the posterior margin of the shell (Conant and Collins, 1991).

Figure 6-10. (continued)

Each turtle within the selected target species should be measured to determine total carapace length (mm). To be consistent with the convention used by most herpetologists in the United States, carapace length should be measured as shown in Figure 6-10. The maximum carapace length is defined as the straight line distance from the anterior edge of the carapace to the posterior edge of the carapace (Conant and Collins, 1991).

For shellfish, each individual specimen should be measured to determine the appropriate body size (mm). As shown in Figure 6-9, the recommended body measurements differ depending on the type of shellfish being collected. Height is a standard measurement of size for oysters, mussels, clams, scallops, and other bivalve molluscs (Abbott, 1974; Galtsoff, 1964). The height is the distance from the umbo to the anterior (ventral) shell margin. For crabs, the lateral width of the carapace is a standard size measurement (U.S. EPA, 1990c); for shrimp and crayfish, the standard measurement of body size is the length from the rostrum to the tip of the telson (Texas Water Commission, 1990); and for lobsters, two standard measurements of body size are commonly used. For clawed and spiny lobsters, the standard size is the length of the carapace. For spiny lobsters, the length of the tail is also used as a standard size measurement.

6.3.1.4 Sex Determination (Optional)—

An experienced fisheries biologist can often make a preliminary sex determination for fish by visual inspection. The body of the fish should not be dissected in the field to determine sex; sex can be determined through internal examination of the gonads during laboratory processing (Section 7.2.2.4).

An experienced herpetologist can often make a preliminary sex determination of a turtle by visual inspection in the field. The plastron (ventral portion of the carapace) is usually flatter in the female and the tail is less well developed than in the male. The plastron also tends to be more concave in the male (Holmes, 1984). For the common snapping turtle (*Chelydra serpentina*), the cloaca of the female is usually located inside or at the perimeter of the carapace, while the cloaca of the male extends slightly beyond the perimeter of the carapace. The carapace of the turtle should never be resected in the field to determine sex; sex can be determined through internal examination of the gonads during laboratory processing (Section 7.2.3.4.). For shellfish, a preliminary sex determination can be made by visual inspection only for crustaceans. Sex cannot be determined in bivalve molluscs without shucking the bivalves and microscopically examining gonadal material. Bivalves should not be shucked in the field to determine sex; sex determination through examination of the gonads can be performed during laboratory processing if desired (Section 7.2.4.2).

6.3.1.5 Morphological Abnormalities (Optional)—

If resources allow, states may wish to consider documenting external gross morphological conditions in fish from contaminated waters. Severely polluted

aquatic habitats have been shown to produce a higher frequency of gross pathological disorders than similar, less polluted habitats (Krahn et al., 1986; Malins et al., 1984, 1985; Mix, 1986; Sinderman, 1983; and Sinderman et al., 1980).

Sinderman et al. (1980) reviewed the literature on the relationship of fish pathology to pollution in marine and estuarine environments and identified four gross morphological conditions acceptable for use in monitoring programs:

- Fin erosion
- Skin ulcers
- Skeletal anomalies
- Neoplasms (i.e., tumors).

Fin erosion is the most frequently observed gross morphological abnormality in polluted areas and is found in a variety of fishes (Sinderman, 1983). In demersal fishes, the dorsal and anal fins are most frequently affected; in pelagic fishes, the caudal fin is primarily affected.

Skin ulcers have been found in a variety of fishes from polluted waters and are the second most frequently reported gross abnormality. Prevalence of ulcers generally varies with season and is often associated with organic enrichment (Sinderman, 1983).

Skeletal anomalies include abnormalities of the head, fins, gills, and spinal column (Sinderman, 1983). Skeletal anomalies of the spinal column include fusions, flexures, and vertebral compressions.

Neoplasms or tumors have been found at a higher frequency in a variety of polluted areas throughout the world. The most frequently reported visible tumors are liver tumors, skin tumors (i.e., epidermal papillomas and/or carcinomas), and neurilemmomas (Sinderman, 1983).

The occurrence of fish parasites and other gross morphological abnormalities that are found at a specific site should be noted on the field record form. States interested in documenting morphological abnormalities in fish should review the protocols for fish pathology studies recommended in the Puget Sound Estuary Program (1990c) and those described by Goede and Barton (1990).

6.3.2 Sample Packaging

6.3.2.1 Fish—

After initial processing to determine species, size, sex, and morphological abnormalities, each fish should be individually wrapped in extra heavy duty aluminum foil. Spines on fish should be sheared to minimize punctures in the aluminum foil packaging (Stober, 1991). The sample identification label shown in Figure 6-7 should be taped to the outside of each aluminum foil package, each individual fish should be placed into a waterproof plastic bag and sealed, and the

COC tag or label should be attached to the outside of the plastic bag with string or tape. All of the packaged individual specimens in a composite sample should be kept together (if possible) in one large waterproof plastic bag in the same shipping container (ice chest) for transport. Once packaged, samples should be cooled on ice immediately.

6.3.2.2 Turtles—

After inital processing to determine the species, size (carapace length), and sex, each turtle should be placed on ice in a separate burlap or canvas bag and stored on ice for transport to the processing laboratory. A completed sample identification label (Figure 6-7) should be attached with string around the neck or one of the turtle's extremities and the COC tag or label should be attached to the outside of the bag with string or tape. **Note:** Bagging each turtle should not be undertaken until the specimen has been sufficiently cooled to induce a mild state of torpor, thus facilitating processing. The samplers should work rapidly to return each turtle to the ice chest as soon as possible after packaging as the turtle may suddenly awaken as it warms thus becoming a danger to samplers (Frye, 1994). As mentioned in Section 6.3.1, states should analyze turtles individually rather than compositing samples. This is especially important when very few specimens are collected at a sampling site or when specimens of widely varying size or age are collected.

Note: When a large number of individual specimens in the same composite sample are shipped together in the same waterproof plastic bag, the samples must have adequate space in the bag to ensure that contact with ice can occur, thus ensuring proper preservation during shipping. This is especially important when samples are collected during hot weather and/or when the time between field collection and delivery to the processing laboratory approaches the maximum shipping time (Table 6-8).

6.3.2.3 Shellfish—

After initial processing to determine species, size, sex, and morphological abnormalities, each shellfish specimen should be wrapped individually in extra heavy duty aluminum foil. A completed sample identification label (Figure 6-7) should be taped to the outside of each aluminum foil package. **Note**: Some crustacean species (e.g., blue crabs and spiny lobsters) have sharp spines on their carapace that might puncture the aluminum foil wrapping. Carapace spines should never be sheared off because this would destroy the integrity of the carapace. For such species, one of the following procedures should be used to reduce punctures to the outer foil wrapping:

- Double-wrap the entire specimen in extra heavy duty aluminum foil.
- Place clean cork stoppers over the protruding spines prior to wrapping the specimen in aluminum foil.

Table 6-8. Recommendations for Preservation of Fish, Shellfish, and Turtle Samples from Time of Collection to Delivery at the Processing Laboratory

Sample type	Number per composite	Container	Preservation	Maximum shipping time
Fish[a]				
Whole fish (to be filleted)	3-10	Extra heavy duty aluminum foil wrap of each fish.[b] Each fish is placed in a waterproof plastic bag.	Cool on wet ice or blue ice packets (preferred method) or Freeze on dry ice only if shipping time will exceed 24 hours	24 hours 48 hours
Whole fish	3-10	Same as above.	Cool on wet ice or blue ice packets or Freeze on dry ice	24 hours 48 hours
Shellfish[a]				
Whole shellfish (to be resected for edible tissue)	3-50[c]	Extra heavy duty aluminum foil wrap of each specimen.[b] Shellfish in the same composite sample may be placed in the same waterproof plastic bag.	Cool on wet ice or blue ice packets (preferred method) or Freeze on dry ice if shipping time will exceed 24 hours	24 hours 48 hours
Whole shellfish	3-50[c]	Same as above.	Cool on wet ice or blue ice packets or Freeze on dry ice	24 hours 48 hours
Whole turtles (to be resected for edible tissue)	1[d]	Heavy burlap or canvas bags.	Cool on wet ice or blue ice packets (preferred method) or Freeze on dry ice if shipping time to exceed 24 hours	24 hours 48 hours

[a] Use only individuals that have attained at least legal harvestable or consumable size.
[b] Aluminum foil should not be used for long-term storage of any sample (i.e., whole organisms, fillets, or homogenates) that will be analyzed for metals.
[c] Species and size dependent. For very small shellfish species, more than 50 individuals may be required to achieve the 200-g composite sample mass recommended for screening studies.
[d] Turtles should be analyzed as individual rather than as composite samples.

- Wrap the spines with multiple layers of foil before wrapping the entire specimen in aluminum foil.

All of the individual aluminum-foil-wrapped shellfish specimens (in the same composite sample) should be placed in the same waterproof plastic bag for transport. In this case, a COC tag or label should be completed for the composite sample and appropriate information recorded on the field record sheet and COC form. The COC label or tag should then be attached to the outside of the plastic

bag with string or tape. For composite samples containing more than 10 shellfish specimens or especially large individuals, additional waterproof plastic bags may be required to ensure proper preservation. Once packaged, composite samples should be cooled on ice immediately. **Note**: When a large number of individual specimens in the same composite sample are shipped together in the same waterproof plastic bag, the samples must have adequate space in the bag to ensure that contact with ice can occur; thus ensuring proper preservation during shipping. This is especially important when samples are collected during hot weather and/or when the time between field collection and delivery to the processing laboratory approaches the maximum shipping time (Table 6-8).

6.3.3 Sample Preservation

The type of ice to be used for shipping should be determined by the length of time the samples will be in transit to the processing laboratory and the sample type to be analyzed (Table 6-8).

6.3.3.1 Fish, Turtles, or Shellfish To Be Resected—

Note: Ideally fish, turtles, and shellfish specimens should not be frozen prior to resection if analyses will include edible tissue only because freezing may cause some internal organs to rupture and contaminate fillets or other edible tissues (Stober, 1991; U.S. EPA, 1986b). Wet ice or blue ice (sealed prefrozen ice packets) is recommended as the preservative of choice when the fish fillet, turtle meat, or shellfish edible portions are the primary tissues to be analyzed. Samples shipped on wet or blue ice should be delivered to the processing laboratory within 24 hours (Smith, 1985; U.S. EPA, 1990d). If the shipping time to the processing laboratory will exceed 24 hours, dry ice should be used.

Note: One exception to the use of dry ice for long-term storage is if fish or shellfish are collected as part of extended offshore field surveys. States involved in these types of field surveys may employ shipboard freezers to preserve samples for extended periods rather than using dry ice. Ideally, all fish should be resected in cleanrooms aboard ship prior to freezing.

6.3.3.2 Fish, Turtles, or Shellfish for Whole-Body Analysis—

At some sites, states may deem it necessary to collect fish, turtles, or shellfish for whole-body analysis if a local subpopulation of concern typically consumes whole fish, turtles, or shellfish. If whole fish, turtles, or shellfish samples are to be analyzed, either wet ice, blue ice, or dry ice may be used; however, if the shipping time to the processing laboratory will exceed 24 hours, dry ice should be used.

Dry ice requires special packaging precautions before shipping by aircraft to comply with U.S. Department of Transportation (DOT) regulations. The *Code of Federal Regulations* (49 CFR 173.217) classifies dry ice as Hazard Class 9 UN1845 (Hazardous Material). These regulations specify the amount of dry ice

that may be shipped by air transport and the type of packaging required. For each shipment by air exceeding 5 pounds of dry ice per package, advance arrangements must be made with the carrier. Not more than 441 pounds of dry ice may be transported in any one cargo compartment on any aircraft unless the shipper has made special written arrangements with the aircraft operator.

The regulations further specify that the packaging must be designed and constructed to permit the release of carbon dioxide gas to prevent a buildup of pressure that could rupture the package. If samples are transported in a cooler, several vent holes should be drilled to allow carbon dioxide gas to escape. The vents should be near the top of the vertical sides of the cooler, rather than in the cover, to prevent debris from falling into the cooler. Wire screen or cheesecloth should be installed in the vents to keep foreign materials from contaminating the cooler. When the samples are packaged, care should be taken to keep these vents open to prevent the buildup of pressure.

Dry ice is exempted from shipping certification requirements if the amount is less than 441 pounds and the package meets design requirements. The package must be marked "Carbon Dioxide, Solid" or "Dry Ice" with a statement indicating that the material being refrigerated is to be used for diagnostic or treatment purposes (e.g., frozen tissue samples).

6.3.4 Sample Shipping

The fish, turtle, and shellfish samples should be hand-delivered or shipped to the processing laboratory as soon as possible after collection. The time the samples were collected and time of their arrival at the processing laboratory should be recorded on the COC form (Figure 6-9).

If the sample is to be shipped rather than hand-delivered to the processing laboratory, field collection staff must ensure the samples are packed properly with adequate ice layered between samples so that sample degradation does not occur. In addition, a member of the field collection staff should telephone ahead to the processing laboratory to alert them to the anticipated delivery time of the samples and the name and address of the carrier to be used. Field collection staff should avoid shipping samples for weekend delivery to the processing laboratory unless prior plans for such a delivery have been agreed upon with the processing laboratory staff.

SECTION 7

LABORATORY PROCEDURES I — SAMPLE HANDLING

This section provides guidance on laboratory procedures for sample receipt, chain-of-custody, processing, distribution, analysis, and archiving. Planning, documentation, and quality assurance and quality control of all laboratory activities are emphasized to ensure that (1) sample integrity is preserved during all phases of sample handling and analysis, (2) chemical analyses are performed cost-effectively and meet program data quality objectives, and (3) data produced by different states and regions are comparable.

Laboratory procedures should be documented in a Work/QA Project Plan (U.S. EPA, 1980b) as described in Appendix I. Routine sample processing and analysis procedures should be prepared as standard operating procedures (SOPs) (U.S. EPA, 1984b).

7.1 SAMPLE RECEIPT AND CHAIN-OF-CUSTODY

Fish, shellfish, and turtle samples may be shipped or hand-carried from the field according to one or more of the following pathways:

- From the field to a state laboratory for sample processing and analysis
- From the field to a state laboratory for sample processing and shipment of composite sample aliquots to a contract laboratory for analysis
- From the field to a contract laboratory for sample processing and analysis.

Sample processing and distribution for analysis ideally should be performed by one processing laboratory. Transportation of samples from the field should be coordinated by the sampling team supervisor and the laboratory supervisor responsible for sample processing and distribution (see Section 6.3.4). An accurate written custody record must be maintained so that possession and treatment of each sample can be traced from the time of collection through analysis and final disposition.

Fish, shellfish, and turtle samples should be brought or shipped to the sample processing laboratory in sealed containers accompanied by a copy of the sample request form (Figure 6-1), a chain-of-custody form (Figure 6-9), and the field records (Figures 6-3 through 6-6). Each time custody of a sample or set of samples is transferred, the Personnel Custody Record of the COC form must be completed and signed by both parties. Corrections to the COC form should be made in indelible ink by drawing a single line through the original entry, entering

the correct information and the reason for the change, and initialing and dating the correction. The original entry should never be obscured.

When custody is transferred from the field to the sample processing laboratory, the following procedure should be used:

- Note the shipping time. If samples have been shipped on wet or blue ice, check that the shipping time has not exceeded 24 hours.
- Check that each shipping container has arrived undamaged and that the seal is intact.
- Open each shipping container and remove the copy of the sample request form, the COC form, and the field records.
- Note the general condition of the shipping container (samples iced properly with no leaks, etc.) and the accompanying documentation (dry, legible, etc.).
- Locate individuals in each composite sample listed on the COC form and note the condition of their packaging. Individual specimens should be properly wrapped and labeled. Note any problems (container punctured, illegible labels, etc.) on the COC form.
- If individuals in a composite are packaged together, check the contents of each composite sample container against the field record for that sample to ensure that the individual specimens are properly wrapped and labeled. Note any discrepancies or missing information on the COC form.
- Initial the COC form and record the date and time of sample receipt.
- Enter the following information for each composite sample into a permanent laboratory record book and, if applicable, a computer database:
  - Sample identification number (specify conventions for the composite sample number and the specimen number) **Note:** EPA recommends processing and analysis of turtles as individual samples.
  - Receipt date (use Year 2000 comliant format [YYYYMMDD])
  - Sampling date (use Year 2000 comliant format [YYYYMMDD])
  - Sampling site (name and/or identification number)
  - Fish, turtle, and shellfish species (scientific name or code number)
  - Total length of each fish, carapace length of each turtle, or size of each shellfish (mm)

- If samples have been shipped on wet or blue ice, distribute them immediately to the technician responsible for resection (see Section 7.2). See Section 7.2.3 for the procedure for processing turtle samples as individual samples. If samples have been shipped on dry ice, they may be distributed immediately to the technician for processing or stored in a freezer at ≤-20 °C for later processing. Once processed, fillets or edible portions of fish, turtles, or shellfish or tissue homogenates, should be stored according to the procedures described in Section 7.2 and in Table 7-1. **Note**: Holding times in Table 7-1 are maximum times recommended for holding samples from the time they are received at the laboratory until they are analyzed. These holding times are based on guidance that is sometimes administrative rather than technical in nature; there are no promulgated holding time criteria for tissues (U.S. EPA, 1995i). If states choose to use longer holding times, they must demonstrate and document the stability of the target analyte residues over the extended holding times.

7.2 SAMPLE PROCESSING

This section includes recommended procedures for preparing composite homogenate samples of fish fillets and edible portions of shellfish and individual samples of edible portions of freshwater turtles as required in screening and intensive studies. Recommended procedures for preparing whole fish composite homogenates are included in Appendix J for use by states in assessing the potential risk to local subpopulations known to consume whole fish or shellfish.

7.2.1 General Considerations

All laboratory personnel performing sample processing procedures (see Sections 7.2.2, 7.2.3, and 7.2.4) should be trained or supervised by an experienced fisheries biologist. Care must be taken during sample processing to avoid contaminating samples. Schmitt and Finger (1987) have demonstrated that contamination of fish flesh samples is likely unless the most exacting clean dissection procedures are used. Potential sources of contamination include dust, instruments, utensils, work surfaces, and containers that may contact the samples. All sample processing (i.e., filleting, removal of other edible tissue, homogenizing, compositing) should be done in an appropriate laboratory facility under cleanroom conditions (Stober, 1991). Cleanrooms or work areas should be free of metals and organic contaminants. Ideally, these areas should be under positive pressure with filtered air (HEPA filter class 100) (California Department of Fish and Game, 1990). Periodic wipe tests should be conducted in clean areas to verify the absence of significant levels of metal and organic contaminants. All instruments, work surfaces, and containers used to process samples must be of materials that can be cleaned easily and that are not themselves potential sources of contamination. More detailed guidance on establishing trace metal cleanrooms is provided in U.S. EPA (1995a).

Table 7-1. Recommendations for Container Materials, Preservation, and Holding Times for Fish, Shellfish, and Turtle Tissues from Receipt at Sample Processing Laboratory to Analysis

			Storage	
Analyte	**Matrix**	**Sample container**	**Preservation**	**Holding time[a]**
Mercury	Tissue (fillets and edible portions, homogenates)	Plastic, borosilicate glass, quartz, PTFE	Freeze at ≤-20 °C	28 days[b]
Other metals	Tissue (fillets and edible portions, homogenates)	Plastic, borosilicate glass, quartz, PTFE	Freeze at ≤-20 °C	6 months[c]
Organics	Tissue (fillets and edible portions, homogenates)	Borosilicate glass, PTFE, quartz, aluminum foil	Freeze at ≤-20 °C	1 year[d]
Metals and organics	Tissue (fillets and edible portions, homogenates)	Borosilicate glass, quartz, PTFE	Freeze at ≤-20 °C	28 days (for mercury); 6 months (for other metals); and 1 year (for organics)
Lipids	Tissue (fillets and edible portions, homogenates)	Plastic, borosilicate glass, quartz, PTFE	Freeze at ≤-20 °C	1 year

PTFE = Polytetrafluoroethylene (Teflon).

[a] Maximum holding times recommended by EPA (1995i).
[b] This maximum holding time is also recommended by the Puget Sound Estuary Program (1990e). The California Department of Fish and Game (1990) and the USGS National Water Quality Assessment Program (Crawford and Luoma, 1993) recommend a maximum holding time of 6 months for all metals, including mercury.
[c] This maximum holding time is also recommended by the California Department of Fish and Game (1990), the 301(h) monitoring program (U.S. EPA, 1986b), and the USGS National Water Quality Assessment Program (Crawford and Luoma, 1993). The Puget Sound Estuary Program (1990e) recommends a maximum holding time of 2 years.
[d] This maximum holding time is also recommended by the Puget Sound Estuary Program (1990e). The California Department of Fish and Game (1990) and the USGS National Water Quality Assessment Program (Crawford and Luoma, 1993) recommend a more conservative maximum holding time of 6 months. U.S. EPA (1995b) recommends a maximum holding time of 1 year at ≤-10 °C for dioxins/furans.

To avoid cross-contamination, all equipment used in sample processing (i.e., resecting, homogenizing, and compositing) should be cleaned thoroughly before each composite sample is prepared. Verification of the efficacy of cleaning procedures should be documented through the analysis of processing blanks or rinsates (see Section 8.3.3.6).

Because sources of organic and metal contaminants differ, it is recommended that duplicate samples be collected, if time and funding permit, when analyses of both organics and metals are required (e.g., for screening studies). One sample can then be processed and analyzed for organics and the other can be processed independently and analyzed for metals (Batelle, 1989; California Department of Fish and Game, 1990; Puget Sound Estuary Program, 1990c, 1990d). If fish are of adequate size, separate composites of individual fillets may be prepared and

analyzed independently for metals and organics. If only one composite sample is prepared for the analyses of metals and organics, the processing equipment must be chosen and cleaned carefully to avoid contamination by both organics and metals.

Suggested sample processing equipment and cleaning procedures by analysis type are discussed in Sections 7.2.1.1 through 7.2.1.3. Other procedures may be used if it can be demonstrated, through the analysis of appropriate blanks, that no contamination is introduced (see Section 8.3.3.6).

7.2.1.1 Samples for Organics Analysis—

Equipment used in processing samples for organics analysis should be of stainless steel, anodized aluminum, borosilicate glass, polytetrafluoroethylene (PTFE), ceramic, or quartz. Polypropylene and polyethylene (plastic) surfaces, implements, gloves, and containers are a potential source of contamination by organics and should not be used. If a laboratory chooses to use these materials, there should be clear documentation that they are not a source of contamination. Filleting should be done on glass or PTFE cutting boards that are cleaned properly between fish or on cutting boards covered with heavy duty aluminum foil that is changed after each filleting. Tissue should be removed with clean, high-quality, corrosion-resistant stainless steel or quartz instruments or with knives with titanium blades and PTFE handles (Lowenstein and Young, 1986). Fillets or tissue homogenates may be stored in borosilicate glass, quartz, or PTFE containers with PTFE-lined lids or in heavy duty aluminum foil (see Table 7-1).

Prior to preparing each composite sample, utensils and containers should be washed with detergent solution, rinsed with tap water, soaked in pesticide-grade isopropanol or acetone, and rinsed with organic-free, distilled, deionized water. Work surfaces should be cleaned with pesticide-grade isopropanol or acetone, washed with distilled water, and allowed to dry completely. Knives, fish scalers, measurement boards, etc., should be cleaned with pesticide-grade isopropanol or acetone followed by a rinse with contaminant-free distilled water between each fish sample (Stober, 1991).

7.2.1.2 Samples for Metals Analysis—

Equipment used in processing samples for metals analyses should be of quartz, PTFE, ceramic, polypropylene, or polyethylene. The predominant metal contaminants from stainless steel are chromium and nickel. If these metals are not of concern, the use of high-quality, corrosion-resistant stainless steel for sample processing equipment is acceptable. Quartz utensils are ideal but expensive. For bench liners and bottles, borosilicate glass is preferred over plastic (Stober, 1991). Knives with titanium blades and PTFE handles are recommended for performing tissue resections (Lowenstein and Young, 1986). Borosilicate glass bench liners are recommended. Filleting may be done on glass or PTFE cutting boards that are cleaned properly between fish or on cutting

boards covered with heavy duty aluminum foil that is changed after each fish. Fillets or tissue homogenates may be stored in plastic, borosilicate glass, quartz, or PTFE containers (see Table 7-1).

Prior to preparing each composite sample, utensils and containers should be cleaned thoroughly with a detergent solution, rinsed with tap water, soaked in acid, and then rinsed with metal-free water. Quartz, PTFE, glass, or plastic containers should be soaked in 50 percent $HN0_3$, for 12 to 24 hours at room temperature. **Note:** Chromic acid should not be used for cleaning any materials. Acids used should be at least reagent grade. Stainless steel parts may be cleaned as stated for glass or plastic, omitting the acid soaking step (Stober, 1991).

7.2.1.3 Samples for Both Organics and Metals Analyses—

As noted above, several established monitoring programs, including the Puget Sound Estuary Program (1990c, 1990d), the NOAA Mussel Watch Program (Battelle, 1989), and the California Mussel Watch Program (California Department of Fish and Game, 1990), recommend different procedures for processing samples for organics and metals analyses. However, this may not be feasible if fish are too small to allow for preparing separate composites from individual fillets or if resources are limited. If a single composite sample is prepared for the analyses of both organics and metals, precautions must be taken to use materials and cleaning procedures that are noncontaminating for both organics and metals.

Quartz, ceramic, borosilicate glass, and PTFE are recommended materials for sample processing equipment. If chromium and nickel are not of concern, high-quality, corrosion-resistant stainless steel utensils may be used. Knives with titanium blades and PTFE handles are recommended for performing tissue resections (Lowenstein and Young, 1986). Borosilicate glass bench liners are recommended. Filleting should be done on glass or PTFE cutting boards that are cleaned properly between fish or on cutting boards covered with heavy duty aluminum foil that is changed after each filleting. Fillets or tissue homogenates should be stored in clean borosilicate glass, quartz, or PTFE containers with PTFE-lined lids.

Prior to preparing each composite sample, utensils and containers should be cleaned thoroughly with a detergent solution, rinsed with tap water, soaked in 50 percent HNO_3, for 12 to 24 hours at room temperature, and then rinsed with organics- and metal-free water. **Note:** Chromic acid should not be used for cleaning any materials. Acids used should be at least reagent grade. Stainless steel parts may be cleaned using this recommended procedure with the acid soaking step method omitted (Stober, 1991).

Aliquots of composite homogenates taken for metals analysis (see Section 7.3.1) may be stored in plastic containers that have been cleaned according to the

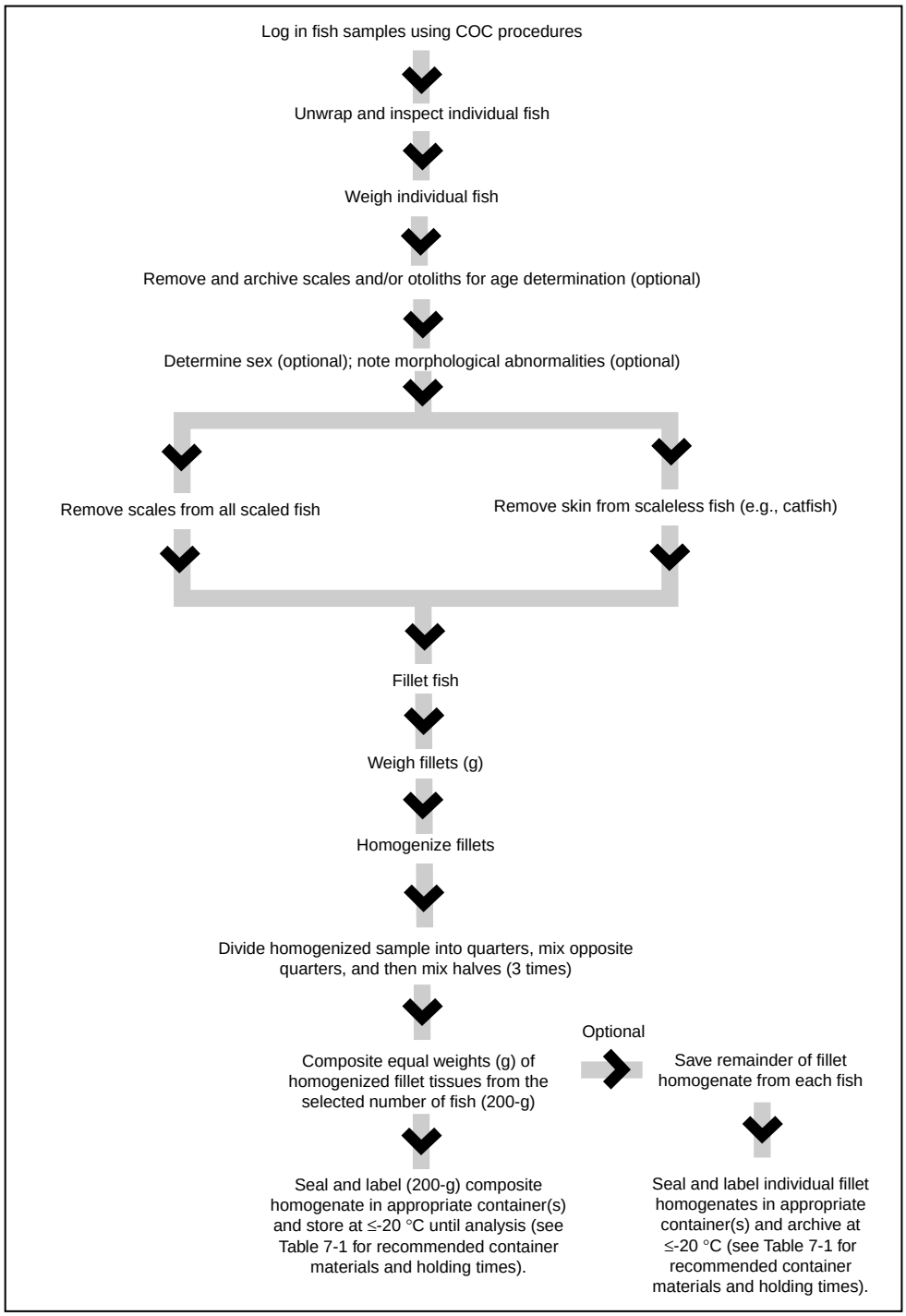
procedure outlined above, with the exception that aqua regia must not be used for the acid soaking step.

7.2.2 Processing Fish Samples

Processing in the laboratory to prepare fish fillet composite homogenate samples for analysis (diagrammed in Figure 7-1) involves

- Inspecting individual fish
- Weighing individual fish
- Removing scales and/or otoliths for age determination (optional)
- Determining the sex of each fish (optional)
- Examining each fish for morphological abnormalities (optional)
- Scaling all fish with scales (leaving belly flap on); removing skin of scaleless fish (e.g., catfish)
- Filleting (resection)
- Weighing fillets
- Homogenizing fillets
- Preparing a composite homogenate
- Preparing aliquots of the composite homogenate for analysis
- Distributing frozen aliquots to one or more analytical laboratories.

Whole fish should be shipped or brought to the sample processing laboratory from the field on wet or blue ice within 24 hours of sample collection. Fillets should be resected within 48 hours of sample collection. Ideally, fish should not be frozen prior to resection because freezing may cause internal organs to rupture and contaminate edible tissue (Stober, 1991; U.S. EPA, 1986b). However, if resection cannot be performed within 48 hours, the whole fish should be frozen at the sampling site and shipped to the sample processing laboratory on dry ice. Fish samples that arrive frozen (i.e., on dry ice) at the sample processing laboratory should be placed in a ≤ -20 °C freezer for storage until filleting can be performed. The fish should then be partially thawed prior to resection. **Note:** If the fillet tissue is contaminated by materials released from the rupture of the internal organs during freezing, the state may eliminate the fillet tissue as a sample or, alternatively, the fillet tissues should be rinsed in contaminant-free, distilled deionized



COC = Chain of custody.

Figure 7-1. Preparation of fish fillet composite homogenate samples.

water and blotted dry. Regardless of the procedure selected, a notation should be made in the sample processing record.

Sample processing procedures are discussed in the following sections. Data from each procedure should be recorded directly in a bound laboratory notebook or on forms that can be secured in the laboratory notebook. A sample processing record for fish fillet composites is shown in Figure 7-2.

7.2.2.1 Sample Inspection—

Individual fish received for filleting should be unwrapped and inspected carefully to ensure that they have not been compromised in any way (i.e., not properly preserved during shipment). Any specimen deemed unsuitable for further processing and analysis should be discarded and identified on the sample processing record.

7.2.2.2 Sample Weighing—

A wet weight should be determined for each fish. All samples should be weighed on balances that are properly calibrated and of adequate accuracy and precision to meet program data quality objectives. Balance calibration should be checked at the beginning and end of each weighing session and after every 20 weighings in a weighing session.

Fish shipped on wet or blue ice should be weighed directly on a foil-lined balance tray. To prevent cross contamination between individual fish, the foil lining should be replaced after each weighing. Frozen fish (i.e., those shipped on dry ice) should be weighed in clean, tared, noncontaminating containers if they will thaw before the weighing can be completed. **Note:** Liquid from the thawed whole fish sample will come not only from the fillet tissue but from the gut and body cavity, which are not part of the final fillet sample. Consequently, inclusion of this liquid with the sample may result in an overestimate of target analyte and lipid concentrations in the fillet homogenate. Nevertheless, it is recommended, as a conservative approach, that all liquid from the thawed whole fish sample be kept in the container as part of the sample.

All weights should be recorded to the nearest gram on the sample processing record and/or in the laboratory notebook.

7.2.2.3 Age Determination (Optional)—

Age provides a good indication of the duration of exposure to pollutants (Versar, 1982). A few scales or otoliths (Jearld, 1983) should be removed from each fish and delivered to a fisheries biologist for age determination. For most warm water inland gamefish, 5 to 10 scales should be removed from below the lateral line and behind the pectoral fin. On soft-rayed fish such as trout and salmon, the scales should be taken just above the lateral line (WDNR, 1988). For catfish and other

Sample Processing Record for Fish Contaminant Monitoring Program — Fish Fillet Composites

Project Number: _____ Sampling Date and Time: _____

STUDY PHASE: Screening Study ☐; Intensive Study: Phase I ☐ Phase II ☐

SITE LOCATION

Site Name/Number: _____

County/Parish: _____ Lat./Long.: _____

Waterbody Name/Segment Number: _____ Waterbody Type: _____

Sample Type (bottom feeder, predator, etc.) _____ **Species Name:** _____

Composite Sample #: _____ Replicate Number: _____ Number of Individuals: _____

					First Fillet (F1) or Combined Fillets (C)			Second Fillet (F2)		
Fish #	**Weight (g)**	**Scales/Otoliths Removed (✓)**	**Sex (M,F)**	**Resection Performed (✓)**	**Weight (g)**	**Homogenate Prepared (✓)**	**Wt. of Homog. for Composite (g)**	**Weight (g)**	**Homogenate Prepared (✓)**	**Wt. of Homog. for Composite (g)**
001										
002										
003										
004										
005										
006										
007										
008										
009										
010										
Analyst										
Date										

Total Composite Weight (g) **(F1 or C)** _____ **(F2)** _____

Notes: _____

Figure 7-2. Sample processing record for fish contaminant monitoring program—fish fillet composites.

scaleless fish, the pectoral fin spines should be clipped and saved (Versar, 1982). The scales, spines, or otoliths may be stored by sealing them in small envelopes (such as coin envelopes) or plastic bags labeled with, and cross-referenced by, the identification number assigned to the tissue specimen (Versar, 1982). Removal of scales, spines, or otoliths from each fish should be noted (by a check mark) on the sample processing record.

7.2.2.4 Sex Determination (Optional)—

Fish sex should be determined before filleting. To determine the sex of a fish, an incision should be made on the ventral surface of the body from a point immediately anterior to the anus toward the head to a point immediately posterior to the pelvic fins. If necessary, a second incision should be made on the left side of the fish from the initial point of the first incision toward the dorsal fin. The resulting flap should be folded back to observe the gonads. Ovaries appear whitish to greenish to golden brown and have a granular texture. Testes appear creamy white and have a smooth texture (Texas Water Commission, 1990). The sex of each fish should be recorded on the sample processing form.

7.2.2.5 Assessment of Morphological Abnormalities (Optional)—

Assessment of gross morphological abnormalities in finfish is optional. This assessment may be conducted in the field (see Section 6.3.1.5) or during initial inspection at the processing laboratory prior to filleting. States interested in documenting morphological abnormalities should consult Sinderman (1983) and review recommended protocols for fish pathology studies used in the Puget Sound Estuary Program (1990c) and those described by Goede and Barton (1990).

7.2.2.6 Scaling or Skinning—

To control contamination, separate sets of utensils and cutting boards should be used for skinning or scaling fish and for filleting fish. Fish with scales should be scaled and any adhering slime removed prior to filleting. Fish without scales (e.g., catfish) should be skinned prior to filleting. These fillet types are recommended because it is believed that they are most representative of the edible portions of fish prepared and consumed by sport anglers. However, it is the responsibility of each program manager, in consultation with state fisheries experts, to select the fillet or sample type most appropriate for each target species based on the dietary customs of local populations of concern.

A fish is scaled by laying it flat on a clean glass or PTFE cutting board or on one that has been covered with heavy duty aluminum foil and removing the scales and adhering slime by scraping from the tail to the head using the blade edge of a clean stainless steel, ceramic, or titanium knife. Cross-contamination is controlled by rinsing the cutting board and knife with contaminant-free distilled water between fish. If an aluminum-foil-covered cutting board is used, the foil should be

changed between fish. The skin should be removed from fish without scales by loosening the skin just behind the gills and pulling it off between knife blade and thumb or with pliers as shown in Figure 7-3.

Once the scales and slime have been scraped off or the skin removed, the outside of the fish should be washed with contaminant-free distilled water and it should be placed on a second clean cutting board for filleting.

7.2.2.7 Filleting—

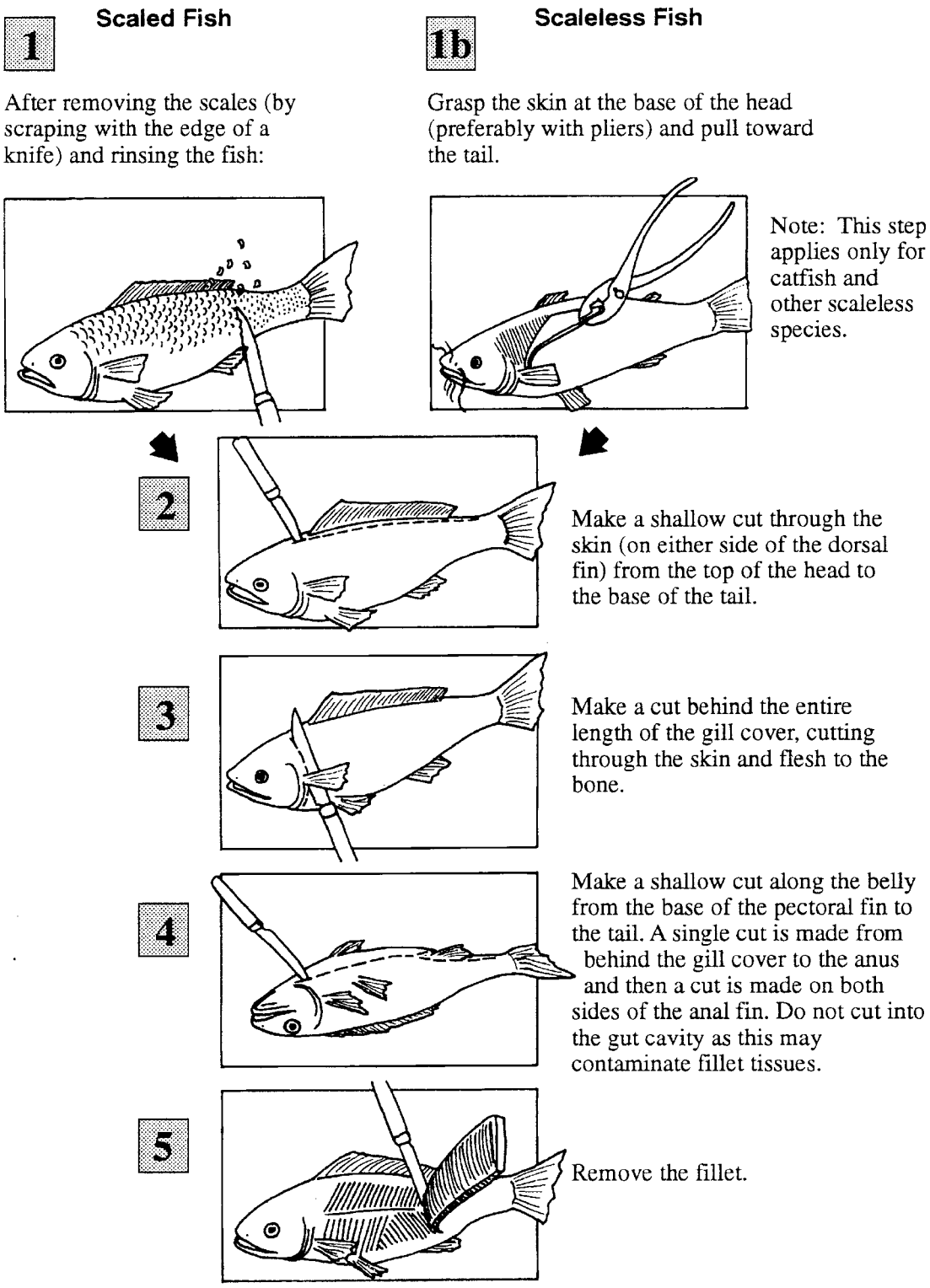
Filleting should be conducted only by or under the supervision of an experienced fisheries biologist. If gloves are worn, they should be talc- or dust-free, and of noncontaminating materials. Prior to filleting, hands should be washed with Ivory soap and rinsed thoroughly in tap water, followed by distilled water (U.S. EPA, 1991d). Specimens should come into contact with noncontaminating surfaces only. Fish should be filleted on glass or PTFE cutting boards that are cleaned properly between fish or on cutting boards covered with heavy duty aluminum foil that is changed between fish (Puget Sound Estuary Program, 1990d, 1990e). Care must be taken to avoid contaminating fillet tissues with material released from inadvertent puncture of internal organs. **Note:** If the fillet tissue is contaminated by materials released from the inadvertent puncture of the internal organs during resection, the state may eliminate the fillet tissue as a sample or, alternatively, the fillet tissue should be rinsed in contaminant-free, deionized distilled water and blotted dry. Regardless of the procedure selected, a notation should be made in the sample processing record.

Ideally, fish should be filleted while ice crystals are still present in the muscle tissue. Therefore, if fish have been frozen, they should not be allowed to thaw completely prior to filleting. Fish should be thawed only to the point where it becomes possible to make an incision into the flesh (U.S. EPA, 1991d).

Clean, high-quality stainless steel, ceramic, or titanium utensils should be used to remove one or both fillets from each fish, as necessary. The general procedure recommended for filleting fish is illustrated in Figure 7-3 (U.S. EPA, 1991d).

The belly flap should be included in each fillet. Any dark muscle tissue in the vicinity of the lateral line should not be separated from the light muscle tissue that constitutes the rest of the muscle tissue mass. Bones still present in the tissue after filleting should be removed carefully (U.S. EPA, 1991d).

If both fillets are removed from a fish, they can be combined or kept separate for duplicate QC analysis, analysis of different analytes, or archival of one fillet. Fillets should be weighed (either individually or combined, depending on the analytical requirements) and the weight(s) recorded to the nearest gram on the sample processing record.



Source: U.S. EPA, 1991d.

Figure 7-3. Illustration of basic fish filleting procedure.

If fillets are to be homogenized immediately, they should be placed in a properly cleaned glass or PTFE homogenization container. If samples are to be analyzed for metals only, plastic homogenization containers may be used. To facilitate homogenization, it may be necessary or desirable to chop each fillet into smaller pieces using a titanium or stainless steel knife prior to placement in the homogenization container.

If fillets are to be homogenized later, they should be wrapped in heavy duty aluminum foil and labeled with the sample identification number, the sample type (e.g., "F" for fillet), the weight (g), and the date of resection. If composite homogenates are to be prepared from only a single fillet from each fish, fillets should be wrapped separately and the designation "F1" and "F2" should be added to the sample identification number for each fillet. The individual fillets from each fish should be kept together. All fillets from a composite sample should be placed in a plastic bag labeled with the composite identification number, the individual sample identification numbers, and the date of resection and stored at ≤-20 °C until homogenization.

7.2.2.8 Preparation of Individual Homogenates—

To ensure even distribution of contaminants throughout tissue samples and to facilitate extraction and digestion of samples, the fillets from individual fish must be ground and homogenized prior to analysis. The fillets from an individual fish may be ground and homogenized separately or combined, depending on the analytical requirements and the sample size.

Fish fillets should be ground and homogenized using an automatic grinder or high-speed blender or homogenizer. Large fillets may be cut into 2.5-cm cubes with high-quality stainless steel or titanium knives or with a food service band saw prior to homogenization. Parts of the blender or homogenizer used to grind the tissue (i.e., blades, probes) should be made of tantalum or titanium rather than stainless steel. Stainless steel blades and/or probes have been found to be a potential source of nickel and chromium contamination (due to abrasion at high speeds) and should be avoided.

Grinding and homogenization of tissue is easier when it is partially frozen (Stober, 1991). Chilling the grinder/blender briefly with a few chips of dry ice will also help keep the tissue from sticking to it (Smith, 1985).

The fillet sample should be ground until it appears to be homogeneous. The ground sample should then be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed together. The grinding, quartering, and hand-mixing steps should be repeated at least two more times. If chunks of tissue are present at this point, the grinding and homogenization should be repeated. **Note:** Skin-on fillets are the fish fillet sample type recommended for use in state fish contaminant monitoring programs. However, skin-on fillets of some finfish species are especially difficult to homogenize completely. No chunks

of tissue or skin should remain in the sample homogenate because these may not be extracted or digested efficiently and could bias the analytical results. If complete homogenization of skin-on fillets for a particular target species is a chronic problem or if local consumers are likely to prepare skinless fillets of the species, the state should consider analyzing skinless fillet samples. If the sample is to be analyzed for metals only, the ground tissue may be mixed by hand in a polyethylene bag (Stober, 1991). The preparation of each individual homogenate should be noted (marked with a check) on the sample processing record. At this time, individual homogenates may be either processed further to prepare composite homogenates or frozen separately and stored at ≤-20 °C (see Table 7-1).

7.2.2.9 Preparation of Composite Homogenates—

Composite homogenates should be prepared from equal weights of individual homogenates. The same type of individual homogenate (i.e., either single fillet or combined fillet) should always be used in a given composite sample.

If individual homogenates have been frozen, they should be thawed partially and rehomogenized prior to weighing and compositing. Any associated liquid should be kept as a part of the sample. The weight of each individual homogenate used in the composite homogenate should be recorded, to the nearest gram, on the sample processing record.

Each composite homogenate should be blended as described for individual homogenates in Section 7.2.2.8. The composite homogenate may be processed immediately for analysis or frozen and stored at ≤-20 °C (see Table 7-1).

The remainder of each individual homogenate should be archived at ≤-20 °C with the designation "Archive" and the expiration date recorded on the sample label. The location of the archived samples should be indicated on the sample processing record under "Notes."

It is essential that the weights of individual homogenates yield a composite homogenate of adequate size to perform all necessary analyses. Weights of individual homogenates required for a composite homogenate, based on the number of fish per composite and the weight of composite homogenate recommended for analyses of all screening study target analytes (see Table 4-1), are given in Table 7-2. The total composite weight required for intensive studies may be less than that for screening studies if the number of target analytes is reduced significantly.

The recommended sample size of 200 g for screening studies is intended to provide sufficient sample material to (1) analyze for all recommended target analytes (see Table 4-1) at appropriate detection limits; (2) meet minimum QC requirements for the analyses of laboratory duplicate, matrix spike, and matrix spike duplicate samples (see Sections 8.3.3.4 and 8.3.3.5); and (3) allow for

Table 7-2. Weights (g) of Individual Homogenates Required for Screening Study Composite Homogenate Sample[a,b]

Number of fish per sample	Total composite weight		
	100 g (minimum)	200 g (recommended)	500 g (maximum)
3	33	67	167
4	25	50	125
5	20	40	100
6	17	33	84
7	14	29	72
8	13	25	63
9	11	22	56
10	10	20	50

[a] Based on total number of fish per composite and the total composite weight required for analysis in screening studies. The total composite weight required in intensive studies may be less if the number of target analytes is reduced significantly.

[b] Individual homogenates may be prepared from one or both fillets from a fish. A composite homogenate should be prepared only from individual homogenates of the same type (i.e., **either** from individual homogenates each prepared from a single fillet **or** from individual homogenates each prepared from both fillets).

reanalysis if the QC control limits are not met or if the sample is lost. However, sample size requirements may vary among laboratories and the analytical methods used. Each program manager must consult with the analytical laboratory supervisor to determine the actual weights of composite homogenates required to analyze for all selected target analytes at appropriate detection limits.

7.2.3 Processing Turtle Samples

Processing in the laboratory to prepare individual turtle homogenate samples for analysis (diagrammed in Figure 7-4) involves

- Inspecting individual turtles
- Weighing individual turtles
- Removing edible tissues
- Determining the sex of each turtle (optional)
- Determining the age of each turtle (optional)
- Weighing edible tissue or tissues
- Homogenizing tissues
- Preparing individual homogenate samples
- Preparing aliquots of the individual homogenates for analysis
- Distributing frozen aliquots to one or more analytical laboratories.

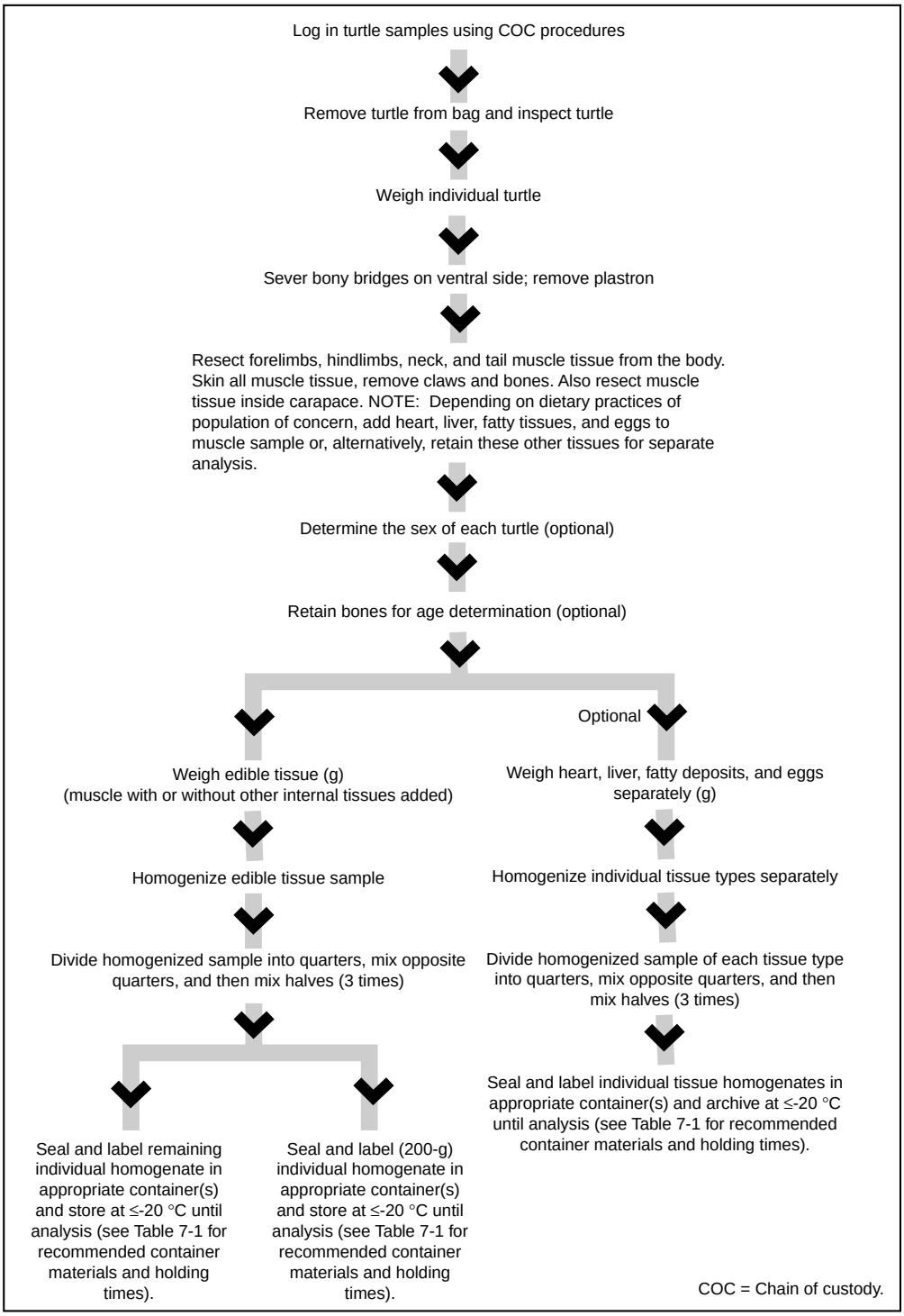


Figure 7-4. Preparation of individual turtle homogenate samples.

Whole turtles should be shipped or brought to the sample processing laboratory from the field on wet or blue ice within 24 hours of sample collection. The recommended euthanizing method for turtles is freezing (Frye, 1994) and a minimum of 48 hours or more may be required for large specimens. Turtles that arrive on wet or blue ice or frozen (i.e., on dry ice) at the sample processing laboratory should be placed in a ≤-20 °C freezer for storage until resection can be performed. If rupture of internal organs is noted for an individual turtle, the specimen may be eliminated as a sample or, alternatively, the edible tissues should be rinsed in distilled deionized water and blotted dry.

Sample processing procedures are discussed in the following sections. Data from each procedure should be recorded directly in a bound laboratory notebook or on forms that can be secured in the laboratory notebook. A sample processing record for individual turtle samples is shown in Figure 7-5.

7.2.3.1 Sample Inspection—

Turtles received for resection should be removed from the canvas or burlap collection bags and inspected carefully to ensure that they have not been compromised in any way (i.e., not properly preserved during shipment). Any specimen deemed unsuitable for further processing and analysis should be discarded and identified on the sample processing record.

7.2.3.2 Sample Weighing—

A wet weight should be determined for each turtle. All samples should be weighed on balances that are properly calibrated and of adequate accuracy and precision to meet program data quality objectives. Balance calibration should be checked at the beginning and end of each weighing session and after every 20 weighings in a weighing session.

Turtles euthanized by freezing should be weighed in clean, tared, noncontaminating containers if they will thaw before the weighing can be completed. **Note:** Liquid from the thawed whole turtle sample will come not only from the muscle tissue but from the gut and body cavity, which may not be part of the desired edible tissue sample. Consequently, inclusion of this liquid with the sample may result in an overestimate of target analyte and lipid concentrations in the edible tissue homogenate. Nevertheless, it is recommended, as a conservative approach, that all liquid from the thawed whole turtle be kept in the container as part of the sample.

All weights should be recorded to the nearest gram on the sample processing record and/or in the laboratory notebook.

Sample Processing Record for Turtle Contaminant Monitoring Program — Individual Samples

Project Number: _____ Sampling Date and Time: _____

STUDY PHASE: Screening Study ☐; Intensive Study: Phase I ☐ Phase II ☐

SITE LOCATION

Site Name/Number: _____

County/Parish: _____ Lat./Long.: _____

Waterbody Name/Segment Number: _____ Waterbody Type: _____

Sample Type (bottom feeder, predator, etc.)_____ **Species Name:** _____

Composite Sample #: _____ Replicate Number: _____ Number of Individuals: _____

Turtle #	Weight (g)	Carapace Length (mm)	Sex (M,F)	Resection Performed (✓)	Tissue Type Used	Tissue Weight (g)	Homogenate Prepared (✓)
001							
002							
003							
004							
005							
006							
007							
008							
009							
010							
Analyst							
Date							

Total Composite Weight (g) _____

Notes: *Define tissues used in edible sample; indicate whether fatty tissues, liver, heart, eggs, or other tissues are being analyzed individually or with muscle tissue as part of the edible sample.*

Figure 7-5. Sample processing record for a contaminant monitoring program—individual turtle samples.

7.2.3.3 Removal of Edible Tissues—

Edible portions of a turtle should consist only of those tissues that the population of concern might reasonably be expected to eat. Edible tissues should be clearly defined in site-specific sample processing protocols. A brief description of the edible portions used should also be provided on the sample processing record. General procedures for removing edible tissues from a turtle are illustrated in Appendix K.

Resection should be conducted only by or under the supervision of an experienced fisheries biologist. If gloves are worn, they should be talc- or dust-free and of noncontaminating materials. Prior to resection, hands should be washed with soap and rinsed thoroughly in tap water, followed by distilled water (U.S. EPA, 1991d). Specimens should come into contact with noncontaminating surfaces only. Turtles should be resected on glass or PTFE cutting boards that are cleaned properly between each turtle or on cutting boards covered with heavy duty aluminum foil that is changed between each turtle (Puget Sound Estuary Program, 1990d, 1990e). A turtle is resected by laying it flat on its back and removing the plastron by severing the two bony ridges between the forelimbs and hindlimbs. Care must be taken to avoid contaminating edible tissues with material released from the inadvertent puncture of internal organs.

Ideally, turtles should be resected while ice crystals are still present in the muscle tissue. Thawing of frozen turtles should be kept to a minimum during tissue removal to avoid loss of liquids. A turtle should be thawed only to the point where it becomes possible to make an incision into the flesh (U.S. EPA, 1991d).

Clean, high-quality stainless steel, ceramic, or titanium utensils should be used to remove the muscle tissue and, depending on dietary or culinary practices of the population of concern, some of the other edible tissues from each turtle. The general procedure recommended for resecting turtles is illustrated in Figure 7-6.

Skin on the forelimbs, hindlimbs, neck, and tail should be removed. Claws should be removed from the forelimbs and hindlimbs. Bones still present in the muscle tissue after resection should be removed carefully (U.S. EPA, 1991d) and may be used in age determination (see Section 7.2.3.5).

To control contamination, separate sets of utensils and cutting boards should be used for skinning muscle tissue and resecting other internal tissues from the turtle (e.g., heart, liver, fatty deposits, and eggs). These other tissue types are recommended for inclusion with the muscle tissue as part of the edible tissue sample because it is believed that they are most representative of the edible portions of turtles that are prepared and consumed by sport anglers and subsistence fishers. Alternatively, states may choose to analyze some of these other lipophilic tissues separately. It is the responsibility of each program manager, in consultation with state fisheries experts, to select the tissue sample

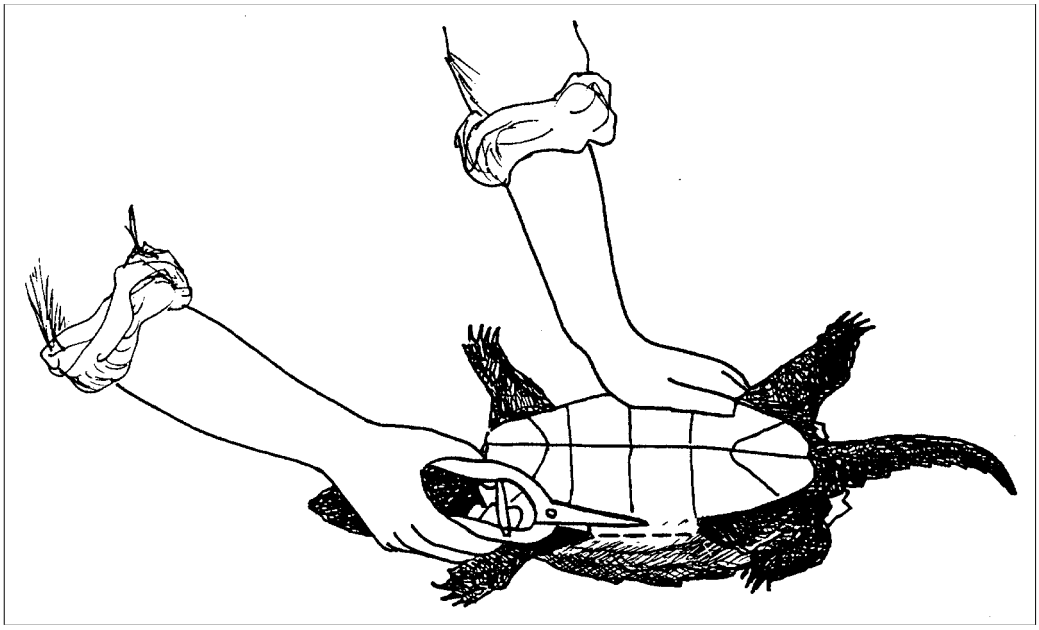


Figure 7-6. Illustration of basic turtle resection procedure.

type most appropriate for each target species based on the dietary customs of local populations of concern.

The edible turtle tissues should be weighed and the weight recorded to the nearest gram on the sample processing record. If the state elects to analyze the heart, liver, fatty deposits, or eggs separately from the muscle tissue, these other tissues should be weighed separately and the weights recorded to the nearest gram in the sample processing record.

If the tissues are to be homogenized immediately, they should be placed in a properly cleaned glass or PTFE homogenization container. If samples are to be analyzed for metals only, plastic homogenization containers may be used. To facilitate homogenization, it may be necessary or desirable to chop each of the large pieces of muscle tissue into smaller pieces using a titanium or stainless steel knife prior to placement in the homogenization container.

If the tissues are to be homogenized later, they should be wrapped in heavy duty aluminum foil and labeled with the sample identification number, the sample type (e.g., "M" for muscle, "E" for eggs, or "FD" for fatty deposits), the weight (g), and the date of resection. The individual muscle tissue samples from each turtle should be packaged together and given an individual sample identification number. The date of resection should be recorded and the sample should be stored at $\leq$-20°C until homogenization. **Note**: State staff may determine that the most appropriate sample type is muscle tissue only, with internal organ tissues analyzed separately (liver, heart, fatty deposits, or eggs). Alternatively, state staff may determine that the most appropriate sample type is muscle tissue with several other internal organs included as the turtle tissue sample. This latter

sample type typically will provide a more conservative estimate of contaminant residues, particularly with respect to lipophilic target analytes (e.g., PCBs, dioxins, and organochlorine pesticides).

7.2.3.4 Sex Determination (Optional)—

Turtle sex should be determined during resection if it has not already been determined in the field. Once the plastron is removed, the ovaries or testes can be observed posterior and dorsal to the liver. Each ovary is a large egg-filled sac containing yellow spherical eggs in various stages of development (Ashley, 1962) (see Appendix K). Each testes is a spherical organ, yellowish in color, attached to the ventral side of each kidney. The sex of each turtle should be verified and recorded on the sample processing form.

7.2.3.5 Age Determination (Optional)—

Age provides a good indication of the duration of exposure to pollutants (Versar, 1982). Several methods have been developed for estimating the age of turtles (Castanet, 1994; Frazer et al., 1993; Gibbons, 1976). Two methods are appropriate for use in contaminant monitoring programs where small numbers of animals of a particular species are to be collected and where the animals must be sacrificed for tissue residue analysis. These methods include (1) the use of external annuli (scute growth marks) on the plastron and (2) the use of growth rings on the bones.

The surface of epidermal keratinous scutes on the plastron of turtle shells develops successive persistent grooves or growth lines during periods of slow or arrested growth (Zangerl, 1969). Because these growth rings are fairly obvious, they have been used extensively for estimating age in various turtle species (Cagle, 1946, 1948, 1950; Gibbons, 1968; Legler, 1960; Sexton, 1959). This technique is particularly useful for younger turtles where the major growth rings are more definitive and clear cut than in older individuals (Gibbons, 1976). However, a useful extension of the external annuli method is presented by Sexton (1959) showing that age estimates can be made for adults on which all annuli are not visible. This method involves visually examining the plastron of the turtle during the resection or tagging the plastron with the sample identification number of the turtle and retaining it for later analysis.

The use of bone rings is the second method that may be used to estimate age in turtles (Enlow and Brown, 1969; Peabody, 1961). Unlike the previous visual method, this method requires that the bones of the turtle be removed during resection and retained for later analysis. The growth rings appear at the surface or inside primary compacta of bone tissues. There are two primary methods for observing growth marks: either directly at the surface of the bone as in flat bones using transmitted or reflected light or inside the long bones using thin sections (Castanet, 1994; Dobie, 1971; Galbraith and Brooks, 1987; Hammer, 1969; Gibbons, 1976; Mattox, 1935; Peabody, 1961). The methods of preparation of

whole bones and histological sections of fresh material for growth mark determinations are now routinely performed. Details of these methods can be found in Castanet (1974 and 1987), Castanet et al. (1993), and Zug et al. (1986). State staff interested in using either of these methods for age determination of turtles should read the review articles by Castanet (1994) and Gibbons (1976) for discussions of the advantages and disadvantages of each method, and the associated literature cited in these articles on turtle species of particular interest within their jurisdictions.

7.2.3.6 Preparation of Individual Homogenates—

To ensure even distribution of contaminants throughout tissue samples and to facilitate extraction and digestion of samples, the edible tissues from individual turtles must be ground and homogenized prior to analysis. The various tissues from an individual turtle may be ground and homogenized separately, or combined, depending on the sampling program's definition of edible tissues.

Turtle tissues should be ground and homogenized using an automatic grinder or high-speed blender or homogenizer. Large pieces of muscle or organ tissue (e.g., liver or fatty deposits) may be cut into 2.5-cm cubes with high-quality stainless steel or titanium knives or with a food service band saw prior to homogenization. Parts of the blender or homogenizer used to grind the tissue (i.e., blades, probes) should be made of tantalum or titanium rather than stainless steel. Stainless steel blades and/or probes have been found to be a potential source of nickel and chromium contamination (due to abrasion at high speeds) and should be avoided.

Grinding and homogenization of tissue is easier when it is partially frozen (Stober, 1991). Chilling the grinder/blender briefly with a few chips of dry ice will also help keep the tissue from sticking to it (Smith, 1985).

The tissue sample should be ground until it appears to be homogeneous. The ground sample should then be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed together. The grinding, quartering, and hand-mixing steps should be repeated at least two more times. If chunks of tissue are present at this point, the grinding and homogenization should be repeated. No chunks of tissue should remain because these may not be extracted or digested efficiently and could bias the analytical results. This is particularly true when lipophilic tissues (e.g., fatty deposits, liver, or eggs) are not completely homogenized throughout the sample. Portions of the tissue sample that retain unhomogenized portions of tissues may exhibit higher or lower residues of target analytes than properly homogenized samples.

If the sample is to be analyzed for metals only, the ground tissue may be mixed by hand in a polyethylene bag (Stober, 1991). The preparation of each individual homogenate should be noted (marked with a check) on the sample processing record. At this time, individual homogenates may be frozen separately and stored at $\leq$-20 °C (see Table 7-1).

The remainder of each individual homogenate should be archived at ≤-20 °C with the designation "Archive" and the expiration date recorded on the sample label. The location of the archived samples should be indicated on the sample processing record under "Notes."

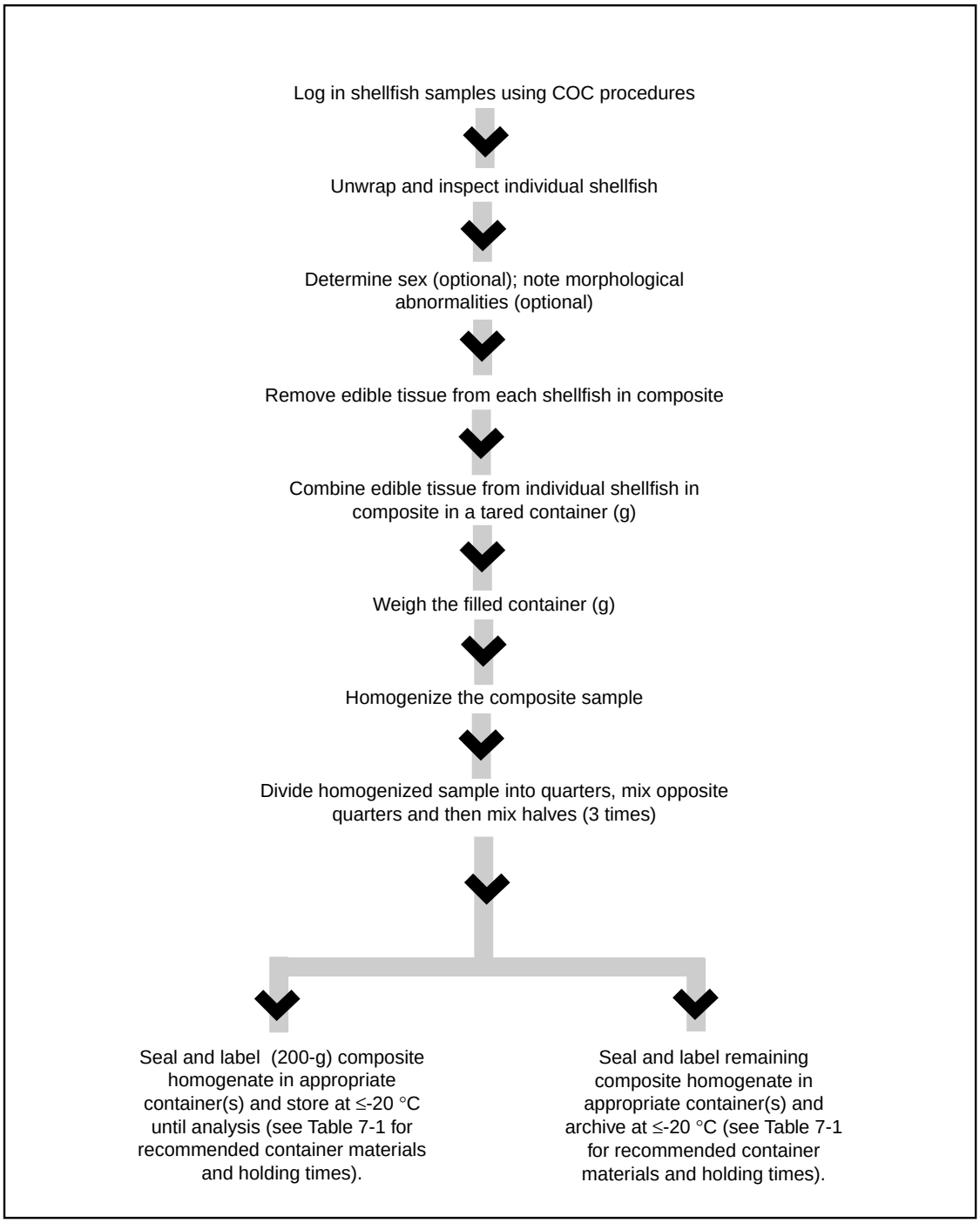
It is essential that the weight of individual homogenate samples is of adequate size to perform all necessary analyses. The recommended sample size of 200 g for screening studies is intended to provide sufficient sample material to (1) analyze for all recommended target analytes (see Table 4-1) at appropriate detection limits; (2) meet minimum QC requirements for the analyses of laboratory duplicate, matrix spike, and matrix spike duplicate samples (see Sections 8.3.3.4 and 8.3.3.5); and (3) allow for reanalysis if the QC control limits are not met or if the sample is lost. However, sample size requirements may vary among laboratories and the analytical methods used. Each program manager must consult with the analytical laboratory supervisor to determine the actual weights of homogenates required to analyze for all selected target analytes at appropriate detection limits. The total sample weight required for intensive studies may be less than that for screening studies if the number of target analytes is reduced significantly.

7.2.4 Processing Shellfish Samples

Laboratory processing of shellfish to prepare edible tissue composite homogenates for analysis (diagrammed in Figure 7-7) involves

- Inspecting individual shellfish
- Determining the sex of each shellfish (optional)
- Examining each shellfish for morphological abnormalities (optional)
- Removing the edible parts from each shellfish in the composite sample (3 to 50 individuals, depending upon the species)
- Combining the edible parts in an appropriate noncontaminating container
- Weighing the composite sample
- Homogenizing the composite sample
- Preparing aliquots of the composite homogenate for analysis
- Distributing frozen aliquots to one or more analytical laboratories.

Sample aliquotting and shipping are discussed in Section 7.3; all other processing steps are discussed in this section. Shellfish samples should be processed following the general guidelines in Section 7.2.1 to avoid contamination. In



COC = Chain of custody.

Figure 7-7. Preparation of shellfish edible tissue composite homogenate samples.

particular, it is recommended that separate composite homogenates be prepared for the analysis of metals and organics if resources allow. A sample processing record for shellfish edible tissue composite samples is shown in Figure 7-8.

Shellfish samples should be shipped or brought to the sample processing laboratory either on wet or blue ice (if next-day delivery is assured) or on dry ice (see Section 6.3.3). Shellfish samples arriving on wet ice or blue ice should have edible tissue removed and should be frozen to ≤-20°C within 48 hours after collection. Shellfish samples that arrive frozen (i.e., on dry ice) at the processing laboratory should be placed in a ≤-20°C freezer for storage until edible tissue is removed.

7.2.4.1 Sample Inspection—

Individual shellfish should be unwrapped and inspected carefully to ensure that they have not been compromised in any way (i.e., not properly preserved during shipment). Any specimen deemed unsuitable for further processing and analysis should be discarded and identified on the sample processing record.

7.2.4.2 Sex Determination (Optional)—

The determination of sex in shellfish species is impractical if large numbers of individuals of the target species are required for each composite sample.

For bivalves, determination of sex is a time-consuming procedure that must be performed after shucking but prior to removal of the edible tissues. Once the bivalve is shucked, a small amount of gonadal material can be removed using a Pasteur pipette. The gonadal tissue must then be examined under a microscope to identify egg or sperm cells.

For crustaceans, sex also should be determined before removal of the edible tissues. For many species, sex determination can be accomplished by visual inspection. Sexual dimorphism is particularly striking in many species of decapods. In the blue crab, *Callinectes sapidus*, the female has a broad abdomen suited for retaining the maturing egg mass or sponge, while the abdomen of the male is greatly reduced in width. For shrimp, lobsters, and crayfish, sexual variations in the structure of one or more pair of pleopods are common. States interested in determining the sex of shellfish should consult taxonomic keys for specific information on each target species.

7.2.4.3 Assessment of Morphological Abnormalities (Optional)—

Assessment of gross morphological abnormalities in shellfish is optional. This assessment may be conducted in the field (see Section 6.3.1.5) or during initial inspection at the processing laboratory prior to removal of the edible tissues. States interested in documenting morphological abnormalities should consult Sinderman and Rosenfield (1967), Rosen (1970), and Murchelano (1982) for

Sample Processing Record for Shellfish Contaminant Monitoring Program — Edible Tissue Composites

Project Number: _____ Sampling Date and Time: _____

STUDY PHASE: Screening Study ☐; Intensive Study: Phase I ☐ Phase II ☐

SITE LOCATION

Site Name/Number: _____

County/Parish: _____ Lat./Long.: _____

Waterbody Name/Segment Number: _____ Waterbody Type: _____

SHELLFISH COLLECTED

Species Name: _____

Description of Edible Tissue _____

Composite Sample #: _____ Number of Individuals: _____

Shellfish #	Included in Composite (✓)	Shellfish #	Included in Composite (✓)	Shellfish #	Included in Composite (✓)
001	_____	018	_____	035	_____
002	_____	019	_____	036	_____
003	_____	020	_____	037	_____
004	_____	021	_____	038	_____
005	_____	022	_____	039	_____
006	_____	023	_____	040	_____
007	_____	024	_____	041	_____
008	_____	025	_____	042	_____
009	_____	026	_____	043	_____
010	_____	027	_____	044	_____
011	_____	028	_____	045	_____
012	_____	029	_____	046	_____
013	_____	030	_____	047	_____
014	_____	031	_____	048	_____
015	_____	032	_____	049	_____
016	_____	033	_____	050	_____
017	_____	034	_____		

Preparation of Composite:

Weight of container + shellfish _____ g

Weight of container (tare weight) _____ g

Total weight of composite _____ g ÷ _____ (# of specimens) = _____ (Average weight of specimen)

Notes: _____

Analyst _____ Date _____

Figure 7-8. Sample processing record for shellfish contaminant monitoring program—edible tissue composites.

detailed information on various pathological conditions in shellfish and review recommended protocols for pathology studies used in the Puget Sound Estuary Program (1990c).

7.2.4.4 Removal of Edible Tissue—

Edible portions of shellfish should consist only of those tissues that the population of concern might reasonably be expected to eat. Edible tissues should be clearly defined in site-specific sample processing protocols. A brief description of the edible portions used should also be provided on the sample processing record. General procedures for removing edible tissues from a variety of shellfish are illustrated in Appendix L.

Thawing of frozen shellfish samples should be kept to a minimum during tissue removal to avoid loss of liquids. Shellfish should be rinsed well with organics- and metal-free water prior to tissue removal to remove any loose external debris.

Bivalve molluscs (oysters, clams, mussels, and scallops) typically are prepared by severing the adductor muscle, prying open the shell, and removing the soft tissue. The soft tissue includes viscera, meat, and body fluids (Smith, 1985). Byssal threads from mussels should be removed with a knife before shucking and should not be included in the composite sample.

Edible tissue for **crabs** typically includes all leg and claw meat, back shell meat, and body cavity meat. Internal organs generally are removed. Inclusion of the hepatopancreas should be determined by the eating habits of the local population or subpopulations of concern. If the crab is soft-shelled, the entire crab should be used in the sample. Hard- and soft-shelled crabs must not be combined in the same composite (Smith, 1985).

Typically, **shrimp** and **crayfish** are prepared by removing the cephalothorax and then removing the tail meat from the shell. Only the tail meat with the section of intestine passing through the tail muscle is retained for analysis (Smith, 1985). Edible tissue for **lobsters** typically includes the tail and claw meat. If the tomalley (hepatopancreas) and gonads or ovaries are consumed by local populations of concern, these parts should also be removed and analyzed separately (Duston et al., 1990).

7.2.4.5 Sample Weighing—

Edible tissue from all shellfish in a composite sample (3 to 50 individuals) should be placed in an appropriate preweighed and labeled noncontaminating container. The weight of the empty container (tare weight) should be recorded to the nearest gram on the sample processing record. All fluids accumulated during removal of edible tissue should be retained as part of the sample. As the edible portion of each shellfish is placed in the container, it should be noted on the sample processing record. When the edible tissue has been removed from all shellfish

in the composite, the container should be reweighed and the weight recorded to the nearest gram on the sample processing record. The total composite weight should be approximately 200 g for screening studies. If the number of target analytes is significantly reduced in intensive studies, a smaller composite homogenate sample may suffice (see Section 7.2.2.9). At this point, the composite sample may be processed for analysis or frozen and stored at ≤-20°C (see Table 7-1).

7.2.4.6 Preparation of Composite Homogenates—

Composite samples of the edible portions of shellfish should be homogenized in a grinder, blender, or homogenizer that has been cooled briefly with dry ice (Smith, 1985). For metals analysis, tissue may be homogenized in 4-oz polyethylene jars (California Department of Fish and Game, 1990) using a Polytron equipped with a titanium generator. If the tissue is to be analyzed for organics only, or if chromium and nickel contamination are not of concern, a commercial food processor with stainless steel blades and glass container may be used. The composite should be homogenized to a paste-like consistency. Larger samples may be cut into 2.5-cm cubes with high-quality stainless steel or titanium knives before grinding. If samples were frozen after dissection, they can be cut without thawing with either a knife-and-mallet or a clean bandsaw. The ground samples should be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed together. The quartering and mixing should be repeated at least two more times until a homogeneous sample is obtained. No chunks should remain in the sample because these may not be extracted or digested efficiently. At this point, the composite homogenates may be processed for analysis or frozen and stored at ≤-20 °C (see Table 7-1).

7.3 SAMPLE DISTRIBUTION

The sample processing laboratory should prepare aliquots of the composite homogenates for analysis, distribute the aliquots to the appropriate laboratory (or laboratories), and archive the remainder of each composite homogenate.

7.3.1 Preparing Sample Aliquots

Note: Because lipid material tends to migrate during freezing, frozen composite homogenates must be thawed and rehomogenized before aliquots are prepared (U.S. EPA, 1991d). Samples may be thawed overnight in an insulated cooler or refrigerator and then homogenized. Recommended aliquot weights and appropriate containers for different types of analyses are shown in Table 7-3. The actual sample size required will depend on the analytical method used and the laboratory performing the analysis. Therefore, the exact sample size required for each type of analysis should be determined in consultation with the analytical laboratory supervisor.

Table 7-3. Recommended Sample Aliquot Weights and Containers for Various Analyses

Analysis	Aliquot weight (g)	Shipping/storage container
Metals	1-5	Polystyrene, borosilicate glass, or PTFE jar with PTFE-lined lid
Organics	20-50	Glass or PTFE jar with PTFE-lined lid
Dioxins/furans	20-50	Glass or PTFE jar with PTFE-lined lid

PTFE = Polytetrafluoroethylene (Teflon).

The exact quantity of tissue required for each digestion or extraction and analysis should be weighed and placed in an appropriate container that has been labeled with the aliquot identification number, sample weight (to the nearest 0.1 g), and the date aliquots were prepared (Stober, 1991). The analytical laboratory can then recover the entire sample, including any liquid from thawing, by rinsing the container directly into the digestion or extraction vessel with the appropriate solvent. It is also the responsibility of the processing laboratory to provide a sufficient number of aliquots for laboratory duplicates, matrix spikes, and matrix spike duplicates so that the QC requirements of the program can be met (see Sections 8.3.3.4 and 8.3.3.5), and to provide extra aliquots to allow for reanalysis if the sample is lost or if QC control limits are not met.

It is essential that accurate records be maintained when aliquots are prepared for analysis. Use of a carefully designed form is recommended to ensure that all the necessary information is recorded. An example of a sample aliquot record is shown in Figure 7-9. The composite sample identification number should be assigned to the composite sample at the time of collection (see Section 6.2.3.1) and carried through sample processing (plus "F1," "F2," or "C" if the composite homogenate is comprised of individual or combined fillets). The aliquot identification number should indicate the analyte class (e.g., MT for metals, OR for organics, DX for dioxins) and the sample type (e.g., R for routine sample; RS or a routine sample that is split for analysis by a second laboratory; MS1 and MS2 for sample pairs, one of which will be prepared as a matrix spike). For example, the aliquot identification number may be WWWWW-XX-YY-ZZZ, where WWWWW is a 5-digit sample composite identification number, XX indicates individual (F1 or F2) or combined (C) fillets, YY is the analyte code, and ZZZ is the sample type.

Blind laboratory duplicates should be introduced by preparing two separate aliquots of the same composite homogenate and labeling one aliquot with a "dummy" composite sample identification. However, the analyst who prepares the laboratory duplicates must be careful to assign a "dummy" identification number that has not been used for an actual sample and to indicate clearly on the

Fish and Shellfish Monitoring Program
Sample Aliquot Record

Aliquot prepared by _____ Date _____ Time _____
(name)

Comments _____

Samples from:

Project No. _____ Site # _____ ☐ Screening study Intensive study ☐ Phase I ☐ Phase II

	Analyte Code ___ ___		Analyte Code ___ ___		Analyte Code ___ ___	
Composite Sample ID	**Aliquot ID**	**Aliquot Weight (g)**	**Aliquot ID**	**Aliquot Weight (g)**	**Aliquot ID**	**Aliquot Weight (g)**
Archive Location:	**Analyze for:** _____ **Ship to:** _____		**Analyze for:** _____ **Ship to:** _____		**Analyze for:** _____ **Ship to:** _____	

Page ____ of ____

Figure 7-9. Example of a fish and shellfish monitoring program sample aliquot record.

processing records that the samples are blind laboratory duplicates. The analytical laboratory should not receive this information.

When the appropriate number of aliquots of a composite sample have been prepared for all analyses to be performed on that sample, the remainder of the composite sample should be labeled with "ARCHIVE" and the expiration date and placed in a secure location at ≤-20 °C in the sample processing laboratory. The location of the archived samples should be indicated on the sample aliquot record. Unless analyses are to be performed immediately by the sample processing laboratory, aliquots for sample analysis should be frozen at ≤-20 °C before they are transferred or shipped to the appropriate analytical laboratory.

7.3.2 Sample Transfer

The frozen aliquots should be transferred on dry ice to the analytical laboratory (or laboratories) accompanied by a sample transfer record such as the one shown in Figure 7-10. Further details on federal regulations for shipping biological specimens in dry ice are given in Section 6.3.3.2. The sample transfer record may include a section that serves as the analytical laboratory COC record. The COC record must be signed each time the samples change hands for preparation and analysis.

Date _____ _____ _____ Time ___:___ (24-h clock)
YYYY MM DD

Released by: _____
(name)

At: _____
(location)

Shipment Method _____
Shipment Destination _____

Date _____ _____ _____ Time ___:___ (24-h clock)
YYYY MM DD

Released by: _____
(name)

At: _____
(location)

Comments _____

_____

Study Type: ☐ Screening — Analyze for: ☐ Trace metals ☐ Organics ☐ Lipid

Intensive Phase 1 ☐ Phase II ☐ — Analyze for *(specify)* _____

Sample IDs:

Laboratory Chain of Custody

Relinquished by	Received by	Purpose	Location

Figure 7-10. Example of a fish and shellfish monitoring program sample transfer record.

APPENDIX J

DEQ FISH KILL PATHOLOGICAL EXAMINATION REPORT

VIRGINIA DEPARTMENT OF ENVIRONMENTAL QUALITY
FISH KILL PATHOLOGICAL EXAMINATION REPORT

POLLUTION COMPLAINT NO._____ COUNTY_____
REGION_____
SITE DESCRIPTION_____
_____
SUBMITTED BY_____
OFFICE/DIV_____
PHONE_____
DATE SHIPPED_____ SHIPMENT METHOD_____
DATE RECEIVED_____
TYPE OF SAMPLES SUBMITTED: Fish___ Water___ Other___

Collection Data

COLLECTED BY_____
OFFICE/DIV_____
PHONE_____
FISH SIZE (Inches) Fry___ 1-6___ 7-12___ 13-18___ >18___
FISH
SPECIES_____
SAMPLE CAPTURED: Alive___ Dead___ Sick___ Feeding___ Unknown___
Hook___ Cast Net___ Seine___ Hand___ Dip Net___
Shot___ Unknown___

NUMBER OF FISH IN SAMPLE_____
SAMPLE CONDITION: Alive___ Dead___ Iced___ Frozen___ Preserved
_____

Pond/Stream Data

DISSOLVED OXYGEN: A.M._____ P.M._____ PH_____ NO_2_____ CO_2_____
HARDNESS_____ ALKALINITY_____ TEMPERATURE_____ SALINITY_____
AMMONIA_____ CONDUCTIVITY_____
OTHER_____
POND ACREAGE_____ STREAM FLOW_____ COLOR_____
OTHER_____
_____
WEATHER: Clear___ Cloudy___ Calm___ Windy___ Rain___ Temp_____

Mortality Data

TOTAL NUMBER DEAD _____ DURATION OF KILL _____

REMARKS _____

_____

_____

_____

_____

_____

_____

_____

APPENDIX K

DEQ SAMPLING TECHNIQUES FOR CHLOROPHYLL AND ALGAE

Sampling Techniques for Chlorophyll

1) Collect chlorophyll samples 1 ft below the surface. Phytoplankton populations are concentrated near the surface and depth composites samples will generally yield too little algal biomass for interpretable results.

2) Chlorophyll samples must be processed by the contract laboratory within 24 hours of collection. Chlorophyll samples are routinely submitted by all regional offices via CEDS/WQM to DCLS as part of the ambient monitoring program. There are two sample collection methods based on whether the samples are field filtered or lab filtered.

   The Group code CHLOR is a lab filtered sample and requires a single sample collected in a 2 liter HDP Amber Bottle preserved at 4 °C with Magnesium carbonate.

   The Group code FCHLR is a field filtered sample which requires a known volume of sample (typically 300 ml) to be filtered through a single 55 mm GF/F Glass fiber filter. The filter is frozen and stored in the dark. **Field filtered are preferred if the filter apparatus is available at the time of sample collection.**

   Multiple collection methods are available to serve different program requirements. These are outlined in CEDS. Charlie Morgan in the DEQ office of Water Quality Programs should be contacted for lab liaison assistance and updates regarding sample procedures.

Sampling Techniques for Algae

Collection

Algae samples for identification are usually collected near the surface in a 1 quart cubitainer or similar container of known volume.

Storage and Preservation

If refrigerated or kept on ice soon after collecting most algae can be kept alive for short periods (a day or two). For long term storage samples should be preserved in a liquid (listed below). Even with ideal preservation, examination of fresh material is sometimes essential for an accurate identification. For example, motile algae should be examined while their flagella and other structures are intact.

Liquid Preservation

A. Formalin

Preserve approximately 1 quart of sample with 40 ml of 2% Formalin and 1 ml of copper sulfate solution (see below). **Formalin is a diluted solution of formaldehyde which is thought to be carcinogenic. Contact with eyes, skin, and air passages should be avoided.** Algae samples can be stored in dilute formalin for a number of years.

B. Lugol's Solution

Add 3-4 ml of Lugol's solution (see below) to a one-quart cubitainer.

Lugol's solution is commonly used for short-term storage (i.e. few months to a year).

Collection Information

The collectors name, date, sample location, and preservation method should be included on the sample tag. Include other information if needed to help identify the algae (e.g. sample number, color and texture, size of algae, floating or submerged, whether the water is saline, brackish, or fresh, and etc.).

Sample Identification

Algae samples may be identified by DEQ RO Staff or outside academic institutions. Recently, Dr. Harold Marshall (phone 757 683-4204, e-mail hmarshal@odu.edu) at Old Dominion University has performed many algae identifications for the Tidewater and Piedmont regional offices. If samples are submitted to an outside institution, a contract and purchase order may be required.

Reagents

The following reagents may be used for chlorophyll or algae preservation.

Magnesium Carbonate
1 gram $MgCO_3$ added to 100 ml distilled water.

Copper Sulfate Solution (Saturated)
21 Grams $CuSO_4$ crystals added to 100 ml distilled water. Stir to dissolve.

2% Formalin Solution:
5 ml 40% aqueous formaldehyde solution (formalin). Formalin is added to 95 ml distilled water. **Formalin is a diluted solution of formaldehyde which is thought to be carcinogenic. Contact with eyes, skin, and air passages should be avoided.**

Lugol Solution

Dissolve 20 g potassium iodide and 10 g iodine crystals in 200 ml distilled water containing 20 ml glacial acetic acid. Preserve sample with 3-4 ml per quart cubitainer.

APPENDIX L

DEQ Chain of Custody Policy and Procedures Guidance Memo No. 00-2016.

MEMORANDUM

DEPARTMENT OF ENVIRONMENTAL QUALITY

SUBJECT: Guidance Memo No. 00-2016
Chain of Custody Policy and Procedures

TO: Regional Directors

FROM: John M. Daniel, Jr.
Air Program Coordination Director

Larry G. Lawson, P.E.
Water Program Coordination Director

Karen Jackson Sismour
Waste Program Coordination Director

DATE: October 23, 2000

COPIES: Dennis H. Treacy, David Johnson, David Paylor, Ralph J. Mayer, Compliance, Enforcement and Monitoring Senior Management Team, Remediation Managers Senior Management Team, Permit and Planning Managers Senior Management Team, Matthew Dullaghan

POLICY STATEMENT

It shall be the policy of the Department of Environmental Quality ("DEQ") that all samples will be collected using the following chain of custody procedures to ensure the integrity of samples so they can be used as admissible evidence to enforce the Commonwealth's environmental laws and regulations.

Samples taken in all cases involving a facility, permit, certificate, order or potential violation of a regulation or law shall follow chain of custody procedures. Samples taken for ambient environmental monitoring do not require chain of custody procedures. Variances to these procedures may be granted for those samples taken for special studies, on a case by case basis, only by the joint written agreement of the

appropriate Compliance and Enforcement Managers ("CEM") and Media Division Director.

The Division of Consolidated Laboratory Services ("DCLS") shall be used for the analysis of all samples except another laboratory may be used upon prior written approval of the appropriate CEM and Media Division Director. The chain of custody procedures used by the alternate laboratory shall be reviewed by the appropriate CEM and Media Division Director to ascertain that it meets DEQ's chain of custody policy and procedure requirements. Only following such review shall authorization be given to use an alternate laboratory.

Samples taken by DEQ's VOC Sampling and Analysis Program shall be sent to the City of Philadelphia Department of Public Health Air Management Services Laboratory, Philadelphia, Pennsylvania or DCLS if they are capable of performing the required testing.

I. PURPOSE AND SCOPE

The following procedures are used by all DEQ employees to ensure accountability for and documentation of sample integrity from the time all samples are collected until receipt by the receiving laboratory. These procedures are intended to document each stage of the sample's life cycle (*i.e.*, collection, transport, and delivery). Only the DEQ Director or his designee can authorize exceptions to this policy.

II. DEFINITIONS

2.1. **Custody-Physical Possession or Control.** A sample is "under custody" if it is in the possession or under the control of the Sample Custodian so as to prevent tampering or alteration of its characteristics. A sample is under custody if:

2.1.1. It is in your possession or in your view after assuming possession,

2.1.2. It was in your possession and then you locked or sealed the sample in a manner to prevent tampering, or

2.1.3. It is in a secured area. A secured area should have restricted access, locked storage facilities and be locked at all times when not attended (*e.g.,* inside a locked cooler, locked vehicle, or locked storage area).

2.2 **Sample.** A portion of an environmental or source matrix that is collected and used to characterize that matrix.

2.3 **Sample Custodian.** The person possessing the sample.

2.4 **Chain of Custody.** A process whereby a sample is maintained under physical possession or control. Chain of custody procedures are one piece of a large quality assurance program to assure data and conclusions are defensible in a legal or regulatory situation.

2.5 **Sample Submission Forms.** DEQ or the laboratory provides the forms used to record sample collection information, test(s) requested and result reporting instructions.

2.6 **Sample Set.** Collection of samples collected during one sampling event.

III. SAMPLE COLLECTION

3.1 **Sampling.** Samples are routinely collected by DEQ employees using standard collection procedures defined by media specific Standard Operating Procedures ("SOPs").

3.2 **Custody Assignment.** The sampler shall ensure proper collection, preservation and labeling of the sample. The sampler will also initiate the chain of custody documentation process, prepare sample submission information, and prepare and store samples for transport to the laboratory. Since as few people as possible should handle samples, the sampler is responsible for the initial custody of the sample.

3.3 **Consultation.** Information regarding the collection, preservation, transport, testing and sample custody may be obtained around-the-clock, seven days a week, from DCLS even when not using DCLS.

3.4 **Sample Kits.** Collection kits with containers, preservatives and sampling instructions may be provided by DCLS. DCLS is available for consultation purposes if there are questions about sample collection and preservation regardless of the laboratory used.

3.5 **Sample Identification.** To ensure samples are traceable, samples shall be clearly labeled immediately upon collection. Labeling information may vary by media SOPs, but labels must be written legibly, using a ballpoint (indelible) pen, unique for the sample/case and firmly fixed to the sample. The sample label shall contain the unique sample number or identification, sample type, name of sampler, and date and time of collection.

3.6 **Sample Preservation.** Sample preservation instructions are provided in sample kit collection instructions and in agency SOPs. Sample preservation actions shall be documented in field logs, on chain of custody forms, on lab sheets, and on sample labels.

IV. SAMPLING DOCUMENTATION

4.1 **Field Logbooks**. In any sampling effort, there are field information and measurements that need to be recorded. This information shall be retained in a sampler's field log. Examples of information entered include: purpose of sampling, producer, type of sample, address, sample composition, description of sampling point, sampling method, date and time of collection, sample identification number, field data, and preservative. This record may be considered evidence and part of the larger aspect of data defensibility. Logbooks shall be kept in a safe place.

4.2 **Custody Forms.** Agency chain of custody forms shall be used when submitting a sample for analysis. Attachment 1 is the General DEQ Chain of Custody Form and Attachment 2 is the DEQ VOC Chain of Custody Form. Chain of custody forms shall be completed by the sampler at the time of sample collection and shall be submitted with each sample set. The

completed form shall be signed by the sampler and dated (chain of custody block) and placed in a waterproof carrier (*e.g.*, zip-lock bag) if it is a water sample. The form shall be packaged with the sample for transport to the laboratory. The original form shall be returned to the sampler along with the results of the tests that are performed. The original chain of custody form and laboratory results is then filed in the appropriate case file.

4.3 **Sample Submittal and Test Request Forms.** With each sample submitted to the laboratory for analysis, the sampler shall include the following information:

- 4.3.1 The analytical request
- 4.3.2 Sample identification
- 4.3.3 Field data
- 4.3.4 The chain of custody form
- 4.3.5 Copies of applicable documents (*e.g.*, MSDS, sample formulations (if applicable))
- 4.3.6 Any other information required to meet laboratory testing and reporting requirements
- 4.3.7 This information may be submitted by:
  - 4.3.7.1 An agency lab sheet that is completed following established agency SOPs and packaged and shipped with the chain of custody form with the sample
  - 4.3.7.2 An electronic file generated by the agency's Comprehensive Environmental Database System ("CEDS")

V. SAMPLE PACKAGING, TRANSPORT AND TRANSFER OF CUSTODY

5.1 **Sample Packaging.** The correct preparation and preservation of samples for transport are critical to ensure sample integrity.

5.1.1 The sampler should contact the laboratory if unsure of any aspect of sample collection, preservation, packaging and transport.

5.1.2 Samples must be labeled, tightly sealed in the appropriate container and double bagged in plastic where applicable.

5.1.3 Evidence tape shall be used to seal sample containers. The tape shall be placed across the container in a manner that tampering attempts would be obvious. In all cases, the initials/mark of the person sealing the evidence must be placed on, across or under the seal.

5.1.4 The chain of custody form and other documentation are to be sealed tightly in a plastic zip-lock bag.

5.1.5 Samples and documentation are then placed in an appropriate transport container (*e.g.,* cardboard mailer, stryofoam box, paint can, plastic box or cooler) and padded (*e.g.,* bubble wrap, styrofoam peanuts) as appropriate.

5.1.6 If not immediately delivered to the laboratory by the sampler, containers shall be locked (personal padlock) and sealed (custody seal). When samples are ready for shipment to DCLS lockable transport containers, locks and custody seals provided by DCLS shall be used. DCLS will retain all keys for locks. Once locked and sealed, containers provided by DCLS may be opened only by DCLS staff. Samples that are ready for shipment to laboratories other than DCLS shall be shipped in tamperproof transport containers.

5.1.7 The sampler is responsible for preservation prior to and during transport. If ice is required, it will need to be contained in a way to prevent leakage. Special shipping measures can be briefly described on the chain of custody form and should be described in detail in the field logbook.

5.2 **Sample Transport.** Samples are to be delivered to DCLS or other prior approved laboratory by one of the following means. Regardless of the mode of transport, arrangements are to be coordinated with the laboratory and delivered as soon as possible.

5.2.1 Immediate Delivery by Sample Custodian. Samples are to be delivered to the Sample Records Management section of DCLS or other prior approved laboratory as soon as possible after collection. The sample shall remain in the Sample Custodian's possession or sight at all times.

5.2.2 Routine and Special Courier. DCLS couriers shall be used to deliver legal and regulatory samples to DCLS. Samples will be packaged as described in § 5.1. DCLS provides special lock boxes and coolers for this purpose. Lock boxes and coolers may be delivered to courier pick-up sites for delivery. The DCLS courier routinely picks samples up in the late afternoon and delivers them to the laboratory the next day. A special pick-up can be arranged for any time of night or day. When received by the laboratory custodian, the package will be inspected to ascertain whether tampering occurred and these actions recorded on the chain of custody form.

5.2.3 Common Carriers. Common carrier delivery services (*e.g.*, United Parcel Service, Federal Express) are often the only practical means of delivering samples to laboratories when DCLS cannot provide the required testing and an alternate laboratory must be used. Common carrier delivery services must commit to safeguard all cargo, assuring that it will not be tampered with and will be delivered promptly. Carriers generally specify the type of shipping container to be used when accepting the carrier's services.

5.2.4 Holding Area. Until the courier or the common carrier picks up the container, the container either must be locked or kept in a locked area.

Attachment 1

Shipping Seal Number

_____

VIRGINIA DEPARTMENT OF ENVIRONMENTAL QUALITY
629 EAST MAIN STREET, RICHMOND, VIRGINIA 23240

CHAIN OF CUSTODY RECORD

SAMPLERS: *(Signatures)* _____

Agency (Print or Type): _____

Address: _____

Phone No.: _____ Fax No.: _____

E-Mail: _____

If this is an Emergency (Priority1) sample, please print 24-hour contact name and number. _____

Date Submitted: _____ Agency Case No.: _____

STATION ID	DATE	TIM'E	STATION DESCRIPTION	COMP.	GRAB	TESTS TO BE RUN IN LAB											Observations, Field Tests, Remarks Made in Field

Relinquished by: *(Signature)*	Date/ Time	Received by: *(Signature)*	Relinquished by: *(Signature)*	Date/ Time	Received by: *(Signature)*
Relinquished by: *(Signature)*	Date/ Time	Received by: *(Signature)*	Relinquished by: *(Signature)*	Date/ Time	Received by: *(Signature)*
Relinquished by: *(Signature)*	Date/ Time	Received in Laboratory by: *(Signature)*	Date/ Time	Shipping seal received n place? ____ Seal No. _____ Lab Remarks:	

Original to Accompany Shipment; copy to Sampler; Copy to Transporter

Attachment 2

COATING SAMPLE CHAIN OF CUSTODY RECORD

INSPECTOR, AGENCY AND ADDRESS: BRENDA EGGLESTON 629 EAST MAIN STREET P.O. BOX 10009 RICHMOND, VA 23240	*SOURCE NAME AND PERMIT #:*

SAMPLE ID NUMBER:	*AMS LAB #*
COATING NAME/TYPE:	
PRODUCT CODE:	
LOT OR BATCH #:	

PROCEDURE:	*ANALYSIS REQUIRED:*	*PROPERTIES:*
[ ] EPA METHOD 24	[ ] VOC CONTENT & DENSITY	[ ] BASE INK
[ ] EPA METHOD 24A	[ ] WATER	[ ] MULTI-COMPONENT COATING
	[ ] EXEMPT SOLVENTS	[ ] SAMPLE PRESERVATION ACTIONS TAKEN DURING SHIPMENT

REMARKS:

PERSON RESPONSIBLE FOR SAMPLE (INSPECTOR'S SIGNATURE):	*TIME:*	*DATE:*

PLANT WITNESS (SIGNATURE):

RELINQUISHED BY:	*RECEIVED BY:*	*TIME:*	*DATE:*	*REASON FOR TRANSFER:*
RELINQUISHED BY:	*RECEIVED BY:*	*TIME:*	*DATE:*	*REASON FOR TRANSFER:*
RELINQUISHED BY:	*RECEIVED BY:*	*TIME:*	*DATE:*	*REASON FOR TRANSFER:*
RELINQUISHED BY:	*RECEIVED BY:*	*TIME:*	*DATE:*	*REASON FOR TRANSFER:*
RELINQUISHED BY:	*RECEIVED BY:*	*TIME:*	*DATE:*	*REASON FOR TRANSFER:*
RELINQUISHED BY:	*RECEIVED BY:*	*TIME:*	*DATE:*	*REASON FOR TRANSFER:*
RELINQUISHED BY:	*RECEIVED BY:*	*TIME:*	*DATE:*	*REASON FOR TRANSFER:*

For Facility Inspections, FI program code.

Run Ids will be: XVA9999999	where X = Regional identifier (e.g. S = SWRO), 9999999 = Permit number minus the outfall number. e.g. SVA0001015 Total of 10 characters
Station Ids will be: VA9999999-999	where 9999999 = Permit number to match run ID and -999 = outfall number e.g. VA0001015-001 Total of 13 characters

For Facility Inspections, FI program code where no permit currently exists,

Run Ids will be LHS120XKKKK	where X = Regional identifier, K = alpha/numeric regionally derived ID e.g. LHS120SJMHP Total of 11 characters
Station Ids will be LHS120KKKKZ	where K = alpha/numeric code regionally derived ID to match run ID Z = alpha/numeric code regionally derived for outfall designation e.g. LHS120JMHP1 Total of 11 characters

For Incident Responses, IR program code,

Run Ids will be IRYYYYX9999	YYYY = four digit year X = Regional identifier 9999 = numeric incident code e.g. IR2000S0089 Total of 11 characters
Station Ids will be IRYYYY9999Z	YYYY = four digit year 9999 = numeric incident code to match run ID Z = alpha/numeric code regionally derived for station designation e.g. IR20000089B Total of 11 characters

For UST, LUST, AST Samplings, UI and UR program codes,

Run Ids will be PCYYYYX9999	YYYY = four digit year X = regional identifier 9999 = numeric incident code e.g. PC2000S3422 Total of 11 characters
Station Ids will be PCYYYY9999Z	YYYY = four digit year 9999 = numeric incident code to match run ID Z = alpha/numeric code regionally derived for station designation e.g. PC20003422B Total of 11 characters

ATTACHMENT 1

SAMPLE COLLECTION CONTACT LIST

DIRECTORS:

Dr. James L. Pearson, DGS Deputy Director for Consolidated Laboratories
Office: 804-786-7905; Pager: 1-800-946-4646 then dial 141-6949.

Dr. Thomas York, Analytical Services Bureau Director,DCLS,
Office: 804-786-7905; Pager: 804-418-9922.

CUSTOMER SERVICE & PLANNING, KITS MANAGEMENT SYSTEM:

Thomas Lindfors, Customer Service-Planning Group, DCLS,
Office: 804-786-0307; Pager: 804-418-9924

Grace LaPlace, Program Support Technician, DCLS,
Office: 804-786-3756

CUSTOMER SERVICE LABORATORY SUPPORT, SAMPLE RECEIVING MANAGEMENT:

Glorious Bennett, Customer Service-Lab Support Group, DCLS,
Office: 804-786-2413; Pager: 804-418-9932

COURIER SERVICE:

Thomas Lindfors, Customer Service-Planning Group, DCLS,
Office: 804-786-0307; Pager: 804-418-9924

EMERGENCY SERVICE OFFICER:

Pager: 804-418-9923

Virginia Department of Environmental Quality - [Field Data]

Action Edit Query Block Record Field Window Help

Field Data

Run Id: IR2001N0202 | Collector ID | Coll. Agency | Survey Pgm | Special Study # | Ship Seal No. | Shipped Date | Print Chain Cust | Get MRS Data

Station ID	Date Time	Depth Desc	Depth	% FRB	Wx	Tide	Temp C	pH	DO	Sp Cond	Salinity
IR2001N0202D	09/20/2000 0950	S	3	50			18.8	3.9	1.12		
IR2001N0202E	09/20/2000 0835	S	3	50			16	4.4	1.45		
IR2001N0202F	09/20/2000 0855	S	3	50			16.5	5	1.67		
IR2001N0202G	09/20/2000 1130	S	3	50			18.2	4.6	1.13		
IR2001N0202H	09/20/2000 1200	S	3	50			23.5	5.5	.44		

Samples

Parameter Group Cd	Blanks / Dups	Cont Id	Lab Name	Lab Num	Lab Status	Ana Recv	Priority Code	Lab Rcvd Date	Lab Code	Volume Filtered	Comp. Aliqt. Hours	Num	Comment
NNE16	R	1	DCLS	645008	38		5	10/27/2000					
NUT1	R	2	DCLS	645009	38		5	10/27/2000					
NUT2	R	3	DCLS	645010	38		5	10/27/2000					
FCMPN	R	4	DCLS	645011	38		5	10/27/2000					

RUN ID (CRUISE) SAMPLING - DEQ ACTIVITY

Record: 1/6

Start | Inbox | Exploring D... | Oracle Forms ... | Oracle SQL*P... | Report Builder | Virginia De... | 9:18 AM

Shipping Seal No.
THUTEST

629 EAST MAIN STREET, RICHMOND, VIRGINIA 23240

CHAIN OF CUSTODY RECORD

SAMPLERS: (Signatures) ALT

Agency: Central Office

Address: 629 E. MAIN STREET

RICHMOND VA 23219

Phone No.:

Fax No: 8046984561

E-mail:

Shipped Date: 11/13/2000

RUN ID IR2001N0202

If this is an Emergency (Priority1) sample, please print 24-hour contact name and number

FIRST LAST M 8041234567

TESTS TO BE RUN IN LAB

STATION ID	Coll Date Time	Group Code	FCMP	INUT1	INUT2	NME1	Observations, Field Tests, Remarks Made i
			Typ	Typ	Typ	Typ	
IR2001N0202B	09/20/2000 09:25		R	R	R	R	
IR2001N0202D	09/20/2000 09:50		R	R	R	R	
IR2001N0202E	09/20/2000 08:35		R	R	R	R	
IR2001N0202F	09/20/2000 08:55		R	R	R	R	
IR2001N0202G	09/20/2000 11:30		R	R	R	R	
IR2001N0202H	09/20/2000 12:00		R	R	R	R	

Relinquished by: (Signature)	Date/ Time	Received by: (Signature)	Relinquished by: (Signature)	Date/ Time	Received by: (Signature)
Relinquished by: (Signature)	Date/ Time	Received by: (Signature)	Relinquished by: (Signature)	Date/ Time	Received by: (Signature)
Relinquished by: (Signature)	Date/ Time	Received in Laboratory by: (Signature)	Date/ Time	Shipping seal received intact ? No of _____	Lab Remarks:

Implementing the Chain of Custody Procedures

The Chain of Custody Record (COCR) shall provide documentation of everyone who has custody of the samples. The sample collector starts the COCR when the samples are collected. The COCR travels with the samples that are listed on the COCR. The COCR must contain the written signature of everyone that has custody of the samples and must document the relinquishment and receipt of the samples between sample custodians.

Transferring COC of samples from person to person

When custody of the samples changes, i.e. when samples are transferred from one person to another, the Custody Record must show the samples being relinquished by one person and received by another in the presence of each other.. The custody of the samples and the responsibility of maintaining sample integrity are transferred during this process. The transfer process is documented on the bottom of the Chain of Custody Record form.

Transferring COC of samples from person to DCLS via courier

This transfer is the same as above except that the transfer is not face to face. Both the collector and receiver document the integrity of the shipping container and the samples therein. The actual sample transfer is via courier in a tamper-proof shipping container.

Preparing the COCR Form

The COCR form may be prepared using one of two methods, CEDS or manually. The preferred method is to use the CEDS system and the COCR form printed from information entered into the CEDS system. When using lab sheets, a multiple-part preprinted carbonless form is used. The COCR does not take the place of a lab sheet or CEDS data shipment. Consideration must be taken of the number of samples being shipped, as **a separate form must be completed for each cooler shipped**. Care must be taken to ensure that the information on the form concerning the samples exactly matches that found on the samples. Samples must be rejected if they do not match the COCR form.

Sample Priority

Using the COCR does not in itself indicate the samples are priority. The priority number should be recorded on the COCR in the space where the 24-hour contact information is located. When using CEDS, this information is also captured in the field data screen and shipped to DCLS. When using lab sheets, each lab sheet must have the priority code entered. Advance notice of priority samples, or samples being delivered on a weekend, should be given the lab by contacting persons on the contact list. Only high priority samples or samples with a short holding time should shipped to the lab for weekend delivery.

SHIPPING SEAL NUMBER

This is number of the wire seal provided by DCLS that will be used to seal the individual coolers. All coolers not delivered in person to DCLS must have a shipping seal in place with the shipping seal number recorded on the COCR. The seal number is unique and entering it on the COCR makes that record unique. When the cooler is received by DCLS they will compare the number of the unbroken seal on the cooler with the seal number that is recorded on the COCR.

FORM NUMBER

The multi-part forms will have a unique form number in the top right hand portion of the page. This number will be used to identify the form from all others when no shipping seal number is used.

SAMPLERS

The person who collects the samples or is present when the samples are collected and labeled and takes initial custody of the samples signs the COCR in this area.

CASE NO OR PC NO OR VPDES NO OR OTHER

This area is used to record the reference number for the sampling event. The number is program specific. Program protocols should be followed when entering this number. When using CEDS this reference number is referred to as the RUN ID.

LAT & LONG (optional)

The latitude and longitude may be entered at the top of the page if known. This is used to identify the location of the sampling event. If more than one site is sampled during the sampling event, a lat/long must be entered for each station and lat/long space at the top of the page should be left empty.

REGION OR UNIT and ADDRESS

Enter the name of the region or central office unit responsible for collecting the samples and the address of the region or unit. This address may be used when returning certificates of analysis.

PHONE & FAX NUMBERS AND E-MAIL

Enter this contact information for the collector. This information will be used to contact the collector if questions arise concerning the samples or analysis.

24-HOUR CONTACT INFORMATION

Enter the sample priority in this area. If the samples have a high priority (1), enter any and all information that can be used 24-hours a day to confer about sample analysis and results. Examples of information are; home, work, pager and cell phone numbers as well as e-mail addresses.

STATION ID

This is the brief description of the station at which the samples listed (on the same line) were collected. Limit the description to information necessary for you to uniquely identify the station from all others collected. This information must match the information on the sample tags and in the field log.

DATE/TIME

This is the date and time at which the samples were collected. The date is in the form MMDDYYYY and time is recorded in military time.

BASIC STATION DESCRIPTION OR CONTAINER TYPE (optional)

This space is used for additional station description information if necessary. For situations where pre-cleaned containers are used, the container description information may be entered here.

COMP/ GRAB (optional)

This space may be used to identify samples other than routine grab samples. In the case of composite samples, the number of samples or time frame of the composite should be entered. If a horizontal or vertically integrated sample is collected, the information may be entered in the station description or under "FIELD OBSERVATIONS".

TESTS TO BE RUN IN THE LAB

Enter the group code from the DCLS catalog of services which contains the analysis desired. If you have questions concerning the appropriate group code, contact DCLS using the contact list.

OBSERVATIONS & FIELD TESTS (optional)

Enter field observations or field tests. These should be entered in the field log and on the lab sheets as well.

RELINQUISHED BY , DATE/TIME, RECEIVED BY

This area is used to record transfers of the sample custody. The person with custody of the samples (initially the collector) must relinquish the sample custody in the presence of the person receiving custody of the samples (except when shipping samples to DCLS). This is accomplished by the custodian signing the COCR form in "relinquished by" section and entering the date and time. The new custodian must sign the COCR form in the "received by" section in the company of the original custodian. This process is followed in any subsequent changes in sample custody. Space is provided for four such transactions plus the final acceptance of custody by DCLS and the date and time the samples are received by DCLS.

SHIPPING SEAL RECEIVED INTACT, NO. OF, LAB REMARKS

This area is used by DCLS to record the number of the seal broken to gain access to the contents of the cooler. They will note if the seal is intact. They will also note any other remarks such as the condition of the samples, ice present, etc.

Using CEDS

CEDS can be used for shipping sample information to DCLS and for printing the COCR from the field data entered in the field data screen.
Since the COCR is printed from CEDS after returning from the field, this method may not be used when sample custody is transferred from person to person in the field.
When using CEDS for shipping sample information to the lab, stations must be established in the station screen and all the pertinent information must be entered in the field data screen using established protocols.
Additionally, you will need to **record the Shipping Seal Number** you will be using on the cooler and the **Shipped Date**. Review the information on-screen with the information on the sample tags and in the field log before printing the COCR form. Information on the COCR form may be changed in CEDS and reprinted if errors are made up until the time that the data is shipped to DCLS.
Click on the <PRINT CHAIN CUST> button on the field data screen to print the COCR form. Three copies will be printed.
Sign one copy as "Relinquished by" and complete the date/time at which the samples are relinquished or locked in the cooler. Place it inside the cooler in a zip-loc type waterproof container.
One copy is put in a zip-loc type waterproof cover and is taped to the outside front of the cooler.
The collector retains one copy with the field log.

Multiple-part Preprinted Form

Complete the multi-part form using the definitions above. All sections not indicated as optional must be completed. Make certain that the information on the form exactly matches the information on the sample tags, the field log, and the labsheets. Minor mistakes made while filling out the form may be corrected by crossing them out and initialing the crossed-out portion. Major mistakes will require a new form to be completed. The form with the mistake must be destroyed.

Sign the bottom portion of the COCR as "Relinquished by:" and complete the date/time at which the samples are relinquished or locked in the cooler.
The original signed copy is to be sent inside the cooler in a zip-loc type waterproof container.
One copy is put in a zip-loc type waterproof cover and is taped to the outside front of the cooler.
The collector retains one copy.

Preparing Samples for Shipment

Before shipping samples make absolutely sure that the information on the sample tags exactly matches the information on the COCR, the field log, and either lab sheets or the CEDS field data entry screen.
Individual samples must be sealed with a custody seal tape or evidence tape in such a way as to prevent the caps from working loose and the sample tags from coming off. This is generally accomplished by wrapping the wire sample tag securely around the neck of the sample container and leaving the ends of the wires exposed. The tape is then used to tightly span between moving and stationary parts of the sample cap securing the ends of the wire under the tape.
Put samples in a large waterproof bag and put into shipping cooler. Ice samples by putting the ice between the cooler and the waterproof bag. The maximum weight of the cooler is 60#.
Record the wire shipping container seal number on the COCR. Put the original signed copy of the COCR form inside the cooler inside of a waterproof zip-loc type bag. If used, include the lab sheets inside the zip-loc bag as well. Close and lock the cooler by closing the hasp (the bail over the eyelet) and securing it using the padlock supplied by DCLS. Put the wire seal individually through both the bail and the eyelet of the hasp. Do not put the wire seal through the eyelet in the same manner as the lock. Before closing the wire seal, make absolutely sure the serial number of the wire seal matches the number entered on the COCR form.

The sealed, locked cooler containing the signed COCR form may be placed in the regionally designated location to be picked up by the DCLS courier. The sealed, locked cooler is considered to now be under the custody of DCLS.

Problems

The sample tags don't match either the CEDS information or the lab sheets, or the COCR form.

The collector can correct these errors only if their field log contains information that will rectify the error. A correction of this type must be meticulously documented in the field log. Only the collector can make changes of this type. If the samples have been shipped, the collector will have to go to DCLS to make the corrections.

Cooler is locked before all the samples are placed inside.

The collector must develop a separate COCR for the additional samples and ship them in a separate COC cooler. The samples listed on the first cooler's COCR but missing will be marked as not received by DCLS. The change in coolers' contents must be documented in the field log.

Cooler is sealed but not locked before all the samples are placed inside.

The seal may be broken to inspect the cooler contents and to add or remove samples prior to padlocking the shipment. If the seal is broken, a new COCR must be developed.

- In CEDS, return to the field data screen (prior to data shipment @ 0900 and 2200, check for the lab send date in the CEDS field data screen), change the seal number, print a new COCR and replace all three of the original COCRs.
- When using lab sheets, you may change the seal number on each of the three copies of the form and initial the change on each sheet. This change should be documented in the field log as well.

You don't have any wire seals.

You or someone else who assumes custody of the samples must accompany the samples to DCLS. Samples delivered by the sample custodian don't require locks or custody seals. They do require container seals.

The wrong wire seal number is recorded on the COCR.

If the cooler is locked, you must accompany the samples to DCLS.
If the cooler is not locked you may change the wire seal. *(see above, breaking the wire seal)*

The Personal Field Log

The personal field log is a legal document used to record information concerning all aspects of an investigation. The log must have bound and numbered pages. The log should be kept in a secure place. Only the owner of book should make notations in the personal field log.
At a minimum, the field log should be used to record information which links that section of the field log with the information found on the COCR. The following information should be used a page headers:

- Investigation identification information such as PC, or permit number
- Date of investigation

The field log should also contain information that supports but does not duplicate information found on the COCR. This includes, but is not limited to:

- Names, addresses, phone numbers of complainant, permit holder, operators, etc.
- Detailed descriptions of the sampling sites
- Variations if any from the WQA SOP manual
- Types of samples collected (grab, straight timed composite including time frame, volume weighted composite, cross-section composite, vertically integrated composite
- Pre and post meter calibration information
- QAQC samples collected
- Detailed observations of the site including physical lay of the land such as upstream, up-gradient, east/west, etc.
- Detailed information included as comments in CEDS or on the lab sheets such as "expect high BOD"
- Documentation of changes to the COCR.

* SHIPPING SEAL #

APPENDIX M

Pfiesteria and the DEQ *Pfiesteria* response and safety plans.

CHESAPEAKE BAY FISH LESIONS AND *Pfiesteria*

Virginia Sea Grant Program • Virginia Institute of Marine Science • September 1997

Recent fish kills and closures in the Pocomoke River have increased public concern over *Pfiesteria piscicida*, a microorganism that has been linked to large fish kills in North Carolina. In response to concerns about an unusual number of fish with lesions reported in Chesapeake Bay, especially the Pocomoke River. A Virginia *Pfiesteria* Task Force was formed in May 1997. The Task Force includes members of the Virginia Marine Resources Commission (VMRC), The Virginia Department of Health (VDH), The Virginia Department of Environmental Quality (DEQ), and research scientists from Virginia Institute of Marine Science (VIMS), and Old Dominion University (ODU). The Task Force has developed an information network that enables members to respond quickly and efficiently to events.

What causes lesions (open sores) on fish?

There are many possible causes for fish lesions including physical injury in nets or traps, bites by other fish or birds, toxic chemicals, and infectious disease agents such as viruses, bacteria and fungi. On the basis of laboratory experiments, we now have to add toxins released by *Pfiesteria* to the list of possible causes. Currently, it is very difficult to determine the original cause of a lesion unless an obvious parasite is present because basic knowledge of the physiologic and environmental factors related to lesions in fish is insufficient.

Open sores that expose the underlying musculature are the most difficult kind of lesion to assess. The skin and mucus of a fish are effective barriers against infection by bacteria, which are always present in Chesapeake Bay waters. However, that barrier can be broken by a variety of causes including injury, general stress to toxic chemicals (including *Pfiesteria* toxins). When the skin/mucus barrier is broken, the area is usually rapidly colonized by bacteria which further erode the tissue and produce an open lesion or sore that may penetrate deep into the musculature. In such cases, the cause of the original break in the skin/mucus barrier that lead to the lesion is difficult to determine with our present state of knowledge.

Have there been unusually high numbers of fish lesions during 1997?

No, not in most areas of Chesapeake Bay. Some fish lesions occur every summer in Chesapeake Bay and based on information from VMRC, DEQ and VIMS and also from agencies in Maryland, the incidence of lesions on Chesapeake Bay fish during 1997 is not unusually high and there is no indication that fish populations are facing serious problems.

The Pocomoke River, located on the Eastern Shore near the Virginia-Maryland border, may be an exception. Commercial fishermen have reported what they consider to be unusually high numbers of fish lesions in the Pocomoke River and there were low- to moderate-level fish kills in the river during August. These lesions and kills have been linked to the toxic dinoflagellate *Pfiesteria piscicida*, but the link is as yet circumstantial.

Have fish kills occurred in Chesapeake Bay in the past?

Small- to moderate-scale fish kills, usually of small menhaden, occur occasionally in tidal creeks during the summer months. These kills are usually caused by low oxygen content of the water, but other possible causes, now including *Pfiesteria*, are routinely investigated.

Who should I call if I see a fish kill or see fish with lesions?

A few fish here and there with lesions, or even a few washed up dead on the beach, is not an uncommon occurrence and there is little cause for concern. However, if large numbers of fish with lesions or dead fish are observed, the appropriate agencies should be notified. The

Virginia Department of Environmental Quality (DEQ) has responsibility for investigating fish kills.

What is *Pfiesteria* and is it related to red tides?

Contrary to some recent press reports, *Pfiesteria piscicida* is not a virus or bacterium and it is not an infectious agent; fish or other organisms cannot become infected with *Pfiesteria*. *Pfiesteria piscicida* is a dinoflagellate, a microscopic, free-floating, single-celled organism with two flagella for locomotion. Most dinoflagellates are plants (called algae or phytoplankton) that gain energy from photosynthesis. However, many species of dinoflagellates, including *Pfiesteria*, do not photosynthesize, but behave like animals and consume algae or bits of organic matter. Normally, *Pfiesteria* feeds on algae cells. However, under certain circumstances that are not understood, and only in the presence of live fish, *Pfiesteria* can release a toxin that can cause sloughing of the surface layer of fish skin and, in high concentrations, can kill fish. *Pfiesteria* has been implicated in fish kills in North Carolina and in the Pocomoke River near the Virginia/ Maryland border on the Eastern Shore.

There are two reasons that the public may connect *Pfiesteria* and "red tides". *Pfiesteria* is a dinoflagellate and red tides are typically, but not always, caused by dinoflagellates. *Pfiesteria* is known to be toxic to fish and red tides are often, but not always, toxic to marine life. Despite these similarities, there are important distinctions to be made between *Pfiesteria* and red tides, especially for the Chesapeake Bay region. *Pfiesteria* is reported to kill fish when it occurs at low concentrations in the water, typically a few hundred cells per milliliter (.00026 gallons) of water. This is not a sufficient concentration of cells to discolor the water and *Pfiesteria* has never been reported to cause discolored water.

Red tides (also called red water or mahogany water) are typically caused by the dense accumulation, typically thousands of cells per milliliter of water, of dinoflagellates near the surface. Red tides are common occurrences in the Chesapeake Bay and its tributaries. They can occur at any time of year but usually are most common during July and August. Unlike other coastal regions of the United States where red tides result in fish death and bans on eating shellfish, red tides in the Chesapeake Bay to date have not been toxic to marine life. This lack of toxicity is because the species of dinoflagellates causing red tides in Chesapeake Bay are not toxic species. Red tides are typically categorized as a type of Harmful Algal Bloom (HAB), whether they are harmful to aquatic life or not. There is increasing interest in HABs worldwide because of the perception that they are becoming much more numerous, are often toxic to marine life, and are likely caused by man's influence on coastal areas. Because of their lack of toxicity to date, there has been less urgency to study red tides in the Chesapeake Bay and it is not clear what causes them and whether they are becoming more numerous.

Does *Pfiesteria* occur in Virginia?

Pfiesteria is known to occur from the Gulf of Mexico along the east coast as far north as Delaware Bay. It has been reported in Virginia waters near the mouth of the Pocomoke River and it is known to occur in Maryland. *Pfiesteria* is probably an ubiquitous organism that occurs all along the east coast in low numbers.

Is there a relationships between *Pfiesteria* and environmental degradation?

Popular press reports of *Pfiesteria* and its possible effect on fish often suggest that nutrient enrichment of estuaries and coastal waters from a variety of land-derived sources is a principal cause of *Pfiesteria* proliferation and activity. Some scientific literature suggests a similar relationship. Manure from hog and chicken production facilities is often identified as a source of nutrients. The association between *Pfiesteria* and nutrient enrichment is also fostered by the tendency to associate *Pfiesteria* with algal blooms, which are well documented to result, in part, from nutrient enrichment of natural waters. However, as discussed above, *Pfiesteria* is not an algae and does not make its own food by photosynthesis and does not require dissolved nitrogen and phosphorous (two typical nutrients) in the water for its nutrition. *Pfiesteria* eats other microscopic plants and animals. Because it is an animal and not a plant it is less likely to respond directly to nutrient enrichment. To the extent that its preferred food is microscopic algae, one

might expect *Pfiesteria* to be more abundant where its preferred food is more abundant. Thus, it might be indirectly linked to nutrient enrichment through its food supply.

In general, the Chesapeake Bay and its tributaries are not as enriched with nutrients as the Pamlico Sound and its tributaries in North Carolina, yet *Pfiesteria* has been reported from various locations in the Chesapeake Bay and has been linked to fish kills and human health problems in the Pocomoke River. However, based on common indices of nutrient enrichment used for the Chesapeake Bay, the Pocomoke River is not considered to be highly enriched. Other, more enriched areas of the Bay have not experienced fish kills or fish with lesions. The carefully controlled scientific experiments which identify nutrient enrichment as a stimulus to *Pfiesteria* are few and others are currently being conducted. Until more results are available it is not possible to say with confidence why *Pfiesteria* occurs where it does and why it becomes toxic when and where it does.

Can Chesapeake Bay expect large-scale fish kills similar to North Carolina?

When fish with lesions were first observed in the Pocomoke River there was doubt about the possible role of *Pfiesteria* as a cause because of the lack of large numbers of dead fish on the surface. In North Carolina, where *Pfiesteria* has been reported to be the cause of fish kills, there are reports of large numbers of dead fish, often hundreds of thousands to millions, during fish kills. Recent fish kills in the Pocomoke River, attributable to *Pfiesteria*, report thousands to perhaps tens of thousands of dead fish, much lower numbers than observed in North Carolina.

One possible explanation for the fewer numbers of dead fish in the Chesapeake Bay region as a result of Pfiesteria may be differences in hydrography between these two regions. The Pamlico Sound and Neuse River estuary in North Carolina are very shallow, poorly flushed estuaries with weak tidal currents. By contrast, the Chesapeake Bay and its tributaries are typically deeper, better flushed and have stronger tidal currents. If Pfiesteria in the sediments are detecting fish in the overlying water column by means of chemical cues from the fish, as some scientists believe, and if fish death is caused by a chemical toxin produced by Pfiesteria, then it is possible that the greater the dispersion of these chemical cues and toxins by water currents and circulation, the fewer fish will be detected and killed. Also, in deeper water fish may be less concentrated.

There is much yet to be learned about Pfiesteria and its role in fish lesions and death, but this is one possible reason that Pfiesteria reported in the Chesapeake Bay region may not be as much as a problem as reported for North Carolina.

Is it safe to eat Virginia seafood?

YES, Chesapeake Bay seafood is safe. Consumers should use common sense and avoid dead fish or fish with sores, but otherwise there is no reason to avoid eating Virginia seafood. There have been no reports of adverse effects on human health from eating shellfish (crabs, oysters, etc.) harvested in the vicinity of fish kills, but little information is available on this subject.

Does Pfiesteria affect humans?

A variety of symptoms have been reported by commercial watermen and other citizens in North Carolina, Maryland and Virginia and by researchers who cultured *Pfiesteria* in the laboratory. Symptoms, including sores, fatigue and short-term memory loss, have only been associated with laboratory exposure, or with large-scale fish kills in North Carolina and with fish kills in the Pocomoke River in Maryland and Virginia. Portions of the Pocomoke River were closed periodically during August because of possible human health concerns. Establishing a definite link between generalized symptoms and *Pfiesteria* is difficult, but health officials are studying the situation carefully. Your local health department has up to date information.

For more information, visit the VIMS web site: http//www.vims.edu

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DEPARTMENT OF ENVIRONMENTAL QUALITY

Operational Response Plan

For *Pfiesteria* Related Fish Kills

Pfiesteria Incident Response Team

PIRT

May 18, 1998

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INTRODUCTION

The purpose of this document is to serve as the operational plan for Department of Environmental Quality (DEQ) staff response to suspected *Pfiesteria* related fish kill events.

DEQ responsibility for fish kill investigations is clearly established by Virginia Code §62.1-44.15(11), and staff guidance on conducting these investigations is provided in the draft revised October, 1997 edition of the Fish Kill Investigation Guidance Manual. Therefore, this plan does not duplicate information in the agency fish kill investigation document but rather provides guidance specific to suspected *Pfiesteria* related events.

The plan establishes a DEQ seven day a week, 24 hour a day *Pfiesteria* response program from May 1 through October 31 of each year. DEQ's Tidewater (TRO), Piedmont (PRO), and Northern Regional (NRO) Offices will each have a *Pfiesteria* response person on call who will handle all calls and reports of fish with lesions, fish acting erratically and fish kills. They will respond with the assistance of enforcement officers from the Virginia Marine Resources Commission (VMRC), who will provide transportation to the fish kill site with their boats for the DEQ *Pfiesteria* responder. The DEQ *Pfiesteria* responder will collect the samples needed to determine if a *Pfiesteria* event is occurring; the Virginia Department of Health (VDH) will use this information to determine if a public advisory warning (yellow light) or water segment closure (red light) decision is necessary (see Appendix B for a discussion of these criteria). DEQ will also have On Scene Coordinators (OSC) on call that will contact all the other agencies and institutions involved and set up a more complete DEQ crew for follow-up work, if a *Pfiesteria* related fish kill event is confirmed. In addition, a Central Office (CO) DEQ response team and OSC staff are available as backup support to the agency regional offices.

OVERVIEW OF THE COMMONWEALTH'S PFIESTERIA PROGRAMS

In addition to the DEQ *Pfiesteria* response plan described in this document, Virginia has developed and is implementing a multi-agency/institution effort for response, monitoring and research on *Pfiesteria piscicida* and *Pfiesteria* Complex Organisms (PCOs). A *Pfiesteria* task force was designated in 1997 and all the agencies and institutions represented on the task force have been involved in the development of Virginia's plan for responding to *Pfiesteria* events. The task force is comprised of 13 members from five agencies and institutions: Virginia Department of Health (VDH), Virginia Institute of Marine Science (VIMS), Department of Environmental Quality (DEQ), Old Dominion University (ODU) and the Virginia Marine Resources Commission (VMRC). In addition, there are a number of auxiliary agencies and academic institutions which share a direct or indirect role in task force efforts: Department of Game and Inland Fisheries (VDGIF), Department of Conservation and Recreation (DCR), the Chesapeake Bay Local Assistance Department (CBLAD), the Marine Products Board (VMPB), Department of Agriculture and Consumer Services (VDACS), Virginia Commonwealth University (VCU) and Virginia Polytechnic Institute and State University (VPISU).

The Commonwealth's *Pfiesteria* monitoring, response, and research activities involve extensive participation by ODU, VIMS, VDH, VCU, DEQ and VMRC. VIMS, ODU and DEQ have been active participants in Virginia's Chesapeake Bay Program for years. ODU brings extensive experience in research and monitoring of phytoplankton and zooplankton. VIMS brings its extensive experience in research and monitoring of phytoplankton and fish health. VDH is undertaking an extensive study funded by the Centers for Disease Control (CDC) of human health effects and is handling the application of health advisories and closures. VIMS will be undertaking a major study of the Great Wicomico River and the fish health monitoring as part of their normal fish surveys in 1998. ODU will be undertaking the screening of water samples by light microscopy for PCOs and will culture sediment samples to facilitate *Pfiesteria piscicida* identification. Both VIMS and ODU are developing scanning electron microscopy capabilities for identification of *Pfiesteria* and similar organisms. Virginia will still rely on North Carolina State University (NCSU) for identification of the toxic forms of *Pfiesteria piscicida* for at least the near term. VIMS is in the process of gearing up for a P-3 laboratory that will enable them to identify the toxic forms.

DEQ, VIMS, and ODU will be supporting VDH's health impacts study through CDC. VIMS and DEQ will perform twice monthly

monitoring of 14 stations identified to support the cohort health study. DEQ will complement the health study with an extensive network of stations for PCO and water quality sampling. A total of 24 stations in small estuaries on the eastern and western shores of the Bay and in the major rivers of the Bay will be monitored for standard water quality parameters, PCOs, chlorophyll "a" and a full range of nutrients. These 24 stations are inclusive of the 14 VDH cohort health study stations.

The data from this water quality and PCO monitoring along with data from *Pfiesteria* event response will complement data from DEQ's 153 ambient water quality monitoring stations on the Bay and data from DEQ's Chesapeake Bay Program monitoring stations. It is believed these data will provide useful information on the spatial distribution of *Pfiesteria* and the environmental factors that result in toxic blooms.

PFIESTERIA RESPONSE

PFIESTERIA INCIDENT RESPONSE TEAMS - PIRT

DEQ has four PIRTs, one from each of the three regional offices (PRO, TRO, NRO and, one from the Central Office). Each team has a initial responder trained in all aspects of *Pfiesteria* response including personal safety. Each region will have an initial responder on call at all times and a list of secondary responders that includes their pager and/or home telephone numbers so they can be activated if needed.

AFTER HOURS

After hours fish kills will be reported by Department of Emergency Services (DES) through the normal regional after hours pollution response protocols. DEQ pollution response staff should obtain as much information as available; but most importantly, the name and telephone number of the person reporting the fish kill to DES. This information will then be referred to the initial response staff member of the appropriate DEQ regional *Pfiesteria* Incident Response Team (PIRT).

ALL FISH KILL REPORTS

All fish kill reports are referred to an initial *Pfiesteria* responder during the "*Pfiesteria* Season" from May through October. Each region shall ensure that at least one initial responder is accessible by pager and cellular phone at all times during regular business hours and after hours. Initial responders will have full training in safety and all required safety equipment (Appendix A); they will be trained in fish species identification.

If a name and telephone number is available, the first step is for the initial responder to call the person who reported the fish kill to DES to get first hand information.

If the event is in brackish or salt water (east of I-95) and there are fish with lesions, fish acting erratically, or the fish are menhaden, then the fish kill should be treated as a possible *Pfiesteria* incident. Any menhaden fish kill should be investigated even if there appears to be an obvious cause.

Any fish kills in waters not likely to have a *Pfiesteria* incident should be handled by existing fish kill response protocols. However, to avoid having the person taking the call make a judgement on whether it is likely to be *Pfiesteria* waters and because the same staff handle both types of fish kills, all fish kills should be referred to the initial responder on the PIRT.

PFIESTERIA RESPONSE - Initial Investigation

Once the *Pfiesteria* responder has determined that the incident should be investigated as a possible *Pfiesteria* incident, the responder will contact VMRC. See Appendix F for the *Pfiesteria* response process and actions.

When requesting assistance from VMRC, call 1-800-541-4646, which is VMRC's twenty-four (24) hour toll free Operations number. If this call is made Monday through Friday between 7:00am and 5:00pm, the dispatcher will contact the appropriate Area Supervisor, who would be responsible for the geographic location in which the incident is occurring.

When calling VMRC, provide them your name, title, department, as well as a number at which you can be reached until contacted. If you have not been contacted within thirty minutes, call the Operations number again and advise the dispatcher that you have not been contacted. It is possible that the page was not transmitted or received due to technical difficulty. The dispatcher will either relay to you pertinent data or will page again.

If the call to VMRC is made on a Friday after 5:00pm and before 7:00am Monday,the dispatcher will contact the on-call Supervisor who will arrange for the appropriate response. The same time-frame parameters for a response will be in effect.

The responder will arrange to meet VMRC at a mutually acceptable boat launch as close as possible to the fish kill. During regular office hours the responder can take a DEQ regional boat and crew, if the crew is readily available; however, VMRC should still be contacted.

The initial *Pfiesteria* responder will always take a complete package of *Pfiesteria* response equipment and supplies. This package of equipment and supplies will already be loaded in a vehicle and ready to go. The vehicle can be at work for after hours response, if the responder is within a short commute of work; if not, the vehicle will be taken home on weekends and holidays.

Pfiesteria Response - Confirmation of a Pfiesteria Event

If a site investigation confirms a possible *Pfiesteria* event, the responder will contact the regional OSC for the appropriate regional PIRT, who will contact VIMS and ODU to arrange for sample delivery and to notify them of the event (Appendix C). The regional OSC will also contact the VDH *Pfiesteria* contact and DEQ management. Contacts with news media shall follow the DEQ guidance to ensure that complete and accurate information is provided to the media.

Next, the responder will determine the extent of the fish kill and take appropriate samples for verification of the presence of *Pfiesteria*.

The regional OSC will activate a follow-up response. The OSC will use the contact list to call members of the PIRT by using their home and/or pager numbers to get three staff members to accompany the initial responder on a follow-up response.

The OSC will call individual PIRT members from his/her own region first, then Central Office and then, if needed, one of the other regions.

A contact list of DEQ PIRT members and OSCs will be maintained and kept current by the Scientific Research Unit (SRU) within the Central Office. It is the responsibility of each regional office to immediately notify SRU of any changes in the regional contact list in order to keep the list current.

Every effort should be made to perform a follow-up response the next morning. Two DEQ staff will serve as the boat crew along with the initial responder and the third will bring an additional vehicle for the delivery of samples. The OSC also may respond to coordinate activities from a local site.

The follow-up response will be for the collection of *Pfiesteria* complex organisms (PCOs) samples, water quality samples, sediment, fish samples and to do a fish lesion percentage count.

RED LIGHT AND YELLOW LIGHT DECLARATION RESPONSE

The authority and responsibility to make the declaration of a Red or Yellow Light incident currently resides with the VDH (Appendix B). DEQ's role is to investigate fish kills and to gather the information (water samples, fish samples and fish with lesion counts) that VDH needs to make a determination.

If a Red light or Yellow light incident is declared, routine monitoring will be required until the "Undo Criteria" are met and the situation returns to normal. During a Yellow light incident, an OSC may not be necessary, but the regional office may need assistance to maintain the routine monitoring.

If an incident is declared a Red light incident, then a DEQ command center should be established and a rotation of PIRT and/or members will be put in place. If there is an active fish kill and the initial responder eliminates other causes and observes fish with lesions, the incident should be treated as a Red Light incident until a formal determination can be made by VDH.

ON SCENE COORDINATOR

An OSC is required when a "Red Light Waters" declaration is issued, i.e. large scale or ongoing *Pfiesteria* fish kill. Each of the four response teams have designated OSCs.

The responsibilities of the OSC include the following:

- contacting VIMS and ODU to arrange for receipt of the samples from the initial response
- setting up the follow-up response
- ensure that all authorities that need to be informed of the incident have been informed including the VDH *Pfiesteria* response contact and DEQ management
- calling in whatever resources are necessary including requesting Virginia Emergency Services's (VES) assistance
- coordinate with VES the notifcation of local governments
- establish a rotation schedule for the response teams
- ensure the availability of all equipment needed for the incident on an ongoing basis
- ensure the availability of all supplies needed on an ongoing basis
- ensure transportation of samples to the appropriate facilities and delivery of supplies

- liaison with the agency public information personnel to deal with media (VDH currently is lead agency for providing *Pfiesteria* information related to human health effects, CO and RD's also have assigned duties).

- ensure PCO counts, fish lesion analysis and other data are forwarded as quickly as possible to the VDH as appropriate.

EQUIPMENT/FUNDS

- **Financial Resources will be critical to the response teams and OSC.** Each regional office shall maintain an adequate reserve of petty cash to purchase supplies and support at the local scene, especially for sustained incidents.

- **Sampling Equipment.** Each region shall ensure that an adequate supply of sampling equipment is on hand in quantities sufficient to handle 30 days of continuous sampling.

- **Safety Equipment.** All safety equipment including decontamination equipment shall be available at each regional office. (The exception is water needed for field decontamination which will be obtained on an as needed basis.)

- **Command Center.** If needed, a command center will be provided by DES. The center will include cell phone, fax, computer with modem, and printer.

- **Decontamination capabilities.** Each regional office shall have some type of spraying capability available. Silt fencing and straw bales will be purchased from a local supplier as needed.

- **Portable sanitary facilities.** Local supplies will be identified through local officials.

- **Large supply of potable water.** A bottled water supplier could provide a source of potable water unless water is also needed for decontamination. If a source of decontamination water is needed, the OSC will first contact local officials and then if necessary contact the National Guard, VDOT, etc.

- **Boat fuel.** Boat fuel will normally be obtained from the nearest VDOT facility. However, fuel will have to be purchased from a commercial service station or marina after hours and on weekends and in situations where the time required to travel to a VDOT facility would adversely impact the investigation.

- **Tools.** Tools for minor repairs of boats, trailers, sampling

equipment will be brought on scene. These are currently available at the regional offices.

- **Meals and drinks.** These can be obtained from a local supplier.

- **Ice and Coolers.** DEQ regional offices should have on hand sufficient coolers for the investigation. Local suppliers can be used for ice.

FIELD SAMPLING DURING RESPONSE TO PFIESTERIA RELATED FISH KILLS

The following sampling protocols are designed for initial and follow-up response to fish kills that may be related to PCOs, which are also referred to as Harmful Algal Blooms (HABs). Due to the possible human health implications from *Pfiesteria*, staff will follow the Safety Plan For Pfiesteria Response, as specified in Appendix A.

INITIAL RESPONSE FOR WATER QUALITY MONITORING

It is important to arrive and sample while the kill is still in progress. Because of the critical nature of the initial response, a protocol has been developed for conducting investigations when available staff may be limited. A single DEQ responder, using vessel transportation provided by VMRC, Shellfish Sanitation (VDH), or VDGIF, will arrive on site and collect the basic samples as needed.

Some common relationships associated with *Pfiesteria* like fish kills are:

1. Oxygen levels are usually not depressed.
2. Water may be discolored around kill.
3. Live fish may exhibit erratic movements.
4. Presence of fish lesions, especially in juvenile menhaden.

First Response Equipment Kits. The following sampling equipment should be maintained in field kits to minimize response time.

1. Quart cubitainers (20)
2. Gallon cubitainers (5)
3. TOC bottles (5)
4. 2 L Chlorophyll bottles fixed with $MgCO_3$ (5)
5. Lugol's solution (25 ml)**
6. Drop bottle with H_2SO_4
7. Lab Tags (30)
8. Lab Sheets (10)
9. Chain of custody sheets (5)
10. Plastic bags of various sizes for fish samples

11. Discrete sampling bottle (Labline, Alpha, Kemmerer, etc.)
12. Personal protective equipment
13. Appropriate meters for field parameters (pH, DO,salinity)
14. Secchi disk
15. GPS
16. Field notebook/pens
17. Cellular phone and contact list
18. Fish Kill Manual
19. Binoculars
20. Nautical charts
21. 3 - 500 ml Open Mouth Glass Jars
22. Stainless Steel Pan
23. Stainless Steel Scoop

**Lugol's Solution:

20 g Potassium iodide (KI)
10 g Iodine crystals
200 ml distilled water containing 20 ml glacial acetic acid

Field Observations. All field measurements and observations will be recorded in a bound and numbered notebook.

1. Note any discoloration of the water.
2. Note any erratic movements or behavior by the fish.
3. Note size, species, and number of fish.
4. Observe fish for any unusual marks such as lesions, net marks, hook marks, abrasions, or parasites. Note the location on the fish and describe these marks.
5. Record tide, weather conditions, and any other pertinent information.
6. Record sampling coordinates using a GPS.

Field Parameters. Measure the following field parameters:

1. Dissolved oxygen
2. Temperature
3. Conductivity
4. Salinity
5. pH
6. Secchi depth

Fish Enumeration. Follow the procedures described in the American Fisheries Society's Fish Kill Counting Guidelines (Appendix F of the 1997 DEQ Fish Kill Investigation Guidance Manual) for determining fish counts.

Fish Samples. Use the following guidelines in collecting fish for lesion analysis.

1. Collect only distressed or dying fish.

2. Place fish in plastic bags and keep on ice (**Do not freeze or preserve with formalin**). Include an ID

tag with date, time, location, collector, and phone number.

3. Deliver fish as soon as possible, preferably the same day, to Dr. Wolfgang Vogelbein or David Zwerner at VIMS - Aquatic Animal Disease Diagnostic Center, 1st floor, Chesapeake Bay Hall. Call first to make arrangements (Appendix C). If Dr. Vogelbein or his associates cannot be reached, keep the fish on ice until the next business day.

4. Include all field information and a map or description of the location with GPS coordinates.

5. If fish are collected by private citizens, verify that they meet the collection criteria and refer them to the Aquatic Animal Disease Diagnostic Center. If the fish were not collected properly or not moribund when collected, they should not be used.

Pfiesteria Samples. For *Pfiesteria* identification use 1 qt. cubitainers to collect from 1 ft. below the surface 3 replicate non-preserved samples and 3 replicate samples preserved with Lugol's solution (3-4 ml per qt.). Samples are to be in labeled containers kept out of the sunlight in a cooler or at room temperature. *Pfiesteria* samples are to be delivered to Dr. Harold Marshall or his alternate at ODU - Phytoplankton Lab. Call first to make arrangements (See Appendix C). Include the field information on the chain of custody form (Appendix D) and a map or description of the location with the Global Positioning Satellite (GPS) coordinates. Copies of this information are to be maintained by DEQ.

Water Quality Parameters. Water samples should be taken in conjunction with the *Pfiesteria* samples. Collect samples for nutrients (LNUT), BOD/suspended solids (NME1), total organic carbon (TOC), chlorophyll, and any additional parameters that may aid in identifying the cause of the fish kill. For depths greater than 4 meters, surface and bottom samples are to be collected for LNUT, NME1, and TOC. Also, field parameters are to be recorded at 2 m intervals. Label all samples with station location, date, time and collector. On the lab tags include a warning to DCLS that the samples may contain *Pfiesteria*.

Sediment Samples. Take 2 grab samples (taken with a 6" X 6" petite ponar) at the shallow areas close to the shoreline. Transfer the top 2 cm of each grab into a stainless steel pan and mix to combine the samples. Using clean 500 ml glass jars, collect 3 replicate sediment aliquots from the stainless steel pan. Label all samples with station location, date, time, and collector. Deliver the sediment samples with the *Pfiesteria* water samples to Dr. Marshall at ODU.

FOLLOW-UP WATER QUALITY MONITORING

Follow-up monitoring protocols are designed to provide additional data (i.e., to VDH for establishing advisories and closures).

Follow-Up Monitoring Equipment. The following equipment is needed.

1. Quart cubitainers
2. Gallon cubitainers
3. TOC bottles
4. 500 ml open mouth short glass jars
5. 2 L Chlorophyll bottles fixed with $MgCO_3$
6. 25 ml Lugol's solution
7. Drop bottle with H_2SO_4
8. Lab Tags
9. Lab Sheets
10. NCSU chain of custody sheets
11. Plastic bags of various sizes for fish samples
11. Discrete sampling bottle (Labline, Alpha, Kemmerer, etc.)
12. Personal protective equipment
13. Appropriate meters for field parameters (pH, DO, Salinity)
14. Secchi disk
15. GPS
16. Field notebook/pens
17. Cellular phone and contact list
18. Fish Kill Manual
19. Binoculars
20. Ponar dredge
21. Stainless steel pan
22. Stainless steel scoop
23. Field filtration equipment
24. 2 16' otter trawls
25. Large barrel for dumping catch
26. Sorting pans for fish ID
27. Fish measuring board
28. Heavy duty gloves
29. Cast net
30. Nautical charts

Pfiesteria Sampling. Follow the same protocols specified for the initial response sampling.

Water Quality Parameters. Water samples should be taken in conjunction with the *Pfiesteria* samples. Collect the samples using the Chesapeake Bay Program nutrient series (NTNP, PP, PNC), unfiltered nutrients (LNUT), BOD/suspended solids (NME1), total organic carbon (TOC), and chlorophyll. For depths greater than 4 meters, surface and bottom samples are to be collected for all parameters except chlorophyll. Also, field parameters are to be recorded at 2 m intervals. Label all samples with station location, date, time, and name of collector. On the lab tags include a warning to DCLS that the samples may contain *Pfiesteria*.

Sediment Samples. Follow the same protocols specified for the initial response sampling.

Fish Trawl Samples. Fish trawl survey results will be needed to provide VDH with the percent lesion data necessary for establishing advisories and closures. Trawls will be taken using a 16 ft. otter trawl with a 1/4" mesh bag on the cod end. It is recommended that two otter trawls be on board in case one is damaged during trawling. The starting and ending location of each trawl should be determined using a GPS and recorded. Trawls should be made on relatively level substrates (check nautical charts for presence of submerged pilings, obstructions, etc.) at 4-10 ft depths for approximately 2.5 to 5 minutes. The duration of the trawl is to be recorded. Enumerate and record species, length, and presence/absence of lesions for each fish. If numerous fish of one species are collected (i.e. several hundred anchovies), record only the minimum and maximum lengths. In areas with no access by boat, cast nets can be used from shore, piers or bridges. Follow the same protocols for storage and delivery of fish for lesion analysis that was specified in the initial response fish sampling guidance.

FIELD DATA RECORDS

All field measurements and observations are to be recorded in a bound and numbered notebook. All results are to be forwarded to the DEQ Central Office Water Quality Assessments (WQA) unit as soon as received.

DATA MANAGEMENT

WQA unit has the responsibility for maintaining a central data base for physical, chemical, and biological data related to *Pfiesteria* events. (Please refer to the data analysis flow chart in Appendix E).

One special study code will be established for these data sets for use by all regions and central office units, to make them easier to track and retrieve from STORET. Physical, chemical and biological data measured in the field and laboratory will be sent via labsheets to WQA where it will be manually entered into an Access database. When the STORETX database application is available, all data will be stored there until such time as a national *Pfiesteria* database is available that meets the needs of the Commonwealth.

Geolocation standards are routinely established for all data collected. The regional and central office planning staffs are now equipped with ARCVIEW software and supporting hardware. The ability to display data on maps could be a critical way to relay information to other agencies and the public.

FIELD SAMPLING TRAINING

All regional and central office DEQ staff are required - prior to their participation in the agency *Pfiesteria* response program - to either receive training or demonstrate competency in five subject areas:

1. Enumeration of dead fish during fish kill events, including knowledge of agency fish kill investigation guidance manual (1997) and American Fishery Society fish kill counting guidelines (Appendix F of 1997 DEQ Fish Kill Investigation Guidance Manual),

2. Collection, preservation and shipment of water and sediment samples for use in laboratory identification of *Pfiesteria*,

3. Use of otter trawl, beach seine, and gill nets to collect fish for fish lesion surveys,

4. Collection and identification of saltwater and estuarine fish species, especially juvenile species, and

5. Field examination of fish for external abnormalities and lesions.

It is the responsibility of the central office Scientific Research Unit (SRU), to ensure PIRT member competency in these five subject areas and to provide remedial training as needed.

Charter members of the regional and central office PIRTs attended a two day field sampling seminar at the Gloucester Point VIMS campus. The first day and half of the winter seminar included lecture and lab instructions in the five subject areas described above. The classroom training was followed by a field exercise in deployment of fish collection gear.

Equivalent training will be scheduled by SRU on an "as needed basis" for replacement PIRT members. Prior to April 1 of each year, SRU staff will evaluate the need for any additional training or refresher courses and arrange for such staff training as resources allow.

CO team members, not routinely involved in field work, must be trained in operation of dissolved oxygen, pH, and conductivity/salinity meters, basic safe boating skills, and procedures for collecting and submitting samples to DCLS. Before May 1 of each year, they are required to participate in at least two regional monitoring boat runs in order to review all aspects of field monitoring.

Members of the CO PIRT will also be required by the DEQ's Safety Officer to train periodically with their respirators and other safety equipment under conditions similar to what would be encountered in an incident response. If a team member's normal work responsibilities do not include field work, this acclimation training may also be accomplished by periodically assisting in regional Chesapeake Bay tributary monitoring trips or other *Pfiesteria* monitoring activities.

APPENDIX A

DEQ SAFETY PLAN FOR PFIESTERIA RESPONSE

DEQ SAFETY PLAN
FOR *PFIESTERIA* RESPONSE

INTRODUCTION

DEQ is charged with investigating major fish kills in Virginia waters. It also has agreements with several state agencies to participate in coordinated responses to events which may be caused by Pfiesteria or Pfiesteria like organisms (PLOs).

These response duties will require the taking of water samples and specimens of fish during or as soon as possible after a fish kill or during an event where fish are in distress from causes which cannot be readily determined. Additional sampling of water, sediment and fish will be required for a period of at least 30 days if the presence of Pfiesteria is confirmed.

The Pfiesteria organism and presumably other similar organisms produce toxins which cause immobilization and death in fish and are also associated with lesions on the fish.

The greatest problem with people has occurred in a laboratory where the organism was cultured for scientific study. It is believed that the contact with the toxins from Pfiesteria came from inhaling aerosol from the culture tanks over a period of at least several weeks. The symptoms included memory loss and problems related to performing such tasks as arithmetic computation. There have been reports of lesions on the skin of exposed persons.

The department must take prudent measures when intentionally requiring its employees to enter areas suspected of being an active site of release of the toxin(s). When using precautions such as foul weather gear and respirators in warm weather conditions, employees will experience stress from heat. The situation must be managed such that the risk to employees from working in hot conditions does not present an unacceptable risk.

This plan is intended only for those responding to incidents and the follow-up to incidents. It is intended to be a living document to be modified as conditions change and more becomes known about the nature of Pfiesteria and the problems associated with it.

SAFETY STRATEGY

I Control exposures to risks from vehicle, normal boating and aquatic work using conventional best practices, such as seat belts, following regulations and laws, avoiding operations in inclement weather.

II Reduce potential exposures to Pfiesteria Toxins by:

A. Restricting area to only necessary personnel.
B. Confining the potential exposure to as short a time as possible.
C. Rotating crews so that the same persons are not exposed day after day.
D. Preventing contact with potential toxins in water using PPE, i.e. foul weather gear
E. Reduce potential respiratory exposure to toxins in aerosols by using proven respiratory protection scheme and devices.
F. Decontaminations procedures used prior to removal of PPE.

III Manage stress caused by using PPE and respirators in hot weather to prevent impact on employee health and safety. The measures include:

A. Medical screening to prevent exposure of susceptible individuals.
B. Hydration prior to use of PPE.
C. Monitoring of individuals while wearing PPE for indications of problems.
D. Use of work/rest scheme to prevent individuals from exceeding safe exposures.
E. Use of multiple crews to reduce the amount of time each individual has to spend in PPE.
F. First aid of all operational staff to allow effective response to heat and other problems encountered by individuals as a result of field activities.
G. Reduction of solar load by using light colored (reflective) PPE. Shade to be provided where possible.
H. Use of aprons and shoulder length gloves instead of full coverage foul weather gear or full coverage gear to reduce heat load when work conditions (calm water and lack of agents causing aerosols) allow.

IV Report problems and modify programs as necessary. Review of entire safety program at least before after the end of the "Pfiesteria Season" in October and before the end of each calender year.

PRE-ACTION PHASE:

A Medical examinations will be conducted for all employees who are directly involved with response to a Suspected Pfiesteria Incident. These medical examinations are intended to accomplish several objectives.

1. Determine the overall state of health of the individual in order to prevent individuals who have medical problems which might be aggravated by conditions such as working in hot protective gear and equipment during the response activity from being exposed to conditions where they might be susceptible.

2. Qualification to wear respiratory protection.

3 Establish a baseline for comparison in the event that there are suspected problems due to exposure to Pfiesteria or other harmful agents during a response.

B. Each employee in the sampling parties (and boat crews) will be "fit tested" for a respirator.

C. First Aid and CPR training to be conducted prior to participating in the active phase of response. This is intended as a general measure to limit the impact of any accident or physical problems which might occur during response activities. In particular, individuals wearing protective clothing and respirators on warm days may have problems tolerating heat.

D. Training in the use of personal protective equipment such as respirators, foul weather gear, aprons, boots, respirators and the use of ensembles while performing specific tasks.

This training will consist of familiarization and use of the equipment in a classroom or wearing the equipment while performing specific tasks where Pfiesteria is not suspected to be present within the boat storage portion of a building and on the water. Instruction will include specific procedures for donning and doffing protective clothing, respirators and gloves. Decontamination of boats, equipment, respirators and other PPE will be demonstrated.

E. Practice use of the PPE including respirators will be conducted on a weekly basis for all boat crews. A record will be kept of the practice which includes the name, date, type, of equipment and duration of use.

F. Personal flotation gear will be tested with protective equipment, including a full face respirator in a po

G. Establishment of SOPs for tasks or groups of tasks by supervisors. These will be established by the supervisor of the field crew or boat crew after a documented risk analysis using worksheet provided by the Safety Officer. They will include a description of the task, operational instructions and will spec

List of SOPs to be available:

1. Sampling During the event (where dying or distressed fish are present)

2. Calm Conditions (apron or bib foul weather pants with respirator)
3. Filtering Samples
4. Sampling After the Event
5. Cleaning Equipment and tools

A copy of the written, approved SOP will be provided to each individual involved before an operation is conducted. This includes VMRC, VIMS and Health Department personnel who are working with DEQ employees on the same task.

H. It is recommended that sampling and boat crews practice use of PPE during activities which are not response operations and that a log is kept of such practices. This practice should occur at least once a week and the time of the practice should be extended as long as possible to obtain experience and allow the body to adapt to the use of the equipment.

I. Equipment/gear malfunction will be evaluated by the person in charge. All malfunctioning PPE required in the SOP (including respirators) will be replaced or repaired before continuing to conduct the mission. Questionable equipment will be taken out of service and repaired, replaced or tagged for evaluation at the base of operations.

DEPLOYMENT PHASE

Deployment will be such that the entry team or vessel will be at the target area in the early morning, late afternoon or other times to prevent exposure to the hottest portion of the day. When a fish kill is reported at night, deployment should be as early as possible the next day. When an active problem is reported during daylight hours and it is necessary to respond as soon as possible to get the best samples possible, entry will be as brief as possible.

Equipment will be selected or drawn from storage for a specific response. It may be stowed together in a vehicle or boat. There will be a checklist for safety equipment, based on the SOPs to be used, which will be completed before responding to the scene or to the point where a boat will be launched.

Sufficient drinking water and liquid replenishment will be aboard each boat and with each work party. This may be stored in coolers which are protected from contamination. Boat and sampling crews are required to decontaminate and remove PPE (except boots and foul weather gear) and to wash hands and face with potable water before eating, drinking or smoking, after entry is made into target areas.

A quantity of potable water will be available for use in decontamination by use of a pump up sprayer. Each boat will be equipped with a pump capable of delivering a stream of water from the body of water in which the boat is floating to be used in decontamination. This pump should have sufficient capacity to provide a stream of water through a device such as a garden hose. The pump should be checked shortly after launching and prior to reaching the target area.

Appropriate sun block and clothing will be available to protect employees for over exposure to sunlight. Sunglasses which meet the requirements for eye protection (ANSI standards for safety glasses and other types of eye protection as appropriate) will be used protect the eyes from sunlight or uv during sampling.

Additional clothing for the crew, food and personal items must be stowed so that they will not become contaminated with waters from the target area. It is recommended that food and beverage should be consumed prior to departing for target area.

Safety equipment and respirators which are missing or malfunction will be replaced or repaired before deployment occurs unless there is means to deliver replacement equipment before launching or usage will occur.

OPERATIONAL PHASE

There will be one person on each boat or in each operational party who is in charge. That person will determine when a specific SOP will be used.

If the OSC is deployed and establishes a command post, the safety designee for that region will be deployed to the command post and James Saunders, DEQ Safety Officer, will be notified (pager 804-246-4137).

Operations during the response will be conducted as specified in the SOPs which apply.

If it is necessary to deviate from SOPs, it is the supervisor's responsibility to document the deviation and the rational for the deviation. (Notice of the deviation must be provided to the Agency Safety Officer and also will be included in the written report of the response.)

When an entry into PPE is made, the time shall be recorded in the safety log or field notes. An entry in the same record will be made at the conclusion of the use of PPE.

Prior to donning of PPE except for the bib portion of foul weather gear and boots, each worker will consume as much water or an electrolyte replenishing fluid such as "GATOR AID" as he or she can tolerate. This should occur several minutes before the active use of full PPE commences.

After entering the target area, breaks for consumption of food or liquid replenishment will only be taken after leaving the target area, washing down of the PPE and boat area with water not contaminated with water from the target area, and washing of hands and face with potable water.

The target area should be approached from up wind if possible. A marker or a distance from the target area which is protected from mist and spray will be established for each site according to the condition encountered. PPE will be donned as required by the SOP before entering the target area.

If it is determined from observation or measurement that the cause of the event is other than Pfiesteria, use of PPE for protection from Pfiesteria toxins can be discontinued.

Work will commence as soon as possible after entering the target area and the work party or vessel will withdraw as soon as possible to an area which is not contaminated by any spray from the target area. After samples are secured, PPE and all surfaces of sampling tools, boat and exposed equipment will be washed with clean ambient water (another fluid or potable water can be used). The exterior of PPE (except the cartridges of respirators) will be washed with potable water and the equipment will be doffed in the manner indicated during training. Respirator cartridges may be used for an entire day unless they become blocked form water.

Containers of samples will be sealed and placed in secure coolers or other containers prior to removal of and PPE designed to prevent splash or skin contact.

If it may be necessary to use the same equipment to re-enter the area or another area the same day, the equipment shall be air dried prior to reuse so that the interior is not wet. At the close of the day equipment shall be placed in a plastic bag, secured by means of tape or a knot, to be taken ashore and cleaned and dried. If conditions do not allow for drying, disposable protective clothing may be used.

POST OPERATIONAL PHASE:

After returning to the base of operations, PPE shall be cleaned and disinfected as specified by the manufacturer or by training. Respirator cartridges will be discarded at the end of each day's operation.

The boat and equipment on the boat will be cleaned and washed down with fresh water and detergent.

All employees who have entered the target area must shower, including shampoo.

A written report of the operation shall include the names of members of the sampling parties , time and duration of use of respiratory protection and full PPE. The report shall also note any unusual event or incident and must note any deviations from SOPs and the rational for these deviations.

Safety equipment which did not function properly will be repaired or replaced.

APPENDIX B

PFIESTERIA-RELATED PUBLIC HEALTH ACTION LEVELS FOR VIRGINIA TIDAL RIVERS AND ESTUARIES

Pfiesteria-Related Public Health Action Levels for Virginia Tidal Rivers and Estuaries -- Virginia Department of Health

"RED LIGHT" WATERS

Action: Closure of a water area to recreational and commercial uses. Pass-through permitted. Geographic limits will take into account availability of easily recognized (by public) existing markers, and provide an appropriate buffer around the kill area based on visual observation of fish and testing of water for (Pfiesteria Complex Organisms) PCOs. Criteria may change as more knowledge is gained. Closure will prohibit water contact in area, including fishing, swimming, water skiing, other recreational activities and commercial fishing and shellfishing. Public service announcements will be made and signs posted where appropriate.

Meaning: It is reasonable to assume that water exposure may pose a risk to human health from toxins produced by PCOs.

Do Criteria: Presence of a fish kill which low DO or other common factors are ruled out as a cause and fish have deep ulcers or, if no ulcers, water samples examined in a VDH recognized laboratory reveal a significant number of PCOs by light microscopy.

Undo Criteria: No ongoing fish kill for 7 days and fish surveys show deep ulcers in less that 20% of a minimum sample of 50 of any one fish species.*

"YELLOW LIGHT" WATERS

Action: Health advisory for water area is issued via media, posters, signs, or handouts for marine police and water related businesses. Advisory will alert public to the current status and trigger more intensive monitoring. Advisory will reinforce avoidance of fish that are dead, dying, or have deep ulcers. Unannounced inspection of commercial edible fishing will verify that fish with deep ulcers are not entering human food chain. Advisory will recommend avoidance of intense exposure to water (primary contact recreation, e.g. swimming, water skiing). Criteria may change as more knowledge is gained. Recreational and commercial harvesting of fish and shellfish will not be affected. Geographic limits will take into account availability of easily recognized (by public) existing markers, and provide an appropriate buffer around the area of concern based on visual observation of fish and testing of water for PCOs.

Do Criteria: Fish surveys show deep ulcers in more that 20% of a minimum sample of 50 fish of any one species* and water samples examined in a VDH recognized laboratory reveal a **signficant** number of PCOs by light microscopy. There is no evidence of a fish kill as described above.

Undo Criteria: Fish surveys show deep ulcers in less than 20% of a minimum sample of 50 fish of any one species* and water samples examined in a VDH recognized laboratory reveal an **insignificant** number of PCOs. There is no evidence of a fish kill as described above.

OTHER WATERS

General Advisory: Look for posted signs and follow the advise on them. Do not swim in water in which there are dead or dying fish or that looks stagnant, muddy or smells unpleasant. Try to avoid swallowing river, stream, bay or lake water, especially if you are immunocompromised. Prevent broken skin from directly contacting natural bodies of water. Bathe skin with soap and water after contact with natural bodies of water. Do not eat fish or shellfish that have sores or do not appear healthy or have been taken from areas with large numbers of dead and dying fish (fish kills). Report symptoms of illness that you believe are related to contact with water to your physician, your local health department or the toll-free Pfiesteria line (888-238-6154).

*If during follow-up monitoring less than 50 fish of a species are collected, a determination of whether to act will be made after consultation with the Task Force.

APPENDIX C

PFIESTERIA RESPONSE CONTACT LIST

Pfiesteria Response Contact List

VESSEL AVAILABILITY FOR INITIAL RESPONSE

Virginia Marine Resource Commission.

Twenty-four hour toll-free operations number, 1-800-541-4646.

Virginia Department of Game and Inland Fisheries.

Gary Martel, (804-367-1004) or regional fisheries biologists.

VHD Division of Shellfish Sanitation:

Norfolk Office (757-683-8461)
Accomack County Office (757-787-5864)
White Stone Office (804-435-1095)

PFIESTERIA COMPLEX ORGANISM ANALYSIS AND VERIFICATION

Old Dominion University:

Dr. Harold Marshall, (757-683-4204, hmarshal@odu.edu), Phytoplankton Lab, Mills Godwin Building, Norfolk, 23529

Virginia Institute of Marine Science, College of William and Mary:

Dr. Larry Haas, (804-684-7258, lhaas@vims.edu)

FISH PATHOLOGY

Virginia Institute of Marine Science, College of William and Mary

Dr. Wolfgang Vogelbein, VIMS Aquatic Animal Disease Diagnostic Center (daytime 804-642-7261; after hours 804-642-9522, wolf@vims.edu)

David Zwerner (804-642-7249), Waterman's Hall, Gloucester Point.

VIRGINIA DEPARTMENT OF HEALTH

Dr. Suzanne Jenkins, State Epidemiologist (804-786-6029, sjenkins@vdh.state.va.us).

APPENDIX D

CHAIN OF CUSTODY FORM

DEPARTMENT OF ENVIRONMENTAL QUALITY - CHAIN OF CUSTODY REPORT

Organization:

Date:

River:

Collected by (signature): _____ _____ _____ _____

River and Atmospheric Condition (daily temp./wind speed & direction/visual appearance/distressed fish/abnormal appearance)-

_____

_____

_____

Sample Location	Sample Time	Sample Depth	Phytoplankton Preserved	Phytoplankton Unpreserved	Other Samples collected	Physical Data Depth Profile	DO mg/L	Salinity ppt	Temp °C	Secchi Depth	Fish Kill Occurring #'s & Type

Relinquished by: Date: Time: Note:

Received in the field by: Date: Time: Note:

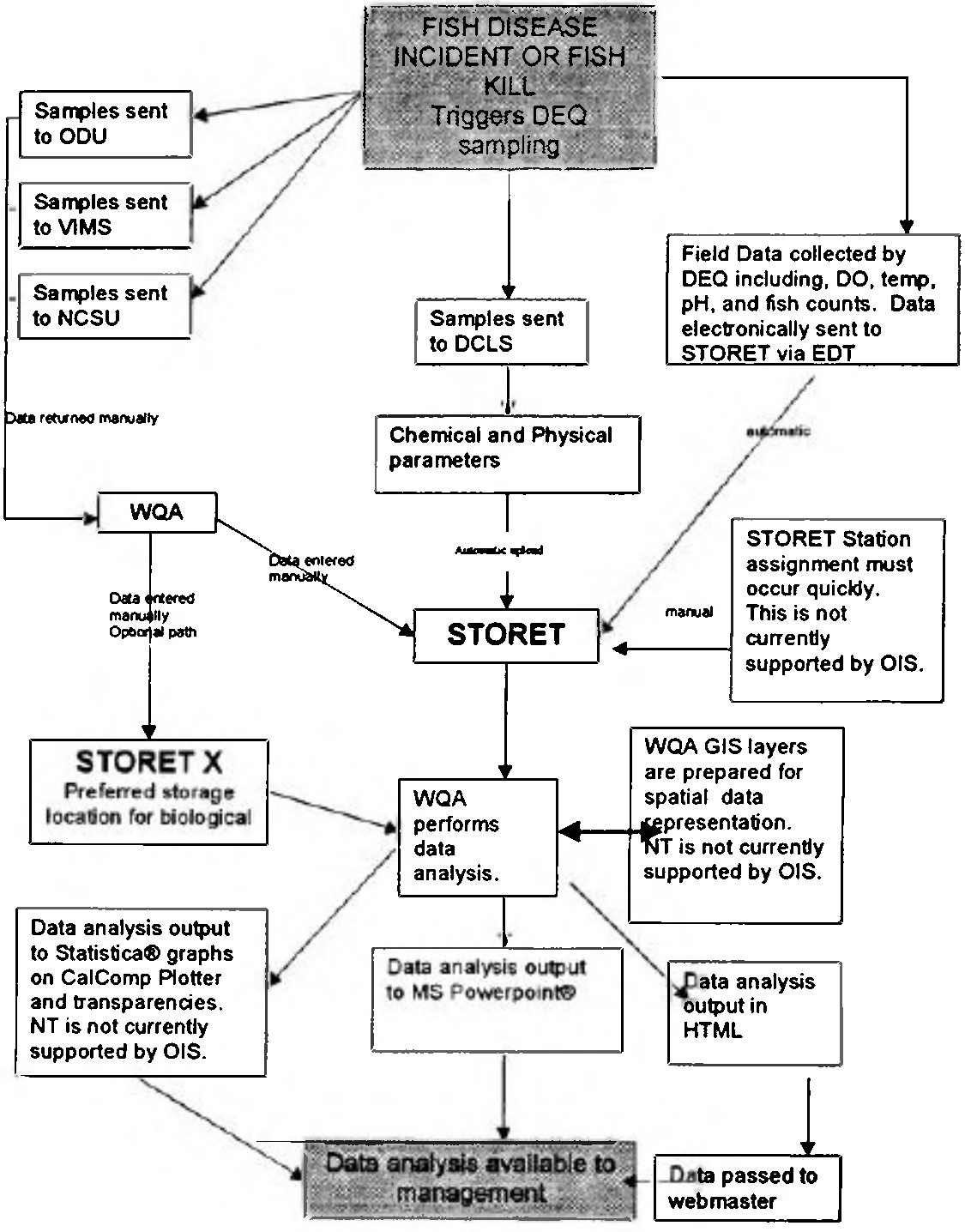
Relinquished by: Date: Time: Note:

Received into the lab by: Date: Time: Note:

APPENDIX E

DATA FLOW CHART FOR *PFIESTERIA* ANALYSIS

NOTE: This flow chart may not print - contact DEQ for a copy.


FISH DISEASE INCIDENT OR FISH KILL Triggers DEQ sampling
Samples sent to ODU
Samples sent to VIMS
Samples sent to NCSU
Samples sent to DCLS
Field Data collected by DEQ including, DO, temp, pH, and fish counts. Data electronically sent to STORET via EDT
Data returned manually
Chemical and Physical parameters
automatic
WQA
Data entered manually
Automatic upload
STORET Station assignment must occur quickly. This is not currently supported by OIS.
Data entered manually Optional path
manual
STORET
STORET X
Preferred storage location for biological
WQA performs data analysis.
WQA GIS layers are prepared for spatial data representation. NT is not currently supported by OIS.
Data analysis output to Statistica® graphs on CalComp Plotter and transparencies. NT is not currently supported by OIS.
Data analysis output to MS Powerpoint®
Data analysis output in HTML
Data analysis available to management
Data passed to webmaster

APPENDIX F

PFIESTERIA RESPONSE PROCESS

COMMONWEALTH OF VIRGINIA
PFIESTERIA RESPONSE PROCESS

INITIAL REPORTS

- ❑ Work Hours - Calls handled at DEQ Regional Offices
  - ✳ Tidewater Regional Office (757-518-2000)
  - ✳ Piedmont Regional Office (804-527-5020)
  - ✳ Northern Regional Office (703-583-3800)

- ❑ After Hours - Calls received at DES (1-800-468-8892)
  - ✳ DES Dispatcher Pages on-call DEQ Responder

- ❑ DEQ Responder decides if field investigation is needed

INITIAL FIELD INVESTIGATION (if needed)

- ❑ DEQ Responder notifies VMRC and arranges field meeting

- ❑ VMRC and DEQ conduct initial field investigation
  - ✳ D.O., pH, and temperature measurements
  - ✳ Visual observations
  - ✳ VMRC and DEQ collect water quality and PCO samples

- ❑ DEQ decides if field response is needed

FIELD RESPONSE (if needed)

- ❑ DEQ On-Scene Coordinator is notified
  - ✳ Activates DEQ Response Team(s) as needed
  - ✳ Notifies Task Force, Secretariats, Local Authorities etc.
  - ✳ Coordinates activities on-site

- ❑ DEQ Response Team(s) collect data
  - ✳ Fish Lesion Counts
  - ✳ Fish Samples sent to VIMS for analysis
  - ✳ PCO Samples sent to ODU for analysis
  - ✳ Water Quality Samples sent to DCLS for analysis

- ❑ All Data sent to VDH as quickly as possible

- ❑ VDH evaluates data and decides action level
  - ✳ Yellow Light - Public Advisory issued
  - ✳ Red Light - water body closed or restricted

- ❑ VMRC enforces water body restrictions

- ❑ VDH decides status changes & handle press relations

FINAL REPORT

USING BENTHIC MACROINVERTEBRATES TO IDENTIFY CAUSES OF FISH KILLS IN THE SHENANDOAH RIVER (2006 and 2007)

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EXECUTIVE SUMMARY

Detailed studies of benthic macroinvertebrates in the Shenandoah River and its tributaries were done in 2006 and 2007. A total of 324 benthic samples were taken, and a total of 179,360 macroinvertebrate organisms were identified and enumerated. The overall purpose of these studies was to determine what the macroinvertebrate assemblage indicated about the biological condition of the Shenandoah River watershed in relation to the fish kills that have been occurring since 2004. There were two major questions: (1) do macroinvertebrate assemblages differ spatially within the watershed; and (2) what environmental variables are responsible for observed differences in macroinvertebrate assemblages? It was anticipated that comparisons of macroinvertebrate assemblage data with measurements of environmental variables would suggest testable hypotheses for causes of the fish kills.

In 2006, the study was confined to "large river" sites on the Shenandoah River and its major tributaries, with one site in the James River basin. In 2007, an intensive study of many smaller tributaries representing subwatersheds of the Shenandoah River was undertaken to examine the effects of specific land uses and environmental stressors. A lesser number of large river sites were also studied in 2007. The observed biological condition of the Shenandoah River, as indicated by benthic macroinvertebrates, was also compared to that of two other similar rivers that support a smallmouth bass fishery in the mid-Atlantic region. Some information about long-term temporal changes in the biological condition of the Shenandoah River was obtained by comparing the present benthic macroinvertebrate assemblages with those observed about 40 years ago. There were eight specific objectives, which are listed below along with a summary of the most important findings.

1) Characterize the benthic macroinvertebrate assemblages in the large river sections of the Shenandoah River system and analyze for differences and similarities in biological condition among the sections.

The benthic macroinvertebrate assemblage was sampled at 11 large river sites in May and August 2006 and 8 sites in May 2007. Nine sites were located where fish kills had occurred previously, and the other two sites were designated as reference sites because there had been no fish kills as of 2006. The fish kill sites were in the Shenandoah River watershed and included sites on the Main Stem (1), North Fork (4), South Fork (3), and major South Fork tributaries (South River, 1; North River, 1). One reference site was in the Shenandoah River watershed (Cedar Creek), and one was in the James River watershed (Cowpasture River). Six replicate samples were taken at each site on all dates. The assemblage was analyzed in terms of the presence of taxa, abundance of taxa, and metrics that summarize the structure and function of the assemblage. Analyses included counts and biomass of invertebrates.

Combining May and August 2006 samples at all sites, 116 taxa were collected. The numbers and kinds of benthic macroinvertebrate taxa do not appear to be unusual at any of the sites that were investigated in 2006. The overall abundance of organisms was very high at sites in the Shenandoah River watershed. The two reference sites (Cowpasture and Cedar) had more taxa than other sites. Two sites on the South Fork (Whitehouse and Front Royal) had somewhat

fewer taxa than the other Shenandoah River sites. Many of the dominant taxa are fairly sensitive to environmental stress.

The taxonomic composition of the benthic macroinvertebrate assemblage was analyzed simultaneously among sites using ordination, a multivariate method used to assess similarities among sample sites with respect to macroinvertebrate composition and density. In addition, the benthic macroinvertebrate assemblage data were condensed into 11 metrics that represented different ecological characteristics. Univariate ANOVA tests were performed for each metric among all sites. The multivariate and univariate techniques both indicated that the two reference sites were different from the nine fish kill sites, with slightly better biological condition at the reference sites. The Cowpasture River assemblage was more strongly different from the other sites than Cedar Creek. When data from the New River were added to the analyses, the benthic macroinvertebrate assemblage there was different from the assemblage at any of the Shenandoah or Cowpasture sites; however, the macroinvertebrate assemblage did not indicate any appreciable difference in the biological condition of the New River. There was no grouping or similarity pattern among site groups that emerged from the analyses. Since the greatest number of fish kills in 2006 occurred in the lower North Fork (Woodstock, Strasburg), it was hypothesized that the macroinvertebrate assemblage at the North Fork site grouping might be different from the other site groupings, but the benthic macroinvertebrate results do not support that supposition. Analyses of benthic macroinvertebrate assemblages have not distinguished any sites on the Shenandoah River with significant reduction in biological condition, nor suggest that any Shenandoah River sites are significantly different from other sites in the basin.

2) Determine the environmental variables responsible for benthic macroinvertebrate assemblage structure in the large river sections.

Ordination (DCA and CCA) and linear regression both suggested that nutrients and substrate conditions influenced macroinvertebrate assemblages in large rivers although no sites appeared differentially influenced compared to others. Available environmental variables at the large river sites included nutrients, periphyton, substrate composition, heavy metals, and the various chemicals measured with passive samplers. There were an appreciable number of significant relationships with moderately strong coefficients of determination. Most of the significant and strong relationships were with various measures of nitrogen and phosphorus compounds, suggesting nutrient concentration as a primary determinant of assemblage structure and function.

Various forms of nitrogen and phosphorus nutrients were positively related to several top taxa densities in many cases. *Optioservus*, in particular, was significantly less abundant in the presence of high nitrate concentration. Conversely, it is possible that nutrient enrichment stimulates primary production which, in turn, stimulates secondary production and invertebrate abundance. Similarly, substrate size and suitability for epilithic primary production appears to be important as quantified by relationships to AFDM and substrate size (*i.e.*, cobble). We speculate that epilithic primary production is stimulated by nutrient enrichment and cascades upward trophically to influence the macroinvertebrate assemblage at sites where nutrient concentrations are higher. In addition, more epilithic primary production creates more microhabitat on rock surfaces for macroinvertebrates.

Heavy metal and passive sampler results were inconclusive, which is at least partially an artifact of small sample sizes available for statistical analyses.

3) Characterize the benthic macroinvertebrate assemblages in a set of subwatershed tributaries that reflect the predominant types of land use within the Shenandoah River system and analyze for differences and similarities in biological condition among the subwatersheds.

After sampling the large river sites in 2006, it became apparent that it would be very difficult to explain any observed differences in benthic macroinvertebrate assemblage structure because all the large river sites are subjected to all of the land uses and all of the potential environmental stressors in the Shenandoah River basin. Thus, we decided to conduct an intensive study of many smaller tributaries representing subwatersheds of the Shenandoah River that could be categorized according to predominant land uses. We speculated that by narrowing the spatial scale of analysis within the Shenandoah River basin we might be able to pinpoint impairment or influence at a smaller more manageable spatial scale or scope. Twenty-six Shenandoah River tributaries were selected using GIS, and various land use variables were calculated, including aspects such as: wetland, forest, pasture/hay, crops, developed land, dairies, beef operations, poultry houses, acres in nutrient management, animal feeding operations, and municipal sewage treatment plants.

Preliminary DCA ordination analysis showed that Passage Creek was a major outlier, being different from other tributary sites based on abundances of *Prosimulium*, a taxa not found in other streams. We removed this site and taxa from a subsequent DCA analysis which allowed us to use a higher level of resolution in detecting differences and relationships between site separation and taxa. The resulting DCA clearly distinguished groups of sites that were characterized by different key taxa. One group of sites was characterized by abundant Planariidae and *Cheumatopsyche*. A second group was comprised of a disproportionate number of Chironomidae. A third group was numerically dominated by *Chimarra*, *Ephemerella*, and *Macaffertium/Stenonema*.

Statistical analyses suggest that tributary sites within the Shenandoah River basin are somewhat distinct based on macroinvertebrate densities. Further, sites likely differ with respect to environmental conditions shown to be significantly related to taxonomic structure.

4) Determine the types of land use and environmental variables responsible for benthic macroinvertebrate assemblage structure in the subwatersheds.

The most informative results of these studies came from using linear regression to assess relationships between macroinvertebrate metrics and top taxa with land use and environmental variables. Regressions supported ordination results and this combination of statistical conclusions strengthens our findings. There were many significant regressions, with macroinvertebrates usually responding positively to increased forest cover and negatively to increases in agricultural or developed area. Non-insect taxa, however, were more abundant in less forested areas having higher agricultural activity in the watershed. The number of dairy farms, cattle farms, and poultry houses was frequently a predictor of reduced biological

condition as quantified by macroinvertebrate metrics. There was not a clear trend for any one of these agricultural land uses to be more important than any others. In addition to summary metric responses, several top taxa responded significantly to land use variables. Planariid worms demonstrated the clearest responses: positively to cropland and negatively to forest cover. Similarly, planariid worms responded positively to poultry houses, animal feeding operations, and nutrient management plans for poultry waste.

A large number of environmental variables were also compared to summary macroinvertebrate metrics and top taxa in tributary streams, which produced an appreciable number of significant and moderately strong relationships. Several forms of nitrogen and phosphorus produced the most relationships. Typically, higher concentrations of nutrients induced a negative taxonomic response, except for planariids, which responded positively. Generally, higher nutrients led to reduced taxa richness, EPT, sensitive taxa, and clingers.

Heavy metals in sediments and clam tissue were also related to macroinvertebrate structure and function as quantified by metrics and top taxa abundance. The most ecologically and statistically significant results showed that higher clam and sediment lead concentrations predicted negative responses in several metrics.

5) Compare the observed biological condition of the large river sections of the Shenandoah River system, as indicated by benthic macroinvertebrates, to that of some other similar rivers that support a smallmouth bass fishery in the mid-Atlantic region.

We were able to make reasonable comparisons to the New River in West Virginia and the Susquehanna River in Pennsylvania. Both drain larger watershed areas than the Shenandoah River, but they have similar rocky bottoms, support smallmouth bass populations, and have not suffered fish kills like the Shenandoah River. Summary taxa lists from the three rivers were analyzed with Jaccard's coefficient, and the benthic macroinvertebrate fauna of the Shenandoah River was not very similar to that of either the Susquehanna or New Rivers, which were also not similar to each other. However, the nature of the difference in the fauna does not indicate that the biological condition of the Shenandoah River is bad. There are appreciably more taxa in the Shenandoah River (68) than the Susquehanna (43) or New (35). Many of the additional taxa in the Shenandoah belong to the insect orders Ephemeroptera, Plecoptera, and Trichoptera, which are mostly comprised of sensitive species. In summary, comparison of the benthic macroinvertebrates in the Shenandoah River to two other somewhat similar rivers in the mid-Atlantic region provides no evidence that the biological condition of the Shenandoah is lower than what would be expected.

6) Compare the observed biological condition of the large river sections of the Shenandoah River system, as indicated by benthic macroinvertebrates, to the historical biological condition of the same sections.

In order to consider long-term temporal differences in the biological condition of the Shenandoah River, we made comparisons to benthic macroinvertebrate data collected by Eugene Surber in the 1960s. He used quantitative methods that made it possible to compare results at seven large river sites sampled in 2006: three on the South Fork (Lynnwood, Whitehouse, Front

Royal), three on the North Fork (Mt. Jackson, Woodstock, Strasburg), and one on the Main Stem (Berryville). Seven of eleven assemblage-level metrics were significantly different between 1960s and 2006 data, and six of those seven metrics indicated better biological condition in 2006. The Bray-Curtis coefficient showed little to moderate similarity between 1960s and 2006 assemblages because there were higher densities and more taxa collected in 2006. Some of the additional taxa are sensitive to environmental stressors, and many of the taxa with much higher densities in 2006 are ones that benefit from the additional algae and fine detritus associated with increased nutrients. The number and types of taxa found in 2006 suggest improvemed biological condition relative to the 1960s.

7) Determine benthic macroinvertebrates that can be identified reliably and feasibly to the species level and can serve as indicator species for the range of biological condition observed in the Shenandoah River system.

We thought that a group of related benthic macroinvertebrates that could be identified to species might demonstrate differential, predictable responses to environmental stressors in the Shenandoah River better than the entire assemblage identified to genus and higher taxonomic levels. Adult riffle beetles (Elmidae) were common in the benthic samples and could be reliably identified, and, thus, were selected as the best candidate species.
In total we collected 14 species of adult elmid beetles. We conducted similar analyses as we did with assemblage-level information including ordination and regression. Elmid adults were useful in detecting differences among sites and relationships with land-use and environmental variables. However, differences among sites generally agreed with assemblage-level analyses, and regression relationships were generally weaker with elmid species data. It does not appear that increased taxonomic resolution of elmid species provides any additional information about the biological condition of the Shenandoah River and its tributaries.

8) Conduct *in situ* toxicity tests with the Asian clam (*Corbicula fluminae*) in the large river sections.

Results from the in-situ bioassays with the Asian clam in June and August 2006 showed significantly lower growth and significantly higher mortality at some sites in comparison to reference sites. However, the sites with significantly different growth or mortality did not follow a consistent spatial pattern and did not correspond with the sites with the greatest fish kills in 2006.

Conclusions

Analyses of benthic macroinvertebrate assemblages have not distinguished any large river sites in the Shenandoah basin with significant reduction in biological condition. The benthic macroinvertebrate assemblages exhibit very high densities and a great deal of taxonomic richness. There are some significant differences among sites, but there are no spatial patterns that correspond with a particular section, such as a fork, or to areas where fish kills have been more prevalent. The benthic macroinvertebrate assemblages at the large river sites fall within the range of what would be expected in similar rivers in the mid-Atlantic region and our comparisons with data from the 1960s show that biological condition has improved temporally.

However, the benthic macroinvertebrate assemblages have become somewhat out of balance, both taxonomically and ecologically. The taxa with exceptionally high densities show strong relationships with various forms of nitrogen and phosphorus that act as nutrients for plant growth. It is likely that high nutrients provide abundant food for macroinvertebrates that are scrapers, collector-filterers, and collector-gatherers. In addition, heavy growth of plant material on solid stable substrate creates excellent microhabitat for macroinvertebrates. While the present benthic macroinvertebrate assemblages at the large river sites do not indicate much, if any, impairment of biological condition, further increases in nutrient concentrations will eventually lead to lower dissolved oxygen, and reduced quantity and quality of food and microhabitat.

Moreover, the results of our benthic macroinvertebrate assemblage analyses at large river sites suggest that macroinvertebrate assemblages are not experiencing any influences similar to fishes. These results lend support to the hypothesis that the fish kills are primarily being caused by a factor specific to fish, probably a biological pathogen. However, results of fish pathology studies seem to indicate a diverse array of fish health problems, including parasites such as trematodes. Some trematodes use snails as intermediate hosts, and snails are one of the taxa whose density has increased greatly and is strongly related to increased nutrients. Other numerically dominant macroinvertebrates may also be involved in the life cycles of trematodes and other parasites that eventually infect fish.

Analyses of tributary streams according to subwatersheds were more informative than the large river studies for elucidating the factors responsible for macroinvertebrate assemblages. Unlike the large river sites, the biological condition of tributary sites ranged from good to poor. Assemblages in these smaller tributaries were strongly related to agricultural land use, including dairy, beef, poultry, and crops. Nutrients derived from land use were the driving force for determining macroinvertebrate assemblages. There was no clear evidence that toxic contaminants of any kind were a major influence on the benthic macroinvertebrate assemblages. It was obvious in some tributary streams that high nutrients had caused an appreciable decrease in biological condition. If nutrient concentrations continue to increase in more tributary streams, the impaired biological condition of the tributaries will eventually be manifested in the large river sections of the Shenandoah basin.

INTRODUCTION

Each spring since 2004, extensive fish kills have occurred in the Shenandoah River drainage. These fish kills have tended to occur at low rates but have lasted for extended periods over great distances. The magnitude of the fish kills has varied spatially each year. In 2004, the fish kills affected nearly the entire length of the North Fork of the Shenandoah River. In 2005, over 100 miles of the South Fork of Shenandoah River were impacted. In 2006, the fish kills were focused in the North Fork, but also occurred in a portion of the South River and the main stem Shenandoah River. In 2007, fish kills spread to the James River basin, including the Cowpasture River, Maury River, and main stem James, and also occurred throughout the Shenandoah River.

Benthic macroinvertebrates are key organisms for assessing the biological condition of freshwaters because: (1) there are many species within the natural assemblages of streams that

fill a wide variety of ecological niches and perform important ecosystem functions, especially in large, relatively shallow, rocky streams such as the Shenandoah River; (2) different species demonstrate a wide range of sensitivity to stressors; (3) they tend to be largely sedentary organisms that do not move away from sudden episodes of poor water quality or toxic substances; (4) their life history is sufficiently long (most require about 1 year for development from egg to adult) that their absence will be noticeable with a reasonable sampling schedule (*e.g.,* biannually); (5) some species are intermediate hosts for important fish pathogens that occur in some Shenandoah River fishes; and (6) their small body size and habitat spatial scale make it possible to take replicate samples, which facilitates detailed statistical analyses for elucidating relationships between organisms and environmental variables.

Beginning March 1, 2006, scientists at Virginia Tech were contracted by the Virginia Department of Game and Inland Fisheries (DGIF) to make a broad assessment of benthic macroinvertebrates in the Shenandoah River basin in coordination with other studies being conducted to explain the fish kills. The overall purpose of this study was to determine what the macroinvertebrate assemblage indicated about the biological condition of the Shenandoah River watershed. The two major questions were: (1) do macroinvertebrate assemblages differ spatially within the watershed; (2) what environmental variables are responsible for observed differences in macroinvertebrate assemblages? It was anticipated that comparisons of macroinvertebrate assemblage data with measurements of environmental variables would suggest testable hypotheses for causes of the fish kills. This report includes analyses and interpretations of samples taken in 2006 and 2007. In 2006, the study was confined to "large river" sites on the Shenandoah River and its major tributaries, with one site in the James River basin. In 2007, an intensive study of many smaller tributaries representing subwatersheds of the Shenandoah River was undertaken to examine the effects of specific land uses and environmental stressors. A lesser number of large river sites were also studied in 2007. The observed biological condition of the Shenandoah River, as indicated by benthic macroinvertebrates, was also compared to that of two other similar rivers that support a smallmouth bass fishery in the mid-Atlantic region. Some information about long-term temporal changes in the biological condition of the Shenandoah River was obtained by comparing the present benthic macroinvertebrate assemblages with those observed about 40 years ago.

The specific objectives of this study were to:

1. Characterize the benthic macroinvertebrate assemblages in the large river sections of the Shenandoah River system and analyze for differences and similarities in biological condition among the sections;
2. Determine the environmental variables responsible for benthic macroinvertebrate assemblage structure in the large river sections;
3. Characterize the benthic macroinvertebrate assemblages in a set of subwatersheds that reflect the predominant types of land use within the Shenandoah River system and analyze for differences and similarities in biological condition among the subwatersheds;
4. Determine the types of land use and environmental variables responsible for benthic macroinvertebrate assemblage structure in the subwatersheds;

5. Compare the observed biological condition of the large river sections of the Shenandoah River system, as indicated by benthic macroinvertebrates, to that of some other similar rivers that support a smallmouth bass fishery in the mid-Atlantic region.
6. Compare the observed biological condition of the large river sections of the Shenandoah River system, as indicated by benthic macroinvertebrates, to the historical biological condition of the same sections;
7. Determine benthic macroinvertebrates that can be identified reliably and feasibly to the species level and can serve as indicator species for the range of biological condition observed in the Shenandoah River system;
8. Conduct *in situ* toxicity tests with the Asian clam (*Corbicula fluminae*) in the large river sections.

METHODS

Study Sites

Large river

A field reconnaissance was conducted throughout the North Fork, South Fork, and Main Stem of the Shenandoah River in April 2006 to establish benthic macroinvertebrate sampling locations and develop sampling protocols. Nine benthic macroinvertebrate sampling sites were established in riffle areas in the immediate vicinity of previous fish kills and near locations where the Virginia Department of Environmental Quality (DEQ) was taking water quality samples. In addition, "reference" sites (sites where there had been no fish kills) were established on Cedar Creek and Cowpasture River. The locations of these sites are shown in Fig. 1, and information about geography, watershed size, and abbreviations for site names is summarized in Table 1.

Prior to the fish kills that began in 2004, no comparable benthic macroinvertebrate data were available from our fish kill sites, and as such, no pre-fish kill data were available for comparison. Therefore, we chose to use similar sites where there have been no reported fish kills as of 2006 as reference sites for comparison to the fish kill sites. The selection of reference sites for this study was challenging because locations in the basin where there had been no reported fish kills drain smaller watershed areas than fish kill sites. Based on discussions with DEQ, DGIF, and our best professional judgment, a large tributary to the North Fork Shenandoah (Cedar Creek) and the Cowpasture River in the James River basin were selected as reference sites. There had been no reported fish kills at these reference sites prior to and during our benthic studies in spring 2006. However, in 2007 and 2008, the fish kills became more extensive and included both reference sites. Thus, Cedar Creek and Cowpasture River can only be considered nominal reference sites.

Previous data from the New River in West Virginia and the Susquehanna River in Pennsylvania were added to our analyses as additional benchmarks for comparison. Also, historical data from the Shenandoah River collected by Eugene W. Surber in the 1960s were used for temporal comparisons.

Tributaries

After sampling the large river sites in 2006, it became apparent that it would be very difficult to explain any observed differences in benthic macroinvertebrate assemblage structure because all the large river sites would be subjected to all of the land uses and all of the potential environmental stressors in the Shenandoah River basin. Thus, we decided to conduct an intensive study of many smaller tributaries representing subwatersheds of the Shenandoah River that could be categorized according to predominant land uses. Twenty-five Shenandoah River tributaries were were selected using GIS, ranked based on poultry operations, dairy operations, and sewage treatment plants (STPs) within the watershed. Flowlines for the entire Shenandoah River watershed were obtained from the National Hydrography Dataset. The 78 subwatersheds (6th level Hydrologic Unit) for the Shenandoah River in Virginia were obtained from the U.S. Department of Agriculture National Resources Conservation Service. These subwatersheds range from 9,800 to 39,800 acres in size, and typically represent the watershed of individual tributaries draining into the Shenandoah River. Locations of permitted poultry operations and STP discharges were obtained from DEQ, and locations of confined animal feeding operations were obtained from the Virginia Department of Conservation and Recreation (DCR). The subwatersheds were ranked for poultry operations based on both the number of poultry houses and the percentage of acreage in a nutrient management plan for poultry litter. Each poultry category was assigned a score of 1-5 based on quantile ranges of the data , and the scores were added to create the final poultry rank (2-10). The dairy operation rank (1-5) was determined using quantile ranges of the number of dairies in each subwatershed. The STP rank for each subwatershed was based on the presence or absence of a discharge. Subwatersheds were first classified based on the poultry rank, followed by dairy, then STP presence or absence. Reference sites (4) had the lowest poultry and dairy ranks and no STP discharges. Downstream (DS) sites typically drained one more subwatershed than the upstream sampling site on the same tributary. These sites were targeted based on the addition of one or more STP discharges. Specific ranks were not calculated for these sites, but the influence of confined animal feeding operations (CAFO) was anticipated to be similar to the upstream sites. The factorial sampling design is presented in Fig. 2. Finally, the land use for each of the 25 tributaries was quantified in more detail by calculating 20 variables using GIS and information from DEQ and DCR. All tributary sampling sites are shown in Fig. 3, and tributary names and site codes are listed in Table 2. Land use variables and codes are listed in Table 3.

Benthic Macroinvertebrate Sampling

In 2006, we sampled exclusively at large river sites. Benthic macroinvertebrates were sampled in spring 2006 during high baseflow conditions (May 11-13) and late summer 2006 during low baseflow conditions (August 15-17). In 2007, we sampled smaller tributaries between March 24 and April 23 and large river sites between May 22 and May 24. All sampling was stratified to riffle areas and was quantified. Six replicate benthic samples were taken with a D-frame dip net (500 µm) by disturbing a standard area of stream bottom (0.09 m^2). Contents of the net were preserved with 95% ethanol.

In the laboratory, benthic samples were sorted in their entirety (no subsampling) and individuals were identified (mostly to genus), enumerated, and measured to the nearest mm. Published

length-mass regressions were used to estimate invertebrate biomass in each sample (Benke *et al.* 1999). Specimens of emergent adult mayflies, adult riffle beetles, and snails were identified to species.

Environmental variables

Periphyton

During the August 2006 benthic collection, periphyton samples were collected from riffles in conjunction with benthic macroinvertebrate samples. Six cobbles were randomly collected from each study site and frozen until analysis. In the laboratory, periphyton was removed from each cobble with a wire brush and deionized water, and the resultant slurry was subsampled for chlorophyll *a* and epilithic biomass analysis. Epilithic subsamples were split and analyzed for chlorophyll *a* and epilithic biomass. The epilithic fraction was filtered onto preweighed glass fiber filters (0.45-µm) and dried to a constant weight at 60°C. After dry weights were obtained, filters were ignited at 550°C for 24 hours, desiccated, and reweighed to obtain ash-free dry mass (AFDM). Chlorophyll *a* was extracted with 90% acetone and then analyzed with a spectrophotometer after correcting for pheophytin following the methods of Lorenzen (1967).

Inorganic substrate

Field estimates of inorganic substrate and deposited sediment occurred in conjunction with the August 2006 benthic collection. Bottom substrate was visually estimated within a 1 m^2 area of streambed at areas immediately adjacent to benthic macroinvertebrate sample locations. The proportion of stream bottom composed of one of four standard size classes (cobble, pebble, gravel, sand) was estimated. Additionally, the proportion of the stream bottom surface covered by fine deposits was visually estimated.

Chemistry

At large river sites, DEQ measured an array of water quality parameters that could potentially act as stressors to the biota (primarily pH, temperature, and various forms of nitrogen and phosphorus). All analyses were performed on samples from the water column. Water quality samples were taken frequently during spring and summer of 2006 and 2007 from all large river sites where macroinvertebrates were sampled. A random temporal sampling design provided water quality measurements from each site approximately every other day during the five usual working days (no weekend samples). For tributary sites sampled in 2007, DEQ provided all available data from their regular ambient water quality sampling program. We screened those data and used only those that were close enough spatially and temporally to provide meaningful comparisons with our macroinvertebrate data. After screening the DEQ data, there were no water quality data available for some of the tributary sites where benthic macroinvertebrate samples were collected. For comparison to large river and tributary macroinvertebrate data, which were collected on only a few dates in 2006 and 2007, it was necessary to condense the water quality data, which were collected on multiple dates, to a single value for each macroinvertebrate sampling date. Using the available data (which varied with each 'sample set') we calculated the average parameter value for the 2-week period preceding sample collection

and the maximum parameter value during the same period to generate two metrics (mean and maximum) for each water quality parameter.

In 2007, other variables were measured at the large river and tributary sites as part of a separate grant from the Virginia Environmental Endowment, including body burdens of metals in mollusk tissue, sediment metal concentrations, and estrogenic activity in sediment. Asian clams were collected during the spring sampling period for analysis of heavy metal body burdens (As, Cd, Cr, Pb, Se, and Hg). Immediately after collection, clams were placed in well water for 24 hr in order to clear their gut contents. The well water was tested by the Virginia Tech Soils Testing Laboratory for concentrations of the metals of interest and all were below the instrument detection limits. After 24 hr, the mollusks were killed by freezing. Prior to analysis, shells were removed and the tissue was freeze dried and homogenized. One sample was analyzed per site; the number of organisms pooled varied according to their size (average of 20). Clam tissue was analyzed at the College of William and Mary, through an agreement with the Virginia Institute of Marine Science (VIMS). The same laboratory analyzes metals in fish tissue for the DEQ. Results are reported as µg/g dry wt.

Sediment was collected from depositional areas of each sampling site for analysis of 23 target analyte list (TAL) metals. Sediment was collected using a stainless steel scoop, homogenized in a stainless steel pot, and placed in pre-cleaned glass jars. Sediment samples were analyzed by Hampton-Clarke Veritech Labs in Fairfield, NJ. Results are reported as mg/kg dry weight (equivalent to µg/g).

Sediment was also analyzed for estrogenic activity using a bioluminescent yeast estrogen screen (BLYES). Yeast (*Saccharomyces cerevisiae*) is bioengineered to contain the human estrogen receptor, plasmid-bound estrogen response elements, and plasmid-bound luminescence reporters (Sanseverino et al 2005). When an estrogen-like compound binds to the receptor, the luminescence reporters produce light. Specific compounds are not quantified, but the data provide an indication of the potential for sediment constituents to contribute to endocrine disruption in organisms. Sediment was collected as stated above and was freeze-dried and homogenized prior to analysis. Sediment was extracted with solvents, and the extracts were subjected to solid phase extraction (SPE) to remove some of the co-extracted organic material. The SPE extract was subjected to the BLYES assay. The assay was performed at the U.S. Geological Survey (USGS) laboratory in Leetown, WV, using a protocol developed at the facility with permission from the Center for Biotechnology at the University of Tennessee. Standards of 17β-estradiol (E2) were analyzed and used to construct a standard curve. Data are reported as pg/g dry wt. estradiol equivalents (E2Eq). Additionally, dried sediment was analyzed for concentrations (%) of total organic carbon (TOC) and total nitrogen (TN) by the Nutrient Analysis Laboratory at VIMS.

In 2007, DEQ provided data from passive samplers at most of the large river sites, except Mt. Jackson and Woodstock on the North Fork (DEQ 2008). Two types of passive samplers were deployed at each site. Semipermeable membrane devices (SPMDs) were deployed to sequester hydrophobic organic chemicals, and polar organic chemical integrative samplers (POCIS) were deployed to sequester more hydrophilic polar organics. Samplers were deployed for 42 days from late March to early May.

Comparison to Other Similar Large Rivers

In addition to examining for differences in benthic macroinvertebrates within the Shenandoah River, it was also the objective of this study to consider the biological condition of the Shenandoah River in comparison to other similar bodies of water in the mid-Atlantic region. It is difficult to find comparable data for large rivers, but we were able to make reasonable comparisons to the New River in West Virginia (Voshell et al. 1990, 1991) and the Susquehanna River in Pennsylvania (Jackson et al. 1994).

Comparison to Historical Shenandoah River Data

In order to consider the trend in the biological condition of the Shenandoah River over time, we made comparisons to benthic macroinvertebrate data collected by Eugene W. Surber in the 1960s (Surber 1965, 1967, 1969). He used quantitative methods that made it possible to calculate and compare the same measures of assemblage structure and function based on density that we used in this study as well as biomass. Surber reported biomass as wet weight determined from specimens recently blotted to remove excess fluid. We divided his wet weights by 4.12 to convert them to comparable dry weights determined in this study (Mason et al. 1983).

Indicator Species

Concurrent with May 2006 benthic sampling, recently emerged subimago and adult mayflies were collected from streamside vegetation. Representative snails were also hand-picked from stones at each study site for consideration as indicator species. All of these specimens were preserved separately in 70% ethanol for later identification to species in the laboratory. Adult riffle beetles (Elmidae) contained in the replicate benthic macroinvertebrate samples were also identified to species and enumerated.

In-Situ Toxicity Tests

Approximately 700 Asian clams, 8-12 mm in length, were collected from the New River at Eggleston, Giles Co., VA. As clams were collected, they were placed in a holding tank at the Freshwater Mussel Lab, operated by the Department of Fisheries and Wildlife Sciences at Virginia Tech. Clams were prepared for deployment to all study sites on June 20, 2006. Preparation for deployment involved measuring clams and placing them in mesh bags (5 clams per bag, 10 bags per site) (Soucek et al. 2000). Immediately after clams were packaged in mesh bags, they were placed in a cooler with water from the holding tank and driven to the study sites. Two riffles were selected at each study site for placement of mesh bags. A 2.5-ft length of rebar was driven into the streambed at each riffle. Five mesh bags were secured to the rebar. This resulted in 10 mesh bags at each study site, or 50 clams at each site.

The 30-day in situ toxicity test with Asian clams that was initiated on June 25, 2006 evolved into a 60-day study because of high flows throughout July and early August, so results from this test are preliminary. Because of the unsuccessful retrieval of clams after 30 days, a new 30-day in situ test was initiated on August 16, 2006. Again, high flows prevented terminating the second

experiment at 30 days, but we were able to retrieve the Asian clams successfully on September 23 (38 days). Upon retrieval, clams were counted for mortality and measured for growth.

Data Analysis

General descriptors

Data from benthic macroinvertebrate samples were organized into four separate data sets for statistical analysis: Large River Spring 2006, Large River Summer 2006, Large River Spring 2007, and Tributary Spring 2007. Raw data (densities of individual taxa) were summarized into 11 metrics that represent different ecological characteristics of assemblage structure and function (Table 4). For metric calculation, each taxon was assigned a pollution tolerance value (PTV), functional feeding group (mode of acquiring food based on morphology and behavior), and habit (how the organism moves or maintains its position in its environment; also called mode of existence). Assignments to these categories were made based on a synthesis of published literature (*e.g.,* Brigham *et al.*, 1982; Barbour *et al.*, 1999) and 30 years of data and professional experience in the aquatic entomology program at Virginia Tech. PTVs are commonly reported on a scale of 0 to 10, with 0 indicating very tolerant. In this study, taxa with PTVs of 0-2 were considered sensitive while taxa with PTVs of 8 – 10 were considered tolerant.

Ordination

Macroinvertebrate taxa composition was analyzed among sites simultaneously using multivariate methods for each data set. Detrended correspondence analysis (DCA), using PC-ORD (McCune and Mefford, 1999), without downweighting or axis rescaling, was used to ordinate benthic samples in species space using taxa densities. Only numerically dominant taxa (those that comprised > 0.2% of total macroinvertebrate abundance) were included in DCA to reduce variability in the dataset (Gauch, 1982). This analysis was used to determine similarity in taxa composition in benthic samples among the study sites during each sampling period and within each spatial category (within large river sites and within tributary sites).

Similarly, Canonical Correspondence Analysis (CCA) was used where possible to determine which, if any, environmental factors contributed to taxonomic ordination. CCA essentially overlays a second matrix onto the same taxa matrices used in DCA and calculates the percentage of total variance in the dataset explained by the second matrix by axes (typically 2). In some cases, CCA was not possible due to limited environmental data.

Linear regression

Linear relationships between benthic macroinvertebrates (metrics and individual densities for the most abundant taxa) and environmental variables were examined using regression analysis. Environmental variables were treated as independent variables and macroinvertebrate metrics and individual densities for the most abundant taxa were considered dependent variables. Habitat variables (*e.g.*, epileptic material, inorganic substrate) were available only for the August 2006 data. As such, 16 environmental variables, which included watershed size, measures of water chemistry, and habitat, were available to analyze relationships with macroinvertebrates in

August 2006, while only 12 environmental variables (watershed size and water chemistry variables) were available to analyze relationships with macroinvertebrates in May 2006. Environmental variables considered for 2007 large river and tributary data sets included DEQ nutrient data, passive sampler concentrations, and metal concentrations in stream sediments and Asian clam tissue. Regressions were tested for significance with $\propto = 0.05$. We used the coefficient of determination (R^2) for interpreting the strength of relationships in significant regressions. We considered $R^2 > 0.3$ to be ecologically relevant and $R^2 > 0.5$ to be especially meaningful.

Analysis of Variance (ANOVA)

ANOVA and Holm-Sidak tests were performed for each metric and the most abundant individual taxa to analyze differences in assemblage structure and function among the study sites. We hypothesized that the reference sites (Cedar and Cowpasture) might be different from the nine sites where fish kills had occurred during 2004-06 and that the North Fork sites might be different from other sites because the fish kills were greater there in 2006. ANOVA and Holm-Sidak tests compared each site to all other sites and evaluated the hypothesis that at least one site mean for a given metric or taxon differed among the other sites. Using the six replicates collected at each site allowed us to consider all possible groupings.

RESULTS AND DISCUSSION

Large River Assemblage Structure and Function

Taxa present

A list of all taxa collected at each of the 11 study sites in May and August 2006 is presented in Table 5. Combining May and August 2006 samples at all sites, 116 taxa were collected. Of that total, 106 taxa were collected in May versus 82 taxa in August. Thirty-one taxa were collected only in May, while five taxa were collected only in August.

The highest numbers of taxa were collected at the two reference sites: 72 at Cedar Creek and 67 at Cowpasture River. The lowest numbers of taxa were collected at two South Fork sites: 44 at Front Royal and 48 at Whitehouse. The remaining sites, including all of the North Fork sites, had intermediate numbers of taxa that were rather uniform among sites, ranging from 54 to 58. Thirty-five taxa occurred at almost all sites (defined as 10 or 11) in either May or August 2006. Forty-five taxa occurred at only a few sites (defined as 1 or 2) in either May or August 2006.

The presence or absence of particular taxa did not demonstrate any consistent trends among sites, either for individual sites or groupings of sites according to large tributaries, forks, main stem, or reference. This table of data did not reveal any trend for certain taxa to be either present or absent at the North Fork study sites below Burnshire Dam where the most severe fish kills were reported in 2006 (Woodstock and Strasburg).

General descriptors

Initial analyses of density by taxonomic composition to discern patterns among sites were restricted to numerically dominant taxa or 'top taxa'. Data from each analysis set are presented collectively in Table 6. Dominant taxa were determined by ranking the taxa at each site according to density from high to low and including only taxa that comprised more than 0.2% of total density for all taxa at a site. In May 2006, there were 36 top taxa, while in August 2006 there were 28 top taxa. In May 2007, the large river sites had 41 top taxa.

Approximately 60 metrics were calculated to summarize macroinvertebrate assemblages for spring and summer 2006 data (separately). These metrics were categorized according to the following ecological characteristics: density, richness/diversity/evenness, composition, tolerance, trophic, and habits. We eliminated redundant metrics (*i.e*., metrics that summarized similar aspects of macroinvertebrate assemblages) using Pearson product-moment correlation. Redundancy analysis is important to avoid repeating information already summarized by other metrics and to ensure accurate depiction of patterns by multivariate ordination techniques. Eleven metrics emerged as non-redundant, or unique, and were considered in further analyses (Table 4). These eleven metrics were calculated for each sample period: May 2006 (Table 7), August 2006 (Table 8), and may 2007 (Table 9).

Ordination

May 2006. Distinct site separation occurred along two axes in DCA for large river top taxa. Cowpasture River was separated from all other sites due to densities of *Rithrogena, Perlesta, Antherix,* Ceratopogonidae*, Chimarra, Protoptila, Optioservus, and Drunella tuberculata*. Strasburg, North River, and Woodstock formed a separate group, and this was attributed to densities of Planariidae, *Berosus*, and *Leptoxis carinata*.

DEQ nutrient measurements were available for spring 2006 including several nitrogen and phosphorus compounds. CCA ordination of taxa using nutrients as the second matrix revealed separation of Mount Jackson, Berryville, Harriston, and Front Royal from other sites due to higher concentrations of total phosphorus, orthophosphate, and lower ammonia (Fig. 4). The first two axes accounted for 36% of variation in the dataset. In this ordination, Cowpasture, White House, and Mount Jackson were separated from other sites, which clustered together. Nutrients explain the broad separation of these (Cowpasture, White House, and Mount Jackson) sites from other sites, the separation among other sites is not explained by nutrient information.

August 2006. Ordination (DCA) of top taxa by density indicated that Strasburg, Front Royal, White House, and Woodstock were separated from other sites due largely to higher densities of *Protoptila* and *Tricorythodes*. Berryville, Harriston, and Cowpasture were separated due to lower densities of *Isonychia* and *Macaffertium/Stenonema*. Lower densities of *Optioservus*, *Promoresia,* and *Leptoxis carinata* accounted for separation of the remaining sites.

CCA of taxa with DEQ nutrient data suggested that Berryville, Cowpasture, and Cedar Creek were different from other sites due to higher ammonia and total phosphorus and lower total nitrogen and nitrate concentrations (Fig. 5). Lynnwood and North River formed another separate

group based on differences in the same nutrients. Strasburg, Woodstock, and Whitehouse were different from other sites due to concentrations of total phosphorus, nitrate plus nitrite-nitrogen, and ammonia. Essentially, there appear to be two subtle nutrient gradients among sites but the absolute differences in nutrient concentrations still qualify as being moderate to high for all sites. We don't believe the subtle differences in these nutrient concentrations are of much value in explaining differences among sties.

CCA of taxa by substrate characteristics indicated that Woodstock, Lynnwood, North River, Whitehouse, and Harriston formed a distinct group separate from other sites due to higher proportions of cobble and higher epilithic biomass (AFDM) (Fig. 6). Other sites were fairly scattered, but differed not only by lower cobble and lower AFDM but also higher proportions of deposited sediment and gravel. This taxa versus substrate ordination suggests that some sites (Cowpasture, Cedar Creek, Strasburg, Berryville, and Mount Jackson) are characterized as having relatively smaller substrata (gravel, depositional sediments such as silt or detritus), whereas other sites (Harriston, Whitehouse, Lynnwood, Woodstock, and North River) were comprised of larger substrata having more epilithic biomass than other sites.

May 2007. Because only limited DEQ nutrient data (4 sites), passive sampler data (6 sites), and clam tissue/sediment heavy metal data (4 sites) were available for some large river sites, ordination analyses were not particularly useful in assessing differences among sites due to environmental conditions. CCA ordination suggested ammonia and phosphorus concentrations were important in separating sites but the absolute difference in concentrations among sites was very low (*e.g.,* nitrate concentration differed by 1 ppm at most among sites). Ordination using heavy metal and passive sampler data did not reveal patterns useful to determining differences among sites based on taxonomic composition. Generally, ordinations of May 2007 macroinvertebrate top taxa were weaker than 2006 analyses because fewer large river sites were sampled to facilitate extensive sampling in tributaries.

ANOVA

May 2006. In general, reference sites had higher Simpson's diversity index and the percent modified EPT indices, Cedar Creek had higher Total richness, although Mount Jackson actually had higher percent modified EPT index than reference sites. Reference sites also had higher percent non -insect taxa. This could be due to higher densities of pleurocerid snails that are algal grazers and were generally higher at North Fork sites. Similarly, reference sites typically had lower percent sensitive organisms, but some sites had very high percentages of sensitive organisms including Berryville, Front Royal, Harriston, and Lynnwood. North Fork sites did not adhere to a distinct pattern with respect to sensitive organisms. Reference sites also had higher percent modified scrapers with the exception of North River and Mount Jackson, which were similar to reference sites. Percent collector-gatherers, percent collector-filterers, and percent modified clinger metrics did not show a distinct pattern among the forks of mainstem. Percent Crawlers was significantly higher at reference sites, Mount Jackson, and North River.

August 2006. In general, New River sites had much lower density than Shenandoah River sites. Total richness, Simpson's diversity Index, percent modified EPT, and percent modified clingers were different between New River and Shenandoah sites but with no particular pattern. Percent

non-insects was higher in the New River except for Lynnwood, which was similar to New River. Other metrics showed no particular patterns with respect to the New River.

For each sampling season there were many significant ANOVAs, but no apparent patterns emerged to suggest that particular Shenandoah River sections were distinctly different. Shenandoah sites, in general, differed taxonomically from the Cowpasture River and Cedar Creek, but these differences could likely be the result of other factors than simple taxonomic composition.

Multimetric index

The Virginia Stream Condition Index (VSCI) is a multimetric index based on benthic macroinvertebrate assemblages that DEQ uses for monitoring biological condition of streams. The range of possible values for the VSCI is 0 to 100. The criterion for acceptable biological condition is a score ≥ 61, whereas a score ≤ 60 indicates impaired biological condition. VSCI scores for the 11 sites sampled in 2006 are plotted in Fig. 7. All scores for the August samples are well above the acceptable criterion and generally uniform. There is a much greater spread in the scores for the May samples, with two sites scoring below the acceptable criterion (Harriston and Berryville). In addition, five sites scored in the 60s just above the acceptable criterion in May (Lynnwood, Whitehouse, Front Royal, Woodstock, and Strasburg). Four sites in May had VSCI scores very close to those for August, all of which were well above the acceptable criterion (North River, Mount Jackson, Cedar Creek, and Cowpasture River).

The individual metrics that compose the multimetric VSCI are presented in Tables 10 and 11 for May and August, respectively. The VSCI was also calculated for two sites on the New River and compared to the August values from the Shenandoah River. Lower VSCI scores in May as compared to August are largely due to three individual metrics: percent top 2 Dominant Taxa, percent Chironomidae, and percent Ephemeroptera. Higher values for the first two metrics indicate lower biological condition, whereas, higher scores for percent Ephemeroptera indicate higher biological condition. The trend for these three metrics was pronounced at Harriston and Berryville in May, leading to the VSCI score at those two sites being slightly below the criterion for acceptable biological condition.

The VSCI scores indicate that the biological condition of the Shenandoah River compares favorably to the New River, for which major fish kills have not been reported. The reference sites for this study demonstrated biological condition consistently well above the acceptable criterion. Considering the sites according to forks and main stem, showed only minor differences. The only sites for which the VSCI fell slightly below acceptable were on the South Fork (Harriston) and the Main Stem (Berryville) in May. All three North Fork sites had very high VSCI scores in August. In May, The two North Fork sites with the greatest fish kills in 2006 (Woodstock, Strasburg) exhibited lower VSCI scores than in August, but the scores remained above the acceptable criterion. The most upstream North Fork site (Mount Jackson) had similarly high VSCI scores in May and August.

VSCI scores for the Shenandoah and New Rivers should be interpreted cautiously because the watershed areas at the study sites (Table 1) are much larger than the sites in the database used to

develop the VSCI. Excluding the reference sites on Cedar Creek and Cowpasture River, the watershed areas of the Shenandoah sites ranged from 131,411 ha (≈ 500 square miles) at Mount Jackson to 746,877 ha (≈ 2,900 square miles) at Berryville. In contrast, the database for the probabilistic reference sites used to develop the VSCI contained only 3 sites with a watershed area ≥500 square miles and the site with the largest watershed area in the database was only 656 square miles. The sites on the New River in West Virginia drain an even larger watershed (≈ 6,600 – 6,700 square miles). Only the reference sites on Cedar Creek and Cowpasture River had watershed areas (157 and 318 square miles, respectively) that were somewhat representative of the database for the probabilistic reference sites.

Biomass

General descriptors. As was done for density, the biomass of the benthic macroinvertebrate assemblage was examined primarily according to dominant taxa (Table 12). Biomass was only measured in May. The grand mean for total biomass in all May samples (n = 66) was 6354 mg dry mass per m^2. The two sites with the highest mean biomass were Whitehouse and Woodstock, while the two sites with the lowest mean biomass were Harriston and Berryville. There did not appear to be any pattern for forks, reference, or fish kill sites.

Ordination. Detrended correspondence analysis explained 31% of the variation in May 2006 invertebrate biomass data. Most of the site separation was along axis 1, which explained 23% of the variation. The groupings that emerged from the ordination based on biomass data supported the results from the ordinations based on density data. For instance, ordinations based on density also indicated that Cowpasture had a different invertebrate composition, and some of the taxa that that separated Cowpasture based on density data (*Rithrogena*, *Acroneuria*, *Corydalus*, *Chimarra*), also separated Cowpasture based on biomass data.

Metrics. Comparisons among various site groups using metrics based on biomass provided very similar results as comparisons using metrics based on density. The most effective use of the biomass data was for comparing historical data from the 1960s with 2006 data.

Large River: Relationships Between Macroinvertebrates and Environmental Variables

Generally, linear regression was used to assess relationships between macroinvertebrate metrics and top taxa with environmental variables. We used linear regression as a follow up analysis to CCA, where ordination suggested key environmental variables to use in regressions and helped guide decisions about which environmental variables might be most important in determining assemblage structure. CCA in essence was used as a screen to guide regression analysis, but we did not restrict ourselves to relationships indicated by CCA. We also relied on our professional expertise in aquatic ecology to choose other regression analyses that had the potential to be ecologically meaningful for the objectives of this study. The types of environmental variables selected changed with sampling time (May 2006, August 2006, and May 2007).

May 2006

Only DEQ nutrient data were available for spring 2006 analysis. Many relationships were significant (*i.e.*, $p > 0.05$) but we chose to focus on relationships where the coefficient of determination (R^2) was greater than 0.3, or more than 30% of the variation in the dependent variable (macroinvertebrate metric or taxon) was explained by the independent variable (environmental parameter).

Five significant regressions fit our criteria during May 2006 (Table 13). Higher total phosphorus concentrations caused a negative response with percent modified EPT index across sites ($p=0.029$, $R^2=0.428$). Similarly, Simpson's diversity index was negatively influenced by ammonia ($p=0.038$, $R^2=0.397$), total Kjeldahl nitrogen ($p=0.031$, $R^2=0.420$), and total phosphorus ($p=0.039$, $R^2=0.395$). Percent Crawlers was also lower when ammonia concentration was higher ($p=0.05$, $R^2=0.362$). Otherwise, no ecologically significant relationships with assemblage metrics existed during May 2006.

Regarding individual taxa, *Optioservus* responded negatively to the maximum N-N concentration ($p=0.003$, $R^2=0.636$) during May 2006. No other top taxa responded significantly to May 2006 environmental conditions.

August 2006

A greater number of significant relationships were detected when we compared August 2006 environmental variables with metric and taxonomic responses (Table 14). Total density and percent non-insect metrics were higher with higher epilithic biomass (AFDM). Higher nitrogen concentrations (as estimated by different nitrogen parameters including N-N, Nitrate, etc.) were associated with higher density, Simpson's diversity, and percent modified clingers. Similarly, higher phosphorus led to higher diversity and percent modified clingers. Lastly, chlorophyll-a concentration had a positive effect on the percent sensitive organisms.

Regarding individual taxa, *Corbicula* responded positively to substrate size, nitrate concentration, total nitrogen, ammonia, and total phosphorus in August 2006. The amount of epilithic AFDM had a positive effect on *Baetis*, *Leptoxis*, *Optioservus*, and Planariidae but a negative effect on *Hydropsyche* in August 2006. Cobbles, which are larger more stable substrata, positively influenced *Isonychia*, while gravel had a negative effect on *Isonychia*, *Hydropsyche*, and Planariidae. Deposited sediments, or silt, had a positive effect on *Tricorythodes*.

In general, biological materials on the substrate (algae and microbes as reflected by epilithic chlorophyll a and AFDM) better predicted macroinvertebrate responses than the physical nature of the substrate (cobble, gravel, fines). The explanation lies in the increased food and microhabitat provided by the growths of biological materials on the substrate, which are stimulated by nutrients.

Several macroinvertebrate summary metrics and top taxa were significantly related to DEQ nutrients (Table 15). *Stenelmis* and *Leptoxis* densities responded positively to nitrite, nitrate-nitrogen, and ammonia. *Baetis* and *Macaffertium/Stenonema* densities were higher with higher Kjeldahl nitrogen, phosphorus, and orthophosphate. *Hydropsyche* were more abundant with higher N-N. All of these relationships can be explained by the nutrients stimulating growths of biological materials on the substrate, which increased food and microhabitat for these particular macroinvertebrates. We also assessed relationships between passive sampler variables and clam/sediment metals, but these analyses were weakened by lack of data for large river sites in 2007. In general, there were no obvious relationships between macroinvertebrates and metals or other potential contaminants, nor were there any signs that these chemicals were affecting biological condition at the large river sites. Typically, concentrations of contaminants were at or slightly above detection limits and we do not know whether macroinvertebrates would respond at these levels.

Comparison to Other Similar Large Rivers

In addition to examining for differences in benthic macroinvertebrates within the Shenandoah River, it was also the objective of this study to consider the biological condition of the Shenandoah River in comparison to other similar bodies of water. It is difficult to find comparable data for large rivers, but we were able to make reasonable comparisons to the New River in West Virginia (Voshell et al. 1990, 1991) and the Susquehanna River in Pennsylvania (Jackson et al. 1994). Both drain larger watershed areas than the Shenandoah River, but they have similar rocky bottoms, support smallmouth bass populations, and have not suffered fish kills like the Shenandoah River. Summary taxa lists from the three rivers are presented in Table 16. Jaccard's coefficient was used to analyze the similarity of the three rivers based on the presence or absence of taxa. Values for this coefficient range from 0 to 1, with 1 indicating high similarity. The results were as follows:

Shenandoah compared to Susquehanna	0.3924
Shenandoah compared to New	0.3973
Susquehanna compared to New	0.3929

Thus, the benthic macroinvertebrate fauna of the Shenandoah River was not very similar to that of either the Susquehanna or New Rivers, which were also not similar to each other. However, the nature of the difference in the fauna does not indicate that the biological condition of the Shenandoah River is degraded. There are appreciably more taxa in the Shenandoah River (68) than the Susquehanna (43) or New (35). The two sites on the South Fork of the Shenandoah River with the lowest numbers of taxa (Front Royal with 44 and Whitehouse with 48) still had higher numbers of taxa than the Susquehanna or New Rivers. Many of the additional taxa in the Shenandoah belong to the insect orders Ephemeroptera, Plecoptera, and Trichoptera, which are mostly comprised of sensitive species. Within the Ephemeroptera, these include two members of the family Ephemerellidae: *Drunella turberculata* and *Ephemerella*. Within the Plecoptera, Chloroperlidae, *Leuctra*, *Agnetina*, and *Perlesta placida* only occur in the Shenandoah River. Among these three orders, Trichoptera contains the most taxa (9) that only occur in the

Shenandoah River. In addition, there are two sensitive genera of Coleoptera that only occur in the Shenandoah River: a riffle beetle, *Promoresia*, and a water penny, *Ectopria*. In summary, comparison of the benthic macroinvertebrates in the Shenandoah River to two other somewhat rivers in the mid-Atlantic region provides no evidence that the biological condition of the Shenandoah is lower than what would be expected.

Comparison to Historical Shenandoah River Data

In order to consider long-term temporal differences in the biological condition of the Shenandoah River, we made comparisons to benthic macroinvertebrate data collected by Eugene Surber in the 1960s (Surber 1965, 1967, 1969). He used quantitative methods that made it possible to compare total taxa richness and density, density of individual taxa, total biomass, and biomass of mollusks versus non-mollusks. We were able to compare seven of our sites sampled in 2006 to sites sampled previously by Surber: three on the South Fork (Lynnwood, Whitehouse, Front Royal), three on the North Fork (Mt. Jackson, Woodstock, Strasburg), and one on the Main Stem (Berryville). We used recent data collected in May 2006 and matched it with data collected by Surber at a similar time of year (April and June). This provided two replicate samples at each of the seven sites for the Surber data. We randomly chose two of our six replicate samples at each site in May 2006 for comparison.

In the 1960s Surber considered the North Fork to be "relatively unpolluted." He further stated: "Unlike the South Fork, the North Fork water is very clear throughout most of the year. Instead of abundant phytoplankton algae growths, the North Fork has been characterized by profuse growths of filamentous algae such a *Hydrodycton*." Although the South Fork from above Elkton to Front Royal received wastes, such as the Virginia Oak Tannery at Luray and inadequate sewage treatment plants at Stanley, Shenandoah, and Luray, the biota was "relatively unaffected by the wastes entering it." However, there were several large sources of pollution at Front Royal that seriously affected about one-third of the 34.9-mile section of the Main Stem in Virginia. These sources of pollution were: a viscose rayon plant (FMC Corp, Viscose Division), an Allied Chemical Company plant, a food processing plant (Old Virginia, Inc.), and an inadequate sewage treatment plant. The effects of pollution from these sources were sporadic among years for benthic macroinvertebrates and fish. Pollution from Front Royal caused serious fish kills in 1966, 1968, and 1969 and small kills in every year in late winter. Surber's macroinvertebrate data that were used for comparison were not associated temporally or spatially with any of the 1960s fish kills.

The same list of non-redundant macroinvertebrate assemblage metrics was calculated for the samples collected by Surber and compared to the samples from 2006 at each site (Table 17). Paired *t*-tests were used to assess differences in summary metrics between the historical samples and Virginia Tech samples. When compared as three site groups (North Fork, South Fork, Mainstem) no significant differences in metrics were detected. When considered as seven individual sites, several significant differences between dates emerged. Simpson's diversity index ($p=0.031$, $t=-36.501$), percent modified EPT ($p=0.003$, $t=-3.710$), percent scrapers ($p<0.001$, $t=-14.953$), and percent modified clingers ($p=0.004$, $t=-20.670$) were higher in 2006. All of the preceding metrics being significantly higher would indicate better biological condition in 2006. Percent Collector-gatherers ($p=0.006$, $t=19.274$), percent collector-filterers (p=0.047, t

=15.620), and percent crawlers (p=0.030, t =8.464) were lower in 2006. The first two of the three preceding metrics being significantly lower would also indicate better biological condition in 2006. In summary, four of the eleven assemblage metrics were not significantly between the 1960s and 2006, and of the seven metrics that were significantly different, six indicated better biological condition in 2006.

We also analyzed the densities of all individual taxa in the historical and Virginia Tech samples (Table 18). We began by calculating the Bray-Curtis similarity coefficient to assess the similarity of the entire assemblage of individual taxa (densities) at each site between the 1960s samples and the May 2006 samples.

	Site	Bray-Curtis Coefficient
NF		
	Mt. Jackson	0.447445
	Woodstock	0.345216
	Strasburg	0.694632
SF		
	Lynnwood	0.290172
	Whitehouse	0.437419
	Front Royal	0.539177
MS		
	Berryville	0.420468

General guidelines for interpreting Bray-Curtis coefficients of similarity are: > 0.7 indicates assemblages are similar; < 0.5 indicates assemblages are not similar; 0.5 – 0.7 indicates no conclusions about similarity. Almost all of the Bray-Curtis coefficients above indicate that the assemblages sampled in May 2006 were not similar to those sampled at the same sites in the 1960s. No site had similar benthic macroinvertebrate assemblages; Strasburg and Front Royal exhibited the most similarity but only fell in the inconclusive zone. However, the lack of similarity does not indicate a decline in biological condition in 2006 as compared to the 1960s, and actually can be interpreted as better biological condition in 2006.

There were considerably more taxa in 2006 as compared to the 1960s (65 versus 52, respectively; Table 18). Most of the additional taxa that appeared on the list in 2006 are somewhat sensitive to environmental stressors. This is especially true for most of the Ephemeroptera (*e.g.*, *Maccaffertium/Stenonema*, *Isonychia*), Plecoptera (*e.g.*, *Leuctra*, *Agnetina*), and Trichoptera (*e.g.*, *Brachycentrus*, *Protoptila*, *Helicopsyche*, *Lepidostoma*, *Chimarra*). A notable exception to the trend of more sensitive taxa is the conspicuous increased density of Planariidae (flatworms), which are very tolerant of environmental stressors. There were also conspicuous increases in densities of quite a few taxa that are considered facultative to environmental stressors: *Leptoxis carinata* (snail), *Baetis*, *Ephemerella*, *Tricorythodes*, *Corydalus cornutus*, *Cheumatopsyche*, *Hydropsyche*, Hydroptilidae, Chironomidae, and Elmidae. With the exception of *Corydalus cornutus*, the increased density of these taxa can be attributed to increased production of algae, either periphyton or plankton, stimulated by increased nutrients. Some feed on periphyton on rocks (*Leptoxis carinata*, *Baetis*, Hydroptilidae, Elmidae), some feed on algae or detritus suspended in the water (*Cheumatopsyche*, *Hydropsyche*), while others feed on fine detritus that is derived from dead algae and deposited on the bottom (Planariidae, *Tricorythodes*, Chironomidae). Most of the

aforementioned taxa also increase because the excess plant growth on rocks stimulated by increased nutrients creates an especially favorable microhabitat for hiding from predators and coping with fast current. The invertebrate predator *Corydalus cornutus*, has likely increased in density because of the increase in prey organisms.

In summary, the available data on macroinvertebrates indicates that the biological condition at large river sites in the Shenandoah River basin is no worse, and may be better, than it was 40 years ago. The number of taxa and most of the types of dominant taxa collected in 2006 suggest improvement in conditions relative to the 1960s. However, most of the dominant taxa are likely to have high densities because of high nutrient concentrations that stimulate high algae production.

Subwatersheds: Assemblage Structure and Function

General descriptors

We calculated the same eleven non-redundant metrics for macroinvertebrate assemblages collected May 2007 in 26 Shenandoah River tributaries representing different subwatersheds (Table 19). Generally, Total density in tributaries varied widely but did not appear different from large river sites. Taxa richness was only slightly lower at most tributary sites but was very low at a few sites (e.g., Cook's Creek, North River headwaters, Naked Creek – Page Co., Stony Creek headwaters, Linville Creek). Simpson's diversity index is not usually as low as 0.5 or below, but this occurred at some tributary sites (e.g., Linville Creek, Mill Creek – South Fork, Long Glade Creek, Naked Creek – Augusta Co., Stony Creek downstream). The percent modified EPT was at on near zero for several tributaries, mostly the same ones mentioned for the previous metrics. In general, assemblage metrics indicated that the tributary sites represented a wide range of biological conditions, from good to poor, which was the goal of the study design.

Ordination

To assess site groupings and general similarities/differences among the tributary sites we used a combination of Detrended Correspondence Analysis (DCA) and Canonical Correspondence Analysis (CCA) ordination techniques. Again we truncated the total taxa list to include only the top 0.2% to eliminate rare taxa that only contribute noise to these types of statistical analyses. In each case tributary sites were arranged according to relative abundances of the top taxa. Then a second matrix (taxa in DCA, environmental variables in CCA) was used to identify variables that contribute to site separation. Using a second matrix, or data set, ordination allows consideration of parameters that explain separation of data in the first matrix.

We first projected top taxa abundances in a DCA ordination and used a biplot (first and second matrices the same) to show which taxa were most responsible for site separation (Fig. 8). *Prosimulium* were absent at most sites but abundant at Briery Branch and Passage Creek (reference site), which contributed to separation of these sites. Other sites formed two groups based on relative abundances of *Simulium* and *Ephemerella*. These two taxa were 'top taxa', present in nearly all samples, and had varied abundances across tributary sites. A second

reference site, Cedar creek headwaters, was separated from the main cluster of sites along axis two due to a much higher abundance of *Simulium* than other sites. We consider axis two to better explain site differences because axis one was important largely due to *Prosimulium* being present at only five sites. Abundances of *Ephemerella* were especially useful in grouping sites based on density. Some sites had *Ephemerella* densities between 0 and 100 individuals per meter squared, whereas another group of sites ranged from 100 to 2500 individuals per meter squared.

As a follow up ordination we reanalyzed the same data as above but eliminated the outlier site Passage Creek and the outlier taxon *Prosimulium* to better observe differences among the remaining sites (Fig. 9). In this ordination the two axes explain nearly 50% of the variation among sites related to taxa abundance and land use. Several more distinct patterns are apparent with six taxa explaining differences in sites and greater resolution of grouping structure among sites. Removal of outliers exposed patterns among sites whereby key taxa explained major differences among sites distinguishing groups. Planariidae were disproportionately more abundant at Cooks creek and Long Meadow Run which caused separation of these two sites. *Cheumatopsyche* were most abundant at Hawksbill Creek downstream, Holmans creek, and Naked Creek – Page Co. *Chimarra*, *Ephemerella*, and *Maccaffertium/Stenonema* were more abundant at Christians Creek, Smith Creek headwaters, Back Creek, and Smith Creek downstream. Chironomid midges were most abundant at a group of sites including Jennings Creek, Linville Creek, and both Mill Creeks (see lower left quadrat Fig. 9) and explained why these sites were distinct.

CCA ordination of tributary sites, top taxa abundance, and land-use variables shows distinct separation of some sites as well as a clustering of a large number of sites (Fig. 10). Axis 1 explains 36% of the variation among sites, which is a high amount for CCA. Cook's Creek and Smith Creek downstream were the most distant from other sites on the left of axis 1, and the most likely land-use variables separating these sites were high number of animal feeding operations, number of dairy farms, and percent of cropland in the subwatersheds. There was another distinct group of four sites on the right side of axis 1: Passage Creek, Gooney Run, Cedar Creek headwaters, and Back Creek. Three of these sites are reference sites. The positioning of these sites by CCA is the result of low number of animal feeding operations, number of dairy farms, and percent of cropland in the subwatersheds. Thus, these land uses reduce the biological condition of tributary streams.

CCA ordination of tributary sites, top taxa abundance, and sediment chemistry (metals, nitrogen, organic) shows two reference sites separating on the left of axis 1 (Back Creek, Gooney Run) and several non-reference streams on the right of axis 1 (Cooks Creek, Muddy Run, Naked Creek – Augusta Co.; Fig. 11). This separation is produced by high copper, lead, nitrogen, and organic carbon in the sediment at the non-reference sites and *vice versa* for the reference sites. Linville Creek, Hawksbill Creek downstream, Christian's Creek, Stony Creek headwaters, Meadow Creek, Jennings Creek, and Cedar Creek headwaters (a reference site) were separated from remaining sites around the middle of the sediment metals/nutrient gradient. It is possible that assemblages at these sites were responding positively to nutrient enrichment and higher total organic carbon but negatively to sediment copper and lead although it is difficult to determine from CCA alone.

CCA ordination of tributary sites, top taxa abundance, and clam metal concentration also showed a large cluster of sites in the middle surrounded by several outlying sites (Fig. 12). Cedar Creek headwaters and downstream (both reference sites), Passage Creek, Hawksbill Creek downstream, and Meadow creek were separate from other sites. Passage and Hawksbill appeared to separate due to higher clam tissue mercury concentrations, whereas Cedar Creek headwaters and downstream sites separated according to concentrations of clam tissue cadmium and selenium. It is not apparent what influence these heavy metals have on macroinvertebrate assemblages, and these results may not have ecological relevance.

In summary, ordination of tributary sites shows distinct gradients that differentiate sites. Land-use appears to be more important in differentiating sites than specific sediment chemistry variables. All forms of agricultural activity in the subwatersheds appear to influence assemblage structure and function, but these ordinations do not explain the mechanistic causes of these differences or whether specific effluent or sediment runoff is responsible.

Subwatersheds: Relationships Between Macroinvertebrates and Land Use, Environmental Variables

Additional data were collected in tributaries in May 2007 including DEQ nutrients, passive sampler chemistry including 'emerging contaminants', sediment metals and nutrients, clam tissue heavy metal concentration, DEQ fecal coliform and *e. coli* concentrations, and subwatershed land-use measures. Our assessment of assemblage responses to environmental conditions was thus more intensive than in previous analyses. Again we allowed CCA results to prioritize our regression analyses by focusing initially on environmental variables that were important in ordination. Secondarily we also compared macroinvertebrate metrics and top taxa with environmental variables where it was reasonable to hypothesize causal relationships.

Fourteen land-use variables were considered potentially influential to macroinvertebrate assemblage structure and function. We use linear regression to assess relationships between eleven macroinvertebrate metrics and 40 top taxa. There were 49 significant regressions ($p < 0.05$) (Table 20). Often, regressions involving percent Forest showed an inverse relationship with regressions involving percent pasture and hay, percent cropland, and percent developed land. Usually macroinvertebrates responded positively to increased percent forest and negatively to increases in percentage of land area used for different types of agriculture or development. Percent Non-insects, however, responded positively to increases in percentage of land area used for different types of agriculture or development and negatively to increased percent forest. The number of dairy farms, cattle farms, and poultry houses was frequently a predictor of reduced biological condition as quantified by macroinvertebrate assemblage metrics.

The strongest regressions showed that percent collector-filterers were more abundant in forested areas and less abundant in areas characterized by pasture and hayfields. Similarly, the percent modified EPT index was lower in pasture and hayfield areas and higher in forested sites. The highest level of prediction showed that higher numbers of cattle farms, dairy farms, poultry houses, and animal feeding operations led to higher percent non-insects.

In addition to summary assemblage metrics, several individual top taxa responded significantly to subwatershed land use (Table 21). Planariidae responded positively to percent cropland and negatively to percent forest. Similarly, Planariidae responded positively to number of poultry houses, animal feeding operations, and nutrient management plans for poultry waste. *Hydropsyche* also responded positively to what is usually considered detrimental watershed conditions, the number of beef cattle and poultry houses.

A number of other environmental variables were also compared to macroinvertebrate assemblage metrics and top taxa producing 42 significant relationships (Table 22). Several forms of nitrogen and phosphorus predicted metric and top taxa responses including nitrate, nitrite, ammonia, and phosphorus. Typically relatively higher concentrations of these nutrients induced a negative taxonomic response (*i.e.*, taxa abundance decreased). Exceptions were Planariidae responded positively to ammonia, and *Antocha* responded positively to phosphorus. It is unknown whether these organisms prefer enriched conditions or are simply better than other organisms at tolerating high nutrient concentrations. Generally, higher nutrients led to reduced Taxa richness, percent modified EPT, percent sensitive organisms, and percent clingers. We believe nutrients are stimulating primary production on rock surfaces which, in turn, contributes to food and microhabitat availability.

Heavy metals in sediments and clam tissue were also related to macroinvertebrate structure and function as quantified by metrics and top taxa abundance (Table 22). In some cases metal concentrations induced positive responses in macroinvertebrate abundance. In all of these cases the concentration of metal was at or barely above the limit of detection and whether any ecological influence would occur is unknown. Although the direction of relationships was mixed, we expected metals to induce negative invertebrate responses and focus on those results here. The most ecologically and statistically significant results showed that higher clam and sediment lead concentrations predicted negative responses in percent modified EPT, percent sensitive organisms, percent collector-filterers, and percent crawlers.

In summary, regression analysis provided further evidence that the presence of agricultural activity in subwatersheds, various forms of nitrogen and phosphorus, and heavy metals can predict macroinvertebrate structure and function.

Indicator Species

Initially, mayflies, especially members of the family Ephemerellidae, were thought to be good candidates for benthic macroinvertebrates that could be identified to species and might demonstrate differential, predictable responses to environmental stressors in the Shenandoah River. However, there were not many species of ephemerellids, and many early instar nymphs could not be reliably identified. Adult riffle beetles (Elmidae) were common in the benthic samples and could be reliably identified to species. There appeared to be a sufficient number of different species to make this group worthwhile for investigating indicator species. It is possible that increased taxonomic resolution identifies stronger relationships with environmental variables.

In total we collected 14 species of adult elmid beetles in 2007 (Table 23). We conducted similar analyses as we did with assemblage-level information including ordination and regression. In large rivers (2007 data), DCA ordination of elmid species and abundance indicated four distinct groups of sites. Cowpasture and Cedar formed one group, Whitehouse, Mount Jackson, Strasburg, and Woodstock formed a second group, and North River and Cootes Store were separated from all other sites and each other (Fig. 13).

Tributary elmids were also projected in a CCA ordination to examine the influence of land-use in separating sites (Fig. 14). Cooks and Long Meadow Run were separated along axis 1 due to higher numbers of animal feeding operations, poultry houses, and beef cattle per acre. No distinct grouping of other sites was apparent.

Elmid species data were also used in linear regression analyses to detect relationships between land use and environmental variables. Although some relationships were significant, most were weak ($R^2 < 0.3$), and patterns appeared to be caused by outlier points.

Elmid adults were useful in detecting differences among sites but usually just agreed with analyses of the entire assemblage. In general, it does not appear that increased taxonomic resolution of elmids to species provided any more ability to detect differences among sites or to enhance our ability to detect relationships between macroinvertebrate assemblages and environmental variables.

In-Situ Bioassays

Growth

Results from the in-situ tests with the Asian clam (June and August) showed significant differences in growth between the Fish kill and Reference site groups. Asian clams in the Fish kill group of sites consistently showed reduced growth rates relative to Asian clams in the Reference group (Fig. 15). However, based on results from ANOVA, when each site was considered an individual group, Mt. Jackson and Front Royal were the only sites that had significant differences in Asian clam growth in June 2006, and Mt. Jackson and Woodstock were the only sites that had significant differences in Asian clam growth during August 2006 (Fig. 16). During both tests, Asian clams at Mt. Jackson had the highest growth rates. Although growth rates at Woodstock were not detected as significantly different from most sites, it appears that growth at Woodstock was reduced.

Mortality

Mortality responses were very similar to growth responses; there was significantly higher Asian clam mortality in the Fish kill site group than the Reference group during both in-situ tests (Fig. 17). However, ANOVA indicated that significant differences in mortality among sites only occurred in June (Fig. 18). During June, highest mortality occurred at White House. Asian clam mortality at White House was significantly higher than mortality at North River, Harriston, Lynnwood, Woodstock, Berryville, and Cowpasture.

SUMMARY AND CONCLUSIONS

Analyses of benthic macroinvertebrate assemblages have not distinguished any large river sites in the Shenandoah basin with significant reduction in biological condition. The benthic macroinvertebrate assemblages exhibit very high densities and a great deal of taxonomic richness. There are some significant differences among sites, but there are no spatial patterns that correspond with a particular section, such as a fork, or to areas where fish kills have been more prevalent. The benthic macroinvertebrate assemblages at the large river sites fall within the range of what would be expected in similar rivers in the mid-Atlantic region. Comparisons of the benthic macroinvertebrate assemblages at large river sites in the 1960s with those of 2006 show that there are now more taxa and higher densities, thus, biological condition has improved temporally. However, the benthic macroinvertebrate assemblages have become somewhat out of balance, both taxonomically and ecologically. The taxa with exceptionally high densities show strong relationships with various forms of nitrogen and phosphorus that act as nutrients for plant growth. It is likely that high nutrients stimulate algae in the water and on solid substrates. Death and decomposition of algae creates abundant fine detritus along with microbes. Thus, high nutrients provide abundant food for macroinvertebrates that are scrapers, collector-filterers, and collector-gatherers. In addition, heavy growth of plant material on solid stable substrate creates excellent microhabitat for macroinvertebrates. While the present benthic macroinvertebrate assemblages at the large river sites do not indicate much, if any, impairment of biological condition, further increases in nutrient concentrations will eventually cause a decline in biological condition because of over production of algae. This will eventually lead to lower dissolved oxygen, and reduced quantity and quality of food and microhabitat.

Moreover, the results of our benthic macroinvertebrate assemblage analyses at large river sites suggest that macroinvertebrate assemblages are not experiencing any influences similar to fishes. These results lend support to the hypothesis that the fish kills are primarily being caused by a factor specific to fish, probably a biological pathogen. However, results of fish pathology studies seem to indicate a diverse array of fish health problems, including parasites such as trematodes. Some trematodes use snails as intermediate hosts, and snails are one of the taxa whose density has increased greatly and is strongly related to increased nutrients. Other numerically dominant macroinvertebrates may also be involved in the life cycles of trematodes and other parasites that eventually infect fish.

Analyses of tributary streams according to subwatersheds were more informative than the large river studies for elucidating the factors responsible for macroinvertebrate assemblages. Subwatershed tributary analysis facilitated spatially explicit analysis of land-use variables in addition to instream environmental variables. Unlike the large river sites, the biological condition of tributary sites ranged from good to poor. Assemblages in these smaller tributaries were strongly related to agricultural land use, including dairy, beef, poultry, and crops. Biological condition declined in relation to increases in these agricultural land uses, but there did not appear to be a difference in the decline of biological condition according to the particular type of agricultural land use. Nutrients derived from land use were the driving force for determining macroinvertebrate assemblages. There was no clear evidence that toxic contaminants of any kind were a major influence on the benthic macroinvertebrate assemblages. It was obvious in some tributary streams that high nutrients had caused an appreciable decrease

in biological condition. If nutrient concentrations continue to increase in more tributary streams, the impaired biological condition of the tributaries will eventually be manifested in the large river sections of the Shenandoah basin.

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FIGURES

Fig. 1 Benthic Macroinvertebrate Study Sites

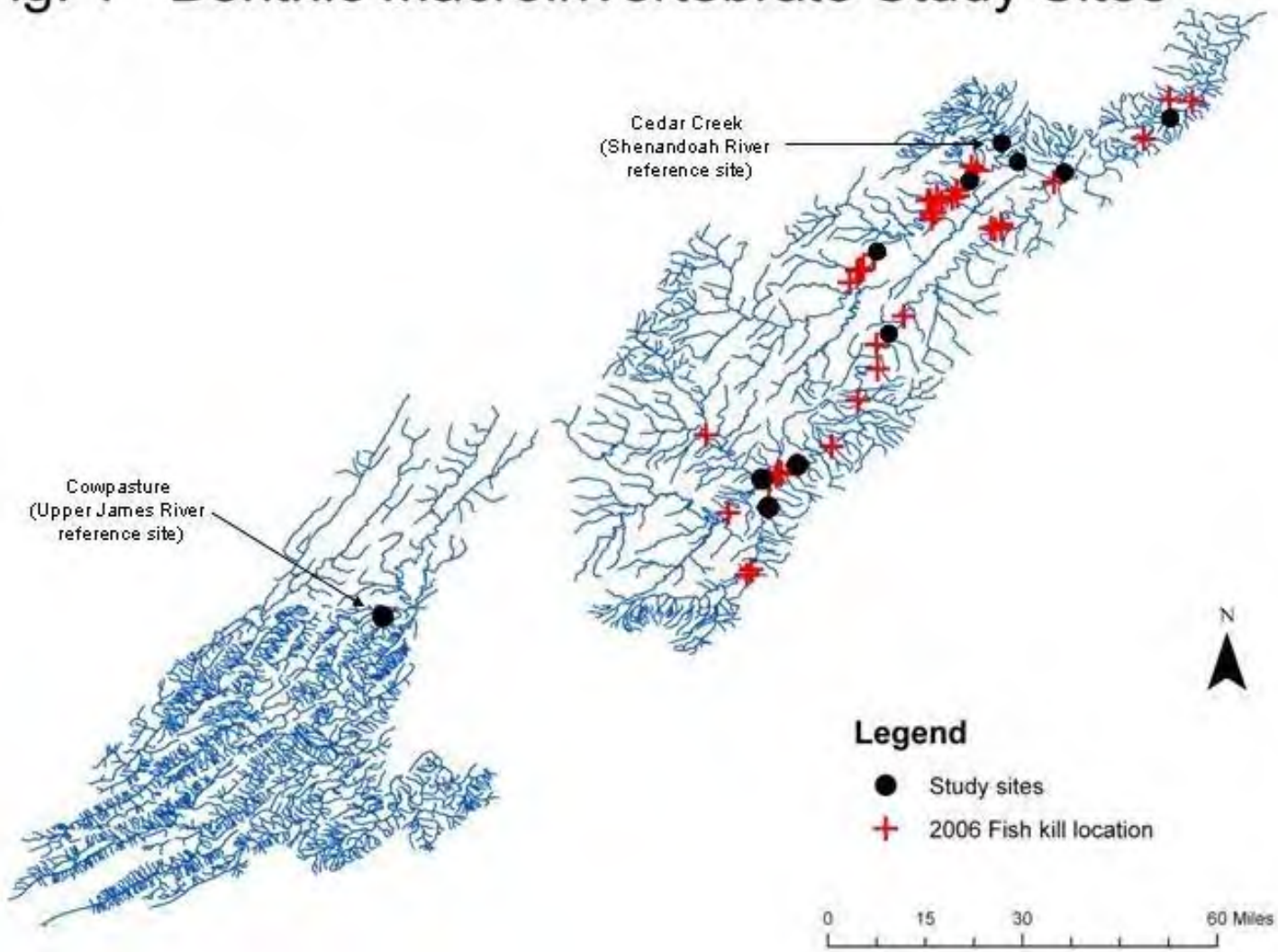
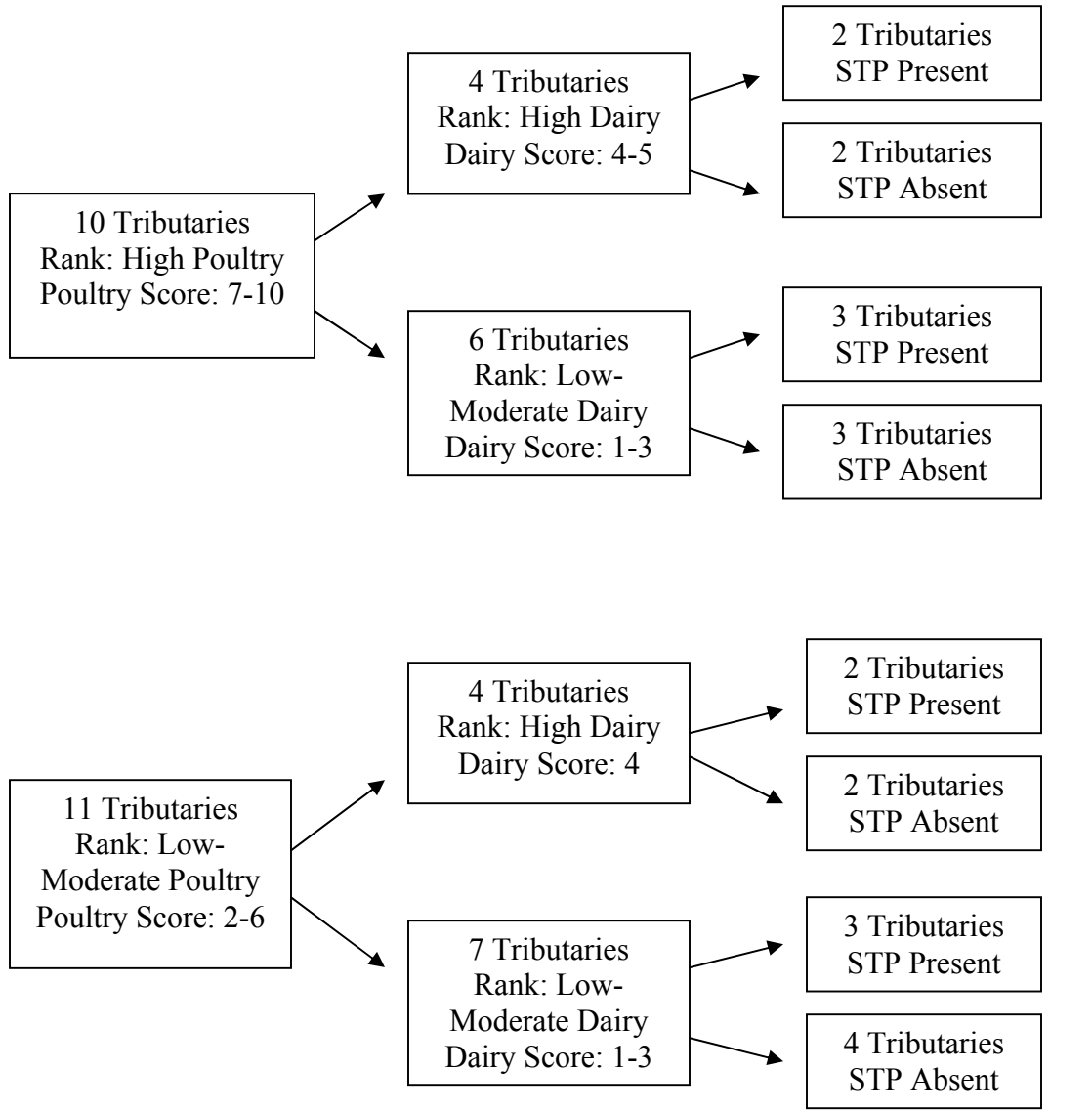


Figure 2. Factorial sampling design for tributary sites within subwatersheds. Boxes illustrate different land-use types used to categorize sites. STP = sewage treatment plant.



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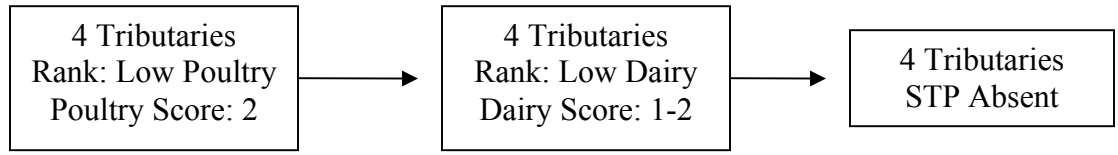


Figure 3. Map of study sites on tributary streams representing subwatersheds of the Shenandoah River basin. Site codes are explained in Table 2.

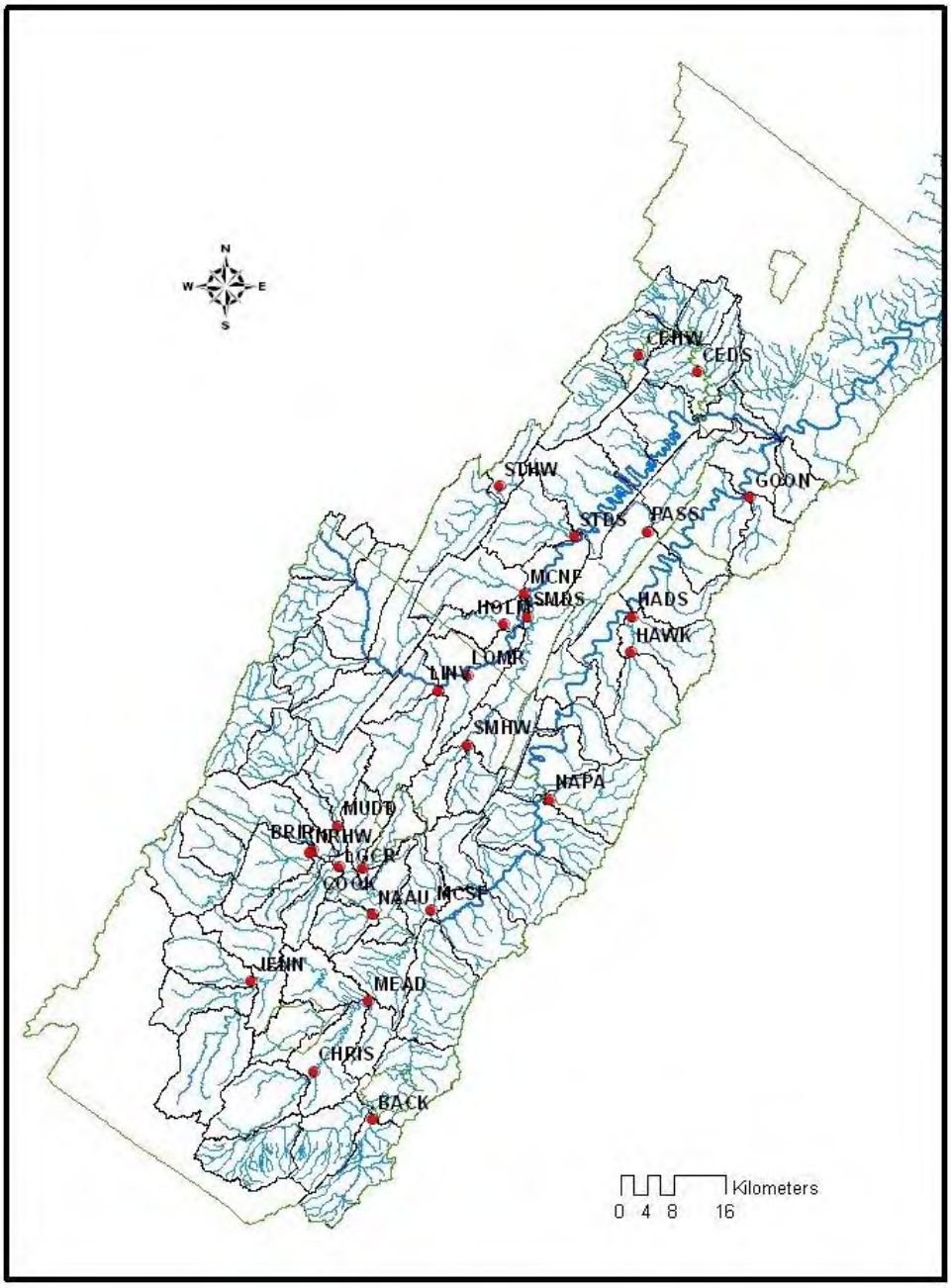


Figure 4. Canonical Correspondence Analysis ordination (CCA) of spring 2006 large river taxa (main matrix) using DEQ nutrient parameters (second matrix) to explain differences in taxa among sites. Axis one explained 18.3% of variation in site separation whereas axis 2 explained 17.2%.. Site codes are explained in Table 1. ORTHP = orthophosphate; TP = total phosphorus. CCA cutoff value for significance R=0.4

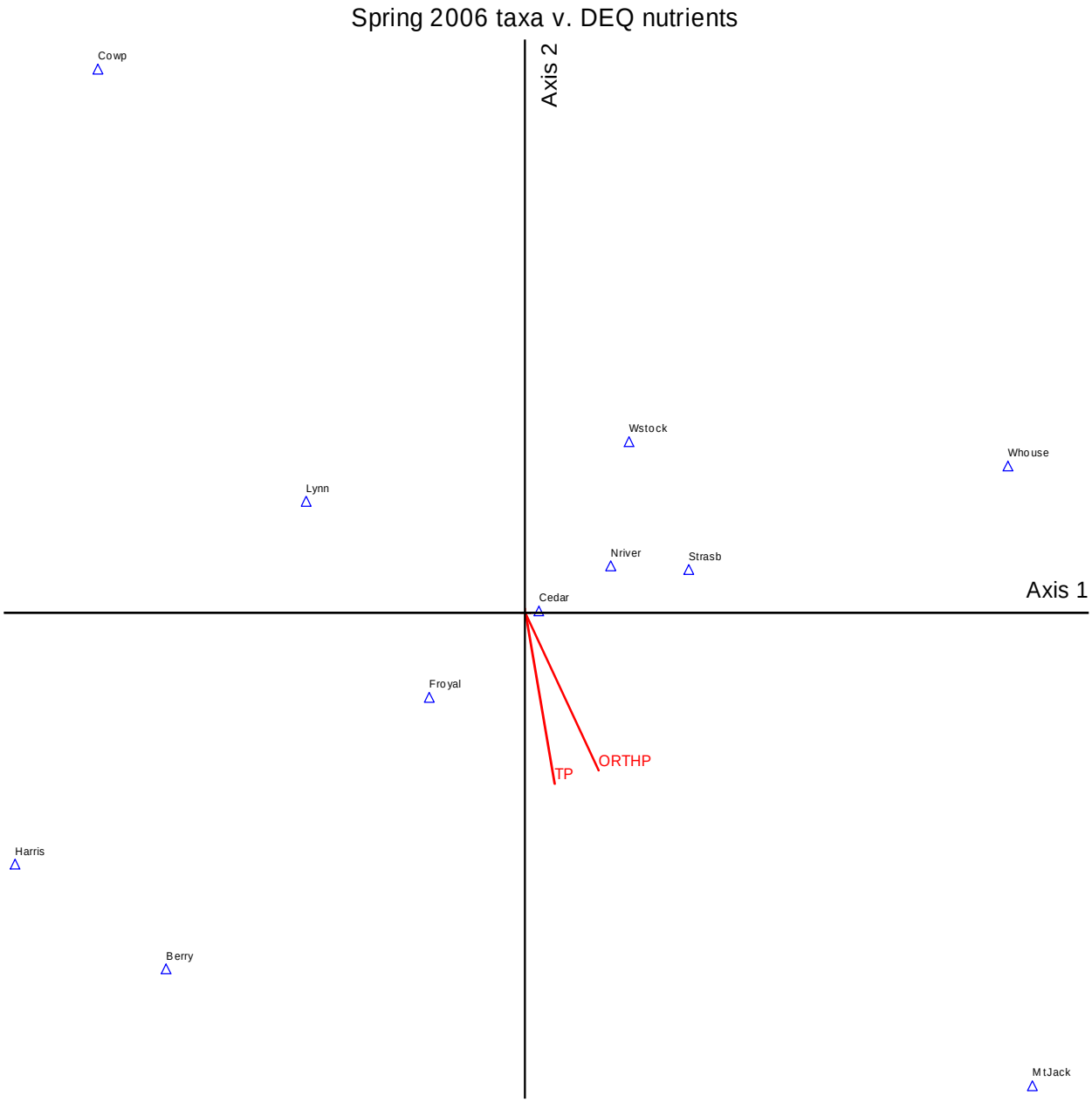


Figure 5. Canonical Correspondence Analysis ordination (CCA) of August 2006 large river taxa (main matrix) using DEQ nutrient parameters (second matrix) to explain differences in taxa among sites. Axis one explained 20% of variation in site separation whereas axis 2 explained 14%. Site codes are explained in Table 1. NN = Nitrate + Nitrite; TP = total phosphorus; AMMON = dissolved ammonia. Cutoff value for significance R=0.5.

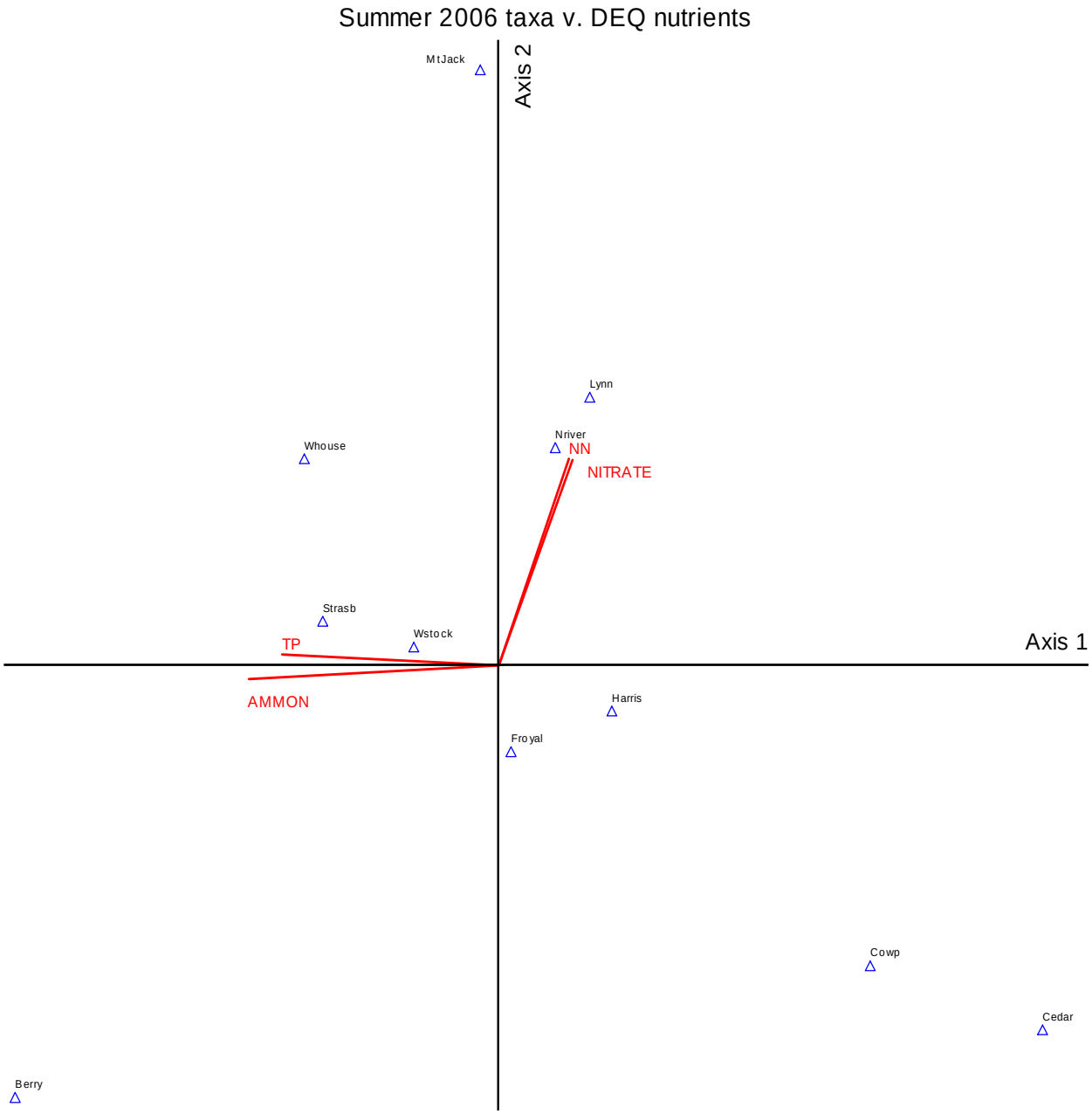


Figure 6. Canonical Correspondence Analysis ordination (CCA) of August 2006 large river taxa (main matrix) using substrate parameters (second matrix) to explain differences in taxa among sites. Axis one explained 21.2% of variation in site separation whereas axis 2 explained 18.6%. Site codes are explained in Table 1. grav = percent gravel; depsed = % deposited sediments; cob = percent cobble; AFDM = ash-free-dry-mass of substrate epilithon. Cutoff value for significance R=0.2.

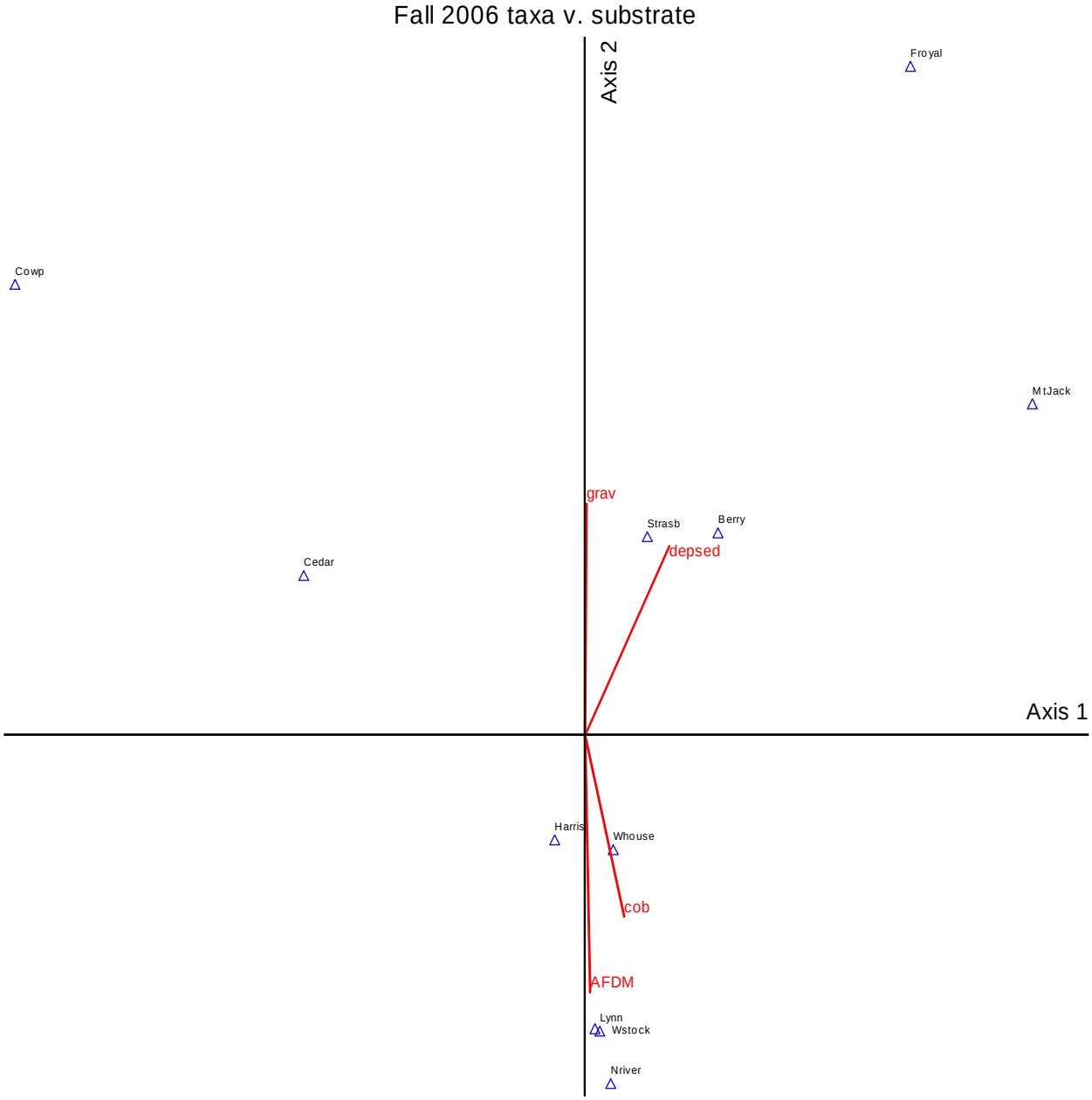


Figure 7. Virginia Stream Condition Index (SCI) by site for large rivers. Black circles are May 2006 data; open circles are August 2006 data. The dashed line indicates that cutoff between impaired and non-impaired. Site codes along x-axis are explained in Table 1.

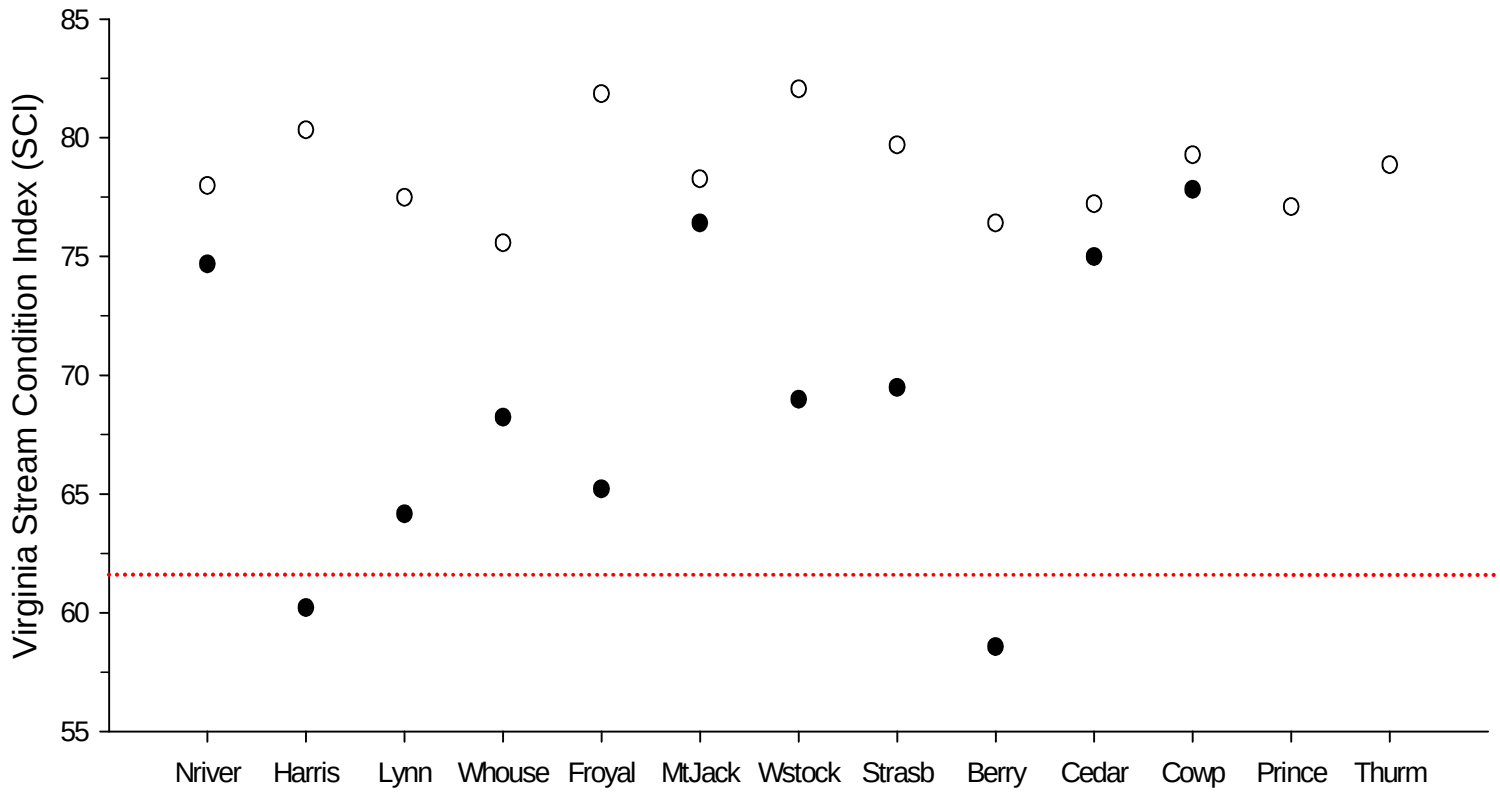


Figure 8. Detrended Correspondence Analysis ordination (DCA) biplot of 2007 tributary top taxa abundance showing taxa most important in site separation. Vector arrows are directly on axes and are difficult to see. Site codes are explained in Table 2. Prosimul = *Prosimulium*; Ephemere = *Ephemerella*.

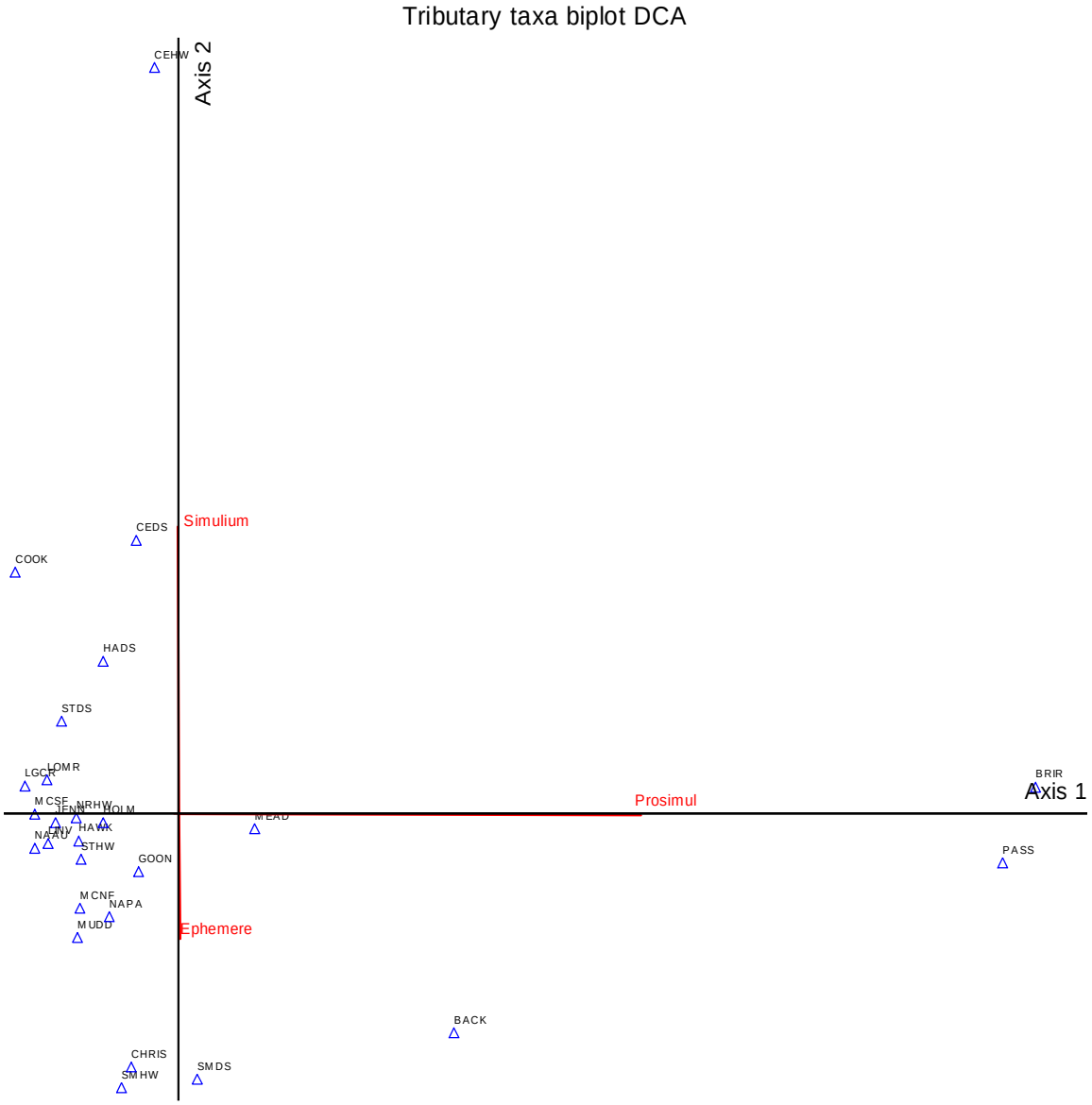


Figure 9. Detrended Correspondence Analysis ordination (DCA) biplot of 2007 tributary top taxa with Passage Creek and *Prosimulium* omitted showing taxa most important in site separation. Site codes are explained in Table 2. Cheumato = *Cheumatopsyche*; Chironom = Chironomidae; Planarii = Planariidae; Ephemere = *Ephemerella*; Maccafe = *Maccaffertium/Stenonema*.

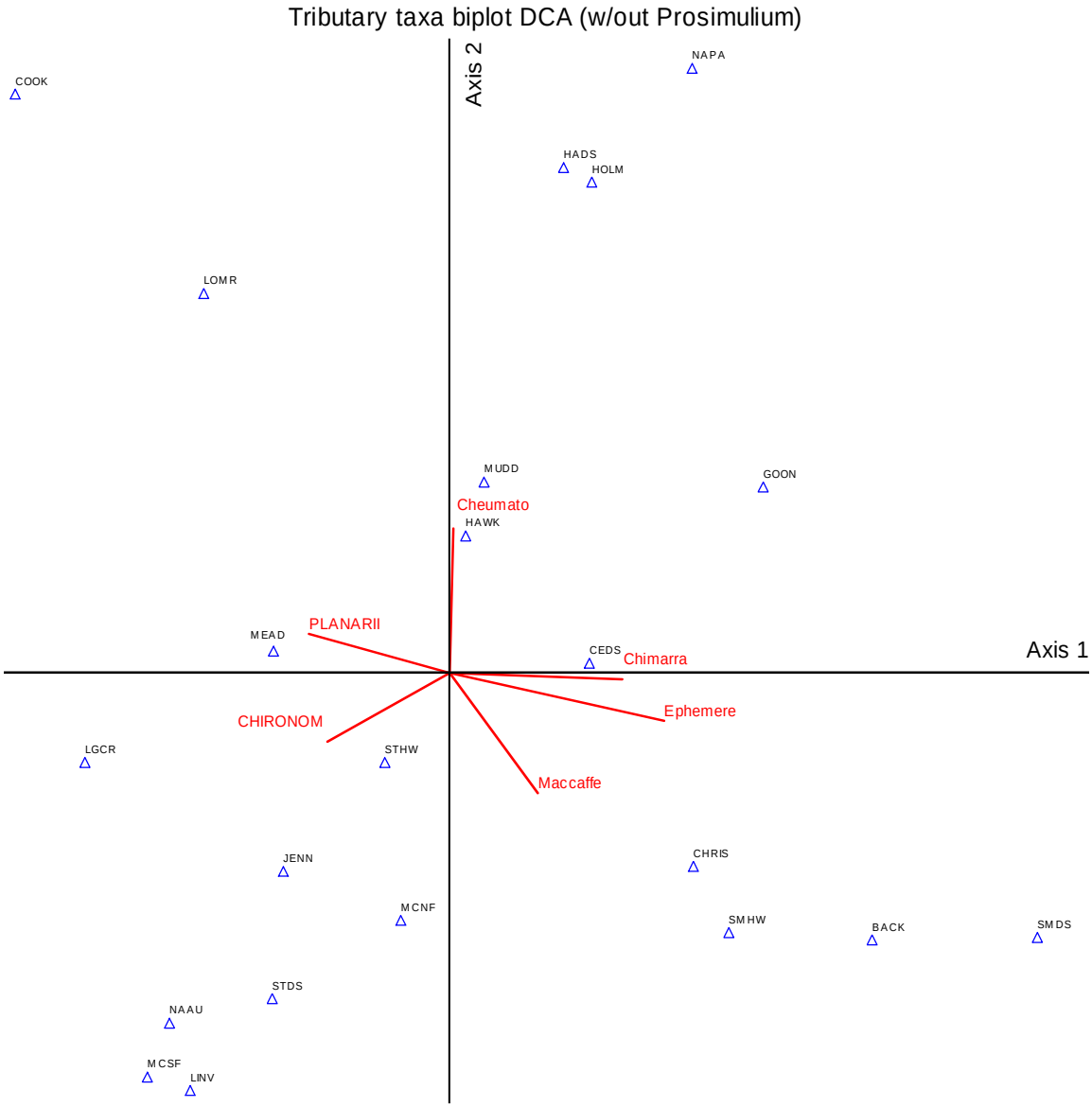


Figure 10. Canonical Correspondence Analysis ordination (CCA) of 2007 tributary taxa (main matrix) using land-use (second matrix) to explain differences in taxa among sites. Axis one explained 16% of variation in site separation whereas axis 2 explained 6%. Site codes are explained in Table 2. #AFO/A = number of animal feeding operations per 1000 acres; %_CROP = percent cropland in watershed; # Dai = number of dairy farms; #_AFO = absolute number of animal feeding operations in watershed. Cutoff value for significance R=0.6.

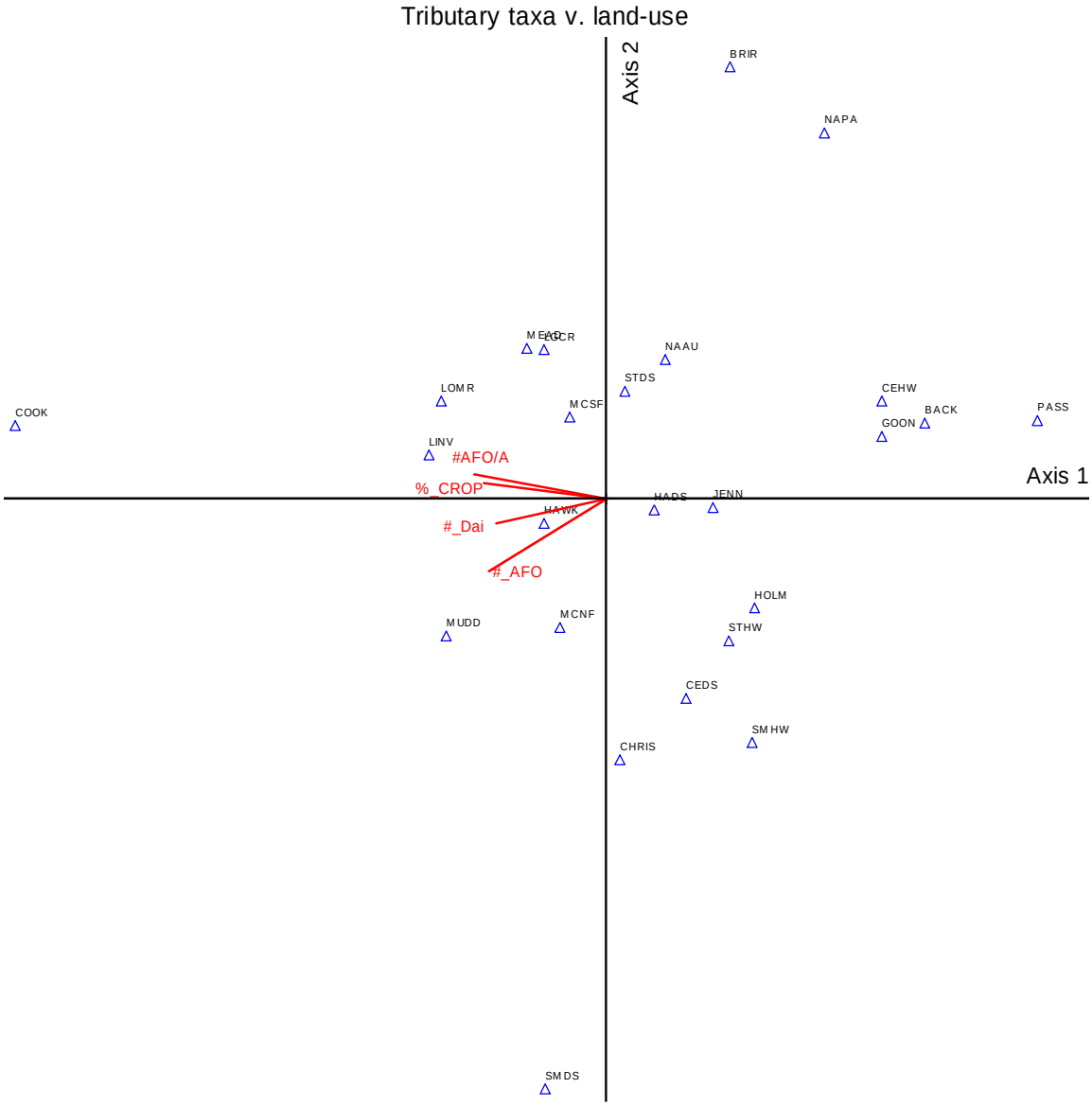


Figure 11. Canonical Correspondence Analysis ordination (CCA) of 2007 tributary taxa (main matrix) using sediment metal, nutrient, and carbon variables (second matrix) to explain differences in taxa among sites. Axis one explained 24% of variation in site separation whereas axis 2 explained 15%. Site codes are explained in Table 2. sed_Cu = sediment copper; TN_% = percent total nitrogen; TOC_% = percent total organic carbon; Sed_Pb = sediment lead. Cutoff value for significance R=0.4.

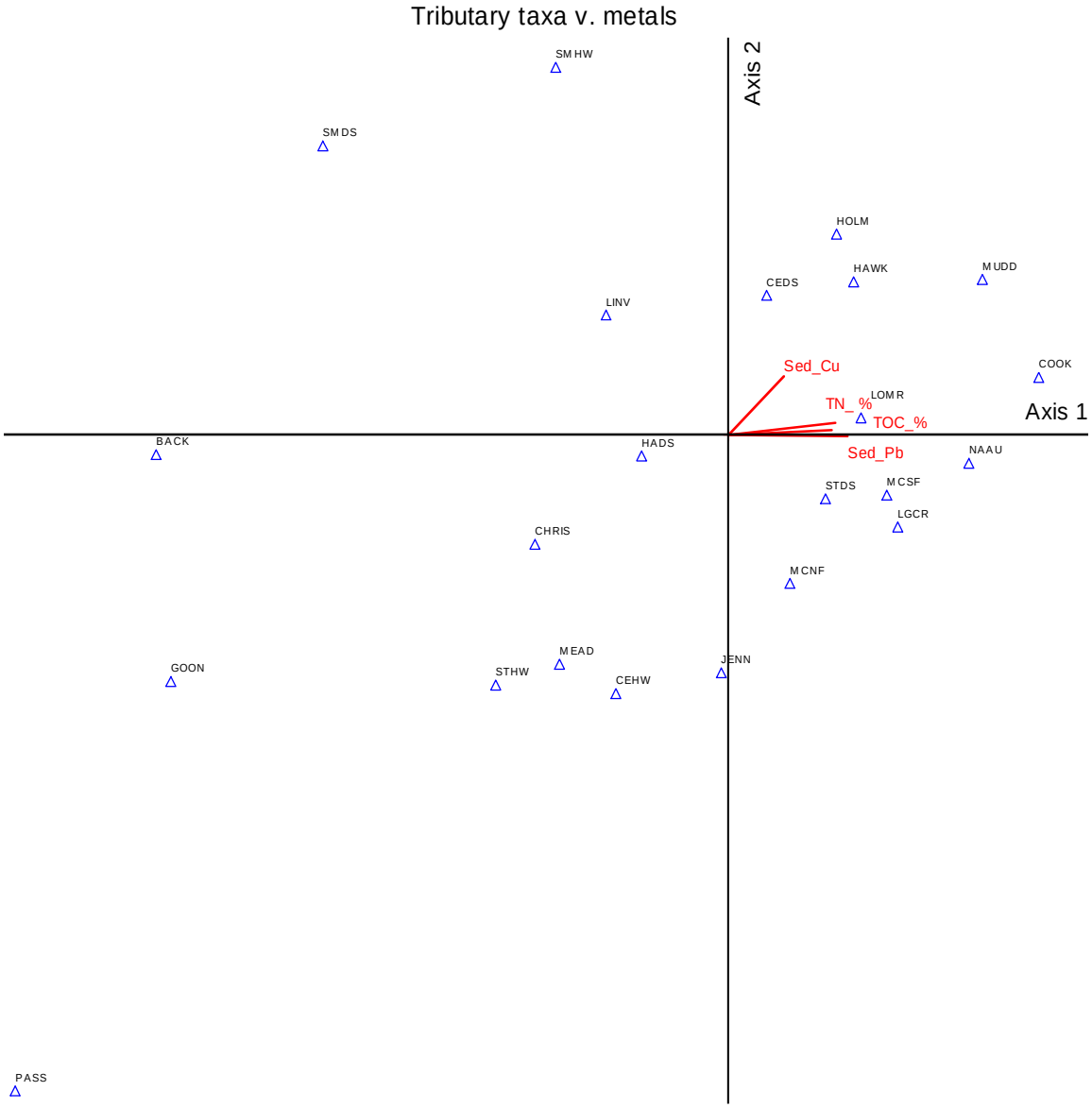


Figure 12. Canonical Correspondence Analysis ordination (CCA) of 2007 tributary taxa (main matrix) using clam tissue metal concentration (second matrix) to explain differences in taxa among sites. Axis one explained 23.4% of variation in site separation whereas axis 2 explained 10.5%. Site codes are explained in Table 2. Clam_Se = clam selenium; Cd = cadmium; Hg = mercury. Cutoff value for significance R=0.4.

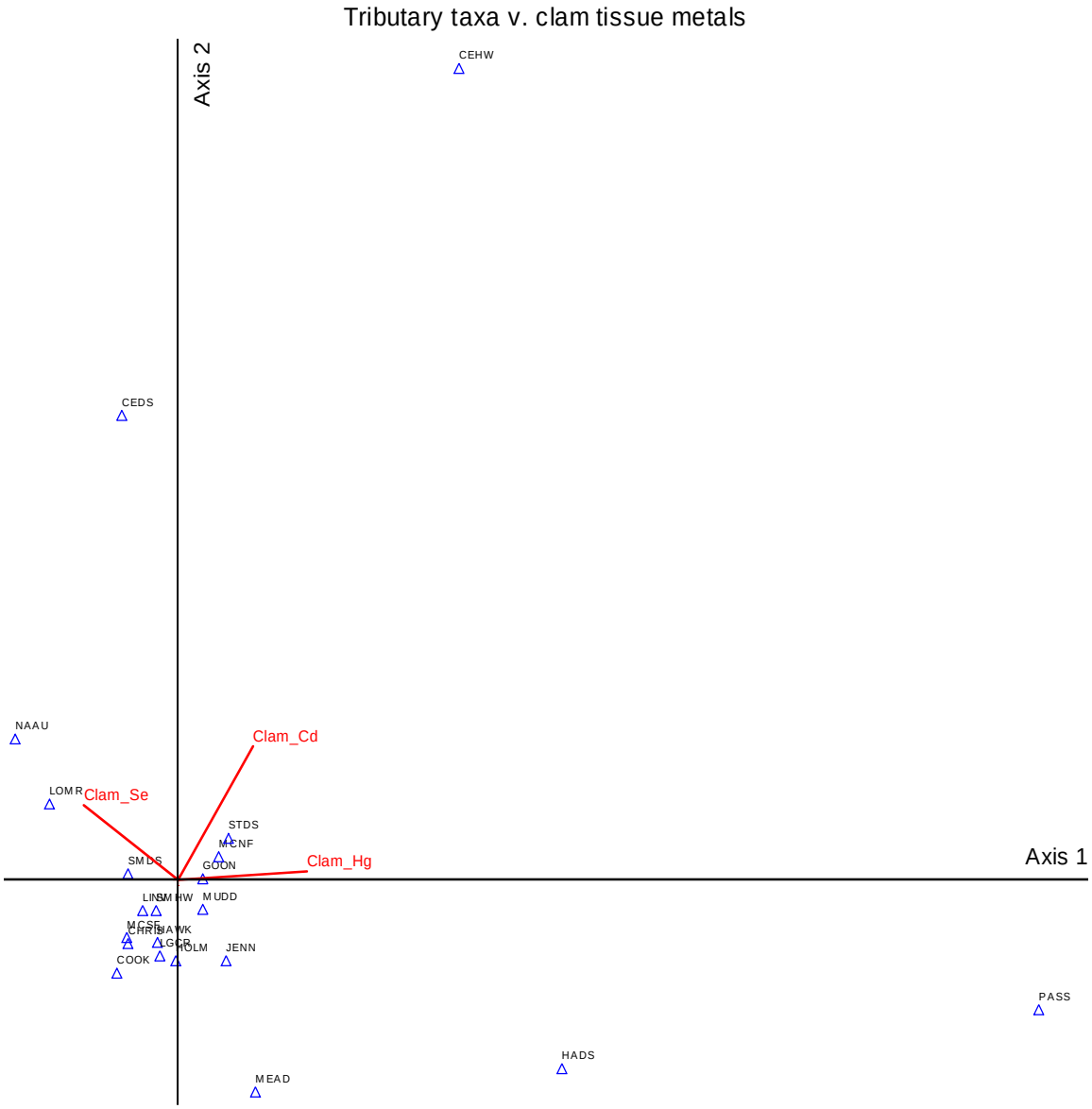


Figure 13. Detrended Correspondence Analysis ordination (DCA) 2007 large river elmid species abundance. Site codes are explained in Table 1.

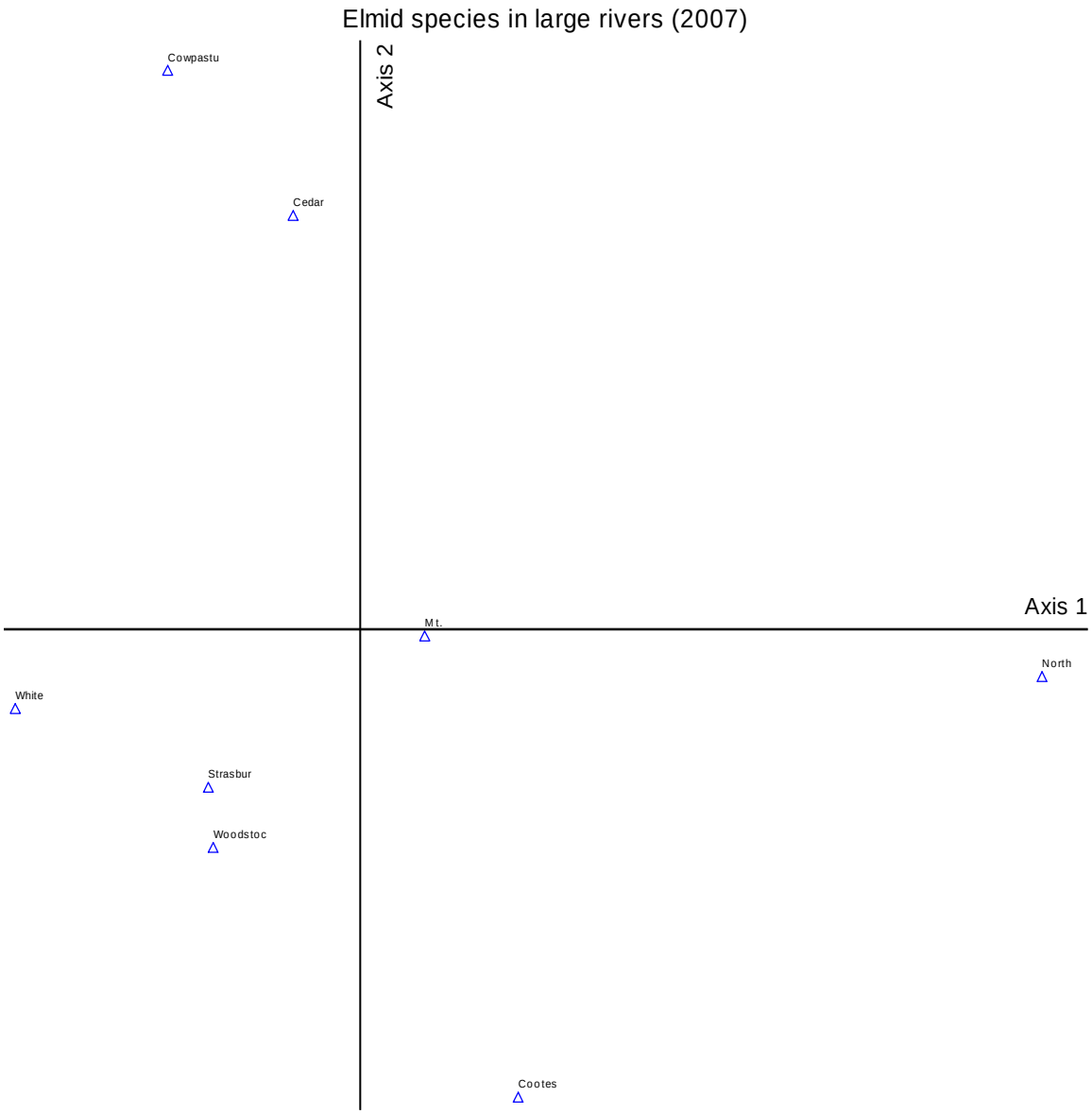


Figure 14. Canonical Correspondence Analysis ordination (CCA) of 2007 tributary elmid species abundance (main matrix) using land-use (second matrix) to explain differences in taxa among sites. Axis one explained 30% of variation in site separation whereas axis 2 explained 17.4%. Site codes are explained in Table 2. #AFO/A = number of animal feeding operations per 1000 acres; #PH/A = number of poultry houses per 1000 acres; #Beef/A = number of beef operations per 1000 acres. Cutoff value for significance R=0.5.

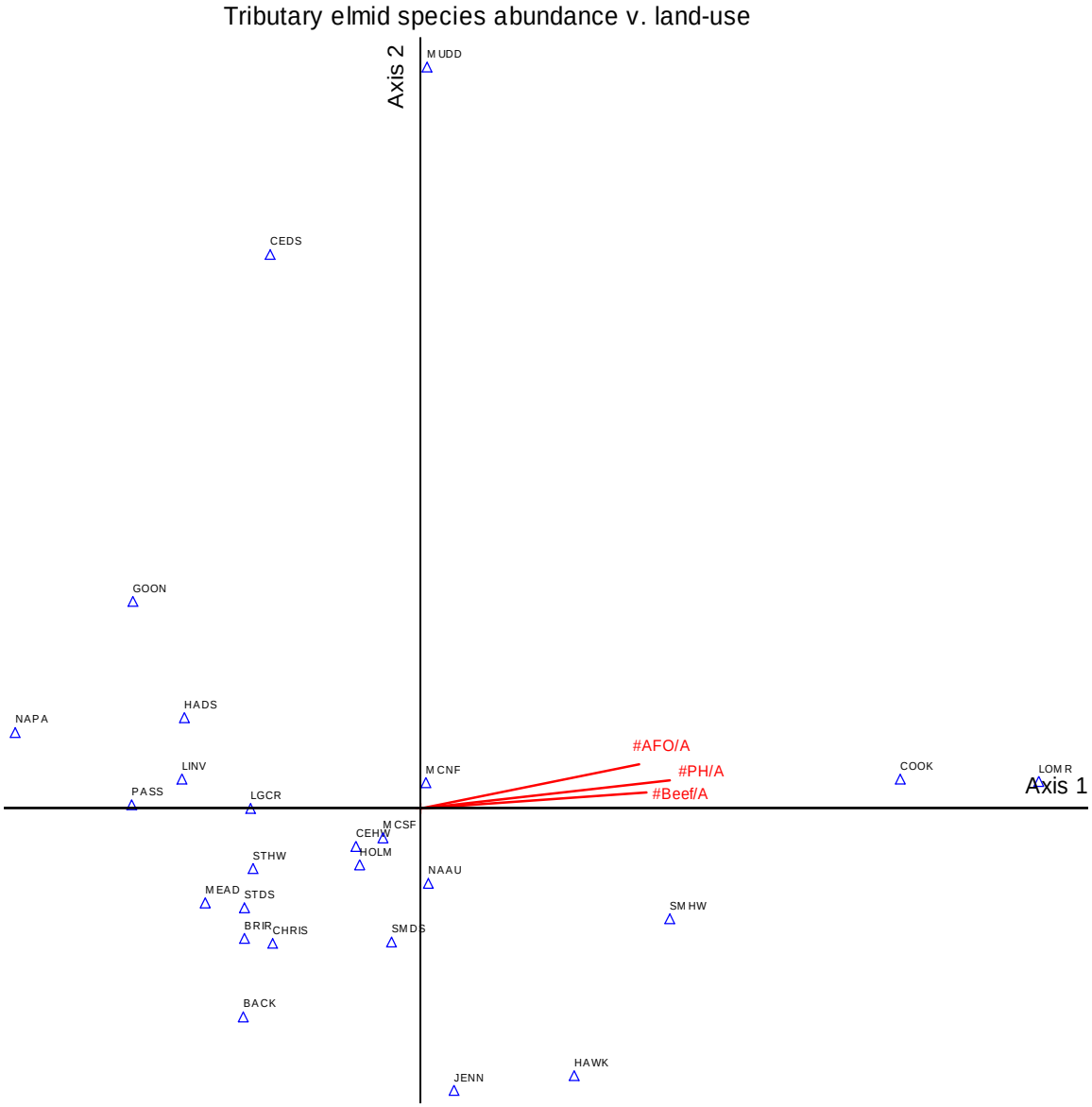
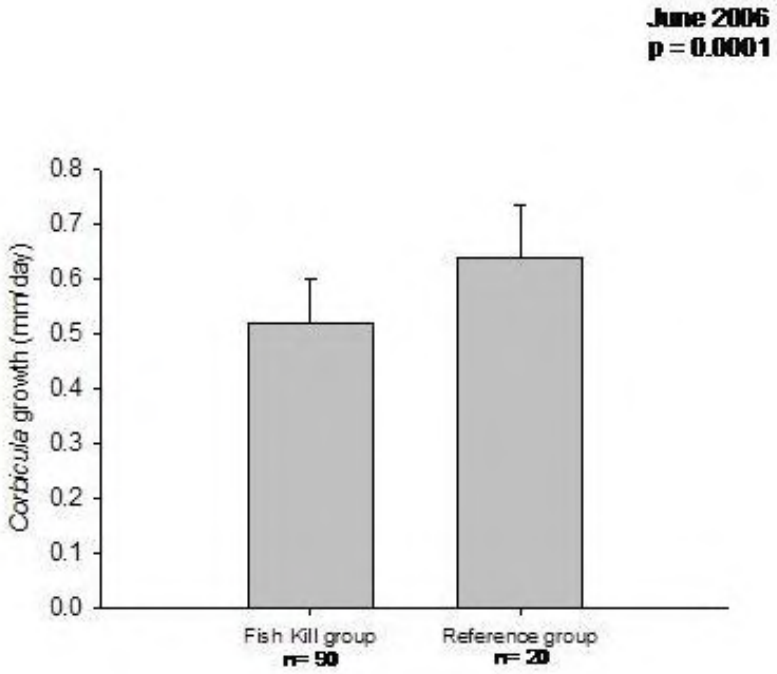


Figure 15. In-situ bioassay results for 2006 *Corbicula* growth (mm/day) between fish kill and reference large river site groups. ANOVA indicated that the site groups were significantly different in June and August.



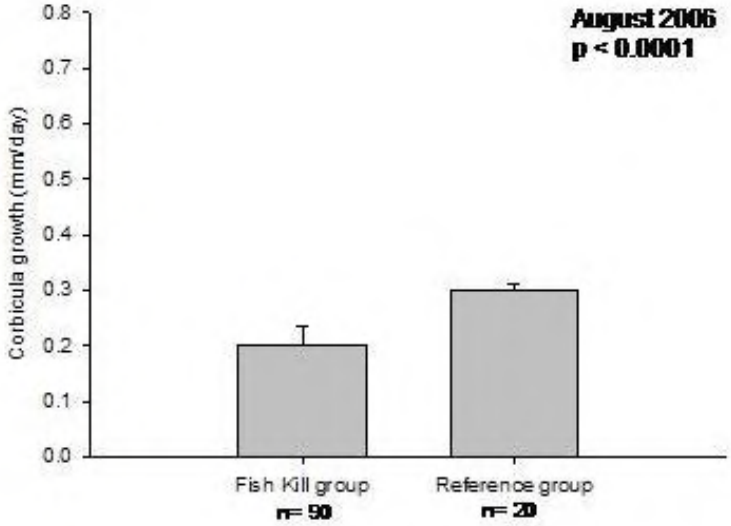


Figure 16. In-situ bioassay results for 2006 *Corbicula* growth (mm/day) among all individual large river sites without any grouping. ANOVA indicated that only Front Royal and Mt Jackson were significantly different in June and only Mt Jackson and Woodstock were significantly different in August.

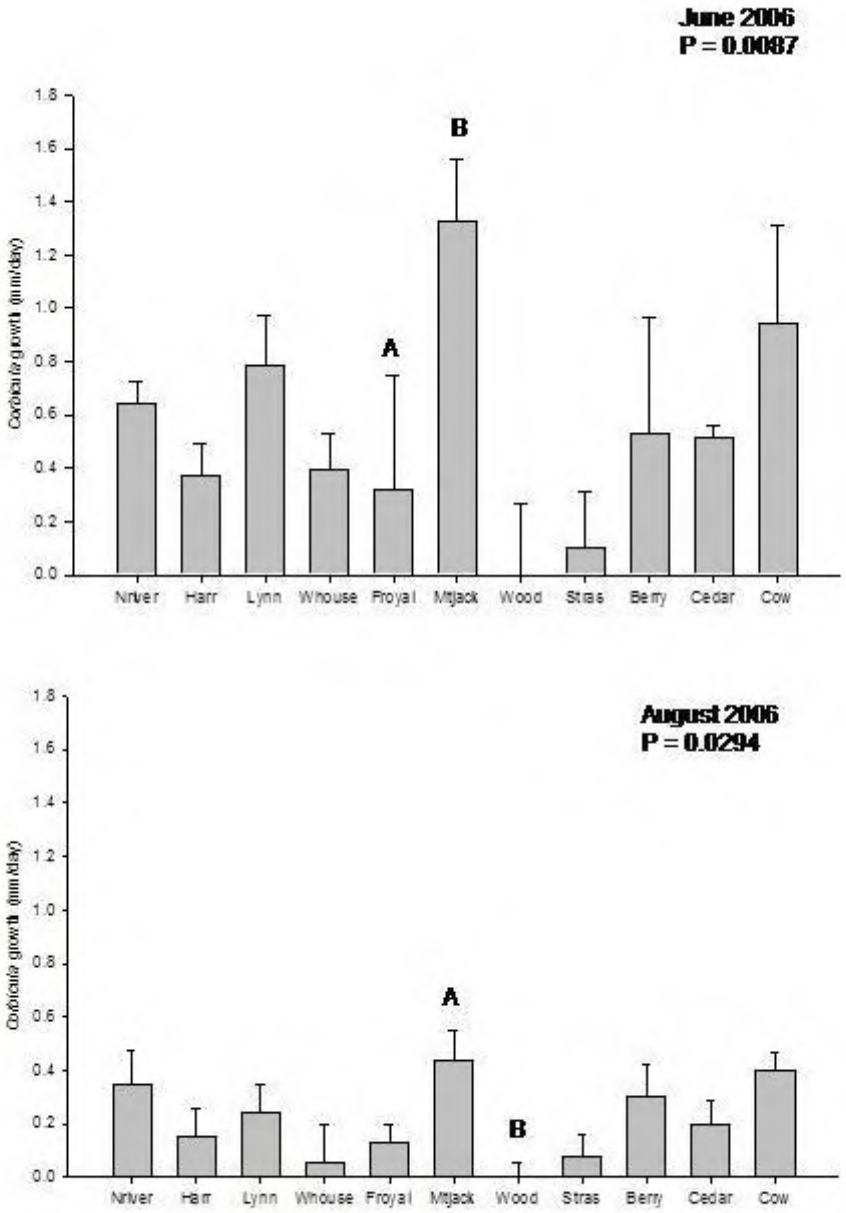


Figure 17. In-situ bioassay results for2006 *Corbicula* mortality between fish kill and reference large river site groups. ANOVA indicated that the site groups were significantly different in June and August.

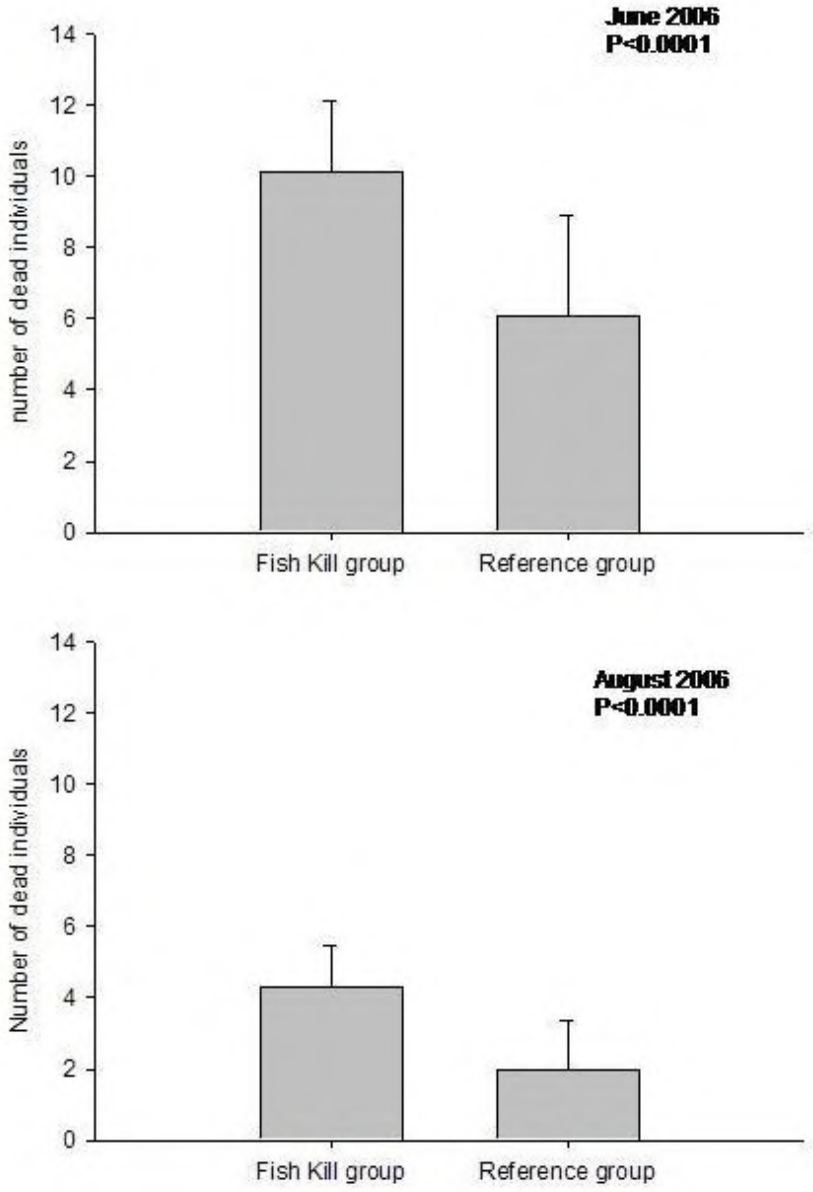
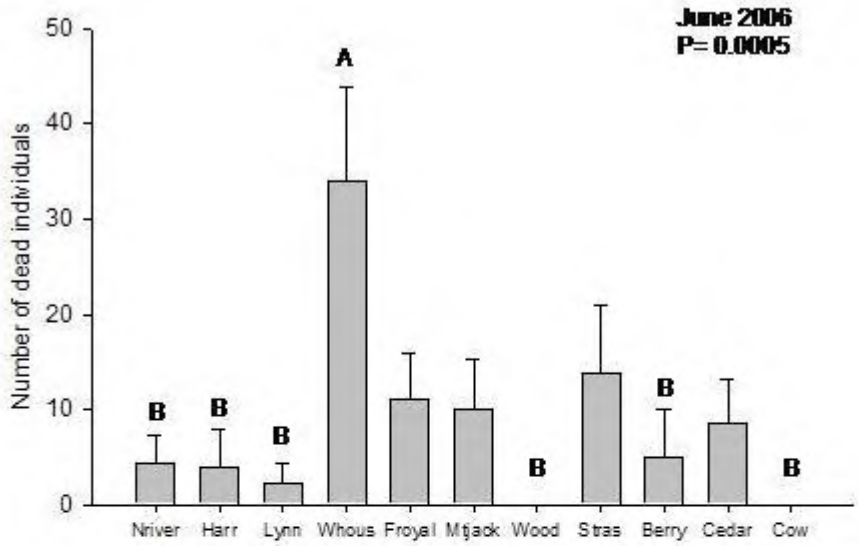
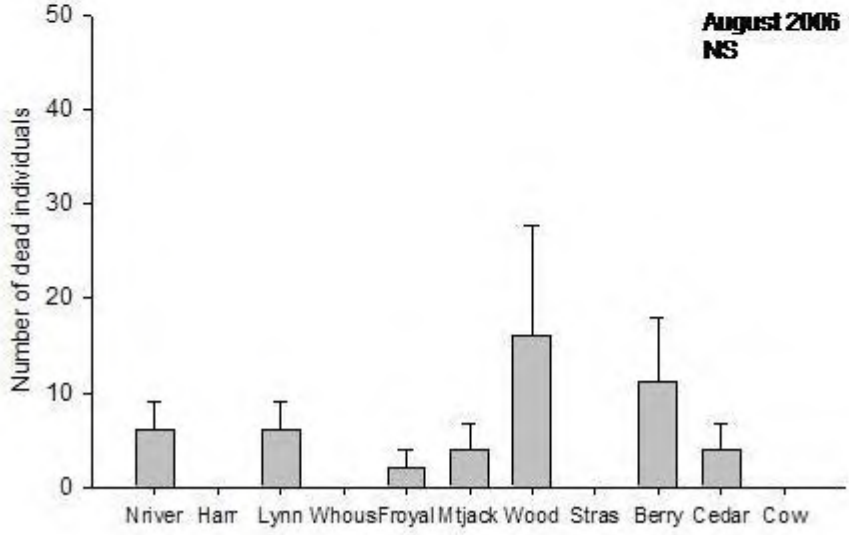


Figure 18. In-situ bioassay results for 2006 *Corbicula* mortality among all individual large river sites without any grouping. ANOVA indicated that only Mt Jackson was significantly different in June, and no sites were significantly different in August.





TABLES

Table 1. Sampling and location information for large river study sites. All are in the Shenandoah River basin, except Cowpasture River, which is in the James River basin. The locations of these sites are shown on a map in Fig. 1.

Site Name	Code	Dates Sampled			Location (latitude/ longitude)	Watershed Area (ha)	Elevation (ft)
		May 2006	Aug 2006	May 2007			
Main Stem							
Berryville	Berry	X	X		39°07'16"N 77°53'40"W	746,877	380
North Fork							
Coote's Store				X			
Mount Jackson	Mtjack	X	X	X	38°44'41"N 78°38'22"W	131,411	840
Woodstock	Wood	X	X	X	38°54'08"N 78°28'50"W	183,652	690
Strasburg	Stras	X	X	X	38°58'21"N 78°21'02"W	199,563	520
South Fork Tribs							
Harriston	Harr	X	X		38°13'06"N 78°50'13"W	53,127	1160
North River	Nriver	X	X	X	38°16'55"N 78°51'05"W	197,059	1060
South Fork							
Lynnwood	Lynn	X	X		38°18'49"N 78°46'18"W	270,134	1040
White House	Whouse	X	X	X	38°38'50"N 78°32'18"W	347,031	740
Front Royal	Froyal	X	X		38°54'53"N 78°12'40"W	418,911	460
Reference							
Cedar Creek	Cedar	X	X	X	39°00'01"N 78°20'00"W	40,756	520
Cowpasture River	Cow	X	X	X	37°58'31"N 78°41'46"W	82,361	1220

Table 2. Tributary sites with codes. Locations of these sites are shown on a map in Fig. 3.

Site Names	Code
Cooks Creek	COOK
Briery Branch	BRIR
Linville Creek	LINV
Muddy Creek	MUDD
Long Meadow Run	LOMR
Mill Creek - N. Fork	MCNF
Naked Creek (Augusta Co.)	NAAU
Hawksbill Creek	HAWK
Hawksbill Creek Downstream	HADS
Holmans Creek	HOLM
Long Glade Creek	LGCR
Mill Creek - S. Fork (Rockingham Co.)	MCSF
Christians Creek	CHRIS
Meadow Run	MEAD
Naked Creek (Page Co.)	NAPA
Jennings Branch	JENN
Smith Creek Headwaters	SMHW
Smith Creek Downstream	SMDS
Stony Creek Headwaters	STHW
Stony Creek Downstream	STDS
Back Creek	BACK
Gooney Run - Ref.	GOON
Passage Creek - Ref.	PASS
Cedar Creek Headwaters - Ref.	CEHW
Cedar Creek Downstream - Ref.	CEDS

Table 3. Land use categories that were quantified for the subwatersheds in the tributary study with codes used in statistical analyses.

Land use categories	Code
# Acres in subwatershed(s)	Area_Acres
% Area as wetland	%_WETL
% Area as forest	%_FOR
% Area as pasture/hay	%_PASTHAY
% Area as crops	%_CROP
% Area as developed land (all types)	%_DEVEL
% Area as barren land	%_BARR
# Dairies	#_Dairy
# Beef operations	#_Beef
# Poultry houses	#_PoultryH
# Acres in a nutrient mgmt plan for poultry litter	SumA_NMP
# Animal feeding operations (total)	#_AFO
# Dairies/1000 acres	#Dairy/1000A
# Beef operations/1000 acres	#Beef/1000A
# Poultry houses/1000 acres	#PH/1000A
# Animal feeding operations/1000 acres	#AFO/1000A
% Acres in a nutrient mgmt plan for poultry litter	%AcresNMP
# Municipal STPs	#MUNSTP
Total flow (MGD) of municipal STPs	MUNFLOW

Table 4. Benthic macroinvertebrate assemblage metrics: definitions and usually expected responses to environmental stressors. The + or – symbols indicate an expected increase or decrease, respectively, in the metric's numerical value.

Metrics (within categories)	Definition	Expected response to environmental stressors
Density		
Total density	Number of all individuals per m^2	+ or -
Richness, Diversity, Evenness		
Total richness	Number of all taxa	-
Simpson's diversity index	Combines richness and abundance with the equation: $\frac{\sum n(n-1)}{N(N-1)}$ Where: n= total number of individuals of a taxon, N=total number of individuals	+ or -
Composition		
% Modified EPT	Proportion of total density consisting of organisms within the orders Ephemeroptera (excluding Baetidae), Plecoptera, and Trichoptera (excluding Hydropsychidae)	-
% Non-insects	Proportion of total density consisting of organisms that are not insects	+
Tolerance		
% Sensitive	Proportion of total density consisting of organisms with pollution tolerance values of 0, 1, or 2	-
Trophic		
% Scrapers	Proportion of total density consisting of organisms that shear the	+ or -

	layer of material growing on firm substrates	
% Collector-gatherers	Proportion of total density consisting of organisms that acquire and ingest fine particles of detritus lying on the bottom	+
% Collector-filterers	Proportion of total density consisting of organisms that acquire and ingest fine particles of detritus suspended in the water	
<u>Habits</u>		
% Crawlers	Proportion of total density consisting of organisms that move around slowly in small spaces within mineral or plant substrate	-
% Modified Clingers	Proportion of total density consisting of organisms that maintain a fixed position on mineral or plant substrate in current (excluding Hydropsychidae)	

Table 5. List of all taxa collected from large river study sites in 2006. (M) indicates the taxon was present in May; (A) indicates the taxon was present in August ; blank cells indicate that a taxon was not collected.

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wood	Stras	Berry	Cedar	Cow
NON- INSECT TAXA											
NEMATODA	A	M A	M A	M	M A	M		M	M		
ANNELIDA											
HIRUDINEA	M						M	M			M
PLANARIIDAE	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
NEMERTEAN	A	M A	M A	M A		M	M A	M A	M A	M A	A
MOLLUSCA											
CORBICULIDAE											
Corbicula fluminea	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
SPHAERIIDAE	M	M A	M A			A		M A	M	M A	M A
unknown gastropod							M A				
PLEUROCERIDAE											
Leptoxis carinata	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
ANCYLIDAE			M A	M A		M A			M A	M A	
PHYSIDAE											
Physa	M A						M A	M A		M A	
PLANORBIDAE				M A			A	M A			
CRUSTACEA											
CAMBARIDAE										M A	
ASELLIDAE											
Caecidotea		M									
CRANGONYCTIDAE											
Crangonyx	A		M								
GAMMARIDAE											
Gammarus							M			M	

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wood	Stras	Berry	Cedar	Cow
ACARI (HYDRACARINA)								M		M	MA
INSECT TAXA											
EPHEMEROPTERA											
BAETIDAE											
Baetis (complex)	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
CAENIDAE											
Caenis	A	M A	M A	A	A	M A	M A	M A	M A	M A	M A
EPHEMERELLIDAE											
Drunella lata		M				M				M	M
Drunella tuberculata	M	M		M		M	M	M		M	M
Ephemerella	M	M	M	M	M A	M	M A	M	M	M A	M A
Eurylophella											M A
Serratella	M A	M A	M A	M A	A	M A	M A	M A	M A	M A	M A
EPHEMERIDAE										M	
HEPTAGENIIDAE											
Leucrocuta	M A	M A		M A		M A	M A	M A		M A	
Maccaffertium/Stenonema	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
Stenacron	M	M A	M		A	M A	M A	A	M A	M	
Rhithrogena								M			M
ISONYCHIIDAE											
Isonychia	M A	M A	M A	M A	A	M A	M A	M A	M A	M A	M A
LEPTOPHLEBIIDAE		M A			A					M A	
LEPTOHYPHIDAE											
Tricorythodes	A	M	M A	A	A	M A	A	A	M A	M A	A
POTAMANTHIDAE											
Anthopotamus	M A	M			A	M			A	M	M A
PLECOPTERA											
CHLOROPERLIDAE					M						

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wood	Stras	Berry	Cedar	Cow
LEUCTRIDAE											
Leuctra							M			M	M
NEMOURIDAE											
Amphinemura		M									
PERLIDAE		M A				M A				M A	M A
Acroneuria	A	M A			A				A	M A	M A
Agnetina	M A	M A	M A	M A	M A	M		A	M A		M A
Neoperla											M A
Paragnetina											A
Perlesta placida (group)	M	M	M A	M A		M	M	M	M	M	M A
PERLODIDAE											
Isoperla				M							
PTERONARCYIDAE											
Pteronarcys										M A	A
ODONATA											
CALOPTERYGIDAE											
Calopteryx							M				
COENAGRIONIDAE											
Argia	M A	M A	M A	M A	A	M A	M A	M A	M	M A	M
GOMPHIDAE											
Gomphus							M			M	
Lanthus		M					A	A		M A	M A
Stylogomphus		A	M						M	A	A
NEUROPTERA											
SISYRIDAE											
Climacia		A								M A	M A
MEGALOPTERA											
CORYDALIDAE											
Corydalus cornutus	M A	A	M A	M A	M A	M A	M A	M A	M A	M A	M A
Nigronia fasciatus		A									M

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wood	Stras	Berry	Cedar	Cow
SIALIDAE											
Sialis						M A					
TRICHOPTERA											
BRACHYCENTRIDAE											
Brachycentrus	M A	M A	M A	M A	A	M	M	M	M	M A	A
Micrasema	M					M	M		A	M A	M A
GLOSSOSOMATIDAE											
Glossosoma nigrior										M	M
Protoptila	M A	M A	M A	M A	M A	M	M A	M A	M A	M A	M A
HELICOPSYCHIIDAE											
Helicopsyche borealis	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
HYDROPSHYCHIDAE											
Diplectrona							M	M			
Cheumatopsyche	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
Hydropsyche	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
Macrostemum											MA
Parasyche		A									
HYDROPTILIDAE											
Agraylea	M A		M A	M A	M A	M A	M A	M A	M A	M A	M A
Hydroptila	M A	M A	M A	M A	A	M A	A	A	M A	M A	
Ochrotrichia	M A										
LEPTOCERIDAE											
Ceraclea		A	M			M		M A	M A	M	M A
Nectopsyche								M			
Oecetis	M		M	M	MA	M	M	M A	A	M	A
Triaenodes	M		M				M	M			
LEPIDOSTOMATIDAE											
Lepidostoma	M A	A	M A		M			A	M A	A	M A

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wood	Stras	Berry	Cedar	Cow
LIMNEPHILIDAE											
Pycnopsyche				A							
UENOIDAE											
Neophylax			M		A	M			M A		
PHILOPOTAMIDAE											
Chimarra	M A	A	M A	M A	M	M A	M A	M A	M A	M A	M A
Dolophilodes	M									M	M
POLYCENTROPODIDAE											
Polycentropus				A							
Neureclipsis	A										
Nyctiophylax						M	M				
PSYCHOMYIIDAE											
Lype diversa	A	M A	M A		M				M	M	
Psychomyia	M									M	
RHYACOPHILIDAE											
Rhyacophila			M			M	M		M	M	M
LEPIDOPTERA											
PYRALIDAE											
Petrophila	A		A	A	A		A	A	A		M A
COLEOPTERA											
ELMIDAE											
Ancronyx				M							
Dubiraphia minima	M A	M A	M A	M A	M	M	M A	M A	M A	M A	M A
Macronychus			A	A				A		M	
Microcylloepus pusillus	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
Optioservus trivittatus	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
Oulimnius latiusculus		M A	A								
Promoresia elegans	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
Stenelmis crenata	M A	M A	M A	M A	A	M A	M A	M A	M A	M A	M

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wood	Stras	Berry	Cedar	Cow
Stenelmis mera	A		M A	M A	A	M A	M A	M A	M A	M A	M A
Stenelmis sandersoni										M A	M A
Stenelmis musgravei			A		M		A		M	A	A
Stenelmis markeli					A						
Stenelmis lateralis											M A
GYRINIDAE											
Dineutus									M		
HYDROPHILIDAE											
Berosus	M	M	M	M	M	M	M	M	M	M	
LUTROCHIDAE											
Lutrochus										M A	
PSEPHENIDAE											
Ectopria			M		M			M	M	M A	A
Psephenus herricki	M A	M A	M A	M A		M A	M A	M	M A	M A	M A
SCIRTIDAE											
Scirtes								M			
DIPTERA											
ATHERICIDAE											
Atherix	A		A							M A	A
BLEPHARICERIDAE											
Blepharicera											M
CERATOPOGONIDAE		M				M	M A	M		M	M A
CHIRONOMIDAE	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
EMPIDIDAE											
Clinocera						M				M	
Hemerodromia	M A		M A	M A	M A	M A	M A	M A	M	M	M
SIMULIIDAE											
Simulium	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A	M A
TABANIDAE										M	

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wood	Stras	Berry	Cedar	Cow
TANYDERIDAE											
Protoplasa fitchii											M
TIPULIDAE											
Antocha	M A	M A	M A	M	M A	M	M	M	M A	M A	M A
Hexatoma									M		
Tipula		M	M	M				M A	M		
Total taxa	55	56	54	48	44	54	55	58	56	72	67

Table 6. 'Top taxa', selected as comprising > 0.2 % of total abundance at a site collected during three sample periods listed in order of descending overall abundance.

Spring 2006	August 2006	2007 Large river
Chironomidae	*Baetis* (complex)	*Stenelmis*
Stenelmis	*Stenelmis*	*Baetis (*complex)
Leptoxis carinata	*Leptoxis carinata*	Chironomidae
Optioservus	*Macaffertium/Stenonema*	*Leptoxis carinata*
Baetis (complex)	*Optioservus*	Empididae
Planariidae	*Isonychia*	Macaffertium/Stenonema
Cheumatopsyche	*Cheumatopsyche*	Ephemerella
Corbicula fluminea	Chironomidae	Hydropsyche
Hydropsyche	*Hydropsyche*	Simulium
Serratella	Planariidae	Optioservus
Macaffertium/Stenonema	*Serratella*	Planariidae
Simuliium	*Tricorythodes*	*Hydropsyche*
Ephemerella	*Leuctra*	*Caenis*
Microsema	*Chimarra*	*Cheumatopsyche*
Psephenus	*Simulium*	*Anthopotamus*
Caenis	*Micrasema*	*Psephenus herricki*
Chimarra	*Corbicula fluminea*	*Microcylloepus*
Helicopsyche	*Agraylea*	*Isonychia*
Perlesta	*Corydalus*	*Stenacron*
Berosus	*Promoresia*	*Eurylophella*
Dubiraphia	*Psephenus*	*Chimarra*
Isonychia	*Helicopsyche*	Dryopidae
Brachycentrus	*Agnetina*	Tabanidae
Protoptila	*Caenis*	*Dubiraphia*
Leuctra	*Protoptila*	*Serratella*
Ceratopogonidae	*Hydroptila*	*Leptophlebia*
Anthopotamus	*Heptagenia*	*Corbicula fluminea*
Promoresia	*Lepidostoma*	Ceratopogonidae
Hemerodromia		Taeniopterygidae
Antocha		*Agnetina*
Drunella tuberculata		*Isoperla*
Diplectrona		*Macrostemum*
Corydalus		*Promoresia*
Rithrogena		*Leucrocuta*
Stenacron		*Acroneuria*
Nemertean		Leptophyidae
		Scirtidae
		Helicopsyche borealis
		Oligochaeta
		Antocha
		Nematoda

Table 7. Spring 2006 macroinvertebrate metrics calculated for eleven large river sites and two New River sites (*). mod = modified; CG = collector-gatherer; CF = collector-filterer.

Site	Total abundance	Taxa richness	Simpson's Diversity Index	%mod EPT	% non-insect	% sensitive	% scrapers	% CG	% CF	% Crawler	Mod % Clinger
North river	714	30	0.884	26	18	24	47	45	15	10	65
Harriston	445	23	0.820	18	9	14	25	72	21	4	53
Lynnwood	566	25	0.819	18	22	19	33	64	11	7	44
Whitehouse	846	28	0.846	12	38	21	43	47	8	6	52
Front Royal	554	25	0.783	18	17	9	33	62	21	5	51
Mount Jackson	441	28	0.870	38	13	24	45	43	4	13	59
Woodstock	637	31	0.846	16	20	19	47	48	11	5	62
Strasburg	598	28	0.797	12	28	23	64	32	10	3	73
Berryville	726	24	0.739	18	4	2	32	63	12	4	52
Cedar	664	35	0.891	27	21	29	51	41	5	12	61
Cowpasture	498	27	0.881	35	11	24	55	29	10	12	72
Prince*	218	19	0.764	36	31	39	40	49	14	8	73
Thurmond*	164	17	0.857	43	17	30	37	54	28	8	77

Table 8. Summer 2006 macroinvertebrate metrics calculated for eleven large river sites and two New River sites. mod = modified; CG = collector-gatherer; CF = collector-filterer.

Site	Total abundance	Taxa richness	Simpson's Diversity Index	%mod EPT	% non-insect	% sensitive	% scrapers	% CG	% CF	% Crawler	Mod % Clinger
North river	929	29	0.868	49	17	21	30	56	14	76	7
Harriston	713	25	0.873	58	8	24	16	66	34	84	6
Lynnwood	913	29	0.875	36	24	25	41	52	17	81	4
Whitehouse	840	25	0.852	29	15	19	49	43	20	84	6
Front Royal	533	25	0.858	69	6	13	30	60	10	67	4
Mount Jackson	188	19	0.818	63	10	22	32	56	12	89	5
Woodstock	691	22	0.863	57	14	31	40	51	16	88	5
Strasburg	393	24	0.861	51	13	15	45	47	5	70	3
Berryville	807	27	0.875	42	5	18	35	52	28	79	8
Cedar	512	27	0.903	31	17	31	56	29	15	86	9
Cowpasture	529	27	0.895	42	6	18	36	51	23	71	9

Table 9. 2007 macroinvertebrate metrics calculated for seven large river sites. mod = modified; CG = collector-gatherer; CF = collector-filterer.

	Total abundance	Taxa richness	Simpson's Diversity Index	% modified EPT	% non-insect	% sensitive	% scrapers	% CG	% CF	% Crawlers	Modified % clingers
Whitehouse	577	20	0.936	9	12	10	71	55	14	3	74
Cedar	533	22	0.956	10	13	12	69	54	15	4	71
North River	412	21	0.946	12	13	14	59	58	15	5	68
Woodstock	343	24	0.949	13	15	16	59	55	12	6	67
Cowpasture	296	23	0.938	13	18	19	68	48	9	7	71
Mount Jackson	334	24	0.945	17	15	18	58	54	12	8	68
Strasburg	289	26	0.948	18	14	20	51	55	13	9	66

Table 10. Virginia Stream Condition Index (VSCI) by site calculated from August 2006 data. (*) indicates data from the New River collected during late summer 1988 and 1989 (Thurm = Thurmond). Site codes are explained in Table 1. E = Ephemeroptera; P = Plecoptera; T = Trichoptera; HBI = Hilsinhoff Biotic Index.

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wstock	Strasb	Berry	Cedar	Cowp	Prince*	Thurm*
Taxa Richness	34	29	32	28	30	23	27	31	29	31	32	24	21
EPT Index	17	15	15	14	16	10	11	13	16	15	17	12	10
% P+T - Hydropsychidae	8.84	7.02	3.95	2.54	5.59	0.89	0.31	1.78	3.70	4.76	17.05	3.60	8.22
HBI (modified family)	4.48	4.34	4.31	4.35	4.42	4.24	4.09	4.35	4.34	3.77	3.96	3.62	4.00
% Top 2 Dominant Family	42.68	32.25	39.70	49.31	41.84	52.88	45.08	49.11	49.22	50.68	39.65	48.05	36.24
% Chironomidae	5.58	4.19	4.45	2.46	2.53	2.13	0.75	1.31	3.91	1.30	15.47	10.42	3.76
% Scraper	36.24	31.78	44.75	53.55	37.66	40.91	46.94	52.50	42.25	63.17	39.84	52.49	42.34
% Ephemeroptera	39.87	48.58	31.05	26.31	62.34	61.93	56.44	46.23	37.26	25.78	26.60	29.04	35.13
SCI	**77.97**	**80.32**	**77.49**	**75.57**	**81.84**	**78.27**	**82.04**	**79.96**	**76.41**	**77.21**	**79.28**	**77.09**	**78.85**
	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired

Table 11. Virginia Stream Condition Index (VCI) by site calculated from May 2006 data. Site codes are explained in Table 1. E = Ephemeroptera; P = Plecoptera; T = Trichoptera; HBI = Hilsinhoff Biotic Index.

	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wstock	Strasb	Berry	Cedar	Cowp
Taxa Richness	34	34	30	27	30	33	35	32	36	46	36
EPT Index	16	17	16	13	15	16	15	12	18	20	17
% P+T - Hydropsychidae	2.68	2.15	3.67	1.98	5.36	4.19	1.25	4.44	2.27	6.01	14.39
HBI (modified family)	4.26	5.10	4.86	5.06	4.99	4.08	4.63	4.34	5.17	4.05	3.64
% Top 2 Dominant	44.87	50.45	50.01	45.92	52.35	45.31	54.61	57.78	67.73	43.62	53.91
% Chironomidae	8.93	36.37	33.68	14.13	27.90	14.09	19.62	12.74	40.78	18.39	4.42
% Scraper	52.43	21.06	32.60	43.84	34.43	54.19	50.27	61.61	29.52	54.64	62.20
% Ephemeroptera	21.20	15.68	13.82	9.18	13.40	28.61	14.40	7.45	15.22	19.80	15.76
SCI	**74.68**	**60.21**	**64.16**	**68.22**	**65.21**	**76.40**	**68.98**	**69.47**	**58.57**	**74.99**	**77.82**
	Not Impaired	Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Not Impaired	Impaired	Not Impaired	Not Impaired

Table 12. Macroinvertebrate mean biomass calculated from large river riffle areas at each study site in spring 2006. Only taxa that dominate the non-mollusk biomass are shown. Values are mean biomass (mg dry mass per m^2) of 6 replicate samples. Site codes are explained in Table 1.

TAXON	Nriver	Harris	Lynn	Whouse	Froyal	MtJack	Wstock	Strasb	Berry	Cedar	Cowp
Stenelmis	610	103	243	700	325	284	605	541	453	113	371
Chironomidae	91	216	301		265	97	230	53	547	188	
Corydalus	141			2133		370	424	43	702	282	117
Isonychia	126			508	94	125	144	90		61	
Maccaffertium/Stenonema	75	200				210	62	57		69	
Agnetina	124		250								96
Hdyropsyche	111	47	151								
Cheumatopsyche					64	97			192		
Ephemerella	278		90			148					
Serratella	100	82								80	
Baetis			87		66		69				
Optioservus										52	82
Leucrocuta						140	111				
Psephenus		61								70	
Chimarra											154
Simulium		47									
Tipula			108								
Berosus				239							
Helicopsyche					44						
Nigronia										52	
Dubiraphia										50	
Acroneuria											154
Rithrogena											96
Number of dominant taxa	9	7	7	4	6	8	7	5	4	10	7

Total biomass (no mollusks)	2225	1018	1597	4728	1094	1850	2136	984	2519	1365	1434
Total biomass	7746	2914	6743	16960	4408	4513	8535	5139	3038	6469	3429

Table 13. Linear regression results showing spring 2006 metrics and top taxa versus DEQ water quality environmental variables. Relationships having coefficients of variation (R^2) > 0.3 are considered ecologically relevant and >0.5 are considered especially meaningful and marked with an "*". EPT = Ephemeroptera, Plecoptera, and Trichoptera.

Response/Metric	Independent variable	p-value	R^2	Direction
Modified EPT	**Total phosphorus**	**0.029**	**0.428**	+
Simpson's Diversity Index	**Ammonia**	**0.038**	**0.397**	-
	Kjeldahl nitrogen	**0.031**	**0.420**	-
	Total phosphorus	**0.039**	**0.395**	-
Percent Crawler taxa	**Ammonia**	**0.05**	**0.362**	-
Optioservus	**Maximum Nitrate + Nitrite (N-N)**	**0.003**	**0.636***	-

Table 14. Linear regression results showing August 2006 metrics versus DEQ water quality, epilithic, and substrate environmental variables. Relationships having coefficients of variation (R^2) > 0.3 are considered ecologically relevant and >0.5 are considered especially meaningful and marked with an "*". AFDM = ash-free-dry-mass of epilithon.

Response/Metric	Independent Variable	p-value	R^2	Direction
Total abundance	**Gravel**	**0.019**	**0.477**	**-**
	AFDM epilithon	**0.005**	**0.594***	**+**
	Ammonia max	**0.040**	**0.427**	+
	Kjeldahl max	**0.022**	**0.500**	+
Taxa richness	**Phosphorus**	**0.049**	**0.403**	**-**
Simpson's Diversity Index	**Nitrate**	**0.033**	**0.452**	**-**
	N-N	**0.033**	**0.453**	**-**
	Phosphorus	**0.011**	**0.577***	**-**
	Total phosphorus	**0.016**	**0.538**	**-**
	Nitrate max	**0.018**	**0.524**	**-**
	N-N max	**0.018**	**0.526**	**-**
	Phosphorus max	**0.015**	**0.545**	**-**
	Orthophosphate max	**0.010**	**0.587***	**-**
Percent non-insect	**AFDM**	**0.010**	**0.536**	**+**
Percent sensitive taxa	**Chlorophyll-a**	**0.007**	**0.576***	**+**
	Kjeldahl nitrogen	**0.035**	**0.444**	**-**
Percent collector taxa	**Orthophosphate**	**0.050**	**0.399**	+
Percent CR taxa	**Phosphorus**	**0.034**	**0.447**	**-**
Modified % clingers	**Nitrate**	**0.018**	**0.524**	**+**
	Nitrite	**0.034**	**0.449**	+
	Nitrate + Nitrite	**0.017**	**0.527**	**+**
	Nitrate max	**0.020**	**0.510**	**+**
	Nitrate + Nitrite max	**0.020**	**0.511**	**+**
	Phosphorus max	**0.011**	**0.573***	**+**
	Orthophosphate max	**0.010**	**0.586***	**+**

Table 15. Linear regression results showing 2007 large river 'top taxa' versus DEQ water quality variables. Relationships having coefficients of variation (R^2) > 0.3 are considered ecologically relevant and >0.5 are considered especially meaningful and marked with an "*". AFDM = ash-free-dry-mass of epilithon.

Response/Metric	Independent Variable	p-value	R^2	Direction
Leptoxis caranita	**Nitrite**	**0.041**	**0.689**	+
	Nitrate + Nitrite	**0.026**	**0.747**	+
	Ammonia	**0.014**	**0.812***	+
Stenelmis	**Nitrite**	**0.017**	**0.797**	+
	Nitrate + Nitrite	**0.008**	**0.856***	+
	Ammonia	**0.031**	**0.725**	+
Baetis	**Kjeldahl nitrogen**	**0.021**	**0.774**	+
	Phosphorus	**0.034**	**0.716**	+
	Orthophosphate	**0.041**	**0.689**	+
Macaffertium	**Phosphorus**	**0.007**	**0.866***	+
	Orthophosphate	**0.010**	**0.845***	+

Table 16. Comparison of taxa present in the Shenandoah River, Virginia (Berryville, Strasburg, Front Royal, 2006) and two other similar rivers in the mid-Atlantic region (New River, West Virginia, 1988-89; Susquehanna River, Pennsylvania, 1989).

	Shenandoah River	New River	Susquehanna River
NON- INSECT TAXA			
NEMATODA	X		X
HIRUDINEA	X		
OLIGOCHAETA	X		X
PLANARIIDAE	X	X	X
MOLLUSCA			
Bivalve	X	X	X
GASTROPODA			
PLEUROCERIDAE	X	X	
ANCYLIDAE	X		X
PHYSIDAE			
Physa	X		
PLANORBIDAE	X		
CRUSTACEA			
CAMBARIDAE	X	X	
ACARI (HYDRACARINA)	X		X
INSECT TAXA			
EPHEMEROPTERA			
BAETIDAE			
Baetis (complex)	X	X	X
Centroptilum			X
Heterocloeon			X
Pseudocloeon			X
Unidentified Baetidae			X
CAENIDAE			
Caenis	X	X	X
EPHEMERELLIDAE			
Drunella tuberculata	X		
Ephemerella	X		
Serratella	X	X	X
EPHEMERIDAE			
Hexagenia			X
HEPTAGENIIDAE			
Epeorus		X	
Heptagenia		X	X
Leucrocuta	X		X
Maccaffertium/Stenonema	X	X	X
Stenacron	X	X	X
Rhithrogena	X		X

	Shenandoah	New	Susquehanna
Unidentified Heptageniidae			X
ISONYCHIIDAE			
Isonychia	X	X	X
LEPTOPHLEBIIDAE	X		
Choroterpes			X
LEPTOHYPHIDAE			
Tricorythodes	X	X	X
POLYMITARCYIDAE			
Ephoron		X	X
POTAMANTHIDAE			
Anthopotamus	X		X
PLECOPTERA			
CHLOROPERLIDAE	X		
LEUCTRIDAE			
Leuctra	X		
PERLIDAE		X	X
Acroneuria	X	X	
Agnetina	X		
Neoperla		X	
Perlesta placida (group)	X		
ODONATA			
COENAGRIONIDAE			
Argia	X	X	
GOMPHIDAE			
Lanthus	X	X	
Stylogomphus	X		
MEGALOPTERA			
CORYDALIDAE			
Corydalus cornutus	X	X	
Nigronia fasciatus		X	
SIALIDAE			
Sialis			X
NEUROPTERA			
SISYRIDAE			
Clinacia	X		
TRICHOPTERA			
BRACHYCENTRIDAE			
Brachycentrus	X		
Micrasema	X	X	
GLOSSOSOMATIDAE			
Protoptila	X		X
HELICOPSYCHIIDAE			
Helicopsyche borealis	X		
HYDROPSHYCHIDAE			
Diplectrona	X		
Cheumatopsyche	X	X	X
Hydropsyche	X	X	X
Macrostemum			X

	Shenandoah	New	Susquehanna
HYDROPTILIDAE			
Agraylea	X		
Hydroptila	X	X	X
LEPTOCERIDAE			
Ceraclea	X	X	X
Nectopsyche	X		
Oecetis	X		
Triaenodes	X		
LEPIDOSTOMATIDAE			
Lepidostoma	X		
UENOIDAE			
Neophylax	X		
PHILOPOTAMIDAE			
Chimarra	X	X	X
PSYCHOMYIIDAE			
Lype diversa	X		
RHYACOPHILIDAE			
Rhyacophila	X		
LEPIDOPTERA			
PYRALIDAE			
Petrophila	X		X
COLEOPTERA			
ELMIDAE			
Dubiraphia	X		X
Microcylloepus	X	X	
Optioservus	X	X	X
Promoresia	X		
Stenelmis	X	X	X
HYDROPHILIDAE			
Berosus	X		X
PSEPHENIDAE			
Ectopria	X		
Psephenus herricki	X	X	X
SCIRTIDAE			
Scirtes	X		
DIPTERA			
CERATOPOGONIDAE	X		X
CHIRONOMIDAE	X	X	X
EMPIDIDAE			
Hemerodromia	X	X	
SIMULIIDAE			
Simulium	X	X	X
TIPULIDAE			
Antocha	X	X	
Tipula	X		
Total number of taxa	68	35	43

Table 17. Mean metric values for benthic macroinvertebrates assemblage in samples taken from Shenandoah large river sites by Virginia Tech in May 2006 compared to samples taken by Eugene Surber in the 1960s. Site codes are explained in Table 1.

	Surber 1960s							VT 2006						
Metrics	MtJack	Wood	Stras	Lynn	White	Froyal	Berry	MtJack	Wood	Stras	Lynn	White	Froyal	Berry
Total Density	702	704	221	489	581	365	352	441	637	598	566	846	554	726
Total Richness	24	25	20	21	19	22	15	28	31	28	25	28	25	24
Simpsons Diversity Index	0.849	0.852	0.844	0.822	0.774	0.837	0.754	0.870	0.846	0.797	0.819	0.846	0.783	0.739
% Modified EPT	11	26	41	22	9	39	3	38	16	12	18	12	18	18
% Non-insects	34	33	19	6	8	7	7	13	20	28	22	38	17	4
% Sensitive	31	29	16	6	2	7	1	24	19	23	19	21	9	2
% Scrapers	41	28	45	13	8	21	13	45	47	64	33	43	33	32
% Collector-gatherers	56	70	48	76	86	76	83	43	48	32	64	47	62	63
% Collector-filterers	38	28	4	25	56	42	35	4	11	10	11	8	21	12
% Crawlers	12	36	38	18	9	25	9	13	5	3	7	6	5	4
% Modified Clingers	9	22	29	8	6	17	4	59	62	73	44	52	51	52

Table 18. List of taxa and mean density (numbers of individuals per m^2) collected by Eugene Surber in the 1960's and in May 2006 by Virginia Tech in the current study. All samples were collected at the same seven large river sites and in the same season (see text).

	Surber (52 taxa)	**VT** (65 taxa)
NON- INSECT TAXA		
NEMATODA	100	22
OLIGOCHAETA	1578	
HIRUDINEA	89	
PLANARIIDAE	1522	16811
NEMERTEAN		589
MOLLUSCA		
CORBICULIDAE		
Corbicula fluminea		562
SPHAERIIDAE	2100	222
PLEUROCERIDAE		
Leptoxis carinata	6956	38511
ANCYLIDAE	556	156
PHYSIDAE		
Physa	211	189
PLANORBIDAE	44	344
UNIONIDAE	11	
CRUSTACEA		
CAMBARIDAE	11	22
CRANGONYCTIDAE		
Crangonyx		322
TALITRIDAE		
Hyalella azteca	500	
ACARI (HYDRACARINA)		22
INSECT TAXA		
EPHEMEROPTERA		
BAETIDAE		
Baetis (complex)	2178	80333
CAENIDAE		
Caenis	322	1467
EPHEMERELLIDAE	6400	14400
HEPTAGENIIDAE		
Maccaffertium/Stenonema	2578	33600
Stenacron		311
Epeorus	11	88
ISONYCHIIDAE		
Isonychia	689	28667
LEPTOPHLEBIIDAE		1
LEPTOHYPHIDAE		
Tricorythodes	144	14922
POTAMANTHIDAE		
Anthopotamus	144	189
POLYMITARCYIDAE		
Ephoron	667	
PLECOPTERA		
LEUCTRIDAE		
Leuctra		13867

PERLIDAE		
Acroneuria	178	656
Agnetina		2011
Neoperla		67
Paragnetina		22
Perlesta placida (group)		167
PERLODIDAE	22	
PTERONARCYIDAE		
Pteronarcys		33
ODONATA		
CALOPTERYGIDAE		
Calopteryx	33	
COENAGRIONIDAE		
Argia	89	855
GOMPHIDAE		
Lanthus		178
Stylogomphus		67
Progomphus	11	
MACROMIIDAE		
Micromia	11	
LEPIDOPTERA		
PYRALIDAE		
Petrophila		522
NEUROPTERA		
SISYRIDAE		
Climacia		178
MEGALOPTERA		
CORYDALIDAE		
Corydalus cornutus	111	5078
Nigronia fasciatus		11
SIALIDAE		
Sialis	122	22
TRICHOPTERA		
BRACHYCENTRIDAE		
Brachycentrus		522
Micrasema		11
GLOSSOSOMATIDAE		
Glossosoma nigrior	244	
Protoptila		1467
HELICOPSYCHIIDAE		
Helicopsyche borealis	222	2156
HYDROPSHYCHIDAE		
Cheumatopsyche	11044	25211
Hydropsyche	8978	16578
Macrostemum	111	33
Parasyche		167
HYDROPTILIDAE	244	7011
LEPTOCERIDAE		
Ceraclea		156
Oecetis		89
LEPIDOSTOMATIDAE		
Lepidostoma		1122
LIMNEPHILIDAE	44	
Pycnopsyche		11
UENOIDAE		
Neophylax		11

PHILOPOTAMIDAE	44	
Chimarra		9411
POLYCENTROPODIDAE		
Polycentropus		11
Neureclipsis	211	11
PSYCHOMYIIDAE		
Lype diversa		56
RHYACOPHILIDAE		
Rhyacophila	156	
COLEOPTERA		
ELMIDAE	6200	113211
DRYOPIDAE		
Helichus	122	
HYDROPHILIDAE		
Berosus	433	
PSEPHENIDAE		
Ectopria		44
Psephenus herricki	1322	3022
HALIPLIDAE	44	
DIPTERA	1622	
ATHERICIDAE		
Atherix	156	444
CERATOPOGONIDAE		144
CHIRONOMIDAE	12756	19422
EMPIDIDAE		
Hemerodromia	78	200
SIMULIIDAE	380	
Simulium		7567
TIPULIDAE		
Antocha	33	611
Hexatoma	122	
Tipula	33	11

Table 19. Eleven metrics summarizing assemblage structure and function at 26 Shenandoah river tributary sites in 2007. EPT = Ephemeroptera, Trichoptera, Plecoptera; CG = collector-gatherer; CF = collector-filterer.

	Total abundance	Taxa richness	Simpson's Diversity Index	% modified EPT	% non-insect	% sensitive	% scrapers	% CG	% CF	% Crawlers	Modified % clingers
HAWK	211	13	0.779	24	4	1	1	23	24	22	18
COOK	231	6	0.502	0	28	0	0	27	8	1	7
LOMR	1157	15	0.673	1	22	7	21	16	10	6	24
SMHW	970	18	0.712	25	2	3	10	36	10	24	26
JENN	290	15	0.530	18	1	10	3	14	13	14	10
LGCR	841	19	0.424	2	8	0	2	8	8	7	5
MUDD	335	14	0.583	13	3	3	6	20	15	10	17
BRIR	228	15	0.750	32	5	21	19	25	25	20	49
GOON	193	15	0.726	34	1	13	8	27	25	27	27
CEHW	288	20	0.728	22	4	15	13	12	46	13	59
STHW	127	11	0.661	27	2	8	11	20	12	23	18
LINV	231	11	0.331	3	2	1	13	4	2	2	15
PASS	184	15	0.664	32	2	6	14	21	29	27	39
HOLM	307	15	0.754	19	2	0	6	22	33	17	21
MCNF	370	15	0.511	15	1	2	10	15	7	12	15
MEAD	303	15	0.531	9	6	7	23	5	6	6	27
BACK	173	12	0.703	53	0	9	7	41	16	47	15
CHRIS	315	18	0.727	28	3	6	22	31	7	28	27
CEDS	91	12	0.805	16	8	9	26	24	25	7	67
NRHW	142	10	0.537	21	1	6	7	16	10	15	14
STDS	906	19	0.471	8	4	8	8	10	11	6	20
HADS	216	13	0.832	20	4	5	7	26	33	15	37
SMDS	771	21	0.755	54	5	17	17	43	16	46	28
NAAU	472	14	0.441	7	5	2	11	10	11	9	14
NAPA	150	10	0.726	34	1	3	7	39	19	26	29
MCSF	1940	23	0.394	6	5	5	9	8	3	6	11

Table 20. Linear regression results showing eleven 2007 tributary metrics versus land-cover variables. Relationships having coefficients of variation (R^2) > 0.3 are considered ecologically relevant and >0.5 are considered especially meaningful and marked with an "*". Beef = beef operations; dairy = dairy operations; PH = poultry houses; AFO = animal feeding operations; NMP = nutrient management plan for poultry litter.

Response/Metric	Independent Variable	p-value	R^2	Direction
Total abundance	% forest	0.025	0.200	-
	% pasture/hay	0.012	0.242	+
	# beef/1000acres	0.022	0.208	+
Taxa richness	# dairy/1000acres	0.048	0.159	-
Simpson's Diversity Index	**% forest**	**0.005**	**0.300**	**+**
	% pasture/hay	0.006	0.288	-
	% crop	0.033	0.183	-
% modified EPT	**% forest**	**<0.001**	**0.427**	**+**
	% pasture/hay	**<0.001**	**0.428**	**-**
	% crop	0.007	0.278	-
	# dairy/1000acres	0.037	0.175	-
	# beef/1000acres	0.019	0.217	-
	# PH/1000acres	0.004	0.302	-
	# AFO/1000acres	0.007	0.273	-
	# acresNMP	0.013	0.240	-
% non-insect taxa	% forest	0.013	0.240	-
	% pasture/hay	0.032	0.185	+
	% crop	**<0.001**	**0.462**	**+**
	% development	0.034	0.180	+
	# dairy/1000acres	**<0.001**	**0.540***	**+**
	# beef/1000acres	**<0.001**	**0.382**	**+**
	# PH/1000acres	**<0.001**	**0.596***	**+**
	# AFO/1000acres	**<0.001**	**0.670***	**+**
	% acres NMP	0.005	0.293	+
% sensitive taxa	**% forest**	**0.004**	**0.305**	**+**
	% pasture/hay	0.005	0.296	-
	% crop	0.025	0.199	-
	% acresNMP	0.010	0.256	-
% collector-filterer	**% forest**	**0.002**	**0.351**	**+**
	% pasture/hay	**0.004**	**0.314**	**-**
	% crop	0.013	0.242	-
	% development	0.012	0.247	-
	# beef/1000acres	0.013	0.240	-
% crawler taxa	% forest	0.010	0.257	+
	% pasture/hay	0.011	0.252	-
	% crop	0.034	0.181	-
	# PH/1000acres	0.024	0.203	-
	# AFO/1000acres	0.025	0.200	-
	% acres NMP	0.041	0.170	-

% modified clingers	Watershed area	0.012	0.246	+
	% forest	**0.005**	**0.301**	**+**
	% pasture/hay	0.008	0.268	-
	% crop	0.012	0.244	-
	% development	0.031	0.187	-
	# beef/1000acres	0.035	0.179	-
	# AFO/1000acres	0.043	0.167	-
	% acres NMP	0.042	0.167	-

Table 21. Linear regression results showing 2007 tributary 'top taxa' versus land-cover variables. Relationships having coefficients of variation (R^2) > 0.3 are considered ecologically relevant and >0.5 are considered especially meaningful and marked with an "*". PH = poultry houses; beef = beef operations; AFO = animal feeding operations; NMP = nutrient management plan for poultry litter; MUNSTP = municipal sewage treatment plant.

Response/eMtric	Independent Variable	p-value	R^2	Direction
Chironomidae	% forest	0.014	0.233	+
	% pasture/hay	0.009	0.262	-
Ephemerella	# beef/1000acres	0.007	0.273	+
	# MUNSTP	**<0.001**	**0.424**	**+**
Hydropsyche	% pasthay	0.019	0.216	+
	# beef/1000acres	**<0.001**	**0.416**	**+**
	# PH/1000acres	**0.002**	**0.336**	**+**
Stenelmis	% forest	0.012	0.246	-
	% pasture/hay	0.007	0.274	+
	# beef/1000acres	0.021	0.211	+
Cheumatopsyche	# PH/1000acres	0.031	0.187	+
Leptoxis carinata	# beef/1000acres	0.017	0.222	+
	# MUNSTP	0.038	0.172	+
Planariidae	**% forest**	**0.002**	**0.334**	**-**
	% crop	**<0.001**	**0.521**	**+**
	# AFO	0.029	0.190	+
	# dairy/1000acres	**<0.001**	**0.391**	+
	# PH/1000acres	**<0.001**	**0.614***	**+**
	# AFO	**<0.001**	**0.588***	**+**
	# acresNMP	**<0.001**	**0.364**	**+**
Oligochaeta	% forest	0.022	0.208	-
	# beef/1000acres	0.035	0.180	+
	# PH/1000acres	0.05	0.157	+
Hemerodromia	% forest	0.017	0.222	-
	% pasture/hay	0.006	0.286	+
	# beef/1000acres	0.042	0.167	+
Macaffertium	Watershed area	0.022	0.209	+
	# AFO/1000acres	0.027	0.195	-
	# PH/1000acres	0.021	0.210	-
	# MUNSTP	0.008	0.270	+

Table 22. Linear regression results showing eleven 2007 tributary macroinvertebrate metrics and 'top taxa' versus other environmental variables. Relationships having coefficients of variation (R^2) > 0.3 are considered ecologically relevant and >0.5 are considered especially meaningful and marked with an "*". Mod = modified

Response/eMtric	Independent Variable	p-value	R^2	Direction
Taxa richness	**Phosphorus**	**0.041**	**0.696***	**-**
% mod EPT	**Nitrate**	**0.04**	**0.475**	**-**
	Nitrogen	**0.033**	**0.499**	**-**
% sensitive taxa	**Nitrate**	**0.009**	**0.345**	**-**
	Nitrogen	**0.011**	**0.628***	**-**
mod% clingers	**Phosphorus**	**0.043**	**0.995***	**-**
Hydropsyche	**Phosphorus**	**0.022**	**0.999***	**-**
Antocha	**Phosphorus**	**0.013**	**0.741***	**+**
Leptoxus caranita	**Total phosphorus**	**0.033**	**0.997***	**-**
Planaria	**Ammonia**	**0.029**	**0.579**	**+**
Taxa richness	Clam arsenic	0.048	0.190	+
Simpson's Diversity	Clam mercury	0.047	0.192	+
	Clam lead	0.022	0.227	-
% mod EPT	Clam arsenic	0.049	0.172	-
	Clam lead	**<0.001**	**0.471**	**-**
% non-insect	Sediment chromium	0.030	0.206	+
	Sediment lead	0.034	0.198	+
% sensitive taxa	Clam cadmium	0.043	0.199	+
	Sediment lead	**0.006**	**0.306**	**-**
	Total Organic Carbon	**0.003**	**0.360**	**-**
% CG	Sediment lead	0.036	0.193	-
% CF	Clam arsenic	0.039	0.205	-
	Clam cadmium	**0.002**	**0.407**	**+**
	Clam mercury	**0.005**	**0.349**	**+**
	Sediment lead	**0.007**	**0.301**	**-**
% crawlers	**Sediment lead**	**0.002**	**0.364**	**-**
Mod % clingers	**Clam cadmium**	**<0.001**	**0.519***	**+**
	Clam mercury	0.011	0.297	+
	Sediment lead	0.038	0.189	-
	Total Organic Carbon	0.015	0.260	-
Stenelmis	Clam arsenic	0.025	0.236	+
	Sediment manganese	0.04	0.185	+
Simuliidae	**Clam cadmium**	**<0.001**	**0.533***	**+**
	Sediment cobalt	0.021	0.227	-
	Clam mercury	0.015	0.345	+
Oligochaeta	Sediment arsenic	0.044	0.179	+
	Sediment chromium	0.023	0.222	+
Baetis	Clam chromium	0.026	0.235	+

Hemerodromia	Index of Estrogenic Activity (E2Eq)	0.037	0.200	+

Table 23. Abundance of elmid adults collected at each tributary sites. Where genus abbreviation is used it is the same as the preceding column reading left to right.

Site Code	*Ancronyx verigata*	*Macronychus glabratus*	*Microcylloepus pusillis*	*Optioservus trivittatus*	*O. ovalis*	*Oulimnius latiusculus*	*Promoresia tardella*	*P. elegans*	*Stenelmis crenata*	*S. mera*	*S .sandersoni*	*S. musgravei*	*Stenelmis markeli*	*Stenelmis lateralis*
HAWK	0	0	1	1	0	0	0	0	0	0	0	0	0	0
COOK	0	0	0	0	0	0	0	0	0	1.3	0	0	0	0
LOMR	0	23.8	0	0	0	0	0	0	2	20.3	0	0	0	0
SMHW	0	1	0	0	2.3	1.5	0	0	0	10.8	0	0	0	0
JENN	3.5	0	1	0	1	0	0	0	0	0	1	0	0	0
LGCR	3.5	0	5.6	0	2.2	1	0	0	0	1	10.2	0	0	0
MUDD	3.5	0	1	0	0	1.5	0	0	0	0	3.7	0	0	0
BRIR	3.5	0	0	0	0	0	0	0	0	0	0	0	0	0
GOON	3.5	0	1	0	0	2	1	0	0	1	1	0	0	0
CEHW	3.5	0	2	0	0	1	0	1	0	0	2	1.5	0	0
STHW	3.5	0	0	0	0	0	0	2	0	0	4	0	0	0
LINV	3.5	0	0	0	0	1	0	0	0	0	2.3	1	0	0
PASS	3.5	0	0	0	1	5	0	1	1	0	0	3	0	0
HOLM	3.5	0	0	0	0	0	4	0	0	0	5.8	0	0	0
MCNF	3.5	0	1	0	0	1	0	0	0	1	2.5	0	0	0
MEAD	3.5	0	1.3	0	0	1.3	0	0	0	0	9.5	0	0	0
BACK	3.5	0	0	0	0	0	0	0	0	0	1	0	0	0
CHRIS	3.5	0	2.5	1	1	2.3	2	0	0	2.5	15.5	0	0	0
CEDS	3.5	0	0	0	0	2	0	0	0	0	0	4	1	0
NRHW	3.5	0	0	0	0	0	0	0	0	0	0	0	0	0
STDS	3.5	0	1	0	0	1	0	0	0	0	2.3	0	0	0
HADS	3.5	0	0	0	0	1	0	0	0	0	4.3	0	0	0
SMDS	3.5	0	1	0	1	3.5	0	0	0	3.7	5.8	0	0	1
NAAU	3.5	0	3	0	0	0	0	0	0	3	3.2	0	0	0
NAPA	3.5	0	0	0	0	1	0	0	0	0	0	0	0	0
MCSF	3.5	1.25	2.2	1	1.7	1.7	1	0	0	4.3	11	1	0	0

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Water Quality Indicators Guide:

Surface Waters

United States Department of Agriculture

Soil Conservation Service

Water Quality Indicators Guide: Surface Waters

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FOREWORD

With more than fifty years of experience in soil and related resources, the Soil Conservation Service (SCS) is actively involved in agriculturally related water quality issues. In recent years as the public has become concerned about surface and ground water problems, SCS has endeavored to meet water quality needs by developing and transferring new and innovative technologies. As part of that new effort the SCS has developed the Water Quality Indicators Guide: Surface Waters to aid in finding water quality solutions to problems from sediment, animal wastes, nutrients, pesticides, and salts.

With the creation of new laws, such as the Food Security Act of 1985 and the Water Quality Act of 1987, and many new regulations relating agriculture and water quality, SCS needs new tools to address water quality situations. The Water Quality Indicators Guide also helps fulfill the needs of educators for information and guidance to teach water quality in a clear and understandable manner.

The Water Quality Indicators Guide employs a simplified approach, allowing the user to learn the fundamental concepts of water quality assessment quickly. The guide extracts basic tenets from many disciplines, such as geology, hydrology, biology, ecology, and wastewater treatment, and focuses those ideas in making decisions about water quality. With the guide the user can assess potential water quality conditions without elaborate chemical testing procedures or intricate species identification. Then, the user can determine possible sources of the problem on adjacent lands, and recommend practices for correcting the condition.

The SCS has backed the guide's qualitative approach with the skills, knowledge, and experience of SCS biologists, hydrologists, water quality specialists and others from across the Nation. Our hope is that the Water Quality Indicators Guide: Surface Waters will prove to be a useful tool in making water quality assessments, leading to improved water quality and a better environment for all of us.

ROBERT R. SHAW
Deputy Chief for Technology
July 18, 1988

Preface

The *Water Quality Indicators Guide: Surface Waters* is dedicated to Vernon M. Hicks, retired Soil Conservation Service (SCS) biologist, who fostered the idea of the guide when he was National Environmental Coordinator for SCS. As a result of many years of service in the South and the Northeastern United States, Mr. Hicks recognized the need for SCS field personnel to have a guide that allowed the user to recognize surface water quality problems easily but reliably, and to select conservation and best management practices that help remedy those problems. To date, few water quality publications have employed the indicator approach where environmental "surrogates" are used to represent pollution potentials. However, Mr. Hicks believed that environmental conditions could be surveyed without elaborate chemical testing procedures, and judgments made based on surrogates, concerning the quality of waters.

The core of the *Water Quality Indicators Guide* is the field sheets and list of associated practices to remedy or abate agricultural nonpoint source pollution. The field sheets are arranged in matrix format with environmental indicators given for sediment, animal wastes, nutrients, pesticides, and salts. Each indicator is divided into descriptions of the environment from excellent to poor, and each description is given a weighted numerical ranking. The user matches the individual description with what is observed in the water or on the land. By totaling the individual rankings, a score is obtained indicating the potential for agricultural nonpoint source problems. Practices can be selected from the list to alleviate problem situations.

With practice, the user of this guide will find that he or she can quickly learn water quality assessment procedures through the use of the guide's field sheets. With experience, the user's ability to assess water quality situations accurately with the field sheets will also increase. The guide is flexible, with places on the field sheets where the user can insert environmental surrogates representing local environmental conditions.

Charles R. Terrell
National Water Quality Specialist
Soil Conservation Service
United States Department of Agriculture
Washington, D.C.
July 18, 1988

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Introduction

Audience and Purpose of This Guide

The *Water Quality Indicators Guide: Surface Waters* is intended for the district conservationists and other field personnel of the Soil Conservation Service (SCS). It is designed to help field personnel recognize agricultural nonpoint source problems and their potential causes, and to give corrective measures. The Indicators Guide is meant to complement SCS's previously published *Water Quality Field Guide* (SCS-TP-160). Together, these two guides provide a comprehensive examination of surface water agricultural nonpoint problems and possible solutions.

The Role of the Soil Conservation Service in Water Quality

Throughout the history of the Soil Conservation Service, Congress has authorized SCS to provide water quality improvements through flood and pollution control. Much of SCS's work in water quality began in the early 1970's as a result of growing public concern about agriculturally related pollution. SCS assisted State and local efforts to develop agricultural plans under Section 208 of the Clean Water Act of 1977.

Both the Soil and Water Resources Conservation Act of 1977 and the Agriculture and Food Act (1981 farm bill) strengthened SCS's role in setting clean water objectives. More water efforts are cited in the Food Security Act of 1985 (1985 farm bill) that has important implications for SCS's future activities concerning water quantity and quality.

A Note to the User of This Guide

The *Water Quality Indicators Guide* examines five major sources of agriculturally related nonpoint source pollution—sediment, nutrients, animal waste, pesticides, and salts. Field sheets are provided to enable the user to assess surface water quality problems easily and accurately and to select appropriate remedial practices. The field sheet concept was adapted from a Wisconsin Department of Natural Resources methodology (ref. 1-1). The field sheets are completed in the field through onsite observations, rather than chemical or physical measurements. Conservation and best management practices (BMP's) are recommended to reduce or eliminate nonpoint source pollution originating from agricultural lands.

This type of approach may be sufficient in *some* instances to *confirm* that a particular nonpoint source pollution problem exists. In *other* instances, it may lead you to *suspect* a given pollutant, which can then be confirmed or denied by additional scientific analysis. When available, dissolved oxygen meters, salinity and conductivity meters, and field test kits may be used to supplement the *Water Quality Indicators Guide* field sheets. However, acceptable determinations can be made by using the field sheets without test kits or meters. When a particular nonpoint source pollutant is identified, the user of this guide is directed to possible solutions (conservation and best management practices), which are listed by number on the field sheets.

There are two types of field sheets: one type for receiving waters, including streams, rivers, lakes, and ponds; and another type for use on agricultural lands draining into the receiving waters. Chapter 1 reviews the overall distribution of agricultural nonpoint source problems. Chapter 2 gives a history of the water quality indicators approach and gives some general limitations of the *Water Quality Indicators Guide: Surface Waters*. Instructions for the water-based "A" type field sheets and for the land-based "B" type field sheets are contained in chapter 2. Chapter 3 presents background ecological information about aquatic ecosystems, especially stream systems.

Chapters 4 through 8 discuss the five major pollutants—sediment, nutrients, pesticides, animal wastes, and salts. These chapters discuss in detail the water quality indicators enumerated in the water-based "A" series of field sheets. It is assumed that Soil Conservation Service district conservationists and other field personnel will be familiar with the terminology given in the land-based "B" field sheets, so few specific instructions are given for the "B" field sheets. The "B" field sheets are designed to assess the pollutant generation potential of a particular field or pasture and are completed in the same way as the "A" field sheets. As an aid, a glossary of terms appears in appendix C.

Chapter 1

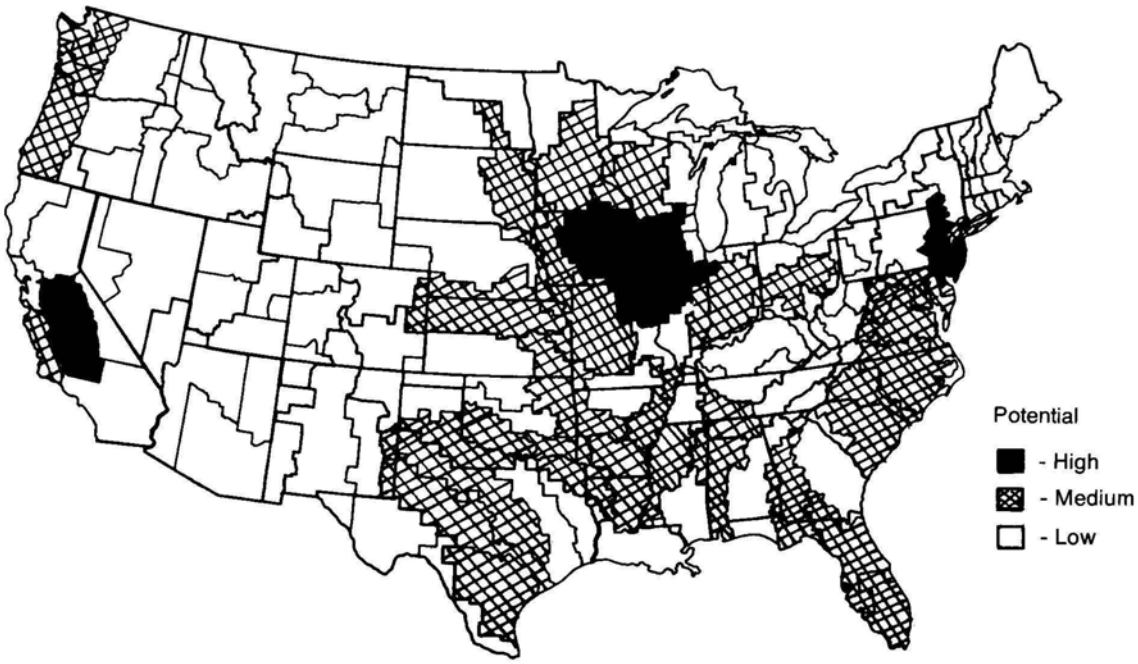
Pollution Related to Agriculture

Recent reports acknowledge that a principal water quality problem in our Nation is nonpoint source pollution. The U.S. Environmental Protection Agency defines nonpoint source (NPS) pollution as precipitation-driven stormwater runoff, generated by land-based activities, such as agriculture, construction, mining, and silviculture. Agricultural nonpoint sources are crop and animal production activities. These activities result in diffuse runoff, seepage, or percolation of pollutants from the land to surface and ground waters (ref. 1-2). Problems relating to agricultural nonpoint source pollution can be observed in the entire range of water bodies from estuaries to lakes and impoundments, to rivers, streams, and even farm ponds. Ground water is also vulnerable to pollution. Contaminated wells and drinking water supplies are now being identified.

In general, water quality problems result from five categories of agriculturally related nonpoint source pollution: sediment, nutrients, animal wastes, pesticides, and salts. Figure 1-1 shows the geographic potential for nonpoint source pollution of surface waters. The potential for agricultural nonpoint source pollution problems, according to SCS's Second Resources Conservation Act (RCA) Appraisal report (ref. 1-3), is shown in figures 1-2 through 1-7:

Figure 1-1

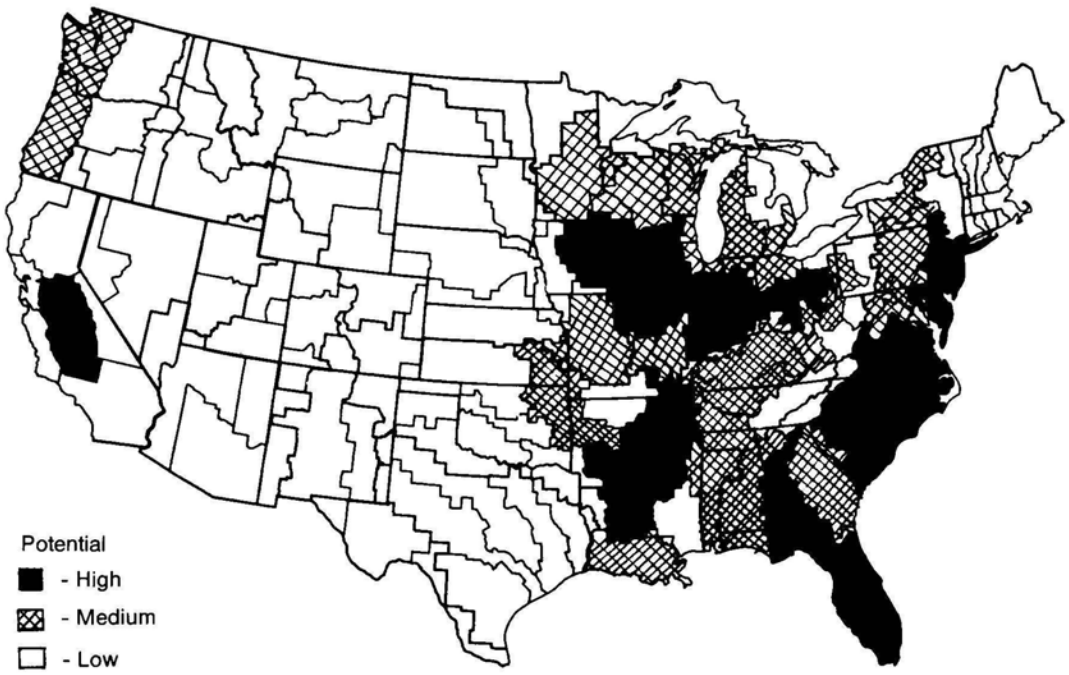
Composite Potential for Nonpoint Source Pollution of Surface Waters.



An area with a "low" composite rating could have a high rating for a specific contaminant. Ratings were made for multi-county watershed areas and do not identify more localized problems.

Figure 1-2

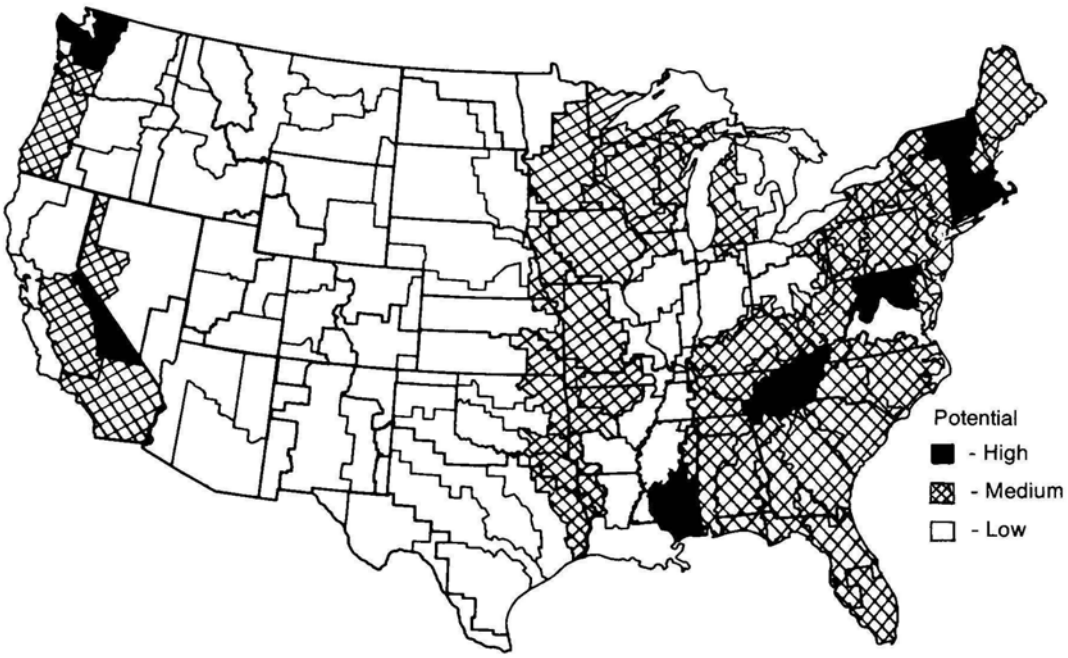
Potential for Pesticide Problems.



The potential for surface water pollution by pesticides was estimated by multiplying the crop acreages in each area by pesticide application coefficients for 184 pesticides. These values were multiplied by an availability factor that estimated the percentage of an application leaving a field and were adjusted by a runoff value for the growing season. Pollution potential is estimated for each watershed as a whole; localized conditions may be masked by aggregation. To confirm the existence of pesticide pollution, stream and lake monitoring would be necessary.

Figure 1-3

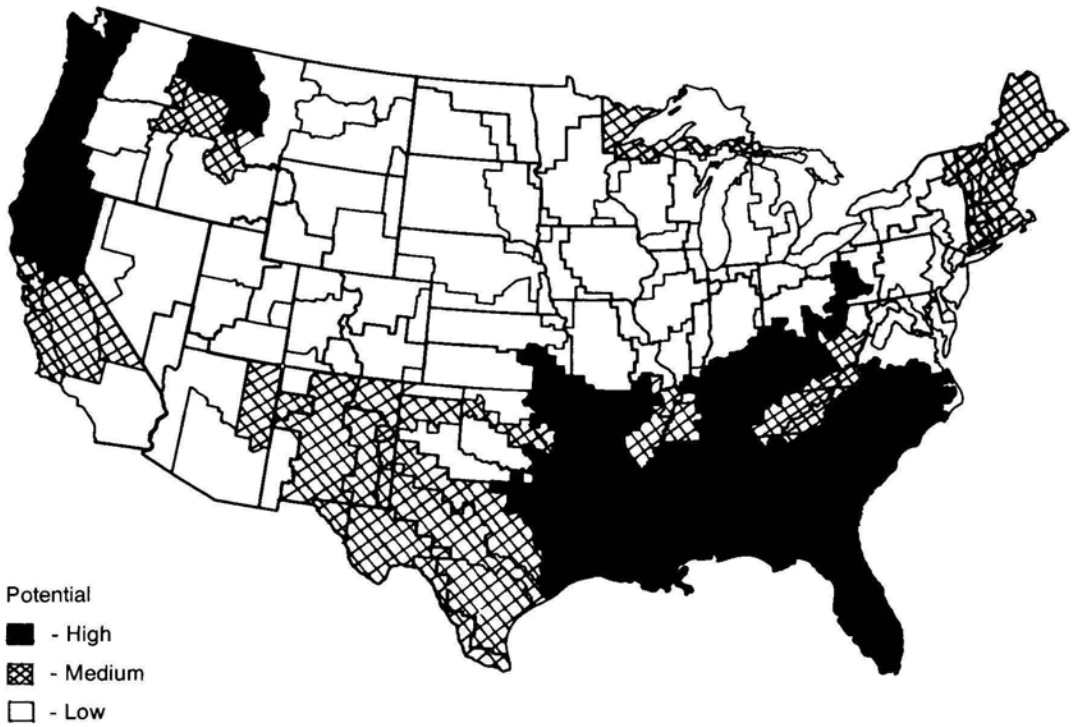
Tons of Manure Per Acre of Cropland and Grassland.



The number of each type of animal in a county (from the 1982 Agricultural Census) was multiplied by the appropriate manure production factor. The amounts of manure produced by all the county's livestock were totaled and aggregated by area; the total was divided by the acreage of cropland plus grassland (from the Agricultural Census) in each area.

Figure 1-4

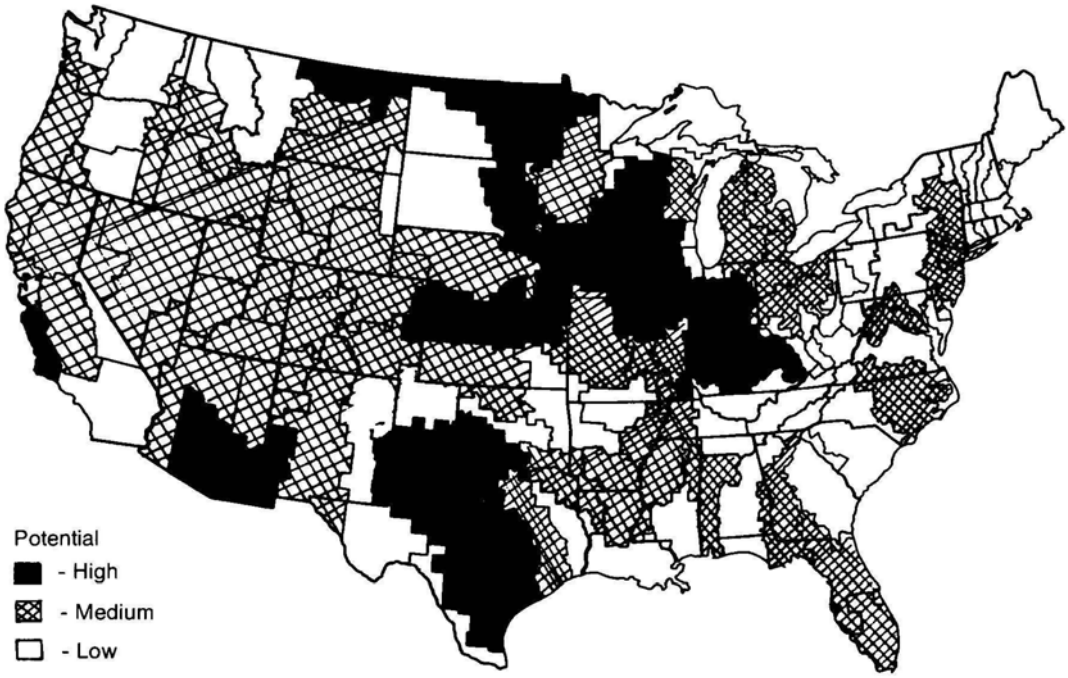
Potential for Animal Waste Problems.



The figure shows potential for pollution resulting from animal wastes, taking into account percentage of manure needing improved management, percentage of cropland and grassland associated with animal enterprises, runoff from precipitation, ratio of feed purchased to feed produced on farm, and ratio of nitrogen and phosphorus available from manure to nitrogen and phosphorus needed by crops.

Figure 1-5

Potential for Nutrient Problems.

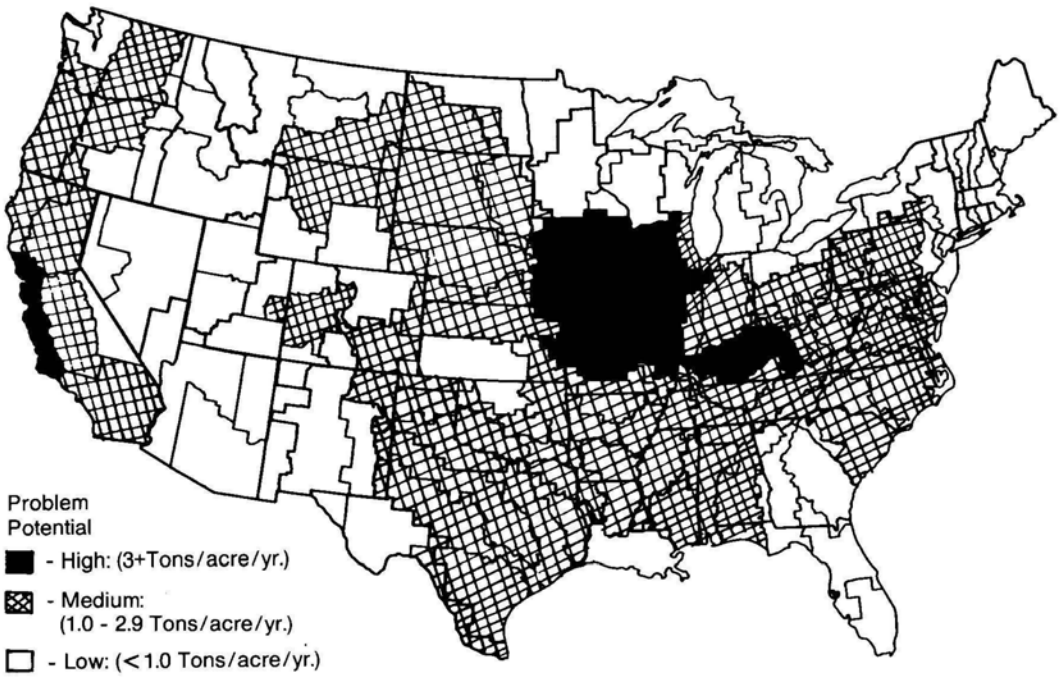


Source: WATSTORE (U.S. Geological Survey data from water quality stations, Ref. 1-4).

The potential for impairment of water quality was estimated by determining nutrient concentrations, by form, and comparing them with the respective threshold levels at which they threaten desired water uses. Data on nutrient concentrations were taken from WATSTORE (U.S. Geological Survey data from water quality stations). Stations were primarily National Stream Quality Accounting Network stations at the downstream and of hydrologic accounting units. Estimates of pollution potential are for the watershed as a whole and may not reflect localized conditions.

Figure 1-6

Estimated Sediment Yield.

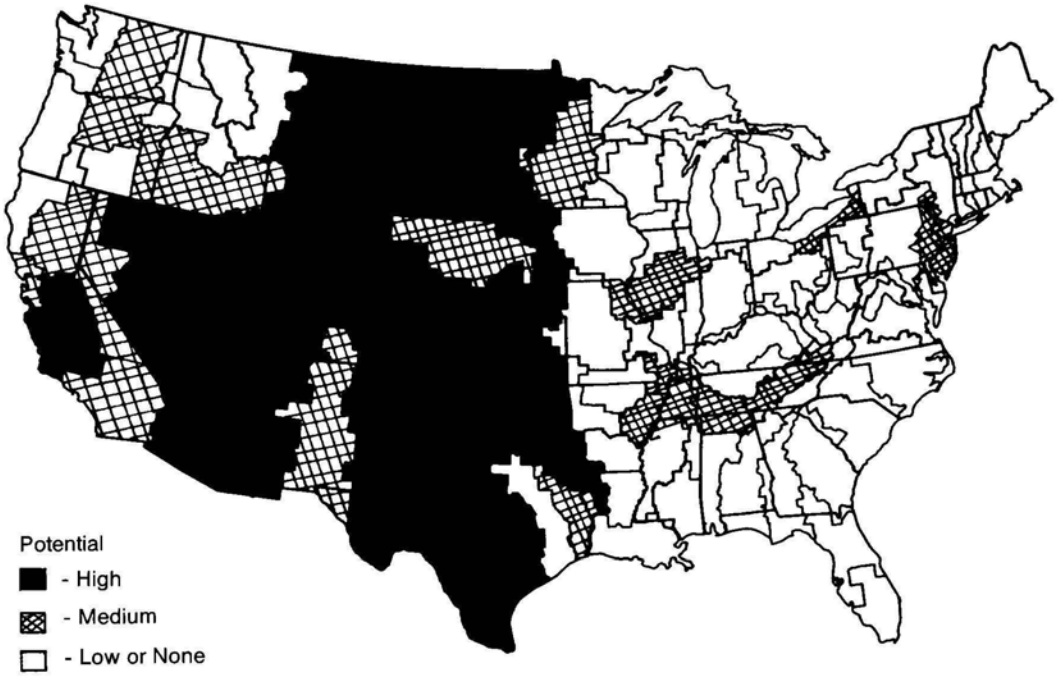


Sources: (1) 1982 National Resources Inventory (USDA-SCS, 1984, Ref. 1-5).
(2) USGS Surface Soil Surveys (Ref. 1-6).
(3) USDA Soil Survey Laboratory Data State Reports (Ref. 1-7).

Estimated sheet and rill erosion rates reported in the 1982 NRI were adjusted to county boundaries. Sediment delivery for each county and land use was estimated using state sediment delivery curves developed for the 1977 NRI. Sediment delivery rates are assumed to be higher in areas where streams are more numerous and closely spaced and where the surface soils have a higher percentage of fine particles (silt and clay). Data from USGS Surface Soil Surveys and USDA Soil Survey laboratory data were analyzed also.

Figure 1-7

Potential for Salinity Problems.



Sources: (1) U.S. Geological Survey National Stream Quality Accounting Network (NASQAN) stations in ASAs (Ref. 1-8).
(2) Published and unpublished data from EPA and USGS.

To assess potential, indicators of total dissolved solids, adjusted sodium adsorption, and chloride concentration were checked and total solid loads were analyzed using data for agricultural acreages, areas affected by saline or sodic soils, and irrigated acres as modifying and/or contributing factors. Data analyzed were taken from the U.S. Geological Survey National Stream Quality Accounting Network stations and published and unpublished data from EPA and USGS.

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Chapter 2

Water Quality Field Analysis

History of the Indicators Approach

Two centuries ago, when the U.S. population was small, the number of farmers and farm animals was also small. Agricultural wastes did not overload streams or other receiving water bodies. In those days, streams cleansed themselves naturally. Today, with the increasing complexity of farms, many watercourses and water bodies are unable to cope with the pollution loads being generated.

The SCS *Water Quality Indicators Guide: Surface Waters* is designed to determine by means of an indicators approach whether farm-generated materials are a problem. Water pollution investigators have used this type of approach since the turn of the century. At the heart of this approach is a comparison of water quality conditions above and below a suspected source of pollution. In most instances, the suspected source may be a "point" source pollution; that is, a type of pollution that can be readily identified as coming from a discrete source, such as a discharging pipe (e.g., a sewage outfall).

The *Water Quality Indicators Guide* adapts this approach for use with nonpoint source pollution—pollutants whose sources are diffuse and not readily identifiable. Nonpoint source pollutants include those substances which run off, wash off, or seep through the ground into receiving watercourses and water bodies. Agricultural nonpoint source pollution tends to wash or run off large tracts of cropland, pastures, feedlots, etc., and the conditions leading to pollution are highly variable.

One of the most important pollution variables is flow. In nonirrigated regions, loadings of the most common nonpoint source pollutants in a small stream tend to be proportional to the amount of runoff. Runoff, in turn, varies with conditions, such as: (1) amount of snowmelt or rainfall; (2) rate of snowmelt or rainfall; (3) soil type, condition, slope, vegetative cover, and land use; (4) time elapsed since the previous storm; and (5) seasonal timing and intensity of storm events.

Not only are the timing and extent of nonpoint source pollution events highly variable, but the effects of nonpoint source pollutants, either singly or in combination, are also variable. The effect of a given pollutant on water quality depends upon local site-specific environmental conditions; that is, on the local geology and the physical/chemical characteristics of the nearby water.

Both water quality and rate of flow influence the types of organisms that inhabit a given watercourse or water body. Organisms respond to many local environmental conditions, including climate, habitat availability, streambed type, etc. The ecology of watercourses is discussed in the next chapter.

Limitations of the Water Quality Indicators Guide

The *Water Quality Indicators Guide* was written to cover the entire United States, so it is general by intent. It can be expected that a particular stream or pond may deviate from the norms presented and will require the user to make adjustments for local situations. However, the guide has been field tested in five States across the Nation and by individual Soil Conservation Service personnel from many other States. The ideas, suggestions, and comments from those tests have been incorporated into this version. The *Indicators Guide* is not a research tool, nor does it give quantitative data, but as a qualitative tool and as an educational or learning device, it will aid the user in evaluating agricultural nonpoint source pollution problems.

This guide is especially limited where water flow rates are excessively low or high. In ephemeral or intermittent streams, some parts of this guide, such as observing fish, vegetation, or bottom invertebrates, cannot be used. The guide's use may be limited in heavily silted, mud-bottom streams, where the silt's presence provides an unsuitable habitat for many species. Also, heavy siltation of the water can "mask" the effects of nutrients that may be present, by shutting out light that normally would reach aquatic vegetation, allowing its growth. Thus, the vegetational part of the nutrient field sheet may not work well in heavily silted waters. In these cases, chemical testing may be necessary to determine nutrient levels.

Description of the Field Sheets

The heart of the *SCS Water Quality Indicators Guide: Surface Waters* is a series of field sheets (appendix F). The field sheets relate to surface water quality and are designed to help field personnel assess the degree of contribution to receiving waters from agriculturally related pollutants, namely sediment, animal waste, nutrients, pesticides, and salts. The receiving watercourses are natural streams, constructed channels, or receiving water bodies, such as ponds or lakes.

The field sheets are of two types: "A" and "B." The five "A" field sheets are designed to assess the effects of pollutants to receiving waters. These are water-based field sheets and should be completed onsite, following visual inspection of the receiving water.

By contrast, the seven "B" field sheets are land-based and are designed to assess the pollutant potential of a particular field or pasture; i.e., how likely it is that an agriculturally produced pollutant will be carried from a given field or pasture to a receiving watercourse or water body, or to ground water. There are more "B" field sheets than "A" sheets, because some land-based activities or environmental conditions required special emphasis.

Procedure for Field Analysis

NOTE: **Do not** write on the original field sheets. Make a copy of each field sheet before proceeding and write on the copies.

Step 1. Begin by completing the background information section (part 1) of the "Watershed Assessment." Although the Watershed Assessment was designed to be used with natural perennial streams, it can be adapted for use on either intermittent or ephemeral streams or on constructed waterways.

Please note that this evaluation cannot be made in the office. It must be made onsite, in the field. If you lack some of the necessary information, seek it from the landowner or operator, county agricultural extension agent, biologist, or other knowledgeable person.

Step 2. The "On-Farm (Ranch) Water Assessment" should be completed for each farm or ranch visited.

Step 3. Next, do a preliminary assessment of possible nonpoint source impacts by answering the questions asked in the "Watercourses" or "Water Bodies" Field Sheet Selection (part 2). If any of the questions in part 2 of the assessment receives a "yes" answer, then it is likely that the receiving water is being adversely affected by the pollutant indicated in the last column under the heading "Probable Cause." You

can verify this by completing the field sheets for this particular pollutant.

Please note that it is much easier to determine nonpoint source (NPS) pollution effects on standing (lentic) water, such as lakes or ponds, than for flowing (lotic) water, because standing water has a longer residence time (time that water remains in the water body), giving pollutants time to react.

Step 4. Proceed to the field sheets. If you are confident of your "no" answers in part 2 of the above assessment, you need to complete only those field sheets corresponding to the questions (pollutants) for which you marked either a "yes" or "can't tell" answer. For example, there will not be an animal waste problem if a particular farm or ranch has no animals and the owner or operator does not import animal waste. Obviously, in this case, none of the animal waste field sheets (2A, $2B_1$, $2B_2$) needs to be completed. If you are not confident that any of the pollutants should be eliminated as possible contributors of NPS pollution in a particular situation, complete all of the field sheets.

To learn how to use the sheets, it is recommended that you go through all of them at least once, including those for pollutants that have just a small possibility of affecting the watercourse or water body. This will allow you to gain familiarity with the sheets. With practice, using the sheets will become second nature to you, and you will complete them very quickly.

Filling Out the Field Sheets

TYPE A FIELD SHEETS

If upon completing part 2 of the watercourse (or water body) assessment you determined that sediment is probably adversely affecting the water, you should begin by focusing on the water-based Field Sheet 1A: "Sediment Indicators for Receiving Watercourses and Water Bodies (fig. 2-1)." Please take time now to look at this sheet. Outlined below is how you should use it. The sheet has answers circled in the way that should be done in the field.

For each field sheet, you are asked to complete the blanks at the top of the sheet which identify you, the evaluator, the county, State, etc. Notice that in the left column, Field Sheet 1A lists six different indicators or rating items with four possible options for item number 3. You will examine one indicator at a time and judge whether the water quality at this particular site ranks as excellent, good, fair, or poor regarding that particular indicator. Please note that these sheets should be completed in the field at the water's edge and *not* in the office.

A standing water body is fairly easy to assess for nonpoint source pollutant impacts. Flowing waters are not as easy to evaluate. The best place to observe a receiving watercourse is downstream of the pollutant sources. The exact point downstream from which to observe varies. If the water flow is very rapid, you may have to make observations at a distance downstream where the flow is slower. This is especially true when using the Nutrient Field Sheet (3A) because the effects from excessive nutrients often do not show in flowing waters until the flow rate is slow.

In completing Field Sheet 1A, it would be best to station yourself beside the stream (fig. 2-2) at the spot indicated by the *A. If the stream is flowing rapidly, flushing away pollutants very quickly, it may be necessary to walk downstream or upstream, observing indicators as you go. For ponds and lakes, it is best to observe from a site that allows a bird's-eye view of the whole water body, as well as from the water's edge.

The first indicator or ranking item on Field Sheet 1A for sediment is turbidity. Note that an indication of nonpoint source sediment pollution can most accurately be assessed only during or immediately following a storm event. Ask yourself, "What does the water look like at this particular site immediately after a storm?" Do you see "conditions normally expected under pristine conditions in your geographic region?" Is the water "clear or very slightly muddy after a storm event" or are "objects visible at depths greater than 3 to 6 feet (depending on water color)," such as described under the EXCELLENT heading? Or do the descriptors under the GOOD category more closely approximate conditions in your area; i.e., the water is "what is expected for *properly* managed agricultural land in your geographic region?" Is the water "a little muddy after a storm event but clears rapidly" or are "objects visible at depths between 1-1/2 to 3 feet (depending on water color)?" Are the conditions at this site better described by the descriptors under the headings of FAIR or POOR? Having read all four definitions under each of the four ratings, decide which of the four BEST describes the condition of the watercourse or body which you are evaluating and circle the number in the bottom of the box for that particular rating.

Follow the procedure outlined above for the turbidity parameter with each of the other five rating items on the Sediment Field Sheet 1A. When you have completed the entire sheet, add the circled numbers to obtain a total for the entire field sheet. This total should fall into one of the four ranking categories (excellent, good, fair, or poor) given at the very bottom of each field sheet. For example, if the total score was "8," record an "8/Poor" in the upper right-hand corner of the field sheet by "Total Score/Rank." What this says is that the water being evaluated is in a "poor" condition relative to sediment—or that sediment is greatly impacting the water at this site.

Design and Tailoring of the Indicator Guide Field Sheets To Fit Your Region

Please note that the field sheets are designed to be used for both flowing water and standing water across the entire United States. To use the sheets throughout this exceedingly diverse geographic area and for flowing and standing waters, it was necessary to include several descriptors per indicator (rating item) in each of the four categories (excellent, good, fair, and poor). These descriptors will rarely fit *all* given situations in a particular geographic area. In fact, some of the options within the same rank might at first appear contradictory if you fail to distinguish between standing and flowing water. Be especially careful when reading these descriptors and be sure to select the option which BEST or most closely matches the site specific conditions of the water you are assessing.

If the condition of the water in your locality *really* falls between two options or has about half of the characteristics of two options, you may "split" a score. You may want to add one or two other descriptors to all four options of a rating item. These

Figure 2-1

Sediment Page 1 of 2

Lat. 40° 37' 30"
Lon. 76° 40' 00"

FIELD SHEET 1A: SEDIMENT
INDICATORS FOR RECEIVING WATERCOURSES AND WATER BODIES

Evaluator Isaacs/Myers County/State Dauphin, PA Date 18 Apr. '88
Water Body Evaluated Pond Water Body Location Lykens, PA Total Score/Rank 24 · Good

(Circle one number among the four choices in each row which BEST describes the conditions of the watercourse or water body being evaluated. If a condition has characteristics of two categories, you can "split" a score.)

Rating Item	Excellent	Good	Fair	Poor
1. Turbidity (best observed immediately following a storm event)	What is expected under pristine conditions in your region. Clear or very slightly muddy after storm event. Objects visible at depths greater than 3 to 6 ft. (depending on water color). OTHER 9	What is expected for properly managed agricultural land in your region. A little muddy after storm event but clears rapidly. Objects visible at depths between 1½ to 3 ft. (depending on water color). OTHER 7	A considerable increase in turbidity for your region. Considerable muddiness after a storm event. Stays slightly muddy most of the time. Objects visible to depths of ½ to 1½ ft. (depending on water color). OTHER 3	A significant increase in turbidity for your region. Very muddy—sediment stays suspended most of the time. Objects visible to depths less than ½ ft. (depending on water color). OTHER 0
2. Bank stability in your viewing area	Bank stabilized. No bank sloughing. Bank armored with vegetation, roots, brush, grass, etc. No exposed tree roots. OTHER 10	Some bank instability. Occasional sloughing. Bank well-vegetated. Some exposed tree roots. OTHER 7	Bank instability common. Sloughing common. Bank sparsely vegetated. Many exposed tree roots & some fallen trees or missing fence corners, etc. Channel cross-section becomes more U-shaped as opposed to V-shaped. OTHER 4	Significant bank instability. Massive sloughing. No vegetation on bank. Many fallen trees, eroded culverts, downed fences, etc. Channel cross-section is U-shaped and stream course or gully may be meandering. OTHER 1
3. Deposition (Circle a number in only A, B, C, or D)	SELECT 3A OR 3B OR 3C OR 3D			
3A. Rock or gravel streams OR	A. For rock and gravel bottom streams: Less than 10% burial of gravels, cobbles, and rocks. Pools essentially sediment free. 9	A. For rock and gravel bottom streams: Between 10% & 25% burial of gravels, cobbles, & rocks. Pools with light dusting of sediment. 7	A. For rock & gravel bottom streams: Between 25% and 50% burial of gravels, cobbles and rock. Pools with a heavy coating of sediment. 3	A. For rock & gravel bottom streams: Greater than 50% burial of gravels, cobbles and rocks. Few if any deep pools present. 1
3B. Sandy bottom streams OR	B. For sandy streambeds: Sand bars stable and completely vegetated. No mudcaps or "drapes" (coverings of fine mud). No mud plastering of banks; exposed parent material. No deltas. 9	B. For sandy streambeds: Sand bars essentially stable and well, but not completely, vegetated. Occasional mudcaps or "drapes." Some mud plastering of banks. Beginnings of delta formation. 7	B. For sandy streambeds: Sand bars unstable with sparse vegetation. Mudcaps or "drapes" common. Considerable mud plastering of banks. Significant delta formation. 3	B. For sandy streambeds: Sand bars unstable and actively moving with no vegetation. Extensive mudcaps or "drapes." Extensive mud plastering of banks. Extensive deltas. 1
3C. Mud-bottom streams OR	C. For mud bottom streams: Dark brown/black tanic-colored water (due to presence of lignins and tanins). Abundant emergent rooted aquatics or floating vegetation. 9	C. For mud bottom streams: Dark brown colored water. 7	C. For mud bottom streams: Medium brown water, muddy bottom. 3	C. For mud bottom streams: Light brown colored, very muddy bottom. 1

Figure 2-1

Sediment Page 2 of 2

FIELD SHEET 1A: SEDIMENT, Continued
INDICATORS FOR RECEIVING WATERCOURSES AND WATER BODIES

Rating Item	Excellent	Good	Fair	Poor
3D. Ponds	Ponds essentially sediment free. No reduction in pond storage capacity. OTHER 9	Ponds with light dusting of sediment. Very little loss in pond storage capacity. OTHER 7	Ponds with a heavy coating of sediment. Some measurable loss in pond storage capacity. OTHER 3	Ponds filled with sediment. Significant reduction in pool storage capacity. OTHER 1
4. Type and amount of aquatic vegetation & condition of periphyton (plants, growing on other plants, twigs, stones, etc.)	Periphyton bright green to black. Robust. Abundant emergent rooted aquatics or shoreline vegetation. In ponds, emergent rooted aquatics (e.g. cattails, arrowhead, pickerelweed, etc.) present, but in localized patches. OTHER 9	Periphyton pale green and spindly. Emergent rooted aquatics or shoreline vegetation common. In ponds, emergent rooted aquatics common, but confined to well-defined band along shore. OTHER 7	Periphyton very light colored or brownish and significantly dwarfed. Sparse vegetation. In ponds, emergent rooted aquatics abundant in wide bank; encroachment of dry land species (grasses, etc.) along shore. OTHER 5	No periphyton. No vegetation. In ponds, emergent rooted aquatics predominant with heavy encroachment of dry land species. OTHER 2
OPTIONAL: 5. Bottom stability of streams	Stable. Less than 5% of stream reach has evidence of scouring or silting. OTHER 9	Slight fluctuation of streambed up or down (aggradation or degradation). Between 5-30% of stream reach has evidence of scouring or silting. OTHER 7	Considerable fluctuation of streambed up or down (aggradation or degradation). Scoured or silted areas covering 30-50% of evaluated stream reach. Flooding more common than usual. More stream braiding than usual for region. OTHER 3	Significant fluctuation of streambed up or down (aggradation or degradation). More than 50% of stream reach affected by scouring or deposition. Flooding very common. Significantly more stream braiding than usual for region. OTHER 1
OPTIONAL: 6. Bottom dwelling aquatic organisms	Intolerant species occur: mayflies, stonefiles, caddisflies, water penny, riffle beetle and a mix of tolerants. High diversity. OTHER 9	A mix of tolerants: shrimp, damselflies, dragonflies, black flies. Intolerants rare. Moderate diversity. OTHER 7	Many tolerants (snails, shrimp, damselflies, dragon flies, black flies). Mainly tolerants and some very tolerants. Intolerants rare. Reduced diversity with occasional upsurges of tolerants, e.g. tube worms and chironomids. OTHER 3	Only tolerants or very tolerants: midges, craneflies, horseflies, rat-tailed maggots, or none at all. Very reduced diversity; upsurges of very tolerants common. OTHER 1

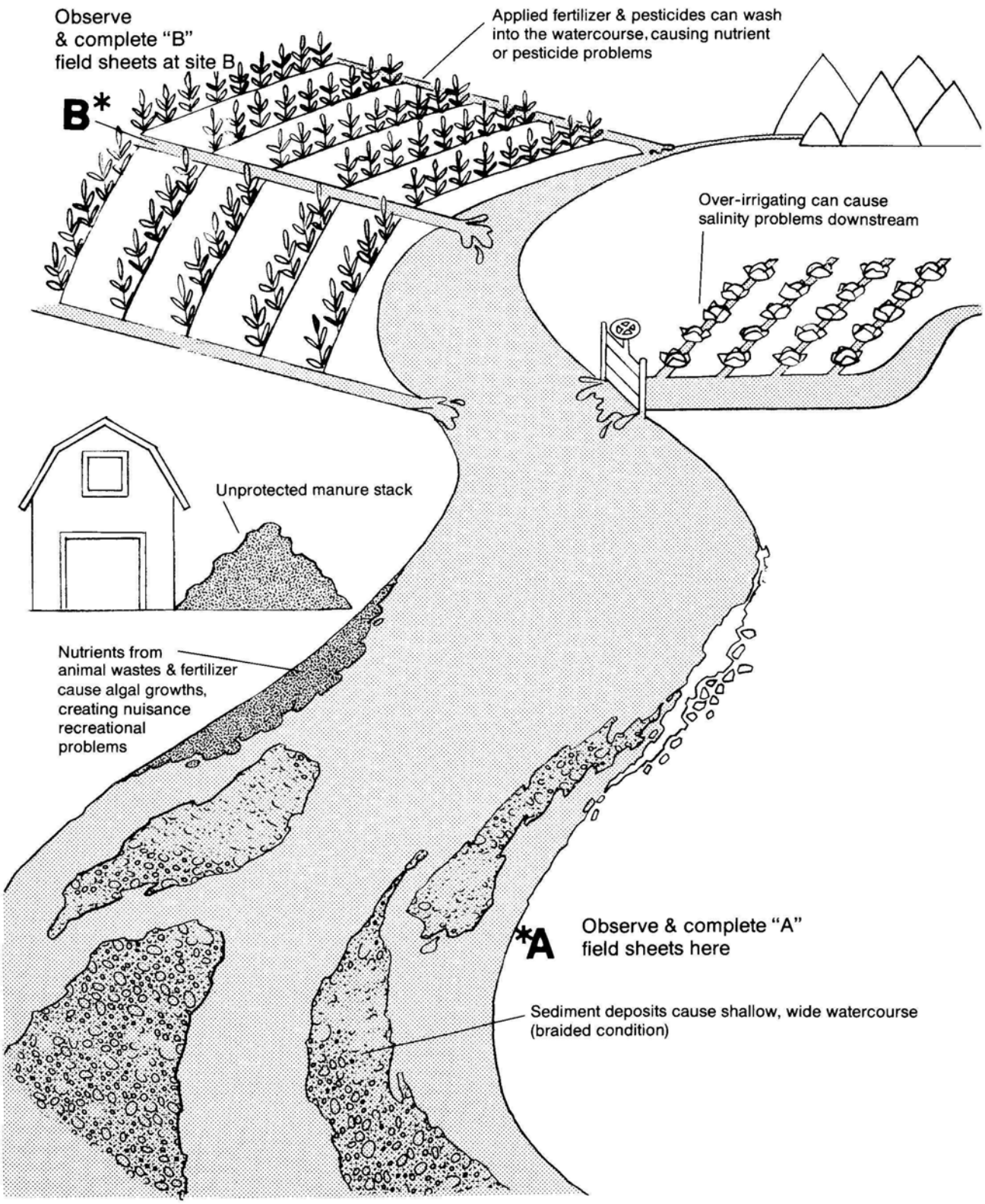
TOTAL [24]

1. Add the circled Rating Item scores to get a total for the field sheet.
2. Check the ranking for this site based on the total field score. (Check "excellent" if the score totals at least 32. Check "good" if the score falls between 21 and 31, etc.). Record your total score and rank (excellent, good, etc.) in the upper right-hand corner of the field sheet. If a Rating Item is "fair" or "poor," complete Field Sheet 1B.

RANKING	Excellent (32-37) [ ]	Good (21-31) [24]	Fair (9-20) [ ]	Poor (8 or less) [ ]
OPTIONAL RANKING (with #5 OR #6)	Excellent (40-46) [ ]	Good (26-39) [ ]	Fair (11-25) [ ]	Poor (10 or less) [ ]
OPTIONAL RANKING (with #5 AND #6)	Excellent (48-55) [ ]	Good (31-47) [ ]	Fair (13-30) [ ]	Poor (12 or less) [ ]

Figure 2-2

Nonpoint Source Pollution Effects.



other options apply to your particular geographic region and precisely define particular water quality situations. The word "OTHER" that has been included in each block on each field sheet means that you are free to adapt the field sheets to your particular region or locale. Note also that if none of the descriptors fit, you can resort to rankings relative to your geographic region, such as the first ones given for the turbidity indicator on Field Sheet 1A: Sediment.

One last point—A field sheet, like any other tool or instrument, is only as good as the person using it. This is true of the use of these field sheets. Those who take the time to learn how to use the *Water Quality Indicators Guide* field sheets will quickly become proficient in their use. Based on your experience with the sheets, you will start to make judgments about water quality and will develop an "intuitive feel" for the water's condition. *Rely on this judgment,* even if it means altering the field sheets.

Remember that the field sheets are only as good a tool as you make them, especially concerning local conditions.

Given that water is severely polluted by sediment, how can we know that the sediment is coming from agriculturally related activities? If it is related to agriculture, how can we correct the problem and improve water quality? To answer these questions, turn to the "B" field sheets.

TYPE B FIELD SHEETS

Assumption. Before using the series "B" field sheets, it is important to recognize that underlying the design of the overall field analysis is the assumption that we are striving for water of fishable/swimmable qualities—a goal established in the Federal Water Pollution Control Act of 1972 and iterated in the 1987 Water Quality Act Amendments. While geographic and site-specific conditions might cause us to accept a "good" rating in some instances, we should not be satisfied with a water quality rating of "fair" or "poor."

The "B" field sheets should be completed in all cases where water quality ranks lower than what is expected regionally under naturally occurring pristine conditions for any of the five major agricultural pollutants. While in many cases the pristine condition will receive an excellent rating, in other cases naturally occurring conditions (geologic, topographic, etc.) prevent the waters from ever being "excellent" (fishable/swimmable). It is important to be able to distinguish between naturally occurring and human-induced limitations to water use. It may be difficult to determine what constitutes "pristine" conditions for your area. If you do not know or are not sure, be sure to consult with local experts in the water quality field. Call the SCS State Office Water Quality Specialist or Biologist or the specialists at the SCS National Technical Centers. Every State has a water pollution control agency, although the names vary. Specialists in these offices are most willing to assist. Additionally, many local colleges and universities have environmental and water quality experts who can be of great help.

The "B" field sheets allow an on-farm or on-ranch assessment (fig. 2–3) of the five major agriculturally related contributors of pollution. Recommendations for improving problem situations are given in the last column of each sheet under "Practices from appendix E" (conservation and best management practices, ref. B–6). Figure 2–3 is completed with circled answers in the way that was done on Field Sheet 1A.

Other than the list of conservation and best management practices (BMP's) in the last column, the format for the "B" series is identical to that for the "A" series. Therefore, the procedure outlined above for use with the "A" field sheets should also be used in completing the "B" sheets.

The "B" sheets should be completed onsite. If a conservation plan exists for a given property, it would be helpful to have it in hand while completing the "B" field sheets. A soil survey of the area would also be helpful if you are not familiar with the land tract. You may want to briefly reconnoiter the tract of land. Previous experience with this particular property owner or manager and prior knowledge of the property will prove invaluable.

Based on your previous knowledge of the land or your recent reconnaissance, define a "representative" field which drains into a watercourse or water body you have judged to be polluted by use of the "A" field sheets. That is, choose an area large enough to give an appropriate numerical weighting to both properly and poorly managed areas. Then proceed to complete the appropriate "B" field sheet relative to the field that you just defined. While a sample field size should be representative, it is recommended to select for your observation site a location where you could expect to find a pollutant. For example, if you were assessing nutrients or pesticides, you might stand in the middle of the row crops, as shown in figure 2–2, where the B* is indicated. If you were interested in sediment pollution, you might position yourself in or near a recently plowed field.

If scores for any of the indicators (rating items) were ranked less than "good" or "excellent," you will want to consider recommending to the property owner or user one or more of the conservation or BMP's listed in the right-hand column of the sheet for that particular rating item. The practices listed are by no means exhaustive and may not be entirely suitable to your locality. Therefore, you will need to evaluate the suggested practices, selecting those that you consider to be appropriate to the given situation and adding others that may be lacking.

Figure 2-3

Sediment

Lat. 40° 37' 30"
Lon. 76° 40' 00"

FIELD SHEET 1B: SEDIMENT
INDICATORS FOR CROPLAND, HAYLAND OR PASTURE

Evaluator Isaacs/Myers County/State Dauphin, PA Date 18 Apr. '88
Field Evaluated Wilson Field Location Lykens, PA Total Score/Rank 30 Good

(Circle one number among the four choices in each row which BEST describes the conditions of the field or area being evaluated. If a condition has characteristics of two categories, you can "split" a score.)

Rating Item	Excellent	Good	Fair	Poor	Practices from Appendix E
1. Erosion Potential	Not significant. Less than T (tolerance); little sheet, rill, or furrow erosion. No gullies. OTHER 10	Some erosion evident. About T; some sheet, rill, or furrow erosion. Very few gullies. OTHER 7	Moderate erosion. T to 2T. Gullies or furrows from heavy storm events obvious. OTHER 3	Heavy erosion. More than 2T. Many gullies or furrows & presence of critical erosion areas. OTHER 0	1,3,5,7,8, 9,10,11, 15,16,17, 18,19,20, 21,22,23, 24,25,26, 27,29,30, 31,32,33, 37,38,40, 45,46,54, 61,62,65, 69,70,73, 75,79,85, 87,95,97, 99,102
2. Runoff Potential	Low: Very flat to flat terrain (0-0.5% slope). Runoff curve number (RCN) 61 - 70. Dry, low rainfall (less than 20"). Even, gentle impact (scattered shower-type) rainfall. OTHER 10	Moderate: Flat to gently sloping (0.5-2.0% slope). RCN 71 - 80. Semidry (20-30"). Even, gentle to moderate intensity rainfall. OTHER 8	Considerable: Gently to moderately sloping (2.0-5.0% slope). RCN 81 - 90. Semiwet (30-40"). Even to uneven intense rainfall. OTHER 4	High: Moderately sloping to steep terrain (greater than 5%). RCN greater than 90. Wet (more than 40"). Intense uneven rainfall, especially in seasons when soil is exposed. OTHER 0	6,9,88,95
3. Filtering effect or sedimentation potential of a vegetated buffer or water/sediment collecting basin	Intervening vegetation between cropland & watercourse greater than 200 ft. Type of intervening vegetation ungrazed woodland, brush, or herbaceous plants. Water & sediment control basins properly installed & maintained. OTHER 8	Intervening vegetation between cropland & watercourse 100 to 200 ft. Type of intervening vegetation grazed woodland, brush, or herbaceous plants or range. Water & sediment control basins properly installed, but poorly maintained. OTHER 6	Intervening vegetation between cropland & watercourse 50 to 100 ft. Type of intervening vegetation high density cropland. Water & sediment control basins poorly installed & poorly maintained. OTHER 4	Cropping from less than 50 ft up to water's edge. Type of intervening vegetation low density cropland or bare soil. No water & sediment control basins. OTHER 2	5,18,25, 27,79,107
4. Resource management systems (RMS's) on whole farm (combined value for all agricultural areas	Excellent management. RMS's always used as needed. OTHER 9	Good management. Most (80%) of the needed RMS's installed. OTHER 7	Fair management. About 50% of the needed RMS's installed. Cropping confined to proper land class. OTHER 3	Poor management. Few, if any, needed RMS's installed. Cropping not confined to proper classes. OTHER 0	Practices same as Rating Item #1
5. Potential for ground water contamination	LOW: Soils rich to very rich in organic matter (greater than 3.0%). Slow to very slow percolation in light textured soils such as clays, silty or sandy clays, or silty clay loams. Perched water table present. In protected bedrock areas (50 ft. of soil & shale cap), well depth is 75-100 ft. In protected bedrock areas overlain with 50 ft. of sand or gravel, well depth is greater than 150 ft. In shallow bedrock areas (25-50 ft. soil & shale cap), well depth greater than 200 ft. In Karst areas, well depth is greater than 1,000 ft., if aquifier is "confined." OTHER 9	MODERATE: Soils rich to moderate in organic matter (3.0 to 1.5%). Slow to moderate percolation in clay loams or silts. Perched water table present. In protected bedrock areas, well depth is 30-74 ft. In protected bedrock areas overlain with 50 ft. of sand or gravel, well depth is 100-149 ft. In shallow bedrock areas, well depth is 50-199 ft. In Karst areas, well depth is 500-999 ft. OTHER 6	CONSIDERABLE: Soils moderate to low in organic matter (1.5 to 0.5%). Moderate to rapid percolation in silty loams, loams, or silts. In protected bedrock areas, well depth is 15-29 ft. In protected bedrock areas overlain with 50 ft. of sand or gravel, well depth is 50 - 99 ft. In shallow bedrock areas, well depth is 25-49 ft. In Karst areas, well depth is 100-499 ft. OTHER 4	HIGH: Soils low to very low in organic matter (less than 0.5%) Rapid percolation in coarse textured loamy sands or sands. In protected bedrock areas, well depth is less than 15 ft. In protected bedrock areas overlain with 50 ft. of sand or gravel, well depth is less than 50 ft. In shallow bedrock areas, well depth is less than 25 ft. In Karst areas, well depth is less than 100 ft. OTHER 0	See animal waste, nutrients, pesticide, & salt "B" Field Sheets for practices

1. Add the circled Rating Item scores to get a total for the field sheet. TOTAL [30]
2. Check the ranking for this site based on the total field score. Check "excellent" if the score totals at least 40. Check "good" if the score falls between 26 and 39, etc. Record your total score and rank (excellent, good, etc.) in the upper right-hand corner of the field sheet. If a Rating Item is "fair" or "poor," find the practices in the right-hand column to help remedy the conditions.

RANKING Excellent (40-46) [ ] Good (26-39) [30] Fair (10-25) [ ] Poor (9 or less) [ ]

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River management: use of macrophyte community structure in UK rivers as an example for ecological assessment for hydromorphological impacts

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Abstract: -The present study used macrophyte community as an assessment tool to provide the basis for the effective management of UK rivers under different degrees of human impacts, i.e. hydromorphological impacts and nutrient enrichment. We developed a river classification scheme based on the gradients of physical habitat modifications and the change of hydromorphology and relate such scheme to the macrophyte community structures. Our overall results suggest that macrophytes are sensitive to hydromorphological changes. At high impact level, transgressive species such as mosses, emergent and amphibious species, including *Sparganium* erectum, *Phragmites australis* and *Berula erecta*, dominanted the macrophyte communities. However, submerged vegetations are the major space occupiers at the low-impacted sites.

Key-words: -aquatic plants, multivariate analysis, habitat structure, community ecology

1 Introduction

Macrophytes are used as indicators for monitoring and assessment of water quality and nutrient enrichment of rivers because of the well-document predictable relationships between their ecological preferences/tolerances and the hydrochemistry of rivers [1]. However, recent studies suggested that the distribution and development of macrophytes are also dependent upon hydromorphology (i.e. hydrological regimes, continuity, channel and bank morphology) which is, in turn, conditioned by underlying catchment geology and physical degradation [2]. As a result of substantial physical alterations of river habitats by human activities for various purposes (e.g. flood control, improvement of drainage for land for agriculture and navigation) in Europe, natural river habitats have been heavily impaired with the hydromorphological characteristics changed [e.g. 3; 4; 5]. The extensive

distribution (in both impacted and unimpacted rivers) and the high diversity (perennial/non-mobile vs. short-lived mobile species) of macrophytes result in the distinct advantage of being good indicators of long-term habitat changes, e.g. capable of indicating long- and short-term physio-chemical changes in the river environment based on the species composition [6; 7].

Given that macrophytes have potential as indicators of hydromorphological degradation [6; 9; 10], macrophytes are highlighted in the WFD as useful for assessment of hydromorphological pressures in rivers [11]. There is therefore a clear need to understand the sensitivity of macrophytes towards the hydromorphological pressures. Limited research has reported such relationships. Until recently, most previous studies have documented the effects of a relatively small subset of river hydromorphological characteristics and/or impacts on macrophyte communities, highlighting the relationships between specific hydromorphological characteristics and individual macrophyte species or families [e.g. 12], or biological groups as by habitat preference: submerged, emerged, and amphibious species [e.g. 13]. The integrated effects of hydromorphological condition (i.e. the combined measure of different hydromorphological elements) upon the macrophyte communities have yet received less attention [9]. The hydromorphological condition of a river is a composite result of many individual physical processes, all of which can be influenced by human activities. If the hydromorphological condition governs community composition of macrophytes and measures of overall hydromorphological condition can be related to macrophyte community structure, then understanding the responses of macrophyte communities to these measures would be useful for management and monitoring.

2 Problem Formulation

In this study, we examined the variation of macrophyte communities with respect to the different degrees of hydromorphological impact and nutrient enrichment across five *apriori* defined river classes, lying along gradients of geographical location and physical degradation, in 251 UK rivers (Figure 1; Table 1) based on both univariate (analysis of variance using Minitab 15.0 software) and multivariate statistical approaches

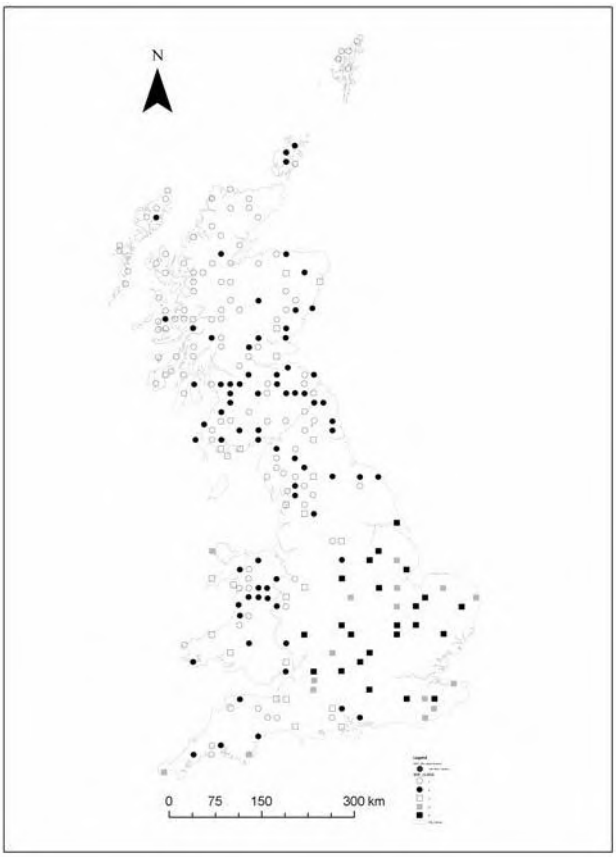

Figure 1. Map showing the distribution of study sites with respect to the five *apriori* defined site classes.

(i.e. principle component analysis, PCA, and non-metric multidimensional scaling, MDS, using PRIMER v6.0 Package) [14]. This classification system was further assessed for its usefulness as site class diagnosis tool for river monitoring purpose. Despite the focus on linking hydromorphological conditions and macrophyte community structure, data on inorganic nutrient levels were also included in the analysis, because of their known influence [e.g. 15] on macrophyte communities. By correlating the environmental and biological dissimilarity matrices, we determined the most important hydromorphological elements characterizing macrophyte community structure and identified the indicator taxa most sensitive to hydromorphological degradation. This work will help understand the relationships between macrophyte communities and hydromorphological alteration in rivers, and contribute to the development of monitoring tools for physical degradation and management decisions in river habitats to physical degradation resulted from human activities.

3 Problem Solution

A strong relationship of geographical location and the impact of hydromorphological modification to the physio-chemical change in UK rivers. This is because most of the environmental variables (89%) including catchment characteristics, measured physical and chemical variables and the three indices of the hydromorphological descriptors (i.e. diversity, score and homogeneity of channel depth, wetted width, flow type and substrate type) were significantly different ($P<0.05$) among the five classes of sites. Principle Component Analysis of both overall variation of environmental variables (hydromorphological + water chemistry variables) and only hydromorphological variables showed that environmental variability was mainly driven by habitat quality and physical modification.

Macrophyte community structure varied as predicted by the River Continuum concept at unimpacted/ low-impacted sites [16]. Species diversity of macrophyte species at individual sites decreased from site class 1 (40 species) to site class 5 (21 species), but the total abundance were higher in lowland rivers (from site classes 4 and 5) than upland rivers (from site classes 1, 2 and 3) (see also Table 1). Species diversity and richness was found negatively correlated to the increasing homogeneity of channel morphological characteristics, flow habitat types and bed substrates. Multidimensional scaling ordinations (MDS) demonstrated a gradational pattern from one major cluster of the sites in an order consistent with increasing hydromorphological impacts from site class 1 to 5. Such a pattern is considered to reflect chronic impacts, hydromorphological and nutrient impacts act over multi-year timescales and the macrophyte communities therefore respond to these impacts continuously.

Multivariate analysis of changes in macrophyte community structure in term of the species abundance indicated that it was highly affected by the habitat modification score (HMS-impact level), homogeneity of channel depth and flow habitat type. This highlighted the importance of hydromorphological variables in characterizing macrophyte community. On the other hand, the influence of nutrient levels on shaping macrophyte community structure was significantly lower than that physical characteristics and hydromorphological modification. Yet, we cannot conclude for the relationship between nutrient enrichment and macrophyte communities as our

results might be the consequence of insufficient sampling for water chemistry analysis in the present study.

Nine macrophyte taxa were identified as bioindicators for the environmental changes in UK rivers by conducting a non-parametric correlation between the environmental and biological dissimilarity matrices using the BIOENV routine from PRIMER v6.0. These species included *Apium nodiflorum*, *Epilobium hirsutum*, *Glyceria maxima*, *Phragmites australis*, *Sparganium erectum*, *Myosotis scorpioides*, *Berula erecta* and the taxonomic group 'all mosses'. Monotonic functions of stress-response relationship with the abundance of the indicator taxa increasing with the levels of physical modifications and decreasing habitat quality were observed. This suggested that the five *apriori* defined site classes are more likely defined by the 'transgressive' species which are either pioneer or opportunistic species usually found in both natural and impacted sites but which never appear as dominant species at unimpacted sites.

4 Conclusion

We concluded that the present river classification scheme is able to distinguish between the macrophyte communities at reference (unimpacted) and impacted sites which were defined based on the gradients of physical habitation modifications and the change of hydromorphology. However, it cannot differentiate the macrophyte community structure between site classes 4 and 5 which showed no difference in hydromorphological condition (Figures 3 & 4). Hence, our results suggested that, due to the sensitivity of macrophytes towards water chemistry, in particular nutrient concentration, as compared to the aquatic fauna, only hydromorphological variables might not be able to discriminate the five site classes. Water chemistry variables (including nutrient concentration) were necessary to be included in the *apriori* site classification systems for effective site class discrimination.

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Table 1. Five classes of sites as divided by the *apriori* arbitrary site classification.

	Site class				
	1	2	3	4	5
Geographical location	N England	N England & Wales	N England & Wales	SE England	SE England
Reference type	Reference site	Non-reference site	Non-reference site	Non-reference site	Non-reference site
Level/ type of impacts	No	Low/ physical	High/ physical and nutrient	Low/ physical	High/ physical and nutrient

EFFECTS OF EUTROPHICATION ON STREAM ECOSYSTEMS

Lei Zheng, PhD and Michael J. Paul, PhD

Tetra Tech, Inc.

Abstract

This paper describes the effects of nutrient enrichment on the structure and function of stream ecosystems. It starts with the currently well documented direct effects of nutrient enrichment on algal biomass and the resulting impacts on stream chemistry. The paper continues with an explanation of the less well documented indirect ecological effects of nutrient enrichment on stream structure and function, including effects of excess growth on physical habitat, and alterations to aquatic life community structure from the microbial assemblage to fish and mammals. The paper also dicusses effects on the ecosystem level including changes to productivity, respiration, decomposition, carbon and other geochemical cycles. The paper ends by discussing the significance of these direct and indirect effects of nutrient enrichment on designated uses - especially recreational, aquatic life, and drinking water.

1. Introduction

1.1 Stream processes

Streams are all flowing natural waters, regardless of size. To understand the processes that influence the pattern and character of streams and reduce natural variation of different streams, several stream classification systems (including ecoregional, fluvial geomorphological, and stream order classification) have been adopted by state and national programs. Ecoregional classification is based on geology, soils, geomorphology, dominant land uses, and natural vegetation (Omernik 1987). Fluvial geomorphological classification explains stream and slope processes through the application of physical principles. Rosgen (1994) classified stream channels in the United States into seven major stream types based on morphological characteristics, including entrenchment, gradient, width/depth ratio, and sinuosity in various land forms. These morphological characteristics affect stream ecosystem processes and community structure and functions. Stream order classification (Strahler 1964) is also widely applied for organizing drainage networks in the United States. These stream classification systems describe hydrology and material transport, which in turn influence physical, chemical, and biological processes.

Another classification scheme is to classify streams based on nutrient conditions (EPA 2001a). EPA divides the country into 14 level III nutrient ecoregions (Omernik 2000) with common land use characteristics to better assess background nutrient concentrations in different geographic regions. This classification reflects spatial and geographic variations that influence nutrient concentrations in streams (Rohm et al. 2002, Wickham 2005) and natural background nutrient concentrations should be established for each region (Smith et al. 2003). Dodds (1998, 2006) proposed classifying streams into trophic state classes similar to those developed for lakes and reservoirs (EPA 2001b).

One of the most important processes in streams is nutrient cycling. Stream channels receive nutrients from upstream, terrestrial runoff, ground water, and the atmosphere. The proportion of each source is variable depending on stream geology, elevation, and regional setting. Different landforms (forest vs. agricultural catchments) and spatial and temporal variables also significantly affect nutrient concentrations and loadings into streams (Arheimer and Liden 2000). Internal nutrient cycling also provides nutrients to streams (Mulholland 1996). Stream biota use nutrients and convert them into biomass; thus, nutrients are important to ecosystem structure and function.

Two major nutrients, nitrogen (N) and phosphorus (P), occur in streams in various forms as ions or dissolved in solution. Aquatic plants convert dissolved inorganic forms of nitrogen (nitrate, nitrite, and ammonium) and phosphorus (orthophosphate) into organic or particulate forms for use in higher trophic production. The right balance of nitrogen and phosphorus is essential for maintaining natural biological communities and ecosystem functions in aquatic systems. In freshwater systems, phosphorus and nitrogen

are limiting nutrients, that is, the levels of these nutrients limit the biological productivity of such systems.

1.2 Limiting nutrients in streams

Stream primary producers, i.e., algae and macrophytes, absorb natural energy from sunlight to fix carbon and convert inorganic forms of N and P into organic forms through photosynthesis, storing the energy produced in their cells. In most streams, either N or P concentrations or both limit this process. Different algae have been reported to require different N and P concentrations for growth. One study found that diatoms require less P (0.3-0.6 µg/L P, Bothwell 1988) to saturate growth than filamentous green algae (25-50 µg/L P, Bothwell 1989). Nitrogen limitation has been reported when ambient N concentration was 55 µg/L in a desert stream in Arizona (Grimm and Fish 1986) and when it was less than 100 µg/L in an Ozark stream (Lohman et al. 1991). Rier and Stevenson (2006) found that algal growth was 90% of maximum rates or higher in nutrient concentrations of 16 µg/L P and 86 µg/L N. The Redfield ratio (molar ratio of 106:16:1 for C:N:P) has been proposed as a community-wide optimum nutrient ratio (Redfield 1958, Borchardt 1996). High ambient or cellular N:P ratios (N:P >20:1) indicate P is limiting growth; low N:P ratios suggest that N is limiting (N:P<10:1). However, levels of nutrient concentrations and ratios for nutrient limitation are also regulated by other abiotic and biotic factors.

Regional differences may determine limiting nutrients for plant growth. Phosphorus used to be and is still considered the sole limiting nutrient in aquatic systems by a number of authors (Huchinson 1957, Correll 1998, Khan and Ansari 2005). With increasing experimental manipulation of nutrient limitation, especially bioassays using nutrient diffusing substrates and artificial streams, N limitation and N and P co-limitation are quite commonly discovered (Grimm and Fisher 1986, Peterson and Grimm 1992). Borchardt (1996) reviewed studies in North America and concluded that roughly the northern half of the United States is P limited while the Southwest and Missouri Ozarks are N limited. The Pacific Northwest may be limited by both N and P. A meta-analysis of 237 nutrient enrichment studies in temperate streams revealed that 16.5% indicated an N response, 18.1% indicated a P response, 23.2% required N and P be added together for a response, 5% had N or P inhibition, and 43% had no response to N or P (Francoeur 2001). These proportions have been confirmed by a similar literature review (Tank and Dodds 2003).

1.3 Eutrophication problems

Eutrophication means "good food". In freshwater systems, eutrophication is a process whereby waterbodies receive excess inorganic nutrients, especially N and P, which stimulate excessive growth of plants and algae. Eutrophication can happen naturally in the normal succession of some freshwater ecosystems. However, when the nutrient enrichment is due to the activities of humans, sometimes referred to as "cultural eutrophication", the rate of this natural process is greatly intensified. Eutrophication was recognized as a pollution problem in North American lakes and reservoirs in the mid-20th century (Rohde 1969). Although nutrient pollution has long been recognized as a major problem in streams and rivers (USEPA 2000), the concept of eutrophication has been less commonly used with respect to nutrient enrichment problems in streams (Dodds 1998, 2006).

Nutrient enrichment of streams in the United States is widespread (Carpenter 1998, Correll 1998, Smith et al. 1999, 2006). EPA assessed approximately 840,000 river and stream miles nationwide and reported that 10% of assessed rivers and streams had nutrient enrichment problems, which contributed to 30% of reported water-quality problems in the impaired rivers and streams (~ 291,000 miles). (USEPA 2002). Nitrate concentration has more than doubled in the Mississippi River since 1965 and concentrations in many major rivers in the Northeast have increased by from 3- to 10-fold since the early 1900s (see reviewed by Vitousek 1997). Smith et al. (1987) found that at 381 riverine sites in the continental United States, the mean total phosphorus concentration was 130 mg/m^3, which is almost double the threshold value for eutrophication (75 mg/m^3) proposed by Dodds (1998) for streams.

1.4 Sources of nutrient enrichment: point and nonpoint sources

Nutrient concentrations in streams and rivers have been strongly correlated with human land use and disturbance gradients. Both N and P enrichment are linked to agricultural and urban land uses in the watershed. Fluxes of total N in temperate-zone rivers surrounding the North Atlantic Ocean are highly correlated with net anthropogenic input of N to their watersheds (Howarth et al. 1996). Total N and nitrate fluxes and concentrations in rivers are also correlated with human population density (Cole et al. 1993, Howarth et al. 1996). Nitrogen fertilization is the main source of N in streams and rivers (Goolsby and Battaglin 2001). Similarly, urbanization generally leads to higher phosphorus concentrations in urban catchments (see review by Paul and Meyer 2001). Increasing imperviousness, increased runoff from urbanized surfaces, and increased municipal and industrial discharges all result in increased loadings of nutrients to urban streams. This makes urbanization second only to agriculture as the major cause of stream impairment, even though the total area covered by urban land in the United States is minor compared to agricultural area (Paul and Meyer 2001).

Nutrient enrichment of aquatic systems from anthropogenic sources includes point and nonpoint sources (Table 1, adapted from Carpenter et al.1998). Both have degraded aquatic systems. Point sources of nutrients include wastewater effluent (both municipal

and industrial) and storm sewer discharge. In contrast to point sources of nutrients that are relatively easy to monitor and regulate, nonpoint sources such as livestock, crop fertilizers, and urban runoff exhibit more spatial and temporal variability. Following strong regulation of point source inputs in response to the Clean Water Act, nutrients from nonpoint sources are now the major source of water pollution in the United States (Carpenter et al. 1998).

Table 1. Characteristics of point and nonpoint sources of chemical inputs to receiving waters recognized by statutes of the United States (from Carpenter et al. 1998).

- Point Sources

-- Wastewater effluent (municipal and industrial)
-- Runoff and leachate from waste disposal sites
-- Runoff and infiltration from animal feedlots
-- Runoff from mines, oil fields, unsewered industrial sites
-- Storm sewer outfalls from cities with a population >100,000
-- Overflows of combined storm and sanitary sewers
-- Runoff from construction sites >2 ha

- Nonpoint Sources

-- Runoff from agriculture (including return flow from irrigated agriculture)
-- Runoff from pasture and range
-- Urban runoff from unsewered and sewered areas with a population <100,000
-- Septic tank leachate and runoff from failed septic systems
-- Runoff from construction sites
-- Runoff from abandoned mines
-- Atmospheric deposition over a water surface
-- Activities on land that generate contaminants, such as logging, wetland conversion, construction, and development of land or waterways

Seasonal variability of nutrient loading into streams is dramatic in both agricultural and woodland streams impacted by fertilization. Fertilization of timberlands with nitrogen to increase production is a common practice for the timber industry in the United States. Typically, concentrations of nitrate-N in streams increase rapidly during rainstorms after fertilization in the fall. Peak concentrations have ranged from less than 100 µg/L to greater than 10,000 µg/L (exceeds EPA drinking water level) during high runoff (Anderson 2002). Ambient NO_3-N concentrations in forest streams are typically elevated as much as from 2- to 10-fold for the entire winter and spring following a fall fertilization (Bisson et al. 1992). Stream uptake reduces NO_3-N concentrations to background levels by summer in most cases (Mulholland 1992, Mulholland and Rosemond 1992). Then a secondary NO_3-N peak is observed in the subsequent fall period, which indicates that applied nitrogen fertilizer remains available for leaching to streams beyond the spring and summer growing seasons. An extreme example of long-term availability is reported at Fernow Experimental Forest (in the Appalachians), where NO_3-N remained elevated relative to control streams 10 years after fertilization with ammonium nitrate at 336 Kg N/ha (Edwards et al. 1991).

1.5 Consequences of eutrophication

Allan (2004) has summarized the various impacts of land use on streams and rivers. Eutrophication, as one of the main causes of stream impairment in the United States, imposes severe threats to ecosystem structure and function. The direct impact of nutrient enrichment is to increase autotrophic production and change species assemblages including proliferation of filamentous algae. Nutrient enrichment also accelerates litter breakdown rates by bacteria and fungi. The dramatic changes at lower trophic levels may also lead to "trophic cascading". As nutrient concentrations increase and destabilize the primary producer assemblage and water chemistry, macroinvertebrates and fish may shift from sensitive species to more tolerant, often non-native species. Changes in the food web may also cause changes in ecosystem function and further alter stream physical habitat and water chemistry, e.g., decreasing dissolved oxygen.

1.6 The objective of this document

The trophic structure of stream ecosystems can be divided into *producers* and *consumers*. *Primary producers* often refers to algae, moss, ferns, and higher plants. They convert inorganic nutrients into organic forms and are the main energy source of streams. Primary producers are consumed by *primary consumers* (herbivores) or after they die, the organic matter is added to the detrital cycle and either decomposed by *decomposers* (bacteria and fungi) or ingested by *detritivores*. *Secondary consumers* (predators) rely on energy sources from primary consumers. Nutrient enrichment of streams directly changes the nutrient supplies for primary producers, which then affects consumers and nutrient cycling processes, leading to changes in ecosystem structure and function (i.e., eutrophication).

The objective of this document is to provide an understanding of the effects of nutrient enrichment on the structure and function of stream ecosystems. Several authors have documented the relationship between stream enrichment and algal biomass (e.g., New England Interstate Water Pollution Control Commission 2001, Virginia Water Resources Research Center 2006, Dodds 2006). And EPA's nutrient criteria technical guidance manual for streams also provides background on nutrient impacts. However, the ecological effects of enrichment on stream structure and function are less well discussed. To develop ecologically sound nutrient criteria to protect streams, it is critical to understand how ecological processes that affect stream nutrient dynamics and ecosystem function will change with elevating nutrient pollution.

Although it is important to understand nutrient cycling processes, including nutrient exchange between terrestrial, aquatic, and atmospheric systems (Mulholland 1992, Valett et al. 1994, Boulton et al.1998, McMahon et al. 1994, Arheimer and Liden 2000), they are beyond the scope of discussion for this document. Readers interested in nutrient cycling should see some of the many reviews on this topic (e.g., Stream Solute Workshop 1990).

2. Effect on physical habitat and water chemistry

2.1 Physical habitat modification

Nutrient enrichment can substantially change stream habitat. The direct effect of nutrient enrichment in streams is excessive accumulation of filamentous benthic algae during the peak summer growing season, altering flow environment; physical benthic habitat used by stream invertebrates and vertebrate organisms (Welch et al. 1989, Chessman et al. 1992). Filamentous green algae, such as *Cladophora, Ulothrix*, and *Rhizoclonium*, favor nutrient enriched environments. Under suitable enrichment, they can be several meters long in high-velocity areas. When they are aging, they often detach and float near the surface (Power 1990). *Vaucheria* and *Chara* can trap sediments to form knolls more than a meter in length in the sandy streams of the north central United States. Chain forming diatoms, such as *Terpsinoe*, can also form long filaments in flowing water. *Cladophora* and other filamentous algae are often habitat for small invertebrates, such as chironomids, amphipods, and many meiofauna (see review by Dodds and Gudder 1992).

Periphyton and macrophytes can also alter water velocity in streams. Dodds and Biggs (2002) found that algae attenuate current velocity more than macrophytes, and of the different types of algae, dense aggregations of diatoms (primarily Cymbella) attenuate velocity more than filamentous green algae or red algae. Benthic aquatic plants can cause an exponential decline in velocity with depth. In urban channelized streams, *Cladophora* and *Ulothrix* attach to concrete irrigation canals, reducing flow rate and capacity. Aquatic plants clogging stream and drainage ditches causes water backup, flooding, and water loss through evaporation from floating or emergent plant surfaces.

Nutrient enrichment can also lead to excessive phytoplankton growth. Excessive planktonic algal growth in slow moving water can reduce light penetration and consequently limit the growth of submerged aquatic plants, decreasing available habitat and shelter for fish and their food organisms (Sand-Jensen et al. 2000). Lack of aquatic plants can also cause erosion and instability of the stream channel.

2.2 Water chemistry

2.2.1 Dissolved Oxygen (DO)

Nutrient enrichment leads to excessive growth of primary producers as well as heterotrophic bacteria and fungi, which increases the metabolic activities of stream water and may lead to a depletion of dissolved oxygen (Mallin et al. 2006). During the day, photosynthesis by primary producers provides a large amount of oxygen to the water. At night, photosynthesis stops and elevated respiration by algae and bacteria continues to consume dissolved oxygen, which can deplete DO. Furthermore, as primary producers die, they are decomposed by bacteria that consume oxygen. Large populations of decomposers consume more dissolved oxygen, which increases the severity of DO depletion.

DO depletion has been reported in many streams through diel studies but is more frequently studied in lowland, slow moving streams (House and Denison 1997, Kaenel et al. 2000, Sabster et al. 2000, Wilcock and Nagels 2001). Daily oxygen fluctuations in enriched streams at low flow were reported to range from a high of approximately 25 mg/L at noon to a low of approximately 3 mg/L at night (Wong and Clark 1976). Decomposition and microbial activities are likely enhanced during summer when rising temperatures lead to lower DO saturation. Sometimes streams become anoxic (House and Denison 1997). Excessive amounts of nutrients in streams and rivers may also negatively impact the dissolved oxygen levels of downstream receiving waters. For example, a zone of hypoxia (< 2 mg/L) in the Gulf of Mexico has been linked to high nutrient inputs from the Mississippi River (USEPA 2000, Mallin et al. 2006).

Long-term nutrient enrichment (of streams) leads to long term declines in average DO concentrations (Vagnetti et al. 2003, Parr and Mason 2003, 2004). One long-term study (Parr and Mason 2004) found a significant decline in DO during1955-1998 in the River Brett, England. That stream has shifted from an autotrophic to a heterotrophic system.

The composition of the aquatic plant community can affect the DO response. Wilcock and Nagels (2001) found that streams dominated by submerged macrophytes exhibited the greatest amplitude swings in DO and pH, and led to DO levels of ~ 86-128% saturation. Parr and Mason (2004) estimated that macrophytes accounted for 45% of community respiration at the study site while sediment accounted for 36%, and suggested that removal of plants would restore DO. Kaenel et al. (2000) suggested that an increase in the oxygen concentration after plant cutting would only be transient in unshaded, nutrient-rich streams.

2.2.2 pH

Diel changes in pH often occur in regions with low acid neutralizing capacity and are related to excessive algal growth and stream metabolism. The “normal” pH range in streams is from 6 to 9 with 7 being neutral, less than 7 indicating acidic conditions, and greater than 7 indicating basic conditions. During photosynthesis, carbon dioxide (CO_2) and water are converted by sunlight into oxygen and carbohydrate. Hydroxyl ions (OH^-) are produced, raising the water column pH. In addition, plants use a large amount of dissolved CO_2 for photosynthesis, resulting in lower levels of carbonic acid (H_2CO_3) in the water column. Thus, photosynthesis increases water column pH. At night, increased respiration from biota increases the release of CO_2 into the water, increasing the production of carbonic acid and hydroxyl ions, which, in turn, increases the acidity.

Extremely high or low pH values in streams are harmful to aquatic organisms. For example, high pH levels can be toxic to fish and other organisms. High pH levels damage fish gills, eyes, and skin, and affect fish reproduction. High pH levels also increase the toxicity of some substances, such as ammonia. Low pH levels can make heavy metals in stream sediment more bioavailable (e.g., Al). pH levels also influence the availability of some nutrients, further exacerbating enrichment problems.

2.2.3 Other chemicals

Toxic effects of chemicals released from certain cyanobacteria have been reported in lakes; very few studies have found cyanotoxins in streams. Pfiesteria, a toxic substance produced by dinoflagellates that cause fish kills, has also been reported in coastal rivers associated with nutrient enrichment (Burkholder 1999). A relatively new golden alga, *Prymnesium parvum*, has been reported to be toxic in Texas. The toxin *prymnesin* affects gill-breathing organisms including fish, tadpoles, and clams (Rhodes and Hubbs 1992) and has been responsible for an estimated 2.5 million dead fish and millions of dead clams in the Pecos, the Colorado, and Brazos river basins in Texas.

Other chemicals can taint drinking water supplies and recreational waters. 2-methylisoborneol and geosmin are two chemicals produced by cyanobacteria that can cause taste and odor problems in drinking water. Livestock that drink water contaminated with cyanobacteria have died (Dodds and Welch 2000). Humans who drink or swim in water that contains high concentrations of toxins from cyanobacteria may experience gastroenteritis, skin irritation, allergic responses, or liver damage (CDC 2004). In 1991, one of the largest recorded riverine blooms of toxic cyanobacteria occurred in the Murray-Darling River Basin in Australia, resulting in a state of emergency being declared to protect water supplies drawn from the river (Oliver et al. 1999).

3. Direct biological responses of streams to eutrophication: primary producers

Although it is generally recognized that nutrient enrichment stimulates algal growth in nutrient limited streams, increased algal productivity may or may not result in accumulation of algal biomass. A number of factors, i.e., light availability, grazer intensity, and physical disturbance, can affect algal biomass accumulation in streams. The fate of periphyton biomass produced in aquatic ecosystems follows four different paths (Lamberti 1996): (1) accumulation as a standing crop of algae, (2) respiration to CO_2 (decomposition), (3) consumption by herbivores (grazing), and (4) exported to suspended matter. The specific fate of biomass will depend heavily on grazing regimes (Lamberti et al. 1987, 1993). In heavily grazed systems, primary production is either consumed or exported, while reduced grazing pressure allows accumulation of algal biomass and its decomposition.

3.1. Responses of algal biomass to nutrient enrichment

A number of authors have documented the positive relationship between benthic algal biomass and nutrient concentrations (see reviews by ENSR 2001, Virginia WRRC 2006, Dodds 2002, 2006). These studies include both field manipulations of nutrient levels to assess benthic algal growth (Bothwell 1989, Walton et al. 1995, Stevenson et al. 2006, Rier and Stevenson 2006) and large scale surveys that investigate relationships between nutrient enrichment and periphyton biomass across different streams (Dodds et al .1997, Biggs and Close 1989, Welch et al. 1992, Biggs 2000, Lohman et al. 1992, Chetelat et al. 1999). These studies established that total N and total P in the water column are significantly related to benthic algal biomass. Empirical models derived from these studies allow one to predict the mean summer chlorophyll *a* level in a stream based on nutrient concentrations (e.g., Biggs 2000).

The positive relationship between algal biomass and nutrient concentrations is also observed in phytoplankton (Jones et al. 1984, van Nieuwenhuyse and Jones 1996, Basu and Pick 1995, Lohman and Jones 1999). Phytoplankton in streams receive less attention because they are low in abundance and frequency. However, in many slow moving embayments, algal concentrations can be more than 40 times that in the main stem of the river (Reynolds and Descy 1996). Van Nieuwenhuyse and Jones (1996) used data from 292 temperate streams and found that summer mean sestonic (suspended) chlorophyll concentration showed a strong curvilinear relationship with summer mean total phosphorus concentration (TP), although much of that sestonic chlorophyll was likely due to dislodged benthic algae technically called tychoplankton.

A fair number of nutrient enrichment studies in streams find no relationship between algal biomass and nutrient concentrations. Many hypotheses have been proposed to explain these results. One possible hypothesis is that nutrients exceeded the maximum concentration to saturate algal growth (e.g., Munn et al. 1989). However, under most circumstances, a number of other factors, such as hydrologic regime (e.g., Welch et al. 1988, Biggs 1989, 1995), light availability (e.g., Lowe et al. 1986), grazers (e.g., Rosemond 1998) and interactions of these factors (Hill et al. 1995) could have contributed to the lack of correlations between benthic algal biomass and nutrient concentrations. Resources and disturbances are the main factors (Figure 1, Biggs 1996) that influence algal accrual in a stream.

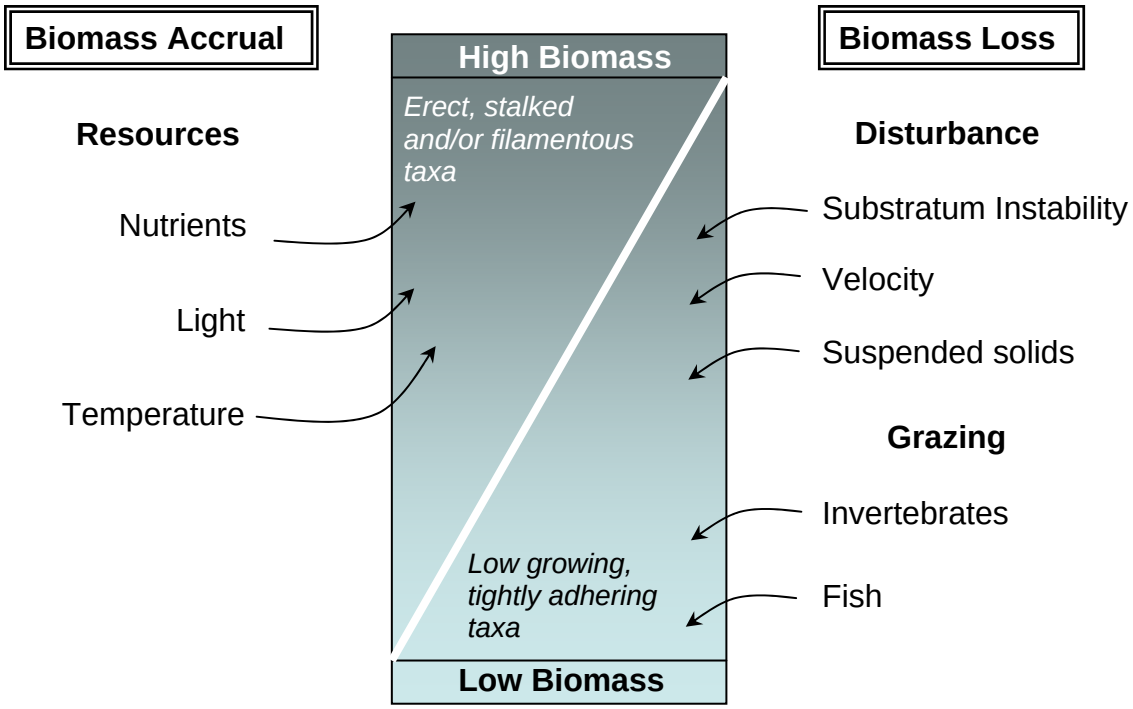

Figure 1. Summary of the disturbance-resource supply-grazer concept for the control of benthic algal development in streams. The relative balance of "biomass accrual" and "biomass loss" is depicted by the width of the triangles that make up the central rectangle. The physiognomy of the community likely to dominate each end of the gradient is also shown. After Biggs 1996.

Hydrological events such as scours significantly reduce the effects of nutrients on periphyton biomass (Welch et al. 1988, Ghosh and Gaur 1994, Bourassa and Cataneo 1998). Biggs (2000) developed a comprehensive model linking hydrologic regime and nutrients to algal accrual. This model was developed using data from New Zealand rivers and streams across a wide range of land use practices and hydrologic patterns. Although the relationship between nutrients and algal biomass was relatively weak (R^2=0.30), including the time of accrual (time since the last scouring flood) increased R^2 values to approximately 70%, indicating that eutrophication effects are stronger under stable flow conditions.

Grazers significantly reduce the amount of algal biomass in streams (Steinman 1996). Rosemond et al. (1993) manipulated both nutrient and herbivore abundances in an experimental mesocosm and found that grazers could moderate or eliminate the observed increase in periphyton biomass with increasing nutrient levels. Other studies also found that increased densities of grazers recruited from nearby areas (Lowe et al. 1986, Welch et al. 1988, Rosemond 1994, Wellnitz et al. 1996, Bourassa and Cataneo 1998, Hillebrand 2002, Roll et al. 2005) could eliminate increases of algal biomass to nutrient additions.

Light is a key factor for algal growth and consequent algal biomass (Hill 1996). Algal biomass can be 4 or 5 times higher in stream segments with open canopies than at sites with more closed canopy cover (Lowe et al. 1986). Light effects increase with trophic state and algal biomass, indicating enhanced importance of light limitation and self-

shading at high nutrient supply (Hillebrand 2005). Light and nutrient limitations also interact, so increased light availability or nutrients alone sometimes do not guarantee an elevated biomass. In a nitrogen limited system, increased light level did not increase accumulation of algal biomass without nitrogen additions (Taulbee et al. 2005). Similarly, several studies have found that nutrient addition alone does not stimulate algal biomass accrual unless light availability is also increased (Bernhardt and Likens 2004, Mallory and Richardson 2005). These results demonstrate that for essential resources such as light and nutrients, the magnitude of the response to enrichment by one resource depends on the relative availability of another.

Interactions of light, nutrients, and grazers may confound the relationship between nutrients and algal biomass in streams. In a meta-analysis of experimental manipulation studies, Hillebrand (2005) found that increased light (light enhancement) increases grazer effect size, whereas grazer presence reduces light effects, which indicates that high light favors algal growth but increased algal biomass is easily offset by increased grazer densities. Similarly, Hill et al. (1995) found light-enhanced primary productivity was reflected by increased grazer densities instead of increased algal biomass.

3.2. Responses of algal species composition to nutrient enrichment

Algal species composition changes with elevated nutrient concentrations (Stevenson 1996, Pan et al. 1996, Stevenson and Smol 2001). Because of their small scale, periphytic algae composition receives less public attention, while problematic macroalgae (e.g., Cladophora) and cyanobacteria receive more. Under most circumstances, a diatom dominated algal community represents healthy, non-enriched stream water quality, while a predominance of filamentous algae may indicate problems with nutrient enrichment. Since algae are often the problem associated with enrichment, a change of taxonomic composition in a stream can show whether nuisance algae are present and can indicate long or short-term changes in point and nonpoint source pollution (Lowe and Pan 1996) that cannot be detected by a one-time sampling of water chemistry. Thus, algal species composition could be considered an important indicator of nutrient pollution.

Diatom species composition is increasingly used as an indicator of environmental conditions, especially nutrient enrichment in streams (Stevenson 1999). European scientists initially used diatoms as indicators of organic pollution and developed tolerance values (e.g., Lange-Bertalot 1979) that were adapted in the United States (Lowe 1974, Bahls 1992, Stevenson and Bahls 1999, KDOW 2002). Recent studies have used a variety of algal attributes to develop biological indices (Hill et al. 2000, 2003, Fore and Grafe 2002, Wang et al. 2005). Diatom indicators are most sensitive to nutrient enrichment, and several authors (van Dam et al. 1994, Kelly and Whitton 1995, Kelly et al. 1998, Winter and Duthie 2000) have reported this sensitivity. A variety of studies have also developed diatom autoecological preferences for nutrients using weighted average regression techniques like those used in paleoecology (Pan and Stevenson 1996, Potapova et al. 2004, Potapova and Charles 2005).

3.3 Responses of macrophytes to nutrient enrichment

The definition of macrophytes varies. According to Wetzel (1975), macrophytes represent a taxonomically diverse group of aquatic plants including vascular plants, mosses, ferns, and macroalgae. However, people generally exclude macroalgae from this group. Most macrophytes (i.e., emergent, floating-leaved, and submerged groups) are rooted in the sediments so they can persist in current flow and absorb nutrients from sediments. Rooted plants live in a more predictable light climate but may experience severe self-shading and generally grow more slowly than algae. The nutrient requirements of rooted macrophytes are lower than those of microalgae because of their low growth rates, high internal C:N:P ratios, and nutrient conserving mechanisms. Nutrient limitation is less important because the plants exploit rich nutrient pools in the sediment (Sandjensen and Borum 1991). One group of macrophytes (e.g., duckweeds), however, are unattached and rely entirely on nutrients from the water column. They are easily affected by current and wind, and are most frequently found in backwaters.

Nutrient effects on macrophytes are poorly studied (Chambers et al. 1999). Several factors have constrained this research. First, light is the most crucial factor regulating species composition and distribution of macrophytes in streams (Chambers and Kalff 1985). Light availability is easily affected by water clarity, riparian canopy cover, and water depth. Competition between phytoplankton and periphyton for nutrients and light may also reduce the direct effects of nutrient enrichment (Sandjensen and Borum 1991). Second, macrophytes mostly use nutrients from sediments. Any nutrient loadings in stream water have to be incorporated into the sediments before being available to the plants. However, nutrient supply can affect plant attributes.

Most studies of nutrient enrichment on plants focus on wastewater effluent. Nutrient enrichment in streams and rivers leads to increasing plant biomass (Chambers and Prepas 1994, Gucker et al. 2006), declines in plant richness (Thiebaut and Muller 1998, San-Jensen et al. 2000), and increases in plant tissue phosphorus (Thiebaut and Muller 2003). Rattray et al. (1991) found that the size and tissue phosphorus content of plants grown on eutrophic sediments was approximately twice that of those grown on oligotrophic sediments, indicating that nutrient supply strongly affects plant stoichiometry. Removal of aquatic plant biomass with its accumulated phosphorus has been proposed as a eutrophication control strategy (Thiebaut and Muller 2003). Reduction of nutrient (particularly N) input from municipal wastewater sources led to macrophyte biomass declines in the Bow River (Alberta) (Sosiak 2002). Other authors have (e.g., Schneider and Melzer 2003) developed plant based trophic indices (Trophic Index of Macrophytes) based on the concentrations of soluble reactive phosphorus in both the water column and the sediment pores of streams in Germany.

4. Indirect biological responses of streams to eutrophication: microbial cycling

In heterogeneous stream reaches, microbial biomass is patchily distributed and is controlled by a number of factors, including light, substrate carbon availability, temperature, nutrients, and current velocity. The main components of stream microbial biomass are bacteria and fungi. Fungi dominate large substrates such as leaves and wood, while bacteria dominate fine organic substrate such as sand (Findley et al. 2002). The organisms are important components of stream food webs and play a key role in carbon cycling.

4.1 Bacteria

Similar to algae, bacteria are also limited by nutrients in aquatic systems, especially in planktonic forms (Cole 1982). Bacteria can outcompete algae for nutrients because of their higher surface area to volume ratio (Fuhs et al. 1972, Currie and Kalff 1984a). Bacterioplankton have substantially higher phosphorus requirements, higher phosphorus contents, and higher net consumption of phosphorus than phytoplankton (Wetzel 2001). Therefore, bacterioplankton can outcompete algae for phosphorus under a wide range of phosphorus supply rates.

Stream periphytic biofilms are mainly composed of algae and bacteria. Bacteria can either inhibit algae by outcompeting it when nutrients are limited (Currie and Kalf 1984b, Grover 2000, Biddanda 2001), or they may interact positively with algae by using its photosynthetic products and decomposing dead plant and algal biomass and recycling nutrients. A number of studies (Geesey et al. 1978, Hudson et al. 1992, Hepinstall and Fuller 1994, Rier and Stevenson 2001, Carr et al. 2005) have demonstrated positive interactions between algae and bacteria in periphyton, though caution should be used when interpreting these correlations due to the complexity of the environment (Findlay et al. 1993, Bott 1996). Some (Rier and Stevenson 2002) speculate the positive correlation between bacteria and algae in biofilms to be a result of the co-dependence between the two for space.

Nutrient enrichment tends to increase both algal and bacteria biomass (Carr et al. 2005). Sobczak (1996) found that interactions between bacteria and algae are weakened in the presence of a labile source of allochthonous DOC, under extreme light limitation, or under extremely oligotrophic conditions where algae are severely nutrient limited. Similarly, Rier & Stevenson (2001) found only a positive statistical relationship between algae and bacteria in streams when streams with periphyton chlorophyll *a* values greater than 5 μg cm^{-2} were included in the analysis. Nutrient addition generally increases the coupling of algae and bacteria biomass (Rier and Stevenson, 2001, 2002, Carr et al. 2005).

Changes in bacteria taxonomic composition and abundance in response to nutrient enrichment are less studied (see Findley et al. 2003, Olapade and Leff 2005). Changes in taxonomic abundance are highly dependent on seasons, level of DOM, and interactions between nutrients and DOM. However, certain bacteria are sensitive to nutrient

enrichment and excessive growth on sensitive insects has been proposed as an indicator of nutrient enrichment (Lemly 1998, 2000).

4.2 Fungi

Similar to bacteria, fungi also play an important role in detrital decomposition in streams. Fungal communities in many streams are also limited by nutrients (Grattan and Suberkropp 2001, Tank and Dodds 2003). This limitation can be released by nutrient additions that lead to significantly higher fungal biomass. Experimental enrichment increased aquatic hyphomycete conidia in the water of a treated stream by 4 to 7 times more than the controlled streams (Gulis and Suberkropp 2004). Other effects observed in the same study included increased number of fungal species detected on each sampling date, and changes in dominance patterns and relative abundances of individual species. Nutrient pollution may also change aquatic hyphomycete diversity and sporulation (Pascoal et al. 2005b).

Bacteria and fungi also compete with each other for nutrients (Gulis and Suberkropp 2003b). Gulis and Suberkropp (2003b) found that fungi inhibit bacterial growth and reduce bacterial biomass by 2-fold at low nutrient concentrations, suggesting that nutrient availability may modify microbial interactions. Fungi seem to be a superior competitor than bacteria on leaves. Fungal biomass can be one or two order of magnitudes higher than bacterial biomass in polluted streams (Gulis and Suberkropp 2003a, Pascoal et al. 2005a).

5. Indirect biological responses of streams to eutrophication: herbivores

Nutrient enrichment accelerates autotrophic production and algal biomass in streams, and consequently changes ecosystem structure at other trophic levels. Long-term (16 years) stream fertilization (P addition) in an arctic stream ecosystem resulted in a dramatic change in the community structure with positive response to fertilization at all trophic levels (e.g., increases in epilithic algal stocks, insect densities, and fish growth). Both top-down and bottom-up control of different trophic levels have been observed in stream ecosystems (Shurin et al. 2002) and has led to development of “trophic cascade” models for streams (Power 1992).

5.1 Invertebrates

A large number of observations and experimental manipulations have shown that invertebrates are food limited in streams (Hill 1992, Rosemond et al. 1993, Biggs and Lowe 1994, Lamerti 1996, Biggs et al. 2000). These studies examined the effect of algal biomass on growth of invertebrates and found that invertebrate abundance could be strongly stimulated by increasing algal availability. Nutrient enrichment, in many cases, did not increase the total biomass of algae in streams; rather the energy was converted into increased invertebrate density through herbivory (Hill et al. 1995).

Changes in macroinvertebrate composition with nutrient enrichment are more complicated than changes of abundance. Mayflies, a group of invertebrates that are considered sensitive to environmental pollutants, show highest relative abundance when algal biomass is at intermediate levels (Miltner and Rankin 1998). The abundance of scrapers, a functional group that is closely related to grazers, is highest when nutrient levels are elevated, indicating positive effects from increased algal availability (Miltner and Rankin 1998). Similarly, scrapers (e.g., *Ancylus fluviatilis*) and detritivores (e.g., Oligochaeta, Lumbriculidae) have shown significant increases in density or biomass on certain substrata with enrichment even while total macroinvertebrate density or biomass did not (Sabater et al. 2005). Benthic invertebrate composition has also been shown to shift from chironomid-amphipod to an oligochaete-gastropod dominated assemblage in response to decreases in DIN and changes in benthic algal abundance and sediment organic carbon concentrations (Chambers et al. 2006).

Enrichment may also alter benthic habitat for macroinvertebrates. In addition to food sources for invertebrates, benthic algae, especially macroalgae, are important habitat for macroinvertebrates (Dudley et al. 1986). Some algal species or growth forms are grazer-resistant (e.g., Oedogonium spp.) and are good habitat for many invertebrates (Steinman et al. 1992). For example, long-term additions of P in an arctic stream resulted in an increase in moss density. The increase in physical habitat associated with this shift was responsible for changes in the macroinvertebrate community (Lee and Hershey 2000).

5.2 Fish

Fish may benefit from increases in food availability when nutrient additions increase primary and secondary production. Enrichment of oligotrophic streams and rivers may result in increased algal biomass, increased benthic invertebrates, and fishes. Long-term enrichment studies in Vancouver Island, British Columbia (e.g., Slaney and Ward 1993, Slaney and Ashley 1997) reported increased fish size up to 2 times. Similar studies (Deegan and Peterson 1992, Slavik 2004) also observed increased fish growth rates in long-term fertilization studies in rivers. However, it is difficult to assess how much of the primary production from nutrient enrichment flows through to fish, and the effect of nutrient addition on fish is largely unpredictable. van Dam et al. (2002) estimate that periphyton ingestion by many herbivorous and omnivorous fish ranges from 0.24 to 112 mg (^{3}dmg fish)$^{-1}$ d^{-1} in a fishpond.

One of the consequences of nutrient enrichment may be loss of sensitive fish taxa and increases in tolerant taxa. The strong correlation between fish metrics and nutrient pollution (Miltner and Rankin 1998) indicates that nutrient enrichment has contributed to changes in the structure of fish assemblages. While nutrient enrichment could potentially benefit fish production in the short term, the ecological consequence of nutrient addition could have severe impacts on stream ecosystems (Stockner et al. 2000). The obvious impact at high nutrient loads is the reduction in DO, which would exclude many sensitive taxa. In addition, excess algal growth would eliminate important feeding and respiration habitat, further reducing survivorship. While it is evident that some nutrient subsidy

benefits the growth of select species, the overall impact is negative, especially at stressful nutrient levels.

5.3 Food web structure

Carpenter et al. (1985) adapted the "cascade of effects" concept developed from top-down marine food webs to lakes and described it as "cascading trophic interactions". They posited that each trophic level is controlled by both predators (top-down control) and resources (bottom-up control). Changes at one trophic level would alter material cycling and other trophic levels in the food web (trophic cascading). Several authors have discussed trophic cascading in streams (e.g., Lamberti 1996, Biggs 2000).

Long-term fertilization studies have demonstrated the cascading effect of nutrient enrichment at several trophic levels. Huntsman (1948) first recognized that fertilizers stimulate downstream algal growth, and lead to increased insect and fish densities. Since then, more quantitative studies (Peterson et al. 1993, Slaney and Ashley 1998) have shown that nutrient additions increase algal biomass at least at the beginning of the enrichment. Later, top-down forces take effect to control primary consumers and consequently algal biomass. Generally, grazing has demonstrated a larger effect than resource limitation in influencing algal biomass and composition (Steiman 1996, Lamberti 1996, Flecker et al. 2002). However, interactions among different trophic levels could be regulated by many factors, which sometimes lead to unexpected responses to nutrient additions at higher trophic levels (Deegan et al. 1997). In either case, plant-herbivore interactions are considered central to food web structure and energy flow in aquatic ecosystems (Lamberti 1996).

While nutrient additions affect higher trophic levels, predators also play an important role in influencing nutrient demand and nutrient supply. Flecker et al. (2002) examined nutrient limitation in the presence and absence of fishes and found that the response to nitrogen enrichment is significantly greater on substrates accessible to natural fish assemblages compared to substrates where grazing fishes are excluded. Many experiments (e.g., Biggs 2000) demonstrate simultaneous and interactive effects of top-down and bottom-up factors in limiting primary producers in streams.

6. Responses of ecosystem function to nutrient enrichment

6.1 Primary production and respiration

Production- Three empirical models have been developed to simulate algal nutrient uptake kinetics: the Michaelis-Menten model, the Monod model, and the Droop model (Borchardt 1996). All three models are similar to each other in form. The Michaelis-Menten model emphasizes nutrient uptake kinetics (Dugdale 1967). The Monod model examines algal specific growth rate along external nutrient concentration (Monod 1950), while the Droop model takes into account intracellular concentration of the limiting nutrient (Droop 1968). These models postulate that algae accrual follows a logistic growth rate and reaches maximum saturation at some concentration.

The Monod equation has been applied in a number of studies to investigate the nutrient-algal production relationship (Rier and Stevenson 2006). The equation is as follows:
$\mu=\mu_{max} * S/(K_{\mu}+S)$,
where μ is the specific growth rate, μmax is the maximum specific growth rate, K_{μ} is the half-saturation constant for growth for the limiting nutrient, and S is the external nutrient concentration (μmole/L).

Experimental approaches have confirmed that periphytic algal growth increases along nutrient gradients (Horner et al. 1983, Bothwell 1989, Walton et al. 1995, Rier and Stevenson 2006, Stevenson et al. 2006). However, while algal biomass accumulation is a reflection of algal production, maximum algal production does not lead to maximum areal biomass. Bothwell (1989) examined both cellular growth rates and maximum areal biomass, and demonstrated that the amount of P needed to saturate cellular growth rates during early stages of colonization was two orders of magnitude lower than concentrations needed to produce maximum areal biomass. Rier and Stevenson (2006) manipulated nutrient concentrations to examine the effect on the growth rate of periphyton in artificial streams, and found that saturating concentrations for algal growth rates were 3 to 5 times lower than concentrations needed to produce maximum biomass. These growth patterns can be fit in to modified Monod models, demonstrating the exponential growth of algal biomass along nutrient gradients.

Correlation analysis between nutrient concentrations and algal biomass and algal productivity in streams also indicate that nutrient enrichment enhances algal production (Dodds et al. 2002, see review by Dodds 2006). Dodds (2006) plotted algal production against biomass using Bott et al.'s (1985) compiled data from the literature and found a positive correlation between algal primary production and algal biomass in streams, though only 24% of the total variance was explained. Field observations are consistent with experimental manipulations of nutrient enrichment and algal production. In addition to algal production, macrophyte production is also stimulated by nutrient enrichment (Gucher and Brauns 2006).

Respiration- It is generally accepted that higher production leads to higher respiration. Dodds (2006) demonstrated that production and respiration by algae in streams are strongly correlated with each other with a correlation coefficient close to 0.90. Similarly, nutrient enrichment increases bacterial and fungal production, which in turn increases aerobic respiration from these decomposers. Thus, the total respiration in enriched streams is higher than production. High respiration:production ratios lead to high biological oxygen demand (BOD) in downstream water (in nutrient enriched streams). Thus, nutrient enrichment will generally lead to elevated gross primary production and whole-stream community respiration, and decreased stream DO concentrations (Chambers and Prepas 1994, Gucker and Brauns 2006).

A good example is blackwater streams in the eastern United States. Studies of nutrient loading on phytoplankton, bacterioplankton, and respiration in blackwater streams indicate that changes in nutrient loading stimulate two different biological pathways

(photosynthetic and heterotrophic activity) (Mallin et al. 2004). Nitrogen additions increase chlorophyll *a* production and significantly stimulate BOD. Combined organic-inorganic phosphorus additions significantly stimulate bacterial abundance, ATP, and BOD on most occasions. In blackwater streams, nitrogen inputs stimulate phytoplankton growth, which in turn dies and decomposes in deeper, higher order streams, becoming a source of BOD and lowering DO. Phosphorus inputs directly stimulate bacterial growth, increase BOD, and lower stream DO concentrations. In some circumstances, hypoxia is expected (Mallin et al. 2006).

6.2 Secondary production and predators

Generally, secondary producers (consumers) in streams are food limited, and their production and biomass are expected to increase in streams with higher primary production due to N and P enrichment. Elwood et al. (1981) found that the initial response to stream nutrient enrichment in a Tennessee woodland stream was increased algal standing crop, which was then consumed by a large increase in grazer abundances. Continuous enrichment of P-limited streams on Vancouver Island, British Columbia led to substantial increases in secondary producers (Perrin et al. 1987, Slaney and Ward 1993). Benthic invertebrate biomass increased by from 2 to 7-fold and fish size by from 1.4 to 2-fold (Slaney and Ward 1993). Macroinvertebrates also ingest bacterial production (Fuller et al. 2004). Benthic invertebrate biomass increased 4.5-fold and fish (cutthroat trout) increased 6.3-fold in a stream enriched by carbon that elevated bacterial biomass (Warren et al. 1964). In another study, enrichment did not cause a general increase in macroinvertebrate density or biomass, but altered assemblage composition in the enriched reach (e.g., Sabater et al. 2005).

Secondary production clearly responds to enrichment and the response may be more clear in oligotrophic than eutrophic streams. Fish, especially herbivores, can obviously benefit from increasing biomass of primary producers. Carnivorous or omnivorous vertebrates also prosper from the increased biomass of primary consumers (e.g., Deegan and Peterson 1992). A recent study (Johnson and Wallace 2005, Johnson et al. 2006) on the growth of salamander larvae, top predators in southern Appalachian streams, indicated that nutrient enrichment stimulates salamander larval production by increasing detrital quality and quantity. Although some enrichment may benefit fish production, a transition region in enrichment from beneficial to detrimental effects has not been defined to the extent that it has for lakes and reservoirs (Welch 1992). It probably exists for different physical types of streams and rivers. Two recent stream studies have provided independent estimates of target nutrient concentrations that should be maintained in order to ensure acceptable water quality needed for fish growth if fish growth is the primary concern (Stockner et al. 2001, Compton et al. 2005).

6.3 Decomposition rates

Microbial decomposition rates are affected by a number of environmental factors, i.e., current velocity, feeding by detritivores, sedimentation, and nutrient enrichment. Microbial decomposition rates are strongly affected by fungal and bacterial productivity and their relative contributions to total microbial biomass (Hieber and Gessner 2002). Findlay et al. (2002) found that bacterial abundance was higher on fine particles, while fungal biomass was significantly greater on larger particle size classes, i.e., on leaves and wood. The relative contribution of reach-scale fungal biomass ranged from 10 to 90% of microbial biomass in this study depending on the quantity of leaves and small wood in the streams. In streams with abundant leaves, fungi dominate the total microbial biomass (98.4 to 99.8%) and cumulative production (97.3 and 96.5%) (Gulis and Suberkropp 2003a). Nutrient enrichment generally increases bacterial and fungal productivity and biomass, but does not change the roles of each assemblage. Gulis and Suberkropp (2003a) found that the fungal yield coefficient exceeded that of bacteria by a factor of 36 and 27 in low- and high-nutrient treatments, respectively.

Nutrient enrichment significantly increases decomposition rate, microbial respiration, fungal and bacterial biomass, and the sporulation rate of aquatic hyphomycetes associated with decomposing leaf material (Gulis and Suberkropp 2003c). Comparisons between one reference site and two downstream polluted sites with high nutrient concentrations in the Ave River, Portugal indicated that bacterial production was greater at the two polluted sites than at the reference sites, while highest fungal biomass and production corresponded to the fastest leaf breakdown. Other studies also support the contribution of fungi and bacteria to leaf litter breakdown and the enrichment of microbial activity by nutrients (Suberkropp 1995, Weyers and Suberkropp 1996, Royer and Minshall 2001, Gulis and Suberkropp 2003a, b, c).

In addition to microbial biomass in streams, decomposition rates can also be regulated by other factors, especially the presence of detritivores. Sponseller and Benfield (2001) found that shredder presence and abundance was critical to leaf breakdown rate in Appalachian headwater streams. Increased sedimentation from agricultural input in some streams may limit the distribution of shredders and thus influence leaf breakdown in these streams. Similarly, Pascoal et al. (2005a) attributed low leaf breakdown rates to low shredder densities in a polluted river.

6.4 Other functional responses

Carbon cycling-Carbon is not considered limiting for periphyton growth in streams because CO_2 can be dissolved in water and provides sufficient inorganic carbon for use in algal production. However, bacteria are limited by carbon in many streams with relatively low DOC inputs. Increases in primary production due to nutrient enrichment increase DOC and the coupling of periphyton and bacteria since bacteria will rely more heavily on organic carbon sources produced by algae and other plants. Microbial decomposition further increases dissolved organic carbon sources. Thus, nutrient enrichment will enhance carbon cycling, especially in slow moving streams.

Nitrogen fixation- Nitrogen fixation by algae and bacteria may be affected by nutrient additions and changes in N:P ratios. Nitrogen fixation by cyanobacteria and other microbes is favored by slow moving or standing water, high temperatures, low DIN and high DON (Dodds 1995). Nitrogen fixation can provide a substantial amount of N to a stream depleted of nitrogen (Grimm and Petrone 1997). Although not much information is available about nutrient enrichment on N fixation in streams, studies in lakes indicate that decreases in the N:P ratio will generally lead to increases in N fixation rates by cyanobacteria (Smith 1990, Hendzel et al. 1994). Grimm and Petrone (1997) found that biomass specific N2 fixation was positively correlated with temperature and light, and negatively correlated with dissolved inorganic nitrogen.

Alkaline phosphatase activity –Aquatic primary producers, especially algae, can excrete an enzyme called alkaline phosphatase in response to P deficiency in the water. Various studies (e.g., Peck et al. 2006) have used the concentration of alkaline phosphatase in the water column and in periphyton as an indicator of P limitation. It is considered the most precise indicator of P limitation over epilithic N:P ratios and algal growth on nutrient-diffusing substrates (Bowman et al. 2005). It is speculated that phosphatase levels of approximately 0.003 mmol/mg chl per hour indicate moderate P limitation, whereas phosphatase levels above 0.005 mmol/mg chl per hour indicate severe P limitation (Steinman and Mulholland 1996).

Silica limitation- Silica limitation is also reported in some streams. Silica is an important component of diatom frustules. A study in eutrophic Lake Okeechobee, Florida indicated that along with N limitation, silica also limited microalgal assemblages dominated by diatoms (Zimba 1998). In streams dominated by diatoms, silica concentrations tended to decrease downstream, implying that diatoms absorb silica from the water (Wall et al. 1998). In some cases, nutrient enrichment generally increases the production rate of green filamentous algae while diatom production decreases. It is possible that diatoms are limited by silica concentrations in these streams. The extent of this limitation is poorly understood in streams.

6.5. Nutrient enrichment impacts on designated uses

Nutrient enrichment may directly lead to excessive increases in algae and other primary producers. The indirect effects of enrichment are to increase stream consumer production, change food web structures, and consequently alter ecosystem function. The deleterious impacts of these structural and functional changes may impair aquatic life uses, limit drinking water resources, and degrade recreational and aesthetic uses of waters. Various impacts of nutrient enrichment in streams have been summarized in Table 2 below.

Table 2. Effect of nutrient enrichment on designated uses of streams.

Excessive nutrient levels allow excessive increases in algae and other primary producers, which, in turn, prevent streams from meeting their designated uses. The adverse effects of either high nutrient levels or the nuisance growth of primary producers include, for example:
• **Impairment of aquatic life uses** -- Diel fluctuation of oxygen concentrations and pH values in streams may negatively impact aquatic life (fish and invertebrates). -- Ammonia toxicity (e.g., at a high level > 1 mg/L) may be derived from high nitrogen concentrations. -- Overgrowth of many algae can lead to algal blooms, several toxins from which have been found to kill fish and other aquatic life. -- Indirect effect of excess growth on physical habitat availability. -- Enrichment may lead to loss of diversity and native taxa, changes in biological community structures (algae, aquatic plant, invertebrate, and fish), and eventually loss of ecosystem function.
• **Negative impact on water resources** -- High nitrate concentration (>10 mg/L) is toxic in drinking water. -- 2-methylisoborneol and geosmin are two chemicals produced by cyanobacteria that can cause taste and odor problems in drinking water. -- Diatoms and filamentous algae can clog intake screens and filters in water treatment plants. -- Decay of algae may lead to taste and odor problems of drinking water. -- Potentially carcinogenic disinfection by-products (trihalomethanes, THMs) may form during treatment of drinking water from eutrophic waters.
• **Degradation of aesthetic and recreational uses** -- Many algae can form large clogs of mats either floating (*Lyngbya, Oscillatoria, Hydrodictyon, Spirogyra*) or attached (e.g., *Cladophora* and *Ulothrix*) to substrata, which are unappealing to swimming, fishing, and boating. -- Large odorous algal masses die off and decay after the growing season, and their presence inhibits recreation and human health. -- Cyanobacteria, such as *Oscillatoria* or *Lyngbya wollei*, can produce chemical compounds that irritate swimmers. -- Fish grown in waters with algal-derived chemicals can suffer impacts on flavor. -- Skin rashes, nasal irritation, or other health effects may result from skin contact with algal toxins. -- Slippery streams are a threat to swimmers. -- *Cladophora* abundance may slow water flow in canals and inhibit navigability.

7. Control of eutrophication: best management practices

Nutrient enrichment poses serious threats to stream ecosystems. Managing nutrient loading into streams will reduce not only the magnitude of maximum algal biomass, but also the frequency and duration of benthic algal problems in streams (Biggs 2000). To better protect and restore streams, control of both point and nonpoint sources of nutrient loadings into streams is essential. Currently, many states have proposed plans to develop nutrient criteria to control nutrient loading into streams. Control of point sources, such as treated wastewater, can be improved with new technology (Arheimer et al. 2004). Still a persistent problem for implementation of criteria will be control of nonpoint sources. It will require innovative technologies and better understanding of stream ecosystems to decrease nutrient loadings from nonpoint sources into streams. Best management practices should be implemented including riparian buffer and wetland protection, and smart use of fertilizers in agricultural and silviculture. New technologies are contributing to some improvement in nutrient pollution from nonpoint sources (Arheimer et al. 2004). More cost-effective practices should be developed to better fulfill this goal.

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The diversity of harmful algal blooms: a challenge for science and management

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Abstract

A broad spectrum of events come under the category of harmful algal blooms (HABs), the common denominator being a negative impact on human activities. Harmful algal blooms involve a wide diversity of organisms, bloom dynamics, and mechanisms of impact. Here we review the effects of natural and man-induced environmental fluctuations on the frequency and apparent spreading of these phenomena. This article highlights the need for interdisciplinary research aimed at shedding light on basic mechanisms governing the occurrence and succession of microalgae in coastal seas. Information integrated from various discipline coupled with improved, sustained monitoring systems, will help predict and manage problems caused by HABs over a wide range of space and time scales. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

The need to exploit marine resources has increased with the growth of the world's population and with enhanced demographic pressure in coastal areas. Harmful phytoplankton events are a serious constraint to the sustainable development of coastal areas. This scenario calls for a co-ordinated scientific and management approach.

The term 'harmful algal blooms' (HABs) covers a heterogeneous set of events that share two characteristics: they are caused by microalgae and they have a negative impact on human activities. Despite these common features, HABs are very diverse in terms of causative organisms, dynamics of blooms and type of impact.

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Numerous publications focusing on harmful algal blooms testify to the increasing interest, both scientific and practical, in these events [1–6]. We refer the reader to these publications for comprehensive descriptions of the current status of knowledge in the field. Here, we review HABs highlighting their heterogeneity and the gaps in our knowledge that impede optimal management and mitigation strategies.

2. The diversity of causative organisms

The list of microalgal species that are potentially involved in HABs comprises about 80 toxic species and about 200 noxious species out of an approximate total of 4000 marine planktonic microalgae described thus far [7]. This list has increased remarkably in recent years because of new cases of harmful events, the development of scientific research in the field, and enhanced human interactions with the coastal zone. Before the first instance of amnesic shellfish poisoning (ASP) in 1987 [8], diatoms were not thought to be toxic, while to date about 10 species are known to produce the toxic compound domoic acid. The total number of potentially harmful species is deemed to be largely underestimated [7,9], given the relatively high number of species (3 or 4) added to the list yearly. This is hardly a surprising finding, considering that several thousand phytoplankton species are still undescribed [10].

It is not an easy task to associate a known species with a harmful phenomenon, as demonstrated by the case of *Protoceratium reticulatum* (Fig. 1). This is a widely

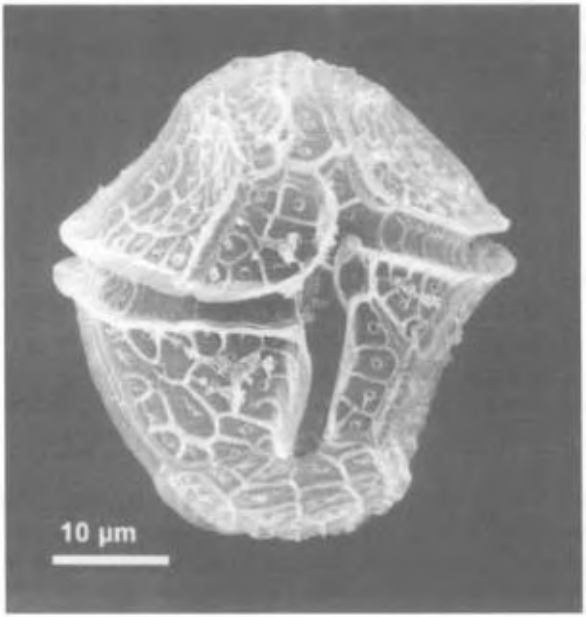

Fig. 1. *Protoceratium reticulatum*.

distributed species which in 1966 was associated with a mass white mussel death on the west coasts of South Africa [11]. At that time, the species was misidentified and subsequently redescribed as a new species, *Gonyaulax grindleyi* [12]. Similar events were subsequently observed in the same area, but the source of toxicity remained obscure. Only 20 yr later [13] was it demonstrated that *P. reticulatum* produces yessotoxin, a toxin that had been known for 10 yr.

Toxicity and other negative effects caused by harmful algae are not limited to a single algal class or to a few genera, but are distributed among several taxonomic groups. This is not surprising: in the terrestrial environment, weeds, pests and poisonous organisms belong to a wide variety of groups, from fungi to flowering plants. The variety of secondary terrestrial plant metabolites and of their ecological effects is amazing [14]. Similarly, the high taxonomical diversity of harmful algae results in a variety of toxins and relative mechanisms of action at different levels of the trophic chain. Ecological requirements and bloom dynamics also vary considerably from one species to another and, like the case of non-harmful species, even within a single species [15,16] different populations of the same species may be toxic or not, exhibit different growth rates under the same conditions and show different life strategies.

Another implication of the taxonomic diversity of HAB species is that the production of toxic substances or other offensive or disturbing features has been selected as an advantageous character more than once in the evolution of these microorganisms. However, the significance of these features in terms of increased fitness or competitive ability is not known.

From a practical viewpoint, the taxonomic diversity of HAB species complicates monitoring operations, which require a high degree of specialised expertise. The automatised detection of harmful species, e.g with specific molecular probes [17], seems an attractive approach that would increase monitoring efficiency; however, the high number of species to be targeted and their genetic diversity impede rapid progress in this field.

3. The diversity of negative effects

Broadly speaking, there are four categories of deleterious effects related to marine microalgae (Table 1):

(i) *Risks for human health*: Some algal toxins, different in structure and toxicity (Table 2), reach humans through specific vector organisms that accumulate these noxious substances in their gastrointestinal tract or body tissues. The most toxic algal species are recorded among dinoflagellates, but a number of diatoms and cyanobacteria also produce neurotoxic substances that can endanger human health (Fig. 2). These toxins induce a number of acute and chronic syndromes i.e. paralytic shellfish poisoning (PSP), diarrhoeic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP), and ciguatera fish poisoning (CFP), which cause a wide variety of neurological and/or gastroenteric symptoms and at times can be fatal. Toxins can accumulate in many species of bivalve molluscs

Table 1
Overview of the deleterious effects caused by harmful algae and harmful algal blooms

Effect	Examples of causative organisms	
Human health		
Paralytic shellfish poisoning (PSP)	Dinoflagellates	*Alexandrium* spp., *Pyrodinium bahamense* var. *compressum*, *Gymnodinium catenatum*
	Cyanobacteria	*Anabaena circinalis*
Diarrhetic shellfish poisoning (DSP)	Dinoflagellates	*Dinophysis* spp., *Prorocentrum* spp.
Neurotoxic shellfish poisoning (NSP)	Dinoflagellates	*Gymnodinium breve*
Amnesic shellfish poisoning (ASP)	Diatoms	*Pseudo-nitzschia* spp.
Ciguatera fish poisoning (CFP)	Dinoflagellates	*Gambierdiscus toxicus*
Allergies, respiratory problems and skin irritation	Dinoflagellates	*Gymnodinium breve*, *Pfiesteria piscicida*
	Cyanobacteria	*Nodularia spumigena*
Hepatotoxicity	Cyanobacteria	*Microcystis aeruginosa*, *Nodularia spumigena*
Natural and cultured marine resources		
Haemolytic, hepatotoxic, osmoregulatory effects and other unspecified toxicity	Dinoflagellates	*Gymnodinium* spp., *Cochlodinium polykrikoides*, *Pfiesteria piscicida*, *Gonyaulax* spp.
	Raphidophytes	*Heterosigma akashiwo*, *Fibrocapsa japonica*
	Prymnesiophytes	*Chrysochromulina* spp., *Prymnesium* spp.
	Pelagophytes	*Aureococcus anophagefferens*
	Cyanobacteria	*Microcystis aeruginosa*
Mechanical damage	Diatoms	*Chaetoceros* spp.
Gill clogging and necrosis	Prymnesiophytes	*Phaeocystis* spp.
	Diatoms	*Thalassiosira* spp.
Tourism and recreational activities		
Production of foams, mucilages, discolouration, repellent odours	Dinoflagellates	*Noctiluca scintillans*
	Prymnesiophytes	*Phaeocystis* spp.
	Diatoms	*Cylindrotheca closterium*
	Pelagophytes	Aureococcus anophagefferens
	Cyanobacteria	*Nodularia spumigena*
Marine ecosystem		
Hypoxia, anoxia	Dinoflagellatesi	*Noctiluca scintillans*, *Heterocapsa triquetra*
	Diatoms	*Skeletonema costatum*
	Prymnesiophytes	*Phaeocystis* spp.
Negative effects on feeding behaviour, reduction of water clarity	Pelagophytes	*Aureococcus anophagefferens*, *Aureoumbra lagunensis*
Toxicity to wild marine fauna	Dinoflagellates	*Gymnodinium breve*, *Alexandrium* spp.
	Diatoms	*Pseudo-nitzschia australis*

(e.g. scallops, mussels, cockles and oysters) as well as in some gastropods, crabs and fish; the characteristics of toxification and de-toxification vary remarkably from one vector to another [18]. In the case of CFP the toxin is accumulated by a number of tropical benthic fish that feed on macroalgae but also ingest harmful microalgae that colonise the macroalgal surfaces. Cyanobacteria are also hazardous for human health. These are generally fresh water organisms but may create problems in low

Table 2
Some algal toxins are extremely potent and may be several times more toxic than for example cobra venom, and more than one thousand times more toxic than cyanide. The table shows the relative toxicity to mice of some algal toxins compared with other biological toxins and with cyanide

Toxin	Source	Toxicity (fold)
Cyanide		1
Muscarin	Amanita muscaria, fungus	9
Okadaic acid	Algae, dinoflagellates (e.g. *Dinophysis* spp.)	50
Domoic acid	Algae, diatoms (*Pseudo-nitzschia* spp.)	80
Prymnesine	Algae, haptophytes (e.g. *Prymnesium parvum*)	350
Cobra toxin	Cobra snake	500
Saxitoxin	Algae, dinoflagellates (e.g. *Alexandrium* spp., *Pyrodinium bahamense*)	1 100
Ciguatoxin	Algae, dinoflagellates (*Gambierdiscus toxicus*)	22 000
Tetanus toxin	Bacterium (*Clostridium tetanii*)	100 000 000

salinity and estuarine coastal waters, where they can produce diverse neurotoxins, including PSP toxins, and such hepatotoxins as mycrocystins and nodularins. Finally, respiratory problems have been reported during blooms of *Gymnodinium breve* along the Gulf coasts of the USA.

About 2000 cases of human poisoning are reported each year [19]. However, this is probably an underestimation because many cases, particularly those involving gastrointestinal symptoms, are incorrectly diagnosed. Fatal episodes are estimated to exceed several hundred cases annually. In some areas affected by PSP, historical reports reveal that coastal populations were well aware of risks associated with eating sea-food in certain periods or under such conditions as a sea-water discoloration [20] or phosphorescence [21]. Although many affected areas are nowadays well controlled thanks to monitoring, the occurrence of toxic events in new areas and the lack of control in others pose a serious risk to human health and life. This is a potentially explosive situation given the pressing need to exploit coastal areas for food production.

(ii) *Impact on living marine resources*: Besides the cases cited above, in which harmful algae may indirectly impinge upon the exploitation of marine resources, several kinds of harmful algae may directly affect wild and cultivated fish (Fig. 3) or marine invertebrates that are valuable seafood. These events are occasionally caused by some of the species that endanger human health when accumulated in filter-feeding animals, such as *Gymnodinium breve* or *Alexandrium* spp. However, most mass mortalities of cultivated fish around the world are generally caused by such ichthyotoxic species as the raphidophytes *Heterosigma akashiwo* and *Chattonella marina*, a number of dinoflagellates and some prymnesiophytes. An alarming case in point is the bloom of *Chrysochromulina polylepis* in May–June 1988 in the Kattegat and Skagerrak area (North Sea) that caused the death of 900 tons of fish, including cod, salmon and trout. This was followed by other extensive prymnesiophyte blooms in 1991 and 1992 [9].

Harmful algae usually affect fish and other invertebrates by producing toxins, but mucilage-producing or spine-bearing algae may cause mechanical clogging or lesions

Fig. 2. The most common harmful microalgae that endanger human health.

of the gills. A reduction in oxygen and hydrogen sulphide production can also cause mass mortalities of huge proportions of commercially valuable species. In March 1994, approximately 60 tons of dead rock lobsters and 1500 tons of fish were stranded on the shores of St. Helena Bay (South Africa) consequent to the decay of a massive bloom of non-toxic algae [22]. Finally, brown tides produced by the picoplanktonic *Aureococcus anophagefferens* in bays of the midwestern Atlantic coasts of the USA led to starvation and recruitment failure in commercially valuable bay scallop populations [23].

Fig. 3. Mass mortality of flat fish in a Korean fish–farm caused by the algal species *Cochlodinium polykrikoides*. Photo by Mr. San Geun Lee, National Fisheries Research and Development Agency of Korea.

(iii) *Impact on tourism and on the recreational use of coastal areas*: A requisite for the exploitation of tourism and of the recreational resources in coastal areas is a high quality of the environment, including unaltered seawater colour, transparency, smell, etc. Algal blooms may cause intense discolorations of seawater, which earned them the name of 'red-tides', but in fact the discolorations can vary from golden brown to emerald green, from reddish to whitish (Fig. 4).

Green–brown-coloured seawater caused by the non-toxic dinoflagellate *Alexandrium taylori* is recurrent along the Mediterranean coasts of Costa Brava since 1982, in coincidence with the annual peak tourists season [24]. Repellent mucilages which are produced by several diatom species have often been reported from the Adriatic Sea [25,26], whereas thick layers of malodorous foams caused by the prymnesiophyte *Phaeocystis globosa* may accumulate along the North Sea shores [27]. Allergic reactions and even more serious disorders caused by cyanobacteria blooms are also a constraint for the recreational use of coastal areas.

(iv) *Damage to the marine ecosystem*: A number of HABs affect organisms that may have no commercial value but nonetheless are functional components of the marine ecosystem. The degradation of high biomass blooms can exhaust oxygen supplies, thus killing not only commercially important species, but also other plants

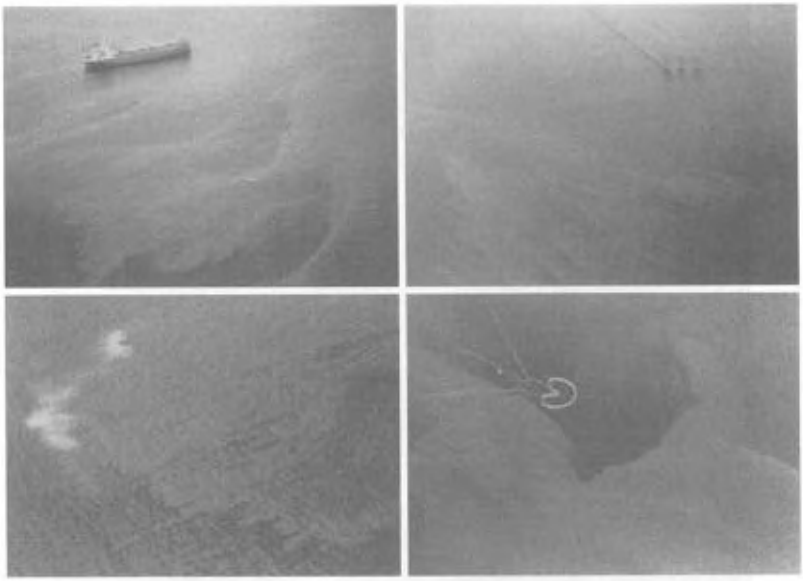

Fig. 4. Sea water discoloration caused by algal blooms in the Gulf of Thailand. Photo by Dr. Thaithaworn Lirdwitayaprasit, Department of Fisheries of Thailand.

and animals that are unable to leave the anoxic area. The co-occurrence of several conditions is required to generate these situations, i.e. non-limiting light and nutrient supplies and local physiographic and oceanographic conditions that entrap the bloom, thereby amplifying the effects of biomass degradation. Generally, anoxic events tend to recur more or less regularly at the same sites, but increased nutrient availability and reduced advection and mixing may cause these events to spread to other different sites.

Anoxia is not the only mechanism whereby HABs exert a deleterious effect on different components of the coastal ecosystem. The aforementioned brown tides of *Aureococcus anophagefferens* caused starvation and recruitment failure in a number of suspension-feeders, including bay scallops. They also reduced grazing by microzooplankton and decimated eelgrass beds through severe light attenuation [23]. In other cases, specific toxins seem to affect a single population of marine mammals, fish, birds, or other organisms. In 1987, 14 humpback whales died in Cape Cod Bay after eating Atlantic mackerel containing saxitoxin [28]. *Gymnodinium breve* has been frequently associated with mortalities of sea turtles, birds, dolphins and manatees in Florida waters [29]. In May 1998, the diatom *Pseudo-nitzschia australis* caused the death of over 400 California sea lions [30]. During this outbreak the neurotoxic domoic acid was detected in sea lion body fluids and in planktivorous fish. Taken together, these findings clearly show that the pathways of the toxins through the food web are diverse and may involve animals other than molluscs as vectors.

4. The diversity of bloom dynamics

The taxonomic diversity of HAB species suggests that each species is adapted to some set of preferred environmental conditions or, in ecological terms, to a defined niche. Conceivably, once the ecological requirements for each species are known, it would be easy to predict its occurrence [4]. One might also predict that several harmful species share a similar niche, thus leading to the identification of a few, basic, unifying bloom mechanisms and, consequently, to the construction of bloom models.

Unfortunately, the real world is more complicated. The ecophysiological requirements of most harmful species are poorly known, and laboratory studies are required to learn more about this field. However, results from laboratory studies are not sufficient to predict the succession of phytoplankton species and blooms of specific harmful organisms in the sea. Indeed, the net growth performance of a species is affected by complex interactions with other organisms, which are scarcely reproducible in laboratory experiments. These include negative interactions, such as grazing, competition, and viral infections, and a positive feedback from predator's excretion, bacterial nutrient regeneration and viral lysis. In fact, it is becoming increasingly evident that phytoplankton life strategies and their interactions with the surrounding environment may reach a degree of complexity unexpected for unicellular organisms. As a result, the capability of a species to cope with environmental conditions apparently not matching its optimal ranges is notably expanded and its occurrence is more difficult to predict.

Numerous phytoplankton species have complex life cycles, i.e. alternation of stages that differ in terms of physiology, motility, resistance to adverse conditions and life styles. Non motile, benthic resting stages (Fig. 5) are widely distributed among HAB dinoflagellates and raphidophytes, and may play a significant role in bloom initiation by inoculating seed populations when conditions are favourable. At the end of a bloom, cyst formation may represent a mechanism whereby organisms are rapidly removed from the water column so as to prepare the 'seed bank' to inoculate the next bloom [31]. Within this already complex framework, it appears that encystment rates, dormancy length, and cyst germination triggers can be species-specific and at times strain-specific. The transitions between different life stages of a species are presumably controlled by the interplay of endogenous factors with the physical and chemical environment, but the role and relative importance of these factors for different species are poorly known [32].

Regarding common mechanisms of bloom dynamics, one might expect the bloom dynamics of a cyst-forming species to differ significantly from that of an ASP-producing pennate diatom, a foam-producing *Phaeocystis* or a benthic dinoflagellate. Less obvious is the notable diversity that may exist among species with apparently homogeneous life strategies, such as cyst-forming dinoflagellates, and even within single species. *Heterosigma akashiwo*, a widely distributed fish killer, may bloom in eutrophic waters or at moderate nutrient levels, in both stratified and mixed waters, in high-salinity waters and in areas influenced by river runoff, in areas with 'natural' trophic chains as well as in places where aquaculture has altered the

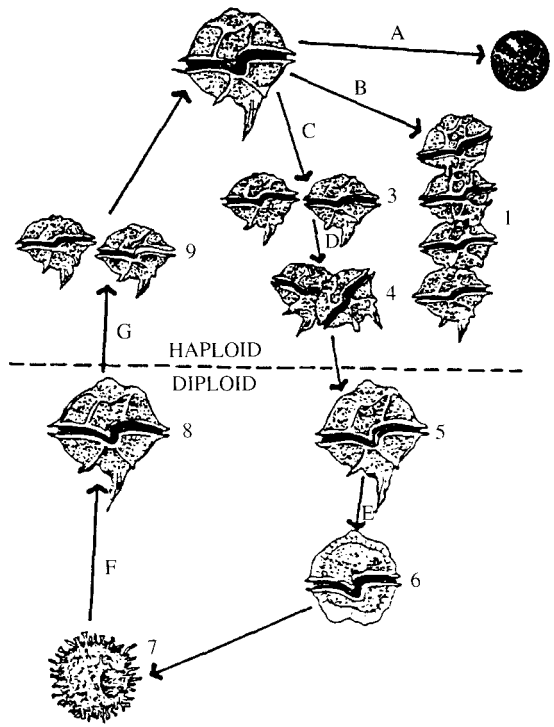

Fig. 5. The life cycle of *P. bahamense* (from Usup & Azanza [68]): (1). Vegetative cells. (2). Pellicle cyst. (3). Gametes. (4). Fusing gametes. (5). Planozygote. (6). Hypnozygote. (7). Resting cyst. (8). Planomeiocyte. (9). Products of meiosis: (A). Ecdysis. (B). Binary fission. (C). Gamete formation. (D). Gamete fusion. (E). Cyst formation. (F). Germination. (G). Meiosis.

trophodynamic balance [33]. Clearly, different ecotypes or cryptospecies must be involved in generating blooms in such diverse environments.

Gymnodinium catenatum (Fig. 6), a naked dinoflagellate responsible for PSP, is another case of a species blooming under different hydrographic conditions. In Tasmanian waters, blooms develop in estuarine waters possibly after inoculation from the extensive cyst beds present on the bottom of these areas [34]. In the Rias Bajas of Galicia, blooms have been related to the relaxation of upwelling events in the coastal North Atlantic, and to the advection of seed populations from offshore waters [35]. Not only the areas affected, but also the bloom mechanisms appear to be different, i.e. very strong biological regulation of the population through cyst germination in one case, and hydrographic control in the other.

These examples clearly demonstrate that the dynamics of harmful algal blooms cannot be elucidated by research on one or a few organisms. The diversity of harmful species at interspecific and intraspecific level is high enough to require a case focused, analytical study, which nonetheless may reveal common features, if any, for mechanisms of bloom initiation, development and termination. The examples

Fig. 6. *Gymnodinium catenatum*.

provided also indicate that it is difficult to define one kind of coastal area which is more prone to HABs, even when a single species is considered. This contrasts with the widespread idea that HABs prefer sheltered areas characterized by nutrient enrichment, possibly of anthropogenic origin, offering more or less prolonged periods of stratification. Indeed, HABs may occur in a variety of areas and hydrographic conditions, including upwelling regions, pristine coastal waters with moderate nutrient levels, and coastal areas affected by shelf-scale circulation.

5. HABs and biomass

The concept of high abundance is implicit in the term 'bloom', however it has a dynamic and relative meaning: a bloom is a 'significant population increase' [36], which leads to a peak. The magnitude of this peak is specific for each species and may vary considerably in space and time, being dependent on environmental conditions. In this sense, a bloom does not imply a high biomass. Nonetheless, the impact of HABs depends on the concentration of the harmful species, even the most toxic species must have a minimum cell concentration to exert the harmful effect. In

fact, most harmful species are probably ubiquitous, and they become hazardous only when their concentration exceeds a certain threshold for a sufficiently long period.

At barely detectable concentrations (10^2–10^3 cells l^{-1}), some harmful species can have dramatic effects; this is the case of highly toxic species like the PSP producers *Pyrodinium bahamense* var. *compressum* and *Alexandrium tamarense*, which however may also form much denser blooms (10^6–10^7 cells l^{-1}). The DSP-producer species of the genus *Dinophysis* (Fig. 7) rarely reach concentrations higher than 10^4–10^5 cells l^{-1}, but are nevertheless dangerous at much lower concentrations. Concentrations as low as 5 cells ml^{-1} are also sufficient for some diatoms endowed with long siliceous spines, such as *Chaetoceros concavicornis* and *C. convolutus* [37], to cause fish mortalities by producing lesions in the gill tissues. Because of the difficulty in detecting low concentrations of planktonic algae, it is not always possible to pre-alert to the harmful events, especially in the case of these extremely noxious species.

On the diametrically opposed end of a scale of danger (Fig. 8) are species that are noxious only when they reach very high concentrations, as in the case of *Phaeocystis* (Fig. 9). This is a normal component of plankton blooms in temperate areas, which has recently been shown to produce a toxin that has anaesthetic properties [38]. At high concentrations *Phaeocystis* may affect fisheries [39], confer a bad taste to fish (blackberry feeding) [40], deviate the herring migration patterns [41] and in some cases produce unsightly slime and foams [27]. Also, very dense blooms of non-toxic

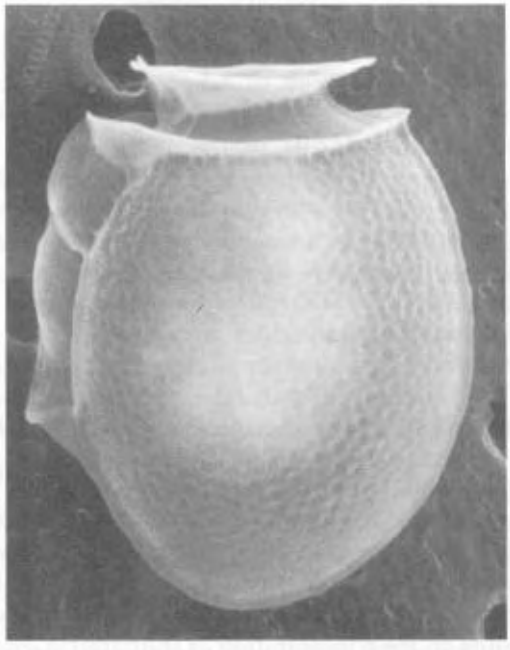

Fig. 7. *Dinophysis acuminata*.

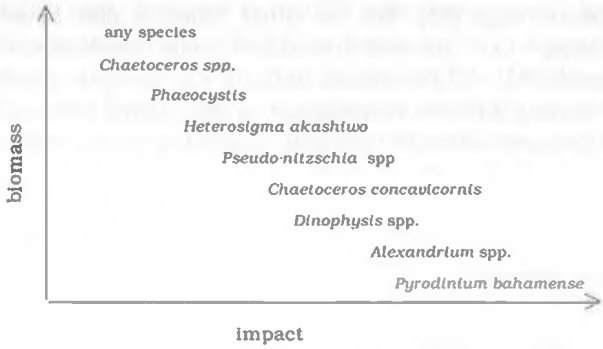

Fig. 8. Schematic representation of the impact/biomass relationships for selected HAB species. Scales for both axes are arbitrary and non-linear.

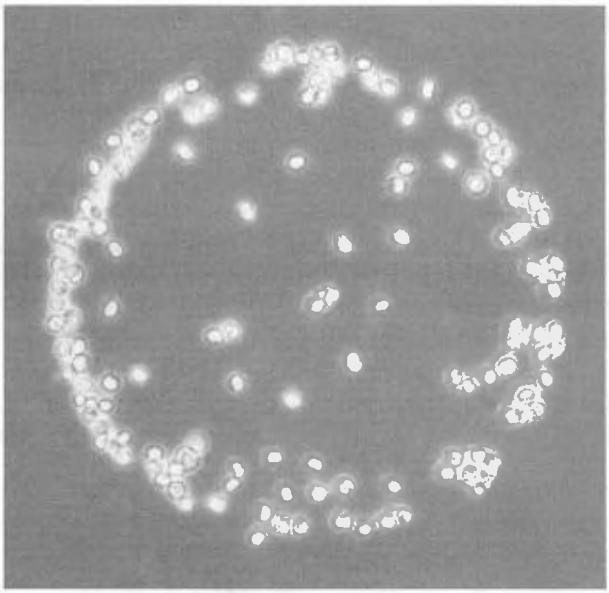

Fig. 9. A sphaerical colony of *Phaocystis* sp.

diatoms (e.g., *Coscinodiscus* spp., *Thalassiosira mala*) have caused discolorations or fish gill clogging [42].

Finally, it could be argued that any phytoplankton species can exert a negative impact when it persistently dominates phytoplankton populations: diatoms have long been considered an optimal food for copepods and identified as the primary producer of the trophic web that sustains exploitable fish species. However, it has recently been demonstrated that diatoms can produce substances that inhibit

viability in copepod eggs [43]. On the other hand, a diet based exclusively on dinoflagellate species that are not considered toxin producers may lower male fertility in copepods [44]. All these cases indicate that complex mechanisms regulate the interactions among different components of the trophic web and that a lowering in diversity of algal population in time and space may pose a serious risk to coastal ecosystems.

6. HABs and eutrophication

The increase of HAB records in coastal waters around the world has often been associated with nutrients derived from anthropogenic activities [1,45]. Recently, such nutrient sources as rainfall have been implicated in the enhanced magnitude of noxious blooms [46]. Cases in point are Tolo Bay, Hong Kong [47,48] and the Seto Inland Sea, in Japan (Fig. 10) [49], where the number of visible blooms, what used to be called 'red tides', increased with eutrophication, and decreased subsequent to restoration practices that reduced nutrient inputs. However, the reduction in bloom frequency and intensity has not been paralleled by the disappearance of harmful species: recently new harmful species have been detected in Japanese waters (e.g. *Heterocapsa circularisquama)*, and the lower impact of high biomass blooms has been counterbalanced by a higher impact of toxic species.

In the laboratory it is easy to demonstrate that algal growth increases with increasing nutrient availability. However, there is scarce evidence for a direct relationship between the spread of harmful events and eutrophication in the sea. High biomass blooms obviously require high nutrient levels. However, a high nutrient supply may not necessarily favour harmful species; an abundance of nutrients could stimulate the development of blooms of non-harmful opportunistic diatoms, flagellates or dinoflagellates, which could outcompete toxic species. On the other hand, species highly toxic at low concentrations, i.e. *Alexandrium* and *Dinophysis*, presumably do not depend on high nutrient concentrations to reach the

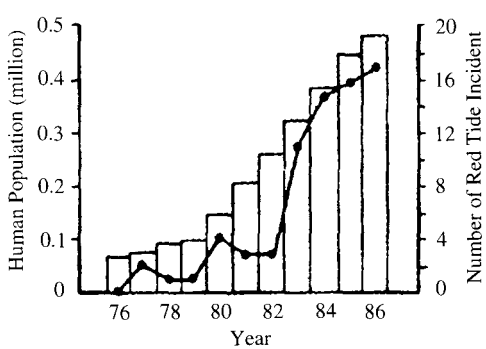

Fig. 10. Number of 'Red tides' in Tolo Harbour, Hong Kong, and human population in Hong Kong from 1976 to 1986 [48].

threshold at which they become harmful. Also, specialized behaviours, like facultative or obligate mixotrophy [50,51] or the capability of vertically migrating to nutrient-replete water column layers [52,53], represent very successful adaptations to cope with the vertical segregation of light and nutrients in stratified waters. Finally, at the ecosystem level, complex trophic relationships, including microbial interactions and excretion from grazers, may enhance nutrient availability and growth rates under apparently oligotrophic conditions [54].

Not only nutrient increase, but also nutrient imbalance, could favour certain species versus others [47,55]. Because eutrophication increases nitrogen and phosphorus inputs, the ratio of these nutrients to silicates becomes very high. This would favour non-diatom species including several harmful/toxic species [45]. In an equilibrium system, the nutrient-ratio hypothesis would hold true only when the deficient nutrient becomes limiting, not when both resources saturate the growth of the species [56]. However, in a delayed-effect scenario, nutrient imbalance would affect the development of different algal species. As an example, in a balanced system of 0.7 : 1 Si : N the consumption of silicates by diatoms would lead to a proportional consumption of dissolved N, with very little resources left for the subsequent successional stage, i.e. flagellates. Conversely, when the Si : N ratio decreases, due to increased N, diatom performance would not change but the contribution of flagellates to annual production would increase [57]. However there are cases, such as that of the Black Sea, where increased eutrophication accompanied by a reduction in silicate concentrations has led to an increase of flagellates and also of diatom blooms [5].

Regardless of the controversy about HAB–eutrophication relationships, the impact of high nutrient supplies and of massive inputs of inorganic and organic compounds in coastal systems undoubtedly merits detailed investigation. In fact, besides the above-mentioned cases of high biomass blooms leading to hypoxic or anoxic events, other negative effects of eutrophication might be worth assessing, e.g. the loss of phytoplankton diversity and shifts in the specific composition of microalgal communities. These changes could influence the fate of primary production and modify the shape of the trophic web in coastal areas, with grave consequences for fisheries and for the exploitation of natural resources. Also, this calls for an interdisciplinary and across-boundary scientific approach that includes data from the watershed to the offshore boundary and that integrates the physical, chemical and biological components of the system analysed at their matching scales of variability.

7. HABs and climatic fluctuations

It is not possible to demonstrate a worldwide increase in HABs because we are unable to detect long-term trends of algal blooms due to the lack of time series at the correct scale [58]. However, at a local scale there are numerous examples of HABs in areas where they were previously unknown. For instance, the first massive *Alexandrium* bloom along the coasts from southern Maine to Massachusetts was

recorded in 1972 and has been followed by PSP outbreaks every year [15]. *Gymnodinium catenatum* was not known in Galician waters before 1976 [59] and it was found in Australian waters only after 1979 [35]. Compositional shifts, changes of dominance and sudden emergence of previously unrecorded species are well known phenomena in long-term phytoplankton dynamics. The passive introduction of species from other sites by means of natural (currents, storms) or man-mediated events (ballast waters, keels, mussel strains transport, etc.) has been implicated in variations in the geographic range of a species, which has also been supported by the absence of cyst records prior to certain dates [60].

The biodiversity of no local phytoplankton population is thoroughly known, therefore it cannot be excluded that a previously unrecorded species was already in an area, although at undetectable concentrations or confined to unsampled microlayers, or that it bloomed for a very brief period. Sediment investigations on resting cysts provide further information on planktonic organisms with benthic stages, and may lead to the detection of rare species that eluded planktonic sampling [61]. Similar investigations on fossilized resting cysts provide data on the distribution of a species over a wider time scale, which covers different hydrographic situations alternating over the geological periods in given areas. As an example, cyst investigations on sediment cores showed that microreticulate cysts attributed to the toxic species *Gymnodinium catenatum* were abundant in the Kattegat area (North Sea) at time windows that corresponded to periods of a relatively warmer climate [62]. Recently, a new non-toxic species that produces a microreticulate cyst has been described from the Kattegat area, *Gymnodinium nolleri* (Fig. 11), that is probably the same species that produced past blooms [63,64]. Even though the Scandinavian species was non-toxic, this case clearly shows the value of sediment core investigations for tracking long-term trends of specific phytoplankton blooms.

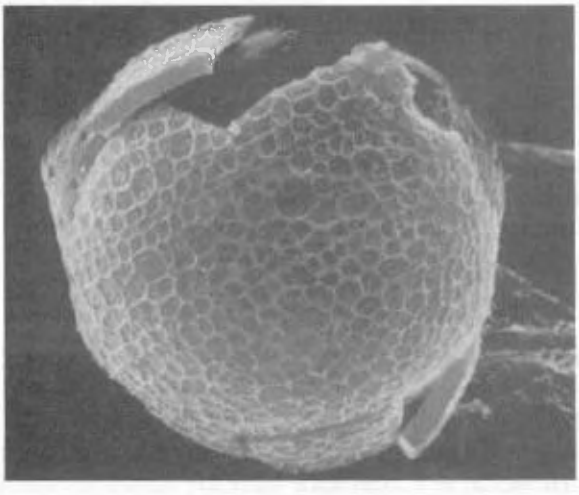

Fig. 11. The microreticulate cyst of *Gymnodinium nolleri*. Photo by Dr. Marianne Ellegaard, University of Copenhagen.

Climatic variations can also affect the spatial distribution of a species. The PSP-producer *Pyrodinium bahamense* is presently known only from tropical seas, but the geographic range of its fossil cysts is much wider and expands over temperate regions of both hemispheres (Fig. 12) [65]. Presumably, these records reflect periods of different climatic conditions, including those warmer than nowadays. In view of climatic changes, either natural or man-enhanced, which possibly lead to global warming, it could be envisioned that the distribution range of this species will tend to widen again. A recent finding of a living cyst of *P. bahamense* off the coasts of Portugal [66] supports this idea. Germination experiments are required to assess whether this is indeed the same species and to test its toxicity.

A relationship between the interannual variability of HABs and climatic variations has been suggested for several cases. The periodicity of blooms of *Pyrodinium bahamense* var. *compressum* in south-east Asia is related with El Niño Southern Oscillation (ENSO) events (Fig. 13) [67,68]. The recent outburst of fish killing species in Hong Kong [69], which caused US$ 32 million damage, has been associated with the high magnitude of El Niño in 1997–1998. A correlation between interranual variations of occurrence and climatic variability has also been hypothesized for *Gymnodinium catenatum* blooms in Australian waters [34] and for *Phaeocystis* sp. in the Atlantic [70].

Temperature variations not only directly affect the geographic range and the magnitude of certain blooms, but can also induce changes in circulation patterns, prolong stratification periods and cause variations in the physical structure of the water column that can favour particular species, including dinoflagellates and their potentially harmful representatives [69,71,72]. These effects highlight the links between long-term variations of HABs and physical oceanography, and point to the

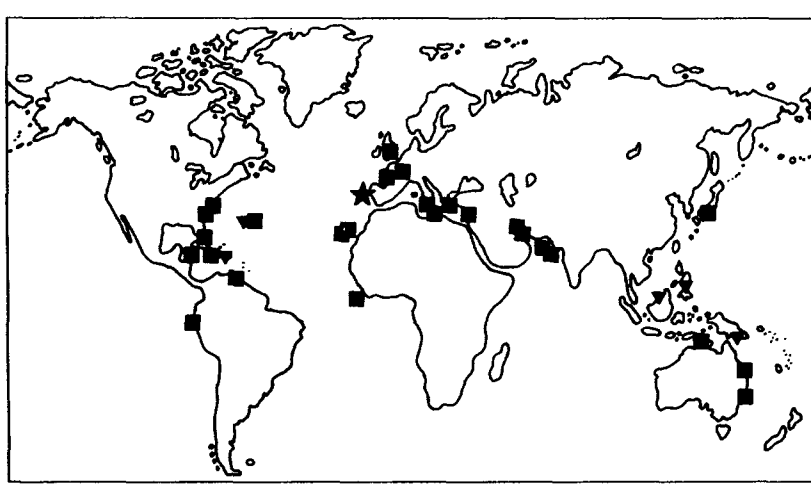

Fig. 12. Distribution of recent (triangles) and fossil (squares) *P. bahamense* cysts (redrawn from Matsuoka [65]). The star marks the recent finding of a living cyst off Portugal [66].

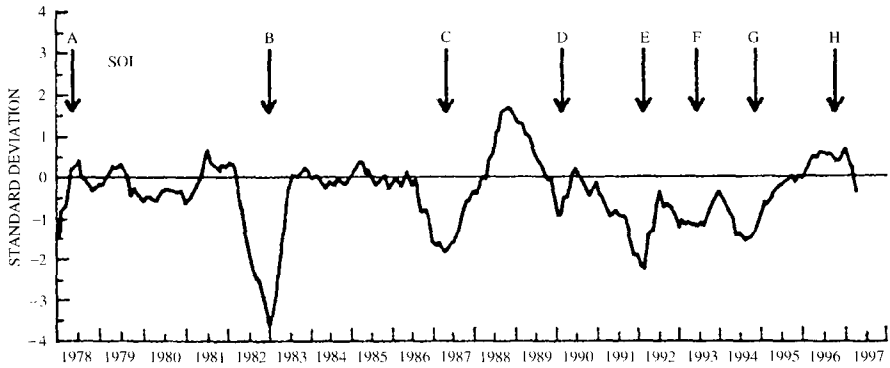

Fig. 13. The relationship between ENSO and blooms of *Pyrodinium bahamense* var. *compressum* (from [68]).

need to predict scenarios in coastal circulation and water mass distribution so as to be able to assess the risk of HABs in coastal areas.

8. Science and management perspectives

Harmful algal blooms are apparently an antithesis to the concept of 'health of the ocean'. However, we have argued that in most cases HABs do not damage marine ecosystems, nor impair their sustained biological functioning. Though extremely dangerous for human health and deleterious for the commercial exploitation of coastal areas, these blooms are natural phenomena with barely evident negative effects on coastal oceans. On the other hand, the health of the oceans and the sustainable development of coastal marine ecosystems can be endangered by human activities that reduce habitat and species diversity. This generally leads to dire consequences for the trophic web, and in some cases may divert energy entrapped by primary production towards non-exploitable living marine resources. Within this category, high biomass blooms in eutrophied areas indeed represent a menace, especially when they are associated with biodiversity loss.

The problems arising from HABs are very diverse, the causes are many and they are on the increase. Like other natural catastrophic phenomena, such as earthquakes, tsunamies and storm surges, correct management is required to mitigate the threat posed by HABs to the economic development of coastal areas and to human health. But what can be done in a practical sense? What information is needed to manage efficiently marine resources, protect public and ecosystem health, encourage and support aquaculture development, and contribute to policy decisions about coastal zone issues, such as waste and sewage disposal, aquaculture development, and dredging? Do human activities exacerbate HABs? How can this be verified, and what steps must be taken to minimise the effects of human activities?

Thus far, early warning when harmful species or toxins in sea-food reach critical concentrations is the most widely used management strategy. An early alert means that specific contingency plans can be put in action to avoid health problems and limit economic losses. The next step, as yet feasible in only a few cases, is to forecast harmful events with a lead time of 1–7 days, so as to mitigate more effectively the economic impact, e.g. harvesting mussels before they become toxic or moving fish-cages to safer depths or areas. At a long time scale, it would be necessary to assess the risk for harmful events when planning the use of coastal areas. In addition, the role of human activities in enhancing these phenomena through eutrophication, transport of harmful species and modifications of coastal areas should be thoroughly assessed. Finally, ad hoc procedures designed to prevent harmful events or to accelerate their termination are the ultimate goal for HAB management.

At present, capillary monitoring seems to be the only tool we have to protect ourselves and food resources of marine origin from the deleterious effect of HABs. To move from early alert and contingency plans to prediction and mitigation, we need basic knowledge about the distribution, species succession, long-term trends and population dynamics of HAB species. Much is known about harmful algae, yet our ability to describe the factors controlling the dynamics of individual species is limited by gaps in our knowledge about how the physiological, behavioural and morphological characteristics of algae (including HAB species) interact with environmental conditions to promote the proliferation of one species versus another. For example, we do not know enough about the environmental cues for encystment and germination, or about the interactions of life cycles with hydrography, to quantify the role of resting stages in the population dynamics of cyst-forming harmful algae. Also, it is often difficult to assess the role of nutrients and light in algal population dynamics and toxicity because some phytoplankton migrate vertically to exploit deep-water sources of nutrients at night, and light near the surface during the day.

The key to predicting HAB phenomena is to identify and quantify adaptations of HAB species that lead to their selection in particular hydrodynamic and ecological conditions. Thus, the central research problem and a challenge for biological oceanographers is to understand the critical features and mechanisms underlying the population dynamics of HAB species. This understanding will serve as a basis for improved monitoring and to build models that predict the occurrence, movement, toxicity, and environmental effects of harmful algal blooms. These predictions are essential for the efficient management and mitigation procedures mentioned above.

Another gap in our knowledge of phytoplankton, including potentially harmful species, are the long-term trends of occurrence and abundance of these organisms, which are related with climatic factors and with their influence on coastal hydrography. To bridge this gap, there is the need for a global network of intercalibrated observations, integrated in a multidisciplinary monitoring framework like that envisioned by the Global Ocean Observing System (GOOS). This would provide the data needed to detect trends and patterns of occurrence and distinguish the effects of natural variability from those of anthropogenic modifications of the environment.

Progress in oceanographic research should be paralleled by progress in the field of toxicology. Newly discovered toxins should be characterised, and detection and analytical methods devised in order to clarify the different types of toxins. Potential new toxin producers should also be identified. Lastly, enzymatic processes involved in the metabolism and elimination of HAB toxins from shellfish should be elucidated.

Targeted studies and technological innovations are essential to improve our understanding of HABs. For example, we need better tools for the detection and monitoring of harmful algae and their biologically active products, more sensitive approaches with which to study the nutrition of planktonic protists, integrated techniques to be able to observe physical, chemical, and biological variability on the scales relevant to physical forcing, improved representations of the physical processes that influence HAB dynamics, advances in physical–biological coupled models (including data assimilation models), and more effective observation systems for the detection and characterisation of phytoplankton community dynamics (including HABs). Rapid advances in bio-technology and instrumentation with which to measure physical and bio-optical variability in the sea, together with more sensitive and selective methods for chemical analysis and improvements in computational abilities, will ensure rapid progress if efforts are well focussed and co-ordinated.

Recently, three initiatives have addressed the question of HAB research and have pinpointed the most pressing research issues for the next decade in the field of oceanography. The first was the formulation of a USA national research plan, ECOHAB. The second is an international initiative by IOC and SCOR devoted to the Global Ecology and Oceanography of Harmful Algal Blooms, (GEOHAB).

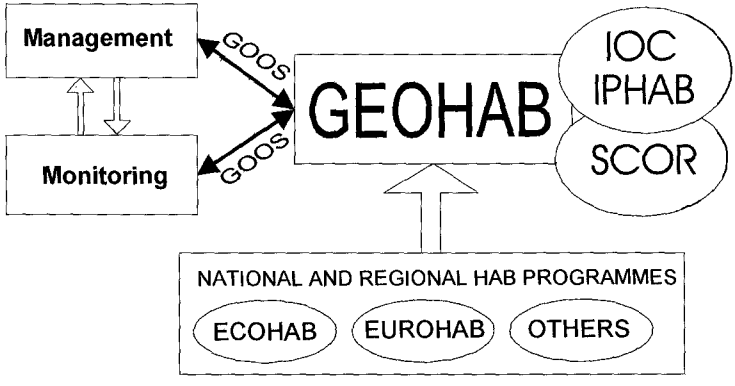

Fig. 14. The relationships between national and international scientific initiatives concerning harmful algal blooms: GEOHAB and its two mother organizations SCOR and the IOC; the linkage to the Intergovernmental Panel on HAB (IPHAB) as the intergovernmental mechanism; the linkage of national HAB research to the GEOHAB Programme; and how GOOS will be an important vehicle to convey the scientific results of GEOHAB to user groups and to the improvement of operational monitoring of HABs. Links with other international science programmes are also present e.g. for establishing ecosystem models.

Lastly, the European Commision sponsored a European initiative on harmful algal blooms (EUROHAB) (Fig. 14). These integrated, multidisciplinary, across-boundaries initiatives will lead to a better understanding of the factors that regulate the dynamics of HABs in the context of physical and chemical forcing, ecosystem dynamics, and human influences. This in turn will result in more effective management and mitigation of the effects of HABs, thus safeguarding the intrinsic and commercial value of coastal marine ecosystems.

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Shenandoah Riverkeeper
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540.837.1479
keeper@shenandoahriverkeeper.org
www.shenandoahriverkeeper.org

November 7, 2013
Ms. Benita Best-Wong
USEPA HQ, Ariel Rios Building
1200 Pennsylvania Avenue, N.W.
Mail Code: 4501T
Washington, DC 20460
RE: Official Evidence for Virginia's 2012 303D/305B Water Quality Integrated Report

Hi Mrs. Best Wong,
I would like to officially place the following information in the record for Virginia's 2012 303D/305B Water Quality Integrated report, for EPA's consideration. As you may be aware Shenandoah Riverkeeper has been producing information for EPA about the problematic algae blooms which occur on the Shenandoah Riverkeeper every year, in an effort to compel EPA to list the Shenandoah River as impaired due to loss of use because of these blooms.

Introduction:
It has been our position that the citizen complaints constitute sufficient record to show that algae reduces use and enjoyment of the entire public river section. But to help EPA understand the special and temporal extent of the algae blooms, we have worked over the past year to develop and share more evidence of the impairment. You may recall seeing our last batch of DVD's, which combined with our previous submissions show hundreds of examples of extreme blooms, during many different times of year, over hundreds of miles of river.

I'm sending this email to add yet more information. Most recently, we contracted with the private company Blue Water Satellite (BWS) in order to use their remote satellite sensing technology to "look" at the chlorophyll and blue green algae/cyanobacteria signatures over approximately 70 miles of the North Fork Shenandoah River on July 1st, 2010. We chose this date out of very few publicly available images of the recreational summer months because this image showed the least amount of cloud cover. Clouds obscure the view of the river and create shadows which can diminish the ability to "see" the river. But the date is an average representation of the river's condition in the summer. It was our goal to determine whether or not algae and blue-green algae/cyanobacteria were present at high levels and/or whether it was present through the entire river reach as we have tried to show with our photographic images.

BWS technology evaluates the unique Spectral Reflective Signature of things like chlorophyll, phycocyanin (the pigment in blue-green algae/cyanobacteria), temperature, nitrate levels etc., in water. They run proprietary algorithms, which have been extensively ground-tested, on both publicly available images and privately obtained images. The July 1st image was publicly available.

Results:
The results showed not only that the blue-green algae/cyanobacteria was present throughout the 70 miles of the North Fork we evaluated, but it was present at high levels. In comparison to the Chlorophyll analysis we did, the values for phycocyanin, which is the surrogate for blue-green algae/cyanobacteria were often higher than chlorophyll. Blue-Green algae/cyanobacteria are ecological indicators of over-nitrification of a waterbody,

negatively affect the ecosystem, present a potential danger to river users if they are developing toxins, but always diminish user use and enjoyment because they are slimy, smelly and unappealing looking. Their physical presence in any significant quantity begins to affect user enjoyment of the river, our citizen letters demonstrate this.

At this time we don't wish to assert that the blue-green algae/cyanobacteria is producing toxins or is presenting a risk to users, but we do wish to notify EPA of the concern. The World Health Organization's standard for contact recreation for Microcystis is 20 parts per billion (ppb). Several images we have submitted in the past show very heavy microcystis blooms in parts of the North Fork. One area in particular is mile 50, upstream of Chapman's dam. We are uncertain of the relationship between phycocyanin and Microcystis but we regularly got readings in this analysis between 20 and 50 ppb for phycocyanin in the reaches of concern.

EPA itself was deeply involved in the Shenandoah River Fish Kill Task Force which convened during the years following the devastating fish kills in the Shenandoah River. During our investigations, EPA suggested several times that blue-green algae/cyanobacteria should be evaluated as a potential contributing factor in the kills. Funding was limited and this was not studied in great depth though many of the symptoms that the fish excibited including a) fused gill lamellae b) oxidative liver damage c) skin lesions are all symtoms of blue-green algae/cyanobacteria toxicity.

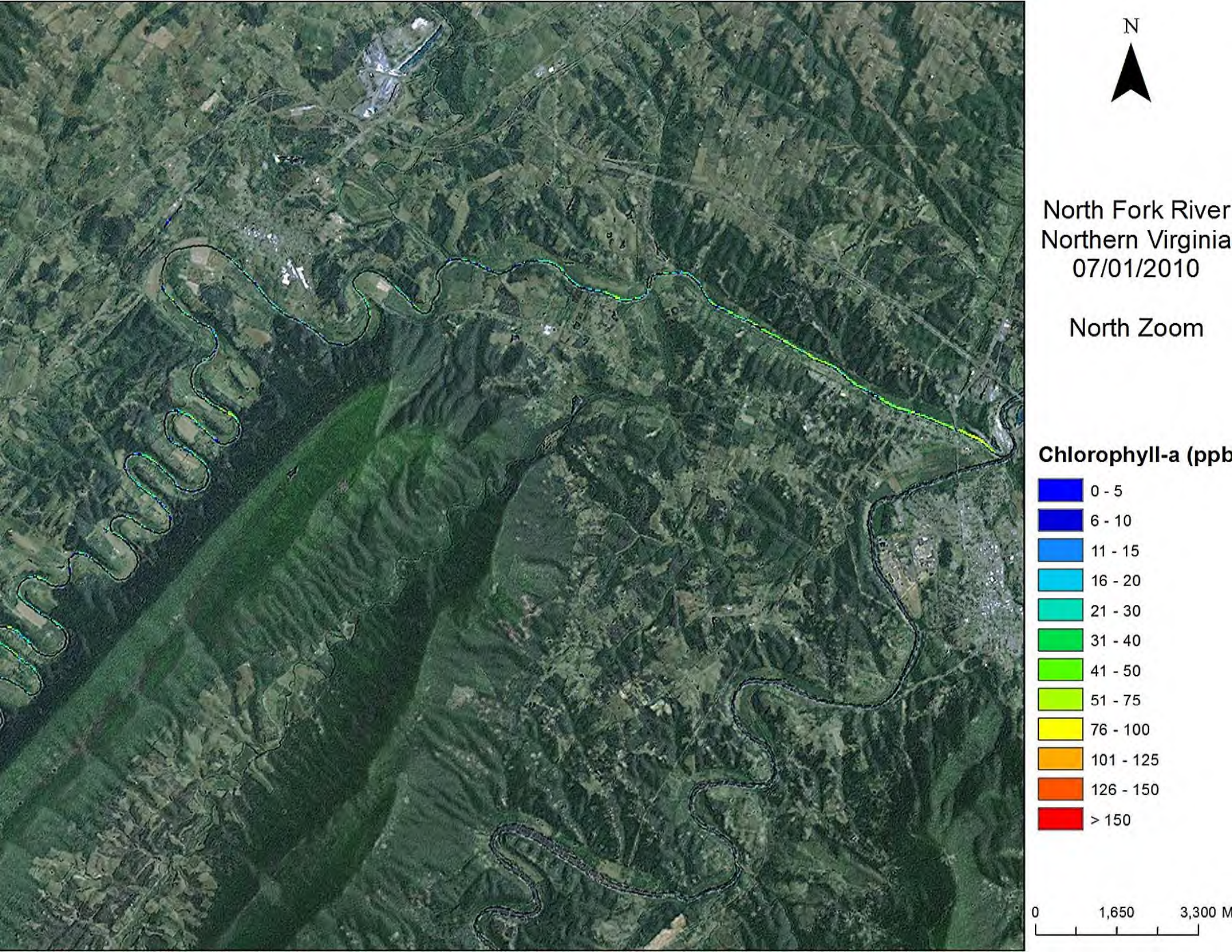
Conclusion:
In conclusion, Shenandoah Riverkeeper submits these satellite images to EPA to show the spacial extent of the algae blooms during the date July 1, 2010. Our records indicate this condition began significant weeks before July 1st and remained through the duration of the summer. We have also provided several images from the river on July 7, 2010, the closest date to that of the satellite images, that we have. We have found that the images help us to see and interpret what it is that is being shown in the spectral reflective signature analysis performed by Blue Water Satellite.

Thank you for your time and attention to these matters, we are happy to discuss these findings with you at you convenience.

Sincerely,

Jeffrey Kelble
Shenandoah Riverkeeper

CC: Bill Richardson- Region III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Northern Virginia
07/01/2010
North Zoom
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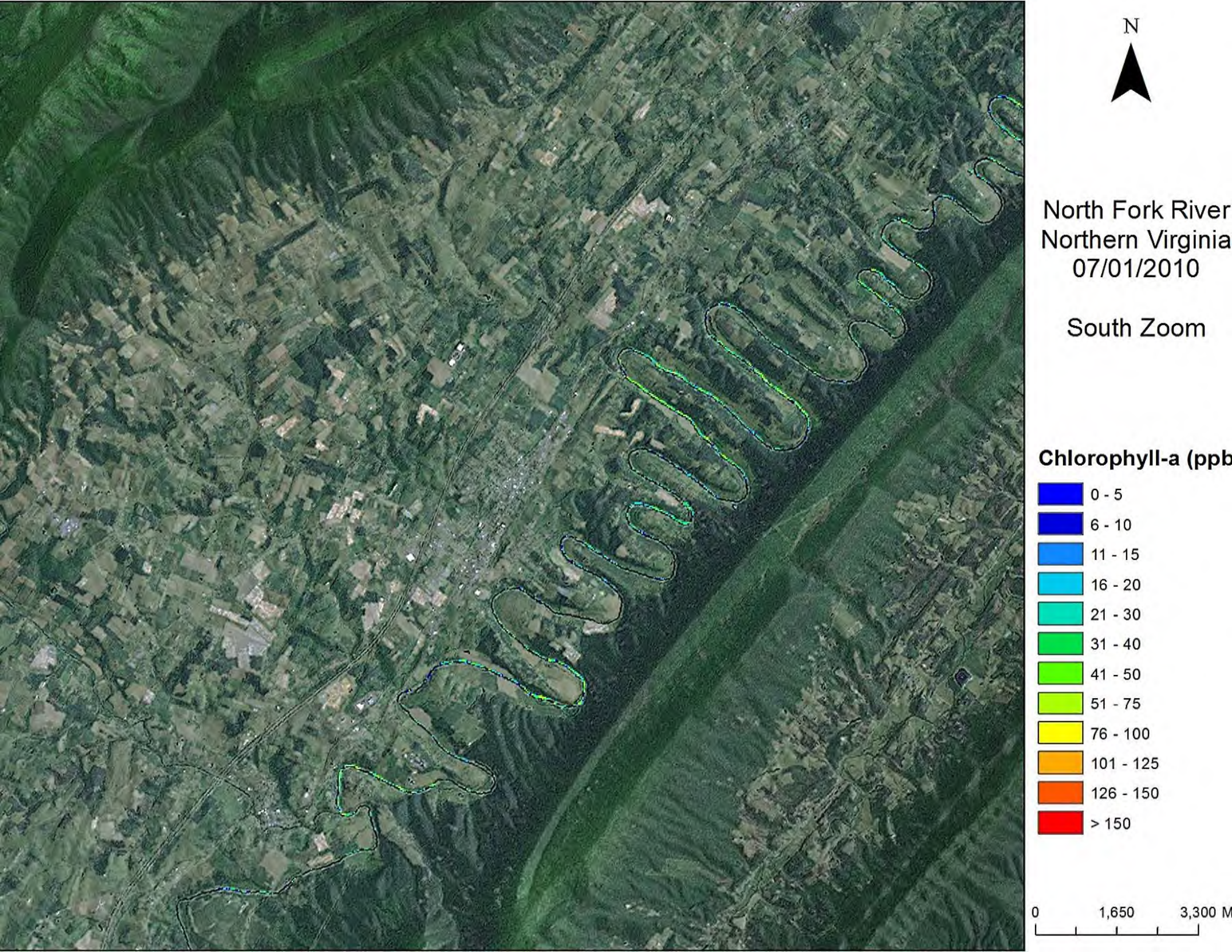
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Northern Virginia
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South Zoom
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North Fork River
Northern Virginia
07/01/2010

Natural Color
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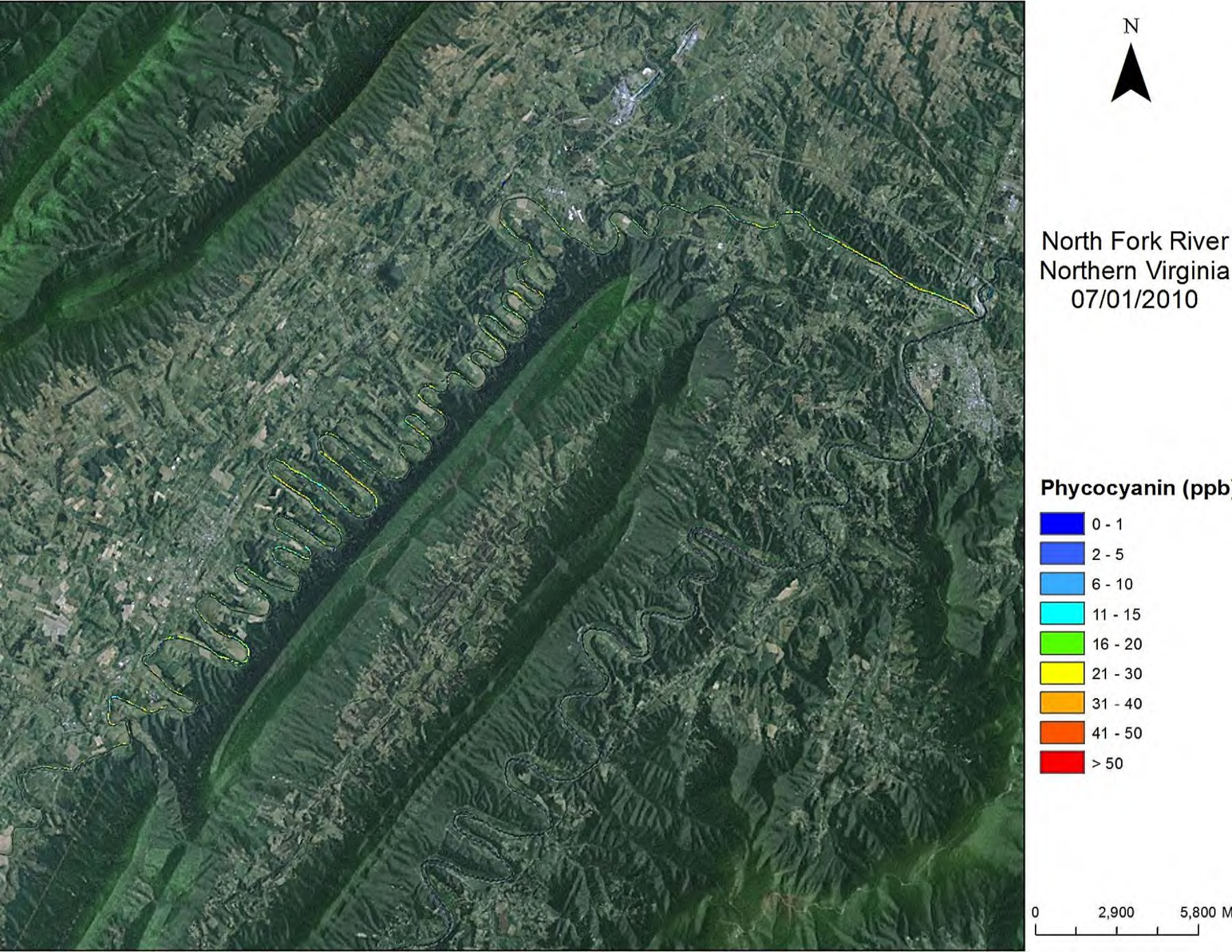
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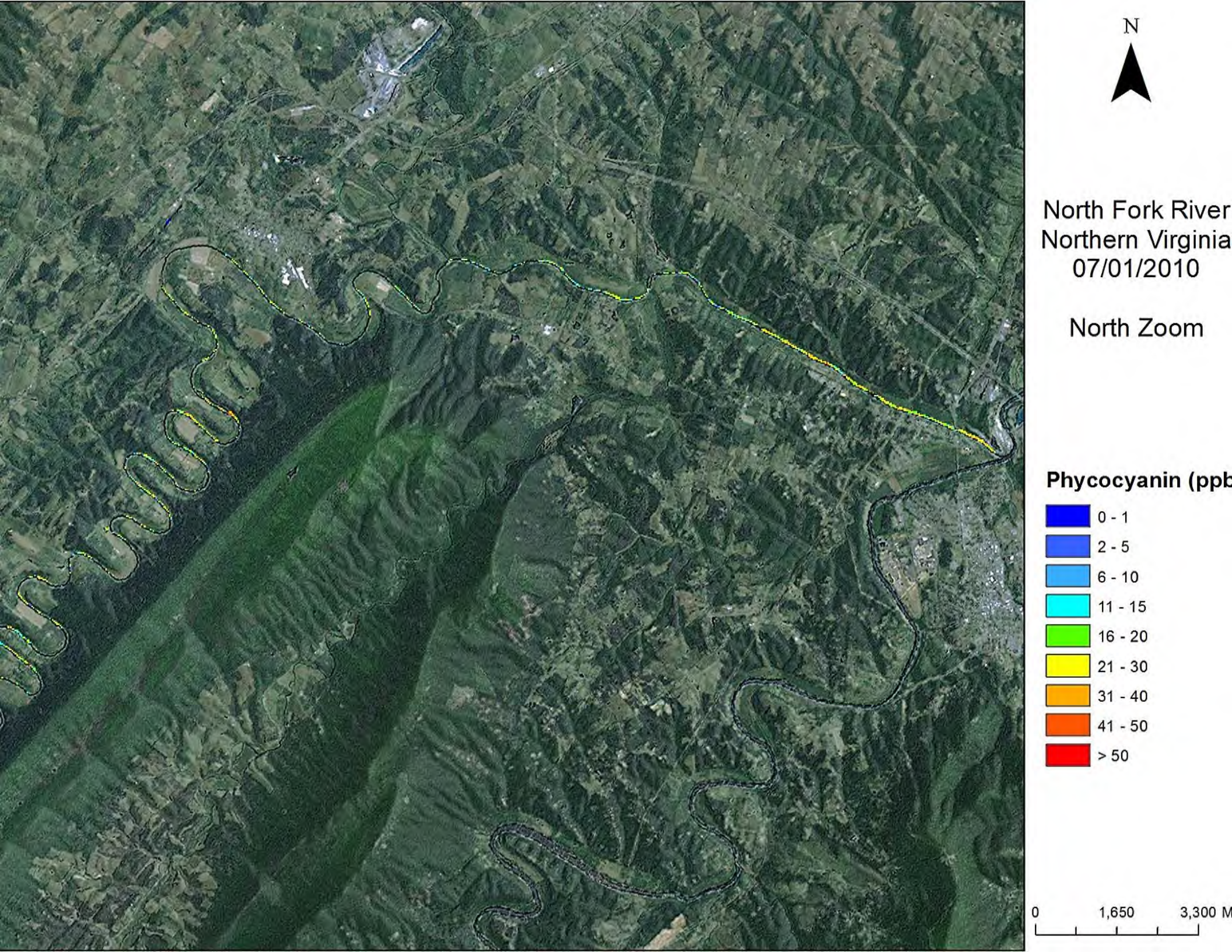
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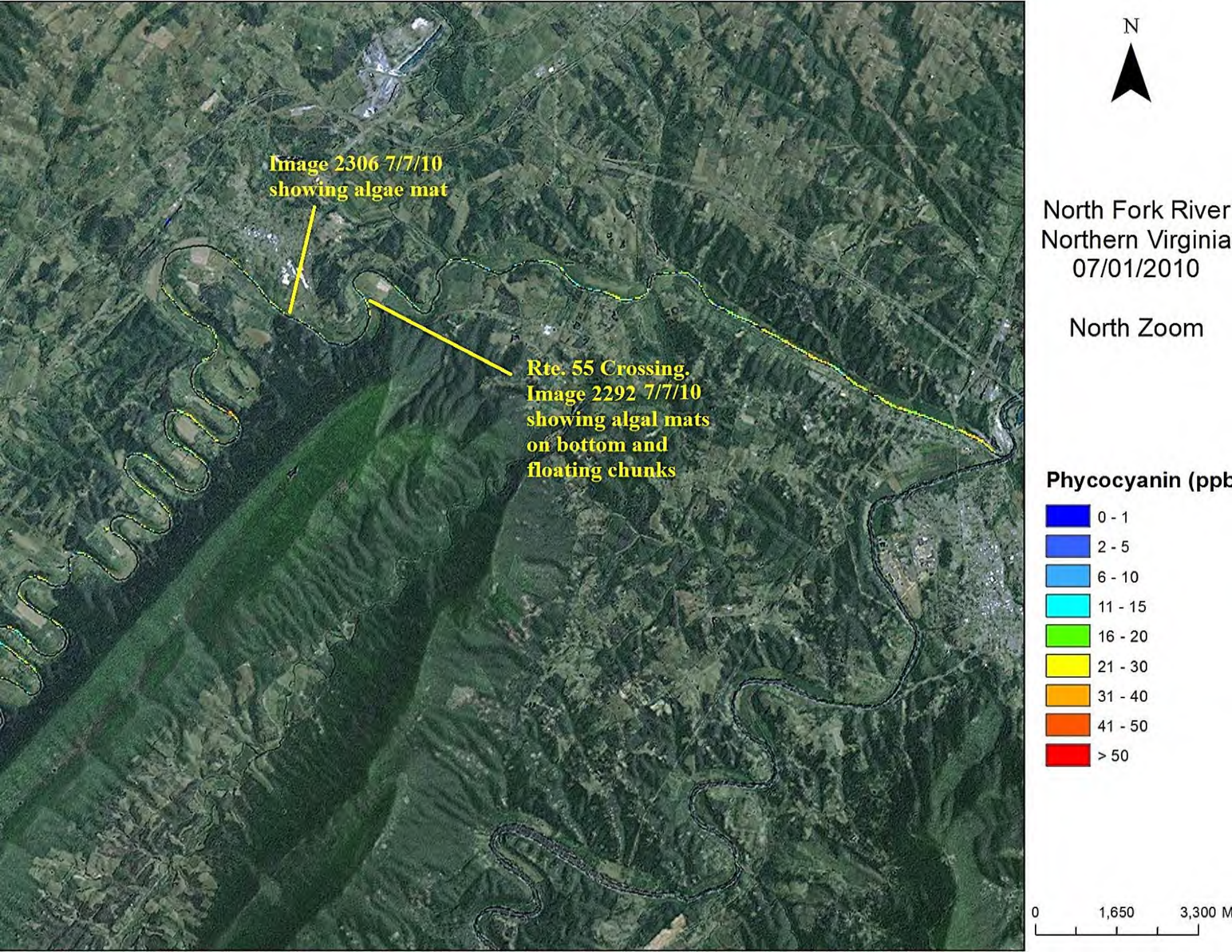
Image 2306 7/7/10
showing algae mat
Rte. 55 Crossing.
Image 2292 7/7/10
showing algal mats
on bottom and
floating chunks
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Northern Virginia
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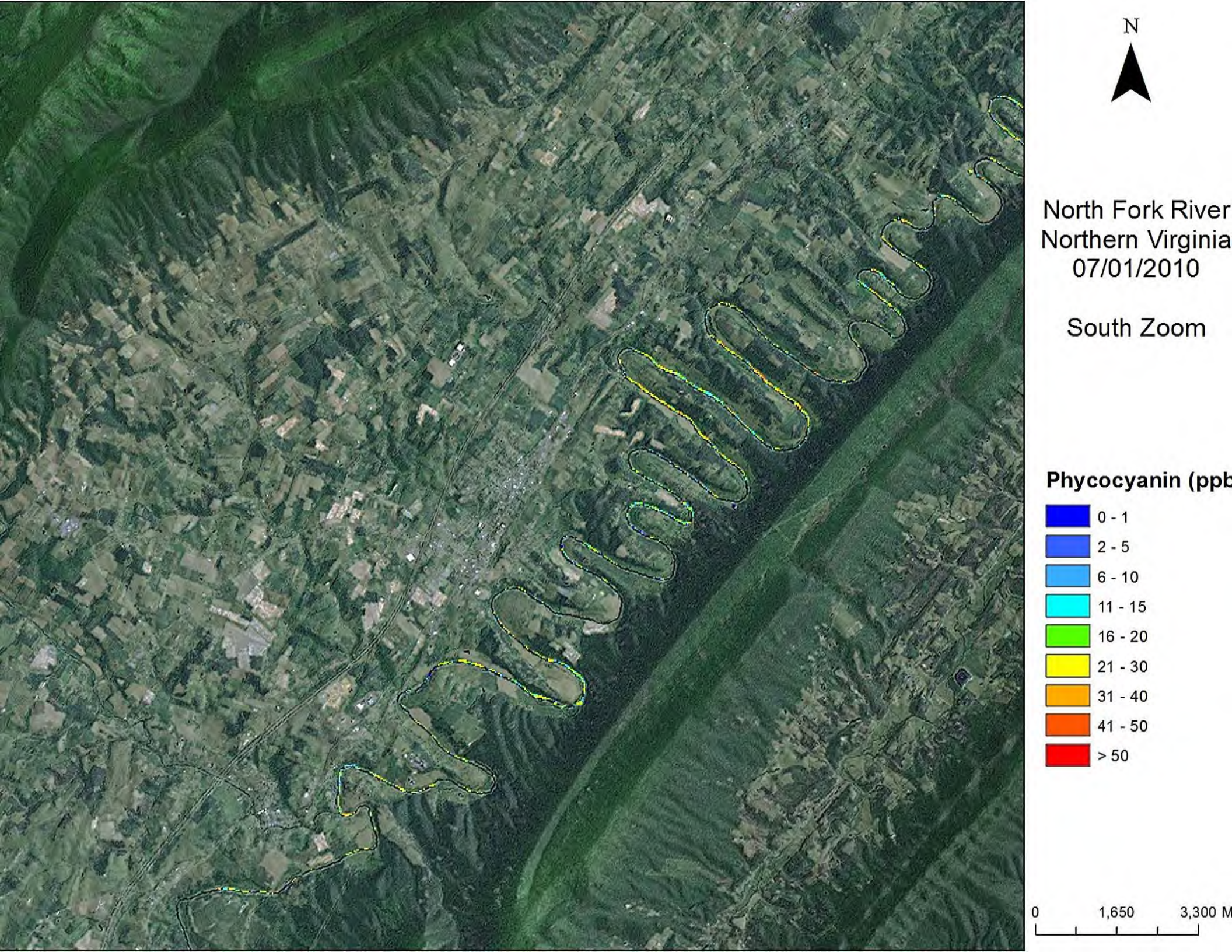
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Northern Virginia
07/01/2010

South Zoom

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January 30, 2015

Via U.S. Mail and email (John.Kennedy@deq.virginia.gov)
John Kennedy
Virginia Department of Environmental Quality
Office of Ecology
P.O. Box 1105
Richmond, VA 23218-1105

Re: Draft 2014 Virginia 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

For years we have documented the presence of excessive algae throughout the North Fork, South Fork, and main stem of the Shenandoah River. In addition to this letter we are submitting a Technical Review that presents and analyzes the evidence of excessive algae, to help the Department of Environmental Quality (DEQ) assess the effect these conditions are having upon the attainment of water quality standards applicable to these three streams.[1] The Technical Review demonstrates beyond any reasonable doubt that excess algae interferes with or diminishes the designated use of these streams for recreation including swimming, wading, floating, canoeing, aesthetic enjoyment, and fishing. This evidence shows that existing effluent limits are not stringent enough to fully implement Virginia's narrative water quality standards or designated uses. The Department must therefore identify the North Fork, South Fork, and main stem of the Shenandoah River on the 2014 list of impaired waters, Category 5, as required by section 303(d) of the Clean Water Act, 33 U.S.C. § 1313(d).

I. Virginia's Mandatory Duty to Identify These Waters as Impaired

The Clean Water Act requires that "[e]ach State shall identify those waters within its boundaries for which the effluent limitations required by section 1311(b)(1)(A) and section 1311(b)(1)(B) of [the Act] are not stringent enough to implement any water quality standard applicable to such waters." 33 U.S.C. § 1313(d)(1)(A). Designated uses are water quality standards by definition. *Id*. § 1313(c)(2)(A). Accordingly, when evidence demonstrates that water quality standards or designated uses are not being attained despite the application of

[1] David Sligh, Technical Review of Evidence to Determine the Presence, Extent, and Consequences of Excessive Algal Growths in the Shenandoah River and its Tributaries (January 2015).

technology-based effluent limitations, the state "shall identify those waters" in its Integrated Report.

A. Relevant Virginia water quality standards

The water quality standards that are applicable to the Shenandoah River and relevant to excess algal growth include the following:

> A. All state waters, including wetlands, are designated for the following uses: recreational uses, e.g., swimming and boating; the propagation and growth of a balanced, indigenous population of aquatic life, including game fish, which might reasonably be expected to inhabit them; wildlife; and the production of edible and marketable natural resources, e.g., fish and shellfish.

9 Va. Admin. Code § 25-260-10.A. (emphasis added).

> A. State waters, including wetlands, shall be free from substances attributable to sewage, industrial waste, or other waste in concentrations, amounts, or combinations which contravene established standards or interfere directly or indirectly with designated uses of such water or which are inimical or harmful to human, animal, plant, or aquatic life.
>
> Specific substances to be controlled include, but are not limited to: floating debris, oil, scum, and other floating materials; toxic substances (including those which bioaccumulate); substances that produce color, tastes, turbidity, odors, or settle to form sludge deposits; and substances which nourish undesirable or nuisance aquatic plant life. Effluents which tend to raise the temperature of the receiving water will also be controlled. * * *

9 Va. Admin. Code § 25-260-20 (emphasis added).

When the Virginia Water Control Board enacted these water quality standards in 1981, its statement of basis and purpose made clear that the Board intended both narrative and numeric limits to be given force and effect:

> Water quality standards consist of narrative statements that describe water quality requirements in general terms, and of numeric limits for specific physical, chemical, biological or radiological characteristics of water. These narrative statements and numeric limits describe water quality necessary to meet and maintain reasonable and beneficial uses such as swimming and other water based recreation, public water supply and the propagation and growth of aquatic life. Standards include general as well as specific descriptions, since not all

requirements for water quality protection can be numerically defined.[2]

The Court of Appeals of Virginia has confirmed recognized that the requirement to protect designated uses has independent force and effect in addition to the requirement to meet other water quality standards. *See State Water Control Bd. v. Captain's Cove Util. Co., Inc.*, 2735-07-1, 2008 WL 2963851 (Va. Ct. App. Aug. 5, 2008) (reinstating water pollution control board's denial of discharge permit on basis that the discharge would impair recreational uses). The court noted that "9 VAC 25–260–20 is written in the disjunctive, prohibiting substances in state waters that <u>either</u> contravene established standards *or* interfere directly or indirectly with designated uses of such water." *Id*. (emphasis in original).

Our Technical Review sets forth extensive evidence of impairment including:

- Over one hundred and twenty citizen complaints identifying algae blooms by location and date, and describing impairment of recreational uses including primary contact recreation, boating, wading, fishing, and general aesthetic enjoyment;
- More than 1,000 photographs and videos, including information on location and date, showing excessive growth of algae;
- Data from a summer 2012 quantitative survey of stream transects for algae conditions in the Shenandoah River; and
- Satellite images in which spectral reflective signatures of several substances in the North Fork Shenandoah River are shown, indicating high concentrations of chlorophyll and phycocyanin (the pigment in blue-green algae or cyanobacteria).

Collectively this evidence provides an overwhelming basis for finding that excess nutrients are present in quantities that, in combination with other environmental factors, cause frequent widespread algae blooms that constitute a nuisance to recreational users.

B. Recent EPA guidance on water quality assessment and listing decisions

In its 2014 guidance on Integrated Reporting the U.S. Environmental Protection Agency (EPA) provided important information that is relevant in this context.[3] Among other things,

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[2] Attachment A, Commonwealth of Virginia State Water Control Board, Water Quality Standards (eff. Dec. 12, 1981) (excerpt). The current water quality standards at 9 Va. Admin Code Ch. 260 are derived from this 1981 enactment.

[3] Attachment B, U.S. Environmental Protection Agency, Office of Wetlands, Oceans, and Watersheds, Memorandum, Information Concerning 2014 Clean Water Act Sections 303(d), 305(b), and 314 Integrated Reporting and Listing Decisions; also available online at:

EPA confirmed that visual assessments provide a valid basis for listing a waterbody as impaired:

> A State can determine whether a waterbody is attaining its applicable narrative nutrient or other relevant narrative criteria and designated uses by using results of visual assessments. For example, field observations of excessive algal growth, macrophyte proliferation, adverse impacts on native vegetation (e.g., eelgrass), presence or duration of harmful algal blooms, unsightly green slimes or water column color, and/or objectionable odors may be a basis to include a waterbody on the State's Section 303(d) list for failing to meet one or more applicable narrative criteria and designated uses.

In addition, EPA affirmed that a state must list waters as impaired if their designated uses are threatened, even if the precise causes are not fully known:

> [I]f a designated use is not supported and the segment currently fails to meet an applicable water quality standard or is "threatened," it must be included on the State's Section 303(d) list even if the specific pollutant causing the water quality standard exceedance is not known at the time.

This guidance is consistent with EPA regulations that govern the Department's listing process, which require that "[e]ach State shall assemble and evaluate all existing and readily available water quality-related data and information to develop the [impaired waters] list…" including, "[a]t a minimum… all of the existing and readily available data and information about the following categories of … (iii) [w]aters for which water quality problems have been reported by local, state, or federal agencies; members of the public; or academic institutions." 40 C.F.R. § 130.7(b)(5).

C. Relevant assessment approaches in other states

Relevant listing approaches in other states provide workable methods for assessing the how excess algal growth prevents attainment of water quality standards. For example, Vermont considers water bodies to be impaired when "[a]n on-going record of public complaint concerning the algal conditions in the water has been established."[4] Montana's approach is

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http://water.epa.gov/lawsregs/lawsguidance/cwa/tmdl/2014-memo.cfm (last visited Jan. 28, 2015).

[4] Attachment C, Vermont Department of Environmental Conservation, Vermont Surface Water Assessment and Listing Methodology (excerpt); also available online at: http://www.vtwaterquality.org/mapp/docs/mp_assessmethod.pdf (last visited Jan. 28, 2015) (in

similar: "Some circumstances related to excess nutrient pollution are severe enough that a rigorous data collection effort is not required. Photo documentation will suffice."[5] These approaches are appropriate for assessing nonattainment of Virginia's water quality standards, since the designated use and the general criteria prohibiting "undesirable or nuisance" both implicate visual impacts of algae.

The Technical Review submitted in support of these comments provides additional background demonstrating the validity of visual assessments and user reports in assessing nonattainment of water quality standards for recreational and aesthetic uses.

II. The Department's Rationale For Declining To List These Streams As Impaired In Previous Years Is Not Legally Or Technically Valid

The Department rejected requests to list these waters as impaired in the 2010 and 2012 Integrated Reports, citing a range of technical and legal interpretations. In September 2014 EPA approved Virginia's 2012 Integrated Report, including the decision not to identify the Shenandoah River main stem, North Fork or South Fork as impaired and requiring TMDL development.[6] While we disagree with EPA's rationale, we note that EPA expressly rejected several of the Department's reasons for deciding not to list these waters as impaired. For example, the 2012 Integrated Report stated that the Department lacks a methodology for assessing what constitutes a nuisance; that "[d]eterminations of 'fully supporting' versus 'impaired' [under the recreation designated use] are based on violations of the e-coli bacteria in freshwater"; and that the Department cannot or will not base a listing on "anecdotal and subjective observations." To the contrary, EPA stated that in considering "whether recreational uses are impaired due to excess algae," "EPA does consider visual observation and statements by water users regarding algal blooms to be relevant to a determination whether one or more narrative criteria or designated uses are being achieved." EPA also stated that the Department should "evaluate spatial and temporal extent of algal growth and its impact on River enjoyment." As noted above, and in the Technical Review, a number of methodologies do exist and can readily be applied to the Shenandoah River.

The 2012 Integrated Report also discussed a number of TMDLs for Shenandoah River tributaries for sediment, and a smaller number of TMDLs for nitrogen or phosphorus, which the

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addition: ("For cyanobacteria (blue-green algae), waters display on-going summer blooms of toxin-producing cyanobacteria and have microcystin concentrations at elevated levels in excess of the World Health Organization guideline of 1 ug/l. Invasive non-native aquatic species are not applicable in this category.")

[5] Attachment D, Montana Dept. of Environmental Quality, Assessment Methodology for Determining Wadeable Stream Impairment Due to Excess Nitrogen and Phosphorus Levels (Dec. 2011); also available online at: https://www.deq.idaho.gov/media/937622-assessment_methodology_determining_wadeable_stream_impairment_excess_nitrogen_phosphorus_levels.pdf (last visited Jan. 28, 2015).

[6] Attachment E.

Department believes to begin to address some of the factors that contribute to excess algae growth on these waters. However, these TMDLs were not developed to address algae impairment and there is no basis for speculating that they would resolve the problem. The Department can avoid listing these waters on its Category 5 impaired waters list only based upon a demonstration that existing effluent limitations are already in place to address the problem. Only then might the Department validly consider listing the streams as impaired but not in need of total maximum daily load development, Category 4.

III. The Department Has Not Advanced Efforts to Monitor or Assess Algal Growth in the Shenandoah River

Despite a long history of evidence that the Shenandoah River is suffering from excess algae, the Department has made no apparent effort to monitor or assess the algae problem in these waters. Our request to list the river as impaired in 2010 was rejected in that year's Integrated Report.[7] In April 2012 Shenandoah Riverkeeper nominated the Shenandoah River main stem, North Fork, and South Fork for inclusion in the Department's annual water quality monitoring plan. The Department rejected this request in July of the same year, citing a lack of assessment criteria.[8] In October 2012 the Department concluded that the Shenandoah River is "having an observed effect of aquatic algae," and on that basis decided to list these waters under VA Category 2B, explaining that "[t]his designation means that these areas will remain a priority for monitoring and assessment in the future and will be evaluated when water quality standards related to nutrients and supporting indicators are available for free flowing rivers and streams." The Department also mentioned that it had received a report from the agency's Academic Advisory Committee outlining a "screening approach" for nutrients, and stated that it was, as of October 2012, reviewing the report to determine its relevance for assessing the presence of nuisance aquatic plants.[9] Yet, more than two years later, the draft 2014 Integrated Report provides no information on what steps the Department has taken, if any, to make monitoring and assessment for algae in the Shenandoah River a priority. We request an update on the Department's efforts as part of the response to comments on this year's report.

[continued next page]

[7] *See* Attachment F (Shenandoah Riverkeeper comments on 2010 Integrated Report).

[8] Attachment G (nomination form and response letter).

[9] Attachment H (2012 Integrated Report, Response to Comments by Shenandoah Riverkeeper).

IV. Conclusion

We have provided overwhelming evidence that the Shenandoah River, including the North Fork and South Fork, are impaired by excessive algal growth and as a result are not supporting their designated use for recreation. We urge the Department to fulfill its duty under the Clean Water Act to now list these waters as impaired in the final 2014 Integrated Report.

Sincerely,

/s/ Jennifer C. Chavez
Jennifer C. Chavez
Staff Attorney, Earthjustice

/s/ Jeff Kelble
Jeff Kelble
President, Potomac Riverkeeper

Sept. 3, 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218
RE: Draft 2014 305(b)/303(d) Water Quality Integrated Report

Dear Mr. Kennedy,

I am writing to ask if you could please add the Shenandoah River Main Stem and both the North Fork Shenandoah and South Fork Shenandoah Rivers to the upcoming Impaired Waters list because of the massive algae problems we are having each year.

I own and live in a riverfront house along the North Fork Shenandoah River at Woodstock in Shenandoah County. I paddle my canoe and kayaks on this river as well as the South Fork Shenandoah River, and the Main Stem Shenandoah River on a regular basis. I work for Shenandoah Riverkeeper and boat somewhere on these 3 rivers at least 5 times a month when the weather permits. I rarely want to swim in the Shenandoah Rivers because the waters in these rivers are green like pea soup. This is most obvious in the deeper pools of the rivers. I have noticed large amounts of algae growing on the bottom in most sections of the rivers. The blue-green algae are the most troubling to me, because they apparently produce toxins. Many towns get their drinking water from the Shenandoah Rivers so the thought of toxic algae in the rivers is very disturbing. I don't see these issues and I feel more comfortable when I swim and boat in the Potomac River above Harpers Ferry.

When the river bottom is covered with algae, along with the wet portions of the boat ramps, it gets very slippery and dangerous for wading and launching boats. I also worry about getting the river water into scrapes and cuts for fear of infections. The floating algae mats produce a nasty smell, and some people have told me they thought it was raw sewage in the river. In the late afternoons and early evenings on late summer and fall days, floating globs of algae nearly fill the river at my house. This discourages me and my guests from swimming in the river, since it is extremely gross when it gets on our bodies and in our hair. I wish the water was clear so my friends and family would want to come to my house to swim in the river. This isn't just a problem at my house at Woodstock. I know this is the case everywhere on the Shenandoah River system. I have seen the thick algae and the green water from Fulks Run to Front Royal on the North Fork. The same problems are present on the South Fork from Port Republic to Front Royal and the Main Stem Shenandoah from Front Royal all the way to Harpers Ferry.

I have flown in airplanes over the confluence of the Shenandoah River where it meets the Potomac River in 2011 and in 2013 and photographed the rivers. The difference in the waters of these two rivers is quite dramatic. What I have described is most evident when you see the pea-soup green Shenandoah River mixing with the clear Potomac waters as it leaves Harpers Ferry on its way to the Nation's Capital. It is sad and embarrassing that Virginia cannot do something about this. I could send you a photo of the Harpers Ferry confluence if you haven't seen what I just described.

When the algae are blooming, I find it difficult to enjoy the river. Please add all 3 sections of the Shenandoah River to the 305(b)/303(d) list and try to help solve the problems we are having with algae here in the Shenandoah Valley. Thank you for reading and considering my comments.

Sincerely,

Alan Lehman
84 Chapel Drive
Woodstock, VA 22664
540-542-9926
lehman_4@yahoo.com

April 10, 2012

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, VA 23218

RE: Draft 2012 305B/303D Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

This letter is meant to show my support for adding the Shenandoah River and her North and South Forks onto the 2012 305B/303D Water Quality Assessment Integrated Report because of the persistent algae problems I see while boating there. I have been an employee of Shenandoah Riverkeeper since October of 2009, and have worked as the agricultural manager. I kayak the river as a part of my work responsibility, but have been a lifetime recreational user of the North Fork, South Fork and Main Stem of the Shenandoah as well as most of the floatable major tributaries such as Cedar Creek, Passage Creek, North River, South River, Middle River etc. Outside of my work duties I continue to use the river on a nearly weekly basis taking my family and friends on float trips.

I regularly kayak and canoe on the Main Stem Shenandoah River and both the North and South Forks with my friends and family. Our family (my brothers and myself) owns 10 kayaks and 3 canoes, which we use for recreation on the rivers in the Shenandoah Valley. My wife grew up along the South Fork in Rockingham County. We usually see algae in one form or another on these waterways. We try to get our children involved with our paddling adventures. But when the algae blooms are at their worst in July and August, my daughters resist my invitations because they don't like the smelly soup that is floating in the Shenandoah. They would prefer we travel farther to the Potomac Highlands or elsewhere for cleaner water, since they like to also swim occasionally, while boating on hot days. We fear our children will choose other activities that don't involve the rivers at all unless conditions improve.

I've seen globs of floating algae on the back eddies and channels on the North Fork Shenandoah River in March and April of this and past years, near Woodstock, New Market and Toms Brook. In May and June of each of the past few years, I've seen filamentous algae on the bottom of the North Fork near Toms Brook and Strasburg, and also on the bottom of the South Fork near Island Ford, Elkton, Shenandoah, Luray, and on the Main Stem Shenandoah River near Morgans Ford Landing, Rt. 50 and Rt. 7 in Clarke County. I've seen the smelly floating algae on the North Fork in July, August, and September near New Market, Mt. Jackson, Edinburg, Woodstock, Strasburg, Toms Brook, and Riverton. This smelly floating algae is also persistent on the South Fork in late summer around Port Republic, Island Ford, Elkton, Shenandoah, Newport, Alma, Luray, and near the Andy Guest/Shenandoah River State Park in Warren County. I have also seen it in large globs on the Main Stem Shenandoah River in late summer in Clarke County and all the way downstream to Harpers Ferry.

I flew over Harpers Ferry with a pilot friend in his small airplane last August and noticed how the Shenandoah River looked like a green pea soup compared to the clear water of the Potomac River at the confluence. I could see the river bottom of the Potomac River, but the bottom of the Shenandoah River was not visible through the murky green water. We could see them mix together downstream of the confluence as the Shenandoah River soup slowly got diluted by the clear Potomac. This is rather disconcerting since we get our drinking water from the North Fork Shenandoah here in Winchester.

I feel this algae plague has diminished my family's enjoyment of the Shenandoah River and her Forks in the Shenandoah Valley. Please include these precious rivers on your 303D/305B impaired waters list, and do everything you can to help return them to clear flowing waterways.

Thank you for reading my opinion.

Sincerely,

Alan Lehman

1314 Vanceright Circle

Winchester, VA 22601

lehman_4@yahoo.com

Allan Thomson

709 Cargil Lane

Charlottesville

VA 22902

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Sir,

As a frequent user of the Shenandoah River, I have noticed that there is often in the spring and summer a slimy mat of algae covering the rocks and native grasses which makes the river not only unsightly but also hazardous to walk in. This is especially true in the North fork and the main stem north of Front Royal. I have been told that this algal bloom is caused by excess fertilizer and manure seeping into the waterway which is a severe deterrent from using the river for recreational purposes with friends and family.

I hope that there is something you can do about this to restore the river to being the wonderful resource for recreation and tourism that it should be.

Yours Sincerely

Allan Thomson

Amy Mrstik
220 Silverleaf Drive
Sterling, VA 20164
Gr8imon@Gmail.com

16 September 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to express my concern about water quality on the Shenandoah River. On the 4th of July, I floated the Shenandoah River with my husband Rick near Andy Guest State Park. It was my first time ever floating a river and I was nervous about it. By the time we stopped for lunch, I had settled down enough to fish a little bit.

As we approached the lower end of our trip, near Front Royal, we stopped so Rick could spend some time in an area he said looked fishy. But I noticed the area was full of dark green algae, and it smelled way worse than fishy. I didn't want to get into the water here because of the smell, so I took pictures of some wild flowers growing along the bank. Rick waded in but soon complained that his lure was getting full of algae and his favorite fishing shirt was getting stained green. I was never able to get that gunk completely out of his shirt. The presence of the algae was the only real negative of the trip, but it certainly diminished my enjoyment of the river.

The Virginia Department of Environmental Quality should include the South Fork of the Shenandoah River on its impaired waters 303D/305B list and look for ways to clean up the algae and improve water quality on the river.

Very respectfully,

Amy Mrstik

Andrea Young
604 Boyle Lane
McLean, VA 22102
dreayoung@gmail.com
301-580-9119 mobile

September 7, 2014

Mr. J. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear. Mr. Kennedy,

I recently had the opportunity to visit the Andy Guest Shenandoah River State Park. I had been there a while ago as a member of the DCR Board and was excited to visit as a VA citizen.

I was stunned to learn the severity of the algae problem in the Shenandoah. Most distressing is that the presence of toxic algae, according to what I am reading and hearing from algae scientists working on the River, is increasing dramatically, and unpredictably. In other words, this is a potentially severe and poorly understood phenomenon that poses serious threats to our drinking water, recreational activities, and quiet enjoyment of the river.

I had planned to take my two young grandsons with me for their first fishing trip on the Shenandoah this weekend. We are 'babysitting' them while their parents are away, and I thought this would be a wonderful introduction to the beautiful wilds of the Valley. We went to the museum instead. Although both my husband and I are accomplished boaters, the thought of the boys tumbling into a river that, despite her beauty, harbors lethal toxins, was too much for their parents. This brought us great sadness, and we felt robbed of a wonderful experience with our grandsons.

I am particularly concerned about the probability that toxins from algae are a) not well understood, b) affecting large numbers of people more and more frequently without warning (Toledo is one example) and c) not screened during purification processes at our utility facilities. The presence of algae in the Shenandoah has, in real time, both diminished our enjoyment and diminished our use of this Virginia Treasure.

I am particularly saddened by the thought that our magnificent Shenandoah, subject of song and lore, is one of the rivers most impaired by this creeping menace in Virginia.

I ask you to please list the Shenandoah as 'impaired' and to begin work on remediation. If ever a river served as an icon for Virginia, then this is the one.

And perhaps, before I get too old to climb into a canoe, my grandsons will be able to safely join me on a journey that all of us will treasure.

Thank you for your help in this matter.

Jeff Kelble <jeff@potomacriverkeeper.org>

Draft

Andrew Riccobono <ariccobono@yahoo.com> Fri, Apr 13, 2012 at 9:01 AM
Reply-To: Andrew Riccobono <ariccobono@yahoo.com>
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>

Jeff,

[Quoted text hidden]

==========

401 Sherrow Avenue
Falls Church, Virginia 22046

April 12, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

I am writing to you today on behalf of my family and our concern about the Shenandoah River and it's status on the 305B/303D list. I have been canoeing and fishing on the river since the 1990s and have grown increasingly concerned about our stewardship of the river.

The condition of the river, in my experience, has steadily declined since I first started fishing the South Fork of the Shenandoah. Back then the water was cleaner, the fish were bigger and healthier, and there was rarely any algae at all. In fact, the entire river was so clean that it looked like an aquarium. The conditions started to decline well before the fish kills in 2005.

I still regularly fish the for smallmouth bass and panfish at the Shenandoah River Andy Guest State park near Bentonville and my experiences from spring through summer have become alarmingly predictable. In the spring, when the water is a little higher and a little cooler, the river remains pleasant to fish. By July my flies are covered in green muck after every cast – whether I am fishing on the surface or with a sinking lure. When the algae die off, the decomposing clumps smell pretty nasty. As a result, I will try and fish the spring creeks of the Shenandoah National Park or travel to fish in Pennsylvania or New York where the water remains cooler, cleaner, and more pleasant.

The Shenandoah is an important resource to the Commonwealth. We should do much more to protect it. Please add the Shenandoah River to the 305B/303D list. It is time for us to find out what is causing all this algae and fix our river.

Yours truly,

Andrew Riccobono

John M Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond VA 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am a long time resident and nature photographer in Shenandoah County. The Valley and the beautiful Shenandoah River have been something that I have enjoyed for decades. Several times a year, I will go to the river, either by myself or with friends and/or family to enjoy it. Canoeing is my favorite activity but also swimming, wading, wildlife watching and fishing are things that I enjoy with my daughter as I did when I was her age growing up in Mt. Jackson.

Problems affecting the health of the Shenandoah River have been around for a very long time. On many occasions over the past several years, algae blooms have been observed from Deer Rapids to the Strasburg town park. I live near Strasburg and this stretch of the river is one that I canoe many times. The algae on this section is worse during the summer months.

In 2009, I canoed the entire length of the North Fork and main stem from Fulks Run to Harper's Ferry at its confluence with the Potomac River. During that time on the river in April and early May, I witnessed something at the area known as Shenandoah Shores on the main stem below Front Royal. There were large clumps of green and brown stuff that were floating around. As I passed, the clumps had a sewage-like stench that could be smelled.

Due to the algae that has been occurring in the river for many years, I try to avoid the areas that are plagued by the algae during my visits or I just avoid going to the river altogether. I am only one person with one family and the algae has certainly diminished our enjoyment and our experience of the river to the point where we have cut back on our use of the river. I am sure many other people, locals and tourists, also feel this way and are affected in a similar fashion.

Because of the algae pollution that is affecting the quality of our environment, I am requesting the Virginia Department of Environmental Quality to have the Shenandoah River (North Fork, South Fork and the main stem) be included to the impaired waters 303D/305B list. I believe it is important that the DEQ study this unique algae problem found in the valley then solve it in order to clean it up.

The Shenandoah River has been romanticized and immortalized through songs, poems and verse. The health of the river is important to the wildlife that live within its ecosystem, to the local economy that depends upon the dollars that recreation on the river brings in and the communities that use it for consumption. We should clean and protect this great Virginia and national treasure that is our Shenandoah River.

Andrew Thayer
361 Little Sorrel Drive
Strasburg, VA 22657
(540) 465 – 8266

<<CONTINUED>>

ANTHONY MORRIS
EVERGREEN
P. O. BOX 560
MADISON, VA 22727-0560

E-mail ANTHONYMORRIS@NEXET.NET

April 2, 2012

Mr. John M. Kennedy
DEQ Office of Water Monitoring and Assement
P.O. Box 1105
Richmond, Va. 23218-1105

Re: Draft 2012 305(b)/303(d)
Water Quality Assessment Integrated Report

Dear Mr. Kennedy, I have fished all my life, worked on streams and Rivers, life long member of Trout unlimited including several committees.

Today, I am writing about the Shenandoah River, and the decline of the enjoyment. I fish near Elkton Va., both up and down river (north & south, and prefer to wade, as well as small craft. Recently, it is quite slippery with the Filamentous algae. "rock snot", as it is called, causes danger, and takes away the joy of fly fishing, or spin fishing. There is an odor as the warm days approach and it is plain to see, that some farms, chicken and other have damaging amounts of excrement washing into the River. I could write a volume of problems, but that would be boring! Let me close by saying, when you factor in, smell, algae, the concern for sickness, (I no longer would bring a pet) the River decline removes a way for families to spend time together. Please consider the Shenandoah as if it were a part of your life. Thank you, Anthony Morris

Mr. John Kennedy
Virginia Department of Environmental Quality
Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105

April 9, 2012

Subject: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

I am writing to request that the Virginia Department of Environmental Quality add the North Fork Shenandoah River, South Fork Shenandoah River, and main stem Shenandoah River to the 305(b)/303(d) list of Virginia's impaired waters.

I am an angler and naturalist who buys annual out-of-state Virginia fishing licenses each year. I have floated and fished the Shenandoah River in late spring and early summer, normally near Front Royal and Luray and downstream near the Shenandoah's confluence with the Potomac, for over 30 years. Over the past 5 years or so, the increased predictability of blooms of planktonic and filamentous algae throughout the Shenandoah River system has caused me to avoid the Shenandoah and either go further afield in Virginia or stick to Maryland waters. Excess algae makes river use unpleasant for me by making rocks excessively slippery, fouling lines and lures, and causing the river to look polluted and "dead." The now-routine occurrence of spring and early summer fish kills in the Shenandoah, which are apparently tied to algae blooms and nutrient enrichment, further inhibits my desire to travel to the Shenandoah.

The occurrence of algae blooms and fish kills on the Shenandoah, and using my valuable recreation time to travel to the Shenandoah only to have these environmental insults impinge on my recreation, significantly diminishes my use and enjoyment of that waterway.

The Shenandoah River system's worsening water quality now routinely makes the local and national news and is therefore common knowledge to fishermen and other river users throughout the mid-Atlantic.

The degradation of the Shenandoah's waters needs to be addressed by the Virginia DEQ as quickly as possible by adding the North Fork Shenandoah River, South Fork Shenandoah River, and main stem Shenandoah River to Virginia's 305(b)/303(d) list of impaired waters so that appropriate studies can be conducted and solutions can be found.

Thank you for your attention to this important issue.

B. Peter Yarrington
1809 Crystal Lane
Silver Spring, MD 20906
301-871-3120

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Hello,

I run a local non-profit that removes physical pollution from the river (tires,cars, culverts,stoves, etc,) and I am also a life long fisherman and kayaker of the Shenandoah River. I use the Shenandoah many times a week pretty much year round. I have observed a mass problem with the content of algae growth and contamination over the course of time and I have also noticed that it gets increasingly worst as the year goes on then reappears thicker the next year. I spend most of my time on the North fork of the Shenandoah from New Market to Front Royal, but I have also noticed this issue on the South Fork as well. This seems to start in about May and seems to worsten untill the river is unbearable to use around July. I don't know how much time to spend on the river, but I spend quite a bit with many people and this is a universal problem that seems to be the tip of everyones discussion when it comes to outdoors recreation at these times in the year. The algae is so thick in spots that it becomes a solid mass at the top of the water and one could hardly get a canoe to glide across it. I assure you that most of the public that uses the Shenandoah for whatever they may recognizes this as an issue if not only an eye sore but also a safety issue due to lack of visibility (and trust me there are plenty of sharp man made things of the bottom of the river to cut your self on) and you should do the same and include this on your impaired waters 303D/305B list. Recognize the problem and please do something about it. This stuff isn't going to get rid of itself.

Beau Morgan
President Earth Korps Inc.
93 Short Mountain Road.
Edinburg VA 22824
540-335-8144
earthkorps@yahoo.com
www.earthkorps.org

Jeff Kelble <jeff@potomacriverkeeper.org>

Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Lienard, Benjamin <benjamin.lienard@sesolutions.com> Sun, Aug 31, 2014 at 11:19 PM
To: "jeff@shenandoahriverkeeper.org" <jeff@shenandoahriverkeeper.org>

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, Virginia 23218-1105

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Mr. Kennedy

In the past I have used the river 2-4 times per month to fish and wade with family, including small children although that has diminished in recent years due to the algae. The majority of my river access is from the town of Shenandoah to the public ramp just before the Shenandoah River outfitters. The primary impact has been a deterrent to want to use the river, the algae is a deterrent to the kids wanting to get in the water (e.g. looks more inviting for reptiles than people) and impacting the ability to share in a family tradition of fly fishing on the river and making it harder to navigate the river when water levels drop. It is unfortunate that the algae has become a deterrent to wanting to use the river. The kids have become turned off to the idea of wading in the river and fishing becomes a waste of time at points which has greatly diminished my enjoyment of the river.

I support the initiative and would request that VA DEQ include the North Fork, South Fork and/or Main Stem on its impaired waters 303D/305B list as it seems to be worse on the Shenandoah than others rivers.

Very Respectfully,

Ben Lienard

14201 Helmsley Rd.

Midlothian, Va 23113

Bernard C. Nagelvoort
3043 Parshall Road
Berryville, VA 22611

April 13, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

Please be aware of the very serious condition of the main stem of the Shenandoah River as indicated by the presence of an excessive brown algae presence for most of last summer seriously reducing visibility in the river even during periods of low flows when it would normally appear to be very clear. This was the most unusual and most serious indication of excess nutrient/pollutants I have seen in the River in the twenty four years I have lived on property bordering the River with 400 feet of river frontage.

As an avid fisherman, I am also discouraged by the dramatic reduction in both small mouth bass and red breast sunfish in my stretch of the river as a result of the fish kills in recent years.

This beautiful River has been subjected to gross mis-use over the past 100 years from industrial and agricultural run-off along with inadequate municipal and industrial wastewater treatment and urban run-off. Its potential for healthy recreation has been substantially diminished over time and the condition last summer is a powerful indication that conditions are not improving.

As I look at the River today with the unusually warm spring we've experienced and modest rainfall, I'm inclined to believe the bad algae conditions are again appearing and will diminish the attractiveness and health of the River yet another summer.

Please accept this letter as an indication of my support for whatever actions DEQ may be able to take to develop and implement plans to reduce the pollution problems causing these serious degraded conditions in the River.

Sincerely,

Bernard C. Nagelvoort

Jeff Kelble <jeff@potomacriverkeeper.org>

River algae

Bud Nagelvoort <bernardc@shentel.net> Sun, Aug 5, 2012 at 4:55 PM
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>

Jeff:
Took a grandson down to the river at my place Friday AM early. Water was coming up some and slightly discolored, but the problem was the stringy algae. We could not fish streamers, only poppers. I haven't been on the river in two weeks so was not aware of the algae. I don't remember anything like this stringy stuff before.
There is some rooted vegetation, but not much and it hasn't reached the surface at my place.
May fish above White House Wednesday.
Bud

Bernard Griswold
829 Hickory Lane
PO Box 267
Woodstock, Virginia, 22664
April 12, 2012

Mr. John M. Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2012 30(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

I am writing to request that Virginia's Department of Environmental Quality add the Shenandoah River system as impaired waters to the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of severe and growing algae growth and blooms each year.

I have had riverfront property directly on the North Fork outside of Woodstock since 1991, first as a weekend retreat, then as a permanent new retirement home on expanded land holdings since 2002. Therefore, I have had intimate contact and use of the North Fork for over 20 years, mainly through floating, fishing, some swimming, and regular environmental monitoring both for water quality and benthos for the Friends of the North Fork of the Shenandoah River. Our home sits on a 25-foot bank with deck directly above the river affording a close up river view 24/7. My use of the river includes primarily the stretch from Edinburg to Strasburg. I am amazed at the changes in indicators of water quality over these 20 years, accelerating in the past 6 or 7.

The visible issue is primarily an increase in attached algae, both type and quantity. However, during dry low water spells, planktonic algae also increases dramatically to the point that it covers and clogs grass beds from shore to shore. Fifteen years ago, vegetation in this area consisted primarily of rooted grasses which provided cover and food for a variety of river creatures, especially from mid-June through early fall. Now, beginning in early June, rooted filamentous brown algae begins to coat rocks and rubble in pools and runs and increases by mid-August to provide floating clumps of brown gunk in such quantity as to collect in masses around any object at the surface. This has resulted, in recent years, in much reduced use of the river in late summer and early fall for all our activities and provides a real eyesore from our vantage point on shore. It also provides a severe odor problem during hot dry, low water periods in late summer. It is obvious, from these indicators over a 20 year time frame that significant changes are occurring in water quality that are not indicative of a healthy ecosystem.

Thus, I am asking that DEQ include the Shenandoah River in its 2012 Water Quality Assessment Report to study this issue carefully and develop a plan and strategy to reverse these issues. The Shenandeoah River is historically a national icon and conserving it as a healthy ecosystem should be paramount as an environmental goal.

Sincerely,
Bernard Griswold

April 13, 2012

John M Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond Virginia 23218-1105

Dear Mr. Kennedy,

My son and I fish the Shenandoah River several times a year. The Shenandoah is a wonderful gift and treasurer to be appreciated. However, each year for the past several years we have noticed more and more as well as larger and larger algae blooms. The blooms turn the river a dark murky green color, almost like green paint. Where there are blooms there are less fish and when there are fish there are less of them. The algae sticks to our fishing line, clings to the bottom of our boat, smells bad and looks bad. In short, algae blooms and all there ill side effects have diminished our enjoyment of the river and as a result we find ourselves using the Shenandoah less and less every year. We have heard from other fisherman that during July and August last year the blooms were so thick and had such a bad odor that the areas of Woodstock, Strasburg, Front Royal and Goods Mills were so unpleasant due the algae that they were unfishable.

Please include the three sections of the Shenandoah River on the 305B/303D list to help determine why we are having these algae problems and to develop and implement a plan to make them go away.

Thank you in advance for considering my comments and we both look forward to the day when the Shenandoah is restore as one of our nation's great fisheries.

Bill McGrath

6402 Broad Street

Bethesda Maryland 20816

Duncan McGrath

150 Franks Street

Fayetteville West VA 25840

April 6, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the North Fork, South Fork and Main Stem of the Shenandoah extensively and I also canoeing and swim on the South Fork. I have been doing this for 40 years. However, recently, for much of the year, large sections of each river experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event. Over the course of the year I use the river system from Compton Rapids to Front Royal and from Front Royal to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to swim or fish and I don't go swimming because they don't like the off-color murky water. When I do fish I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, the fish are usually lethargic and often they don't feed at all. For the past two years I simply could not fish the lower south fork or the entire Main Stem from July through August because of the plankton problem.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulate on anything sticking out of the river and in back eddy. This algae smells like sewage or rotting broccoli. When these algaes are blooming, the fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I just cannot use the River due to the odors and annoyance. I found this problem in the following areas last year from July through August: Strasburg, Bentonville, Front Royal, Luray Dam, 211, Shenandoah, Route 50, and Route 7.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river from Broadway to Strasburg. All three of these algaes make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

This annual cycle of algae severely diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305(b)/303(d) list, determine why we are having these algae problems, and to develop and implement a plan restore the health and beauty of our Shenandoah River system.

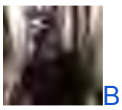
Thank you for your consideration,

Bill Millhouser
3325 Prince Charles Ct.
Falls Church, VA 22044
Email: bmillhouser2010@cox.net
Phone: 703-532-2446

Jeff Kelble <jeff@potomacriverkeeper.org>

comment on Facebook re algae

Robin Broder <robin@potomacriverkeeper.org> Fri, Apr 27, 2012 at 4:09 PM
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>


Bill Minarik
Jeff Kelble is right in pointing out this growing algae problem.

In past years I loved to camp for several days along the Shenandoah and catch 40/50 fish on the fly from my kayak. Two years ago I brought 22 people along and was very disappointed that the alga was horrible and fishing had all but disappeared. Last year was more of the same. The Shenandoah has gone from one of America's great treasures to something that smells as bad as it looks.

For many of us this is the tipping point and action needs and will be taken to make it right. What runs off of our lawns, driveways, houses, roads, farms, etc. winds up in our streams. We can and do make the difference.

--
Robin Broder, Vice President
Potomac Riverkeeper, Inc.
1100 15th Street, NW, 11th floor
Washington, DC 20005
ph 202-222-0706 * fax 202-783-0444
robin@potomacriverkeeper.org * www.potomacriverkeeper.org
www.facebook.com/PotomacRiverkeeper * www.twitter.com/PotomacRiver

SAVE THE DATE! Set Sail for Clean Water, April 28th, Potomac River Gala, June 10th, House of Sweden, & Shenandoah River Rodeo, July 14th

Recognized as "one of the best small nonprofits" by Catalogue for Philanthropy
CFC #87828 * New EarthShare Member #87828

Members of the Virginia Department of Environmental Quality:

I am writing this letter in support of listing the Shenandoah River as a Endangered Waterway. During the past 25 years, my children and I have used the Shenandoah for swimming, kayaking, and fishing. They are grown now, and are off to live in other parts of Virginia. They would be sad to see the condition of the Shenandoah as it is now. Through the years I have seen a steady decline in the quality of the water.

Gone are the days when the Shenandoah was a clear river full of wonderful smallmouth bass of huge size.

Because of the string of years with fish kills, I have limited my use of the Shenandoah during the past five or six summers. Something is killing those fish. Whatever it is couldn't be good for people either.

Last summer, however, I decided to enjoy the river again. I fished the river at least ten ten days. I found it in worse shape than ever. There was a summer-long algae bloom on the Main Stem. The water was an olive-drab green rendering the water nearly opaque. Gone were the lush grass beds that sheltered smaller fish and enriched the river's water with oxygen. Fishing was poor, and I certainly didn't want to stand in that water to fish, let alone swim in it.

Others seem to have found problems with the river, too. There were several days on the Shenandoah last summer when I saw not a single other angler on the river other those with me. The few anglers I encountered reported catching few if any smallmouth bass on the Main Stem, and those caught were of small size.

One day I fished the Main Stem with a friend, an experienced angler, who had never fished the Shenandoah. He was struck by the awful condition of the water. He's not fished the Main Stem since, but continues to tell me of wonderful fishing in less degraded waters elsewhere. Why would he want to spend his fishing money here?

It is obvious to anyone who has spent time on the Shenandoah that the river is in serious trouble. With the help of the DEQ, the Shenandoah can come back to health. Recognizing the serious nature of the problem is a necessary first step.

I hope one day in the future I will be able to enjoy the Main Stem with my grandchildren and show them a river about which we call all be proud.

Sincerely,
Bill Prokopchak
1714 Lewis St.
Winchester, VA 22601

April 18, 2012

FRIENDS OF THE RIVERS OF VIRGINIA FORVA

FORVA DIRECTORS

William Tanger, Chair
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Rick Roth, Treasurer
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Float Fishermen of Virginia
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Erin Miles
Citizens for the Preservation of the River (CPR)
Donnie Mohler
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Trace Noel
Virginia Paddlesports Association
Cole Poindexter
Staunton River Watch
George Santucci
National Committee for the New River
Tom Stutts
Friends of the Staunton
Steven Tingler
Headwaters Watch
John Tippett
Friends of the Rappahannock
Charles Vandervoort
Friends of the Shenandoah
Jeff Wold
Float Fishermen of Virginia

August 19, 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

My wife and I canoe the Shenandoah River once a year or so and I have experienced algae blooms that make it sometimes pretty unpleasant. On hot days if the water is not gunked up with algae we might swim, but only if the water is clear.

My wife and I boated more in the past, but due to fish kills and algae, we tend to go elsewhere. Obviously, algae blooms have diminished my enjoyment of boating on the Shenandoah.

So we would like to request that VA DEQ include the North Fork, South Fork and Main Stem Shenandoah on its impaired waters 303D/305B list.

Thank you,

Bill Tanger email: bill.tanger@verizon.net Cell: 540-266-0237
Chair
FORVA
PO Box 1750
Roanoke VA 24008

Shenandoah Algae – FORVA to DEQ 8-20-14

BOYD W. POST
12158 Cathedral Drive
Lake Ridge, VA 22192

April 6, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P. O. Box 1105
Richmond, VA 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

This is a request that the Virginia Department of Environmental Quality to add all of the Shenandoah River (North Fork, South Fork, and Main Stem) as impaired waters on the 2012 305(b)/303(d) Water Quality Assessment Integrated Report because severe algae problems have occurred over the past several years.

I frequently canoe and fish the above mentioned streams. Very often I find the river so greatly affected by nasty algal blooms that my enjoyment of being on the river is severely impacted and diminished. It is really disgusting to experience retrieving a lure covered with rock snot and have to clean it off. The thought of what that is doing to the fishery is frankly depressing. At other times I experience floating clumps of apparently rotting algae that smell like sewage. This is even more disgusting than the rock snot from the bottom rocks.

I believe the State of Virginia has the responsibility to correct these problems so we citizens can again enjoy this very wonderful resource without the vexing algae problems. Therefore I am asking that DEQ include these mentioned parts of the Shenandoah River in the 305(b)/303(d) list so that a solution to these algae problems can be achieved – the sooner, the better!

Thank you for your consideration of my request.

Sincerely,

Boyd W. Post

Dr. Boyd W. Post
Email: boydpost28@comcast.net
Telephone: 571 408-4227

August 26, 2014

DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report
Dear Mr. Kennedy,

My name is Brian Trow, and I am co-owner of Mossy Creek Fly Fishing in Harrisonburg Virginia. I have been in business for over eleven years, and have one of the largest guide services in the state. I personally float and guide on the Shenandoah River over 75 days a year. I have seen many changes in the 25 years that I have been fishing the river. We experience fish kills annually, some small, and some massive. I have seen use of the river increase, from towns adding a water intakes for drinking, to increased recreational use. I have witnessed stronger efforts by farmers, industry, developers, and towns to work towards solutions that benefit the watershed.

In spite of the efforts there is definitely a "growing" problem that everyone who is in touch with river notices. Algae has been on the river in certain places, and during certain months for as long as I have been fishing it. The last few years we have noticed a huge explosion in the algae. We being, my guide staff, who is on the water more than anyone I know, and our clientele. Our clientele is made up mostly of Virginians from Richmond, DC, and Tidewater, but also many out of staters. There is algae on stretches of the river where we have never seen it before. There are huge mats that cover the sub straight for miles. There is floating algae that can be so bad, that you can't cast a lure or swim without getting covered. We cover the South Fork from Port Republic to Luray. We also fish stretches of the main stem and North Fork.

Half of the beauty of floating the rivers of our state is underwater. Looking into a river and seeing nothing but green water, brown and green rocks, and smelling the awful smells of rotting algae is very discouraging. We already have to deal with poor water quality that takes trophy bass from us every year, and now we can't even enjoy the beauty of looking into the river.

I guide and fish on many other rivers in the state including the James in central Virginia, The Rappahannok, the Cowpasture, and the New. All of these drainages have algae, but not nearly to the degree that the Shenandoah does. Please consider adding the Shenandoah and it's forks to the impaired 303d/305b list. I'm sure you have received many letters of concern on this matter, but for my guides, and my business, there is a much higher level of concern. You don't need 75 days on this river to know that it is sick, and needs help. I would offer a float to anyone in your office who is concerned, and holds the power to make this change. This is a real environmental quality issue that isn't going away, and it can be fixed!

Thanks for your consideration,

Sincerely,

Brian Trow

Mossy Creek Fly Fishing
1790 East Market Street
Harrisonburg Virginia 22801
Store@mossycreekflyfishing.com
Brianetrow@gmail.com
540-434-2444

April 8,2012
Mr. John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing this letter to request that your organization add the North Fork, South Fork and Main Stem of the Shenandoah River as impaired waters on your Water Quality Assessment integrated Report because of the very severe algae problems evident in the river every year.

I fish for small mouth bass in all portions of the river, particularly the North Fork near Edinburg, and the main stem down river from Route 50. I also fish the South Fork near Bentonville. I am experiencing a lot of frustration as the temperature rises during the summer months with regard to cloudiness of the water, and the algae blooms that cause the fishing to be difficult, and the river bottom to be slippery and the surface of the water to be unsightly. This lasts until the fall. It is getting to the point that I do not find it much fun anymore. I cannot even run a lure through the water because of this problem. Also, I am beginning to wonder if there are some things in the water that might affect my skin, should I continue to come in contact with it.

I keep hoping for some improvement, but over the years the problems continue. They continue to the point that it my use of the river will diminish accordingly. It is a shame because it is so beautiful but not so pristine, as it used to be.

Accordingly, I am repeating my request for you to include the waters of the Shenandoah on 305B/303D list to determine why the river is having these problems.

Thank you for taking the time to read this letter. Hopefully, if addressed a plan can be developed and implemented to make the problem go away and make the river more enjoyable for me and future generations.

Sincerely,

Carl Cisky

Carl Cisky
13113 Applegrove Lane
Herndon, VA 20171
Tel. 703-476-2391
Email: cfcisky@verizon.net

April 5, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the North Fork, and Main Stem of the Shenandoah extensively during the summer months for more than 40 years. Recently, however, for much of the summer, particularly the warmer months, large sections of the river experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event, but not always. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to fish as I don't like the off-color murky water. When I do fish I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire Main Stem from July through August because of the plankton green water.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algaes are blooming I literally do not want to be anywhere near the river and I choose to go somewhere else. I have found that if you do fish; the fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance. I found this problem in the following areas last year from July through August;, Deer Rapids, Edinburg, Woodstock, Strasburg, Front Royal, Goods Mill, Shenandoah, Route 50, Route7 among other places.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river from Broadway to Strasburg. All three of these algaes make it nearly impossible to fish and again diminishes and many times eliminates my ability to enjoy the river.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.
Thank you for considering my comments,

Carl Onesty 703 978-4684 conesty@aol.com
8529 Pappas Way
Annandale, VA 22003

April 18, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I was born and raised in Northern VA and lived in this wonderful state my whole life. When it came time for my wife and me to buy our first house we both decided that we would like to settle and make a home in the Shenandoah Valley. So we could be closer to the things we love, for me that was mostly the Shenandoah River. From the early spring to late fall I enjoy floating, fishing and swimming in the river. I take my dogs down to the river for a swim from time to time and I use to take my lab mix fishing with me before she got too old to handle a long day on the river.

I have seen in recent years when on the river that the water has an odd dark greenish color that seems almost like it could glow in the dark to it in the spring. I have witnessed this just last weekend on 4-15-12 when I had floated from Rt.50 to a takeout 4 miles downstream. Then on other floats I have taken in the summer between July and August on the main stem at the low water bridge at Morgan's Ford and between Rt.50 and Rt.7, as well as the South Fork between New Port and Alma also as far up river as Kara there are tons of large clumpy pods of algae that are so bad that it looks like you could walk across it. It is very hard to float this because basically you can't float on top of these algae clumps very well. It has also make it very hard to fish when every cast I'm clearing the algae from my line or lure. Also during the summer there is a strong smell that is not enjoyable at all. This smell seems to stay with you because it is on everything from my canoe and everything I take fishing. I have to spend hours cleaning my gear and bathing my dogs after a trip.

This cycle of algae has diminishes my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305(b)/303(d) list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.
Thank you for considering my comments,

Mr. Charles V Loudermilk II
150 Travis Court
Winchester, VA 22602
Email: Charlie@winchestertu.org
Phone: 571-236-5640

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing this letter today to request that VA DEQ include the North Fork, South Fork and Main Stem on it impaired waters (303D/305B) list because the algae problems have to be addressed.

I'm a fisherman and enjoy fishing and camping along the North Fork, South Fork and Main Stem of the Shenandoah River. I mostly use South Fork and Main Stem between spring, summer and fall, but am always taking long drives to the river to ward off cabin fever during the winter months. For about the last 6 plus years it seems my enjoyment is diminishing because of the algae bloom and sewage smell that it gives off.

I can recall a float from Alma to Whitehouse on the South Fork in July of 2012 that there were section of the river that the algae was so thick that that my canoe in about 2 foot of water would get stuck. I had to use my oar to push myself off the long strings of algae in these sections. I would wade Edinburg area or should I say tried to wade this area. It was very hard to even get in the water and move around because the algae were so thick.

If it is not the thick algae causing problems it is the murky, cloudy greenish stain color it takes on. I fished with a friend on July 22 from his john boat on the main Stem and the water was very murky. It seems like this just about every depth of water we were in. I can recall what seems like 15 plus years ago or more that during the middle of summer the waters were clear and you could see the bottom in 5 and 8 foot depths. It doesn't seem to be the case anymore. When I take my niece fishing she like to take brakes and swim along the way, but doesn't that much or for long because she doesn't feel safe if she can't see her feet. I have seen both of these problems at multiple sections of the rivers between Elkton, town of Shenandoah, Alma, Whitehouse, Luray, Edinburg, Front Royal, Rockland, Rt.50, Locks Landing, Rt. 7 and even as far down river to Bloomery WV.

I do believe that the algae problem is worse in our Shenandoah then other rivers because I floated this July with some friends from Southern West Virginia between Pyramid Rocks boat ramp and White House. All of them too notice of the cloudy murky water and asked if this was normally this bad. Their home water the Greenbrier would get that way but not nearly as bad and would likely run almost Gin clear by July and Aug? My answer sad but true was it is this color all the time now.

These algae blooms truly have diminished my use of the river but will not keep me from using the river. I just feel that we as Virginians need to work together to make the 3 rivers of the Shenandoah better for everyone's use. So for all of these reason I have stated above are why I ask that the North Fork, South Fork, and Main Stem be added to the 305B/303D list, recognize that the river has an algae problem and work to develop a plan to solve it.

Charlie Loudermilk

150 Travis Ct

Winchester, VA 22602

Email: loudermilkcharlie@yahoo.com

Phone: 540-678-0986

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

August 25, 2014

Dear Mr. Kennedy,

I am writing to PLEAD Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2014 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I am the owner and one of the head fishing guides for Mossy Creek Fly Fishing in Harrisonburg, Virginia. My twin brother Brian and I opened our shop and guide service in April of 2003. The NUMBER 1 reason for opening our shop in this location was its proximity to the South Fork of the Shenandoah River. We have been fishing the Shenandoah River for 20 years and guiding on it for 15. We are on the river 12 months a year and almost daily in the spring and summer. We have 7 boats and run more float fishing trips on this river than any other fishing guide service. We do target the James River and New River for smallmouth bass and musky, however we consider the Shenandoah River our home water. Unfortunately claiming the Shenandoah our home water is becoming more and more embarrassing each year as we see constant algae blooms, fish kills, disease, foul smelling water, experience waterborne infections, and more. Some of our guests will not return to fish the Shenandoah or our area again as a result of what they see on the water.

I am amazed at what I see each day and each season I am on the Shenandoah River. I actually grew up in Richmond Virginia about 1 mile from the James River. I was used to seeing crystal clear water through the spring, summer, and fall when of course the river wasn't flooding. Despite water temp and flow the James always looked clean and clear. That also holds true for the middle and upper James River where we now guide and fish. The James, New, Rappahannock, Potomac, and many of their large tributary streams run clean and clear most of the year. I can say the same for possibly 1 major South Fork Shenandoah tributary. The upper South River near Waynesboro seems fairly free of algae most of the year. The North River and Middle River, both major tributaries to the South Fork of the Shenandoah, run green most of the year. I have actually never witnessed a day in my lifetime when the Middle River near Verona, VA has ever not been green. I guess the first time I really started to understand what was wrong with the river was back in 2005 when the first major fish kill occurred. I took pictures that spring, March, April, and May. I noticed that the water actually looked like it belonged in a theme park like King's Dominion rather than in a natural environment. The water was actually a leafy green color and you couldn't see more than about 2 feet into it on a good day. Not only was the color horrendous but the river had a very strong odor of ammonia. So strong some days it would make my eyes and the eyes of my fishing clients water most of the day! Other issues occurred later in the summer time when clumps

of slimy algae would detach from the river bottom and rise up to the surface. You can't even cast a line without getting your gear covered in this slimy, liquidy, brown gunk. That same gunk covers much of the river bottom nowadays. This slimy brownish algae is something I have never seen before until the past few years. It detaches in patches up to the size of a door mat most afternoons and floats down the river. In many eddies and log jams this algae piles up into giant greenish brown mats. The smell is horrendous as if a dead animal carcass was encased in it. Now, I remember 20 years ago seeing the river a "Margarita Green", a color term coined by all the local fishermen and fishing guides, on occasion but not all year. From 2011-present(August 2014) our guides have been on the Shenandoah multiple times each week throughout the year. In the spring and summer we have 3-4 boats out daily. Even in December, January, and February, when most rivers are devoid of plant life and algae, the Shenandoah River had no more than 2 feet of visibility ALL WINTER! What is even more frustrating is that the Main Stem Shenandoah from Front Royal North to Harper's Ferry was so discolored all year you could barely fish! The upper James River, virtually the same size as the South Fork of the Shenandoah was running crystal clear as was the New River, Rappahannock, Potomac, Staunton, and more. I have photos of the river from EVERY month over the past 12 years I have been in business and it is difficult to find 1 photo where the Shenandoah River actually looks clean or clear.

Our busy season on the Shenandoah River is usually from May into September. You can expect to see one of our boats on the South Fork or Main Stem Shenandoah 7 days a week through this time period. We see the same algae problems on the river from Port Republic all the way into West Virginia! Clients are always asking us, 'did it rain recently?' 'Why is the river so dirty?' . Again, the embarrassment sets in as we explain the nutrient loading into the river and the algae problems that exist year round! Clients ask us constantly if the water is safe to swim in or even touch. I recommend they do not swim or even touch the water. I have my clients enter the boat at a boat ramp and exit at a boat ramp. At no time during the day do I allow them out of the boat. If they ask to swim I quote what DEQ officials told me at the first Fish Kill Task Force meeting back in 2005….."Do not enter the Shenandoah River with open cuts or sores and NEVER fully submerge yourself in the Shenandoah River". I used this same quote in a discussion with the General Assembly in Richmond back in 2006 concerning the state of the Shenandoah River. So does this algae problem make me less likely to use the Shenandoah River for recreation? Yes. Unfortunately my business depends on our guide services for me to make a living and support my family. So rather than use our home water, 15 miles down the road from our shop, we are fishing more and more on the James River, over 2 hours away. This 4 hour round trip haul costs us about $80 in fuel per day rather than the $15 it costs us to fish locally. However our customers are much happier and feel safer fishing a river that doesn't look as sick as the Shenandoah.

The current state of the Shenandoah River has diminished my enjoyment of the river. My shop staff and guides love this river and we spend time with VDGIF and DEQ volunteering our efforts to help figure out these problems and issues. However watching fish die as a result of unhealthy water and watching anglers become frustrated as they constantly battle with algae on their lines or catching and handling diseased or dying fish is getting too much to handle on a daily basis. I have been to the doctor twice and

my brother once in the past 3 years with bacterial infections we were told were derived from a water source. The most likely culprit was the Shenandoah River as that was the only body of water we were on throughout the incubation time of our ailments. These infections are obviously not a result of algae in the river, but a direct result of an unhealthy waterway. Back in the 90's articles were written about how the Shenandoah River was one of the top rivers to fish for smallmouth bass in the world! I based my business model around this river system and its history of being such a quality fishery. How did we go from a world recognized fishery to a waterway that is unhealthy to even enter? It not only has diminished my enjoyment of the river but has diminished my business and livelihood.

I am now asking that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and develop and implement a plan to make them go away. I currently do not see major issues on our other large river systems like the James, New, or Rappahannock. This is a Shenandoah River problem. This River is the life of the Shenandoah Valley and it is slowly dying before our eyes. Action should have been taken years ago but we are begging that something be done NOW! Thank you for considering my comments,

Colby Trow

Owner Mossy Creek Fly Fishing

Harrisonburg, VA 22801

Email: store@mossycreekflyfishing.com

Phone: 540-434-2444

Jeff Kelble <jeff@potomacriverkeeper.org>

Form submission from: Take Action!- Help the Shenandoah Riverkeeper Get Rid of Algae

Potomac Riverkeeper <sarah@potomacriverkeeper.org> Mon, Apr 23, 2012 at 2:29 AM

To: jeff@potomacriverkeeper.org

Submitted on Monday, April 23, 2012 - 2:29am
Submitted by anonymous user: [166.248.32.86]
Submitted values are:

Your Letter:
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105 Richmond, Virginia 23218-1105

Dear Mr. Kennedy,

I'm writing in concern of the algae blooms consistently occurring in the North Fork, South Fork, and Main Stem of the Shenandoah River, and request the Virginia Department of Environmental Quality (DEQ) lists these waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report.

I rely on several properties of friends to access the main stem Shenandoah for fishing, and often use public access ramps for canoeing with friends as well. Over the past several years I have found my fishing experiences continuing to decrease along the main stem directly because of algae blooms. I have noticed extensive algae blooms occurring in March and April to the point of not even fishing from frustration of cleaning a green fishing lure, then during May I have been catching less fish than I have recorded in past years. I feel my low fish numbers are in direct relation to the death of these extensive algae blooms decreasing the levels of oxygen in the river when decomposing.

I have also noticed there is less aquatic grass establishing itself in the river along my fishing spots after algae blooms. This is likely an additional response to the less dissolved oxygen from decomposing algae, and has also diminished by experiences and use of the river because there is no cover for fish.

As new years come I find myself using the river lesser than previous years, more recently even safety issues arising with the concern for loved ones. The river use to be a great place to spend time with my girlfriend to fish, and introduce the outdoors to my nephew. During the summer the river bed becomes extensively covered with an algae film on the rocks bank to bank. Both my girlfriend and nephew have had problems keeping upright when wading within the river with the rocks so slippery.

My experiences catching fish on the Shenandoah are not near what they were when I were a kid, and these algae blooms are an issue directly affecting enjoyable leisure. Still I hope one day my nephew gets the same enjoyable opportunities to fish the Shenandoah like I had in the past. To restore what the Shenandoah could be I request the DEQ includes all three sections of the river on the 305(b)/303(d) impaired waters list.

Thank you for considering by suggestion and best regards,

Cory M. Miller
280 Buckhorn Road
Middletown, VA 22645
Email: cmiller082@su.edu
Phone: 540-333-7086

Name: Cory M. Miller
Email: cmiller082@su.edu

The results of this submission may be viewed at:
http://www.potomacriverkeeper.org/node/428/submission/830

Jeff Kelble <jeff@potomacriverkeeper.org>

RE; Draft 2012 305(b)/303(d)Water Quality Assessment Integrated Report

bishbox@comcast.net <bishbox@comcast.net> Wed, Apr 11, 2012 at 9:44 PM
To: jeff@shenandoahriverkeeper.org

To John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O .Box 1105
Richmond Va.23218-1105
RE; Draft 2012 305(b)/303(d)Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I have enjoyed owning property on the South Fork of the Shenandoah River for thirty years now and in two weeks I'll be sitting down at a closing table to purchase the land lot next to mine. I am fifty-one years old and hope to retire there someday as I think its one of the most serene and relaxing places in the state of Virginia.

Some years ago I do remember reading that the river had made the list of the COUNTRYS most endangered rivers and these last few years my friends and family has experienced the decline for ourselves.

The fishing has dropped of dramatically, the turtles are gone and birds seem fewer.
There is an algae that seems to be covering the bottom most of the year, I don't think the science community ever solved the "fish-kill" problem, but the bad news just keeps piling on.

This stubborn and horrible algae will hurt tourism, sport, wildlife and all of us who make Virginia our home, as a result I would like to ask that DEQ include all three sections of the Shenandoah River on 305B/303D LIST, determine why we are having these algae problems, and to develop and implement a plan to bring 'The Daughter of the Stars" back to her great self. I have six Grandkids who are going want to go fishing at Grandpa's country house!
Thank You for considering these concerns,

Mr. Craig Bishop
1000 Canoe River Dr.
Rileyville Va.22650

October 14, 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah River as impaired waters on the Draft 2014 305B/303D list. I am an avid fisherman and outdoorsman, and the algae blooms on the river have seriously diminished mine and my family's use and enjoyment of this beautiful public resource.

In 2005 my family and I became property owners along the North Fork of the Shenandoah River on RT 211 between New Market and Timberville. This was our home for well over 7 years. My children and family dog loved to play in and around the river. We would fish, swim and run our dog only when the river water was clear enough, and didn't smell or show large floating blooms of Algae. We found we needed to plan around and navigate the seasonal drift of Algae blooms and bottom rock snot.

When we found out about the 2005/2006 fish kill on the Shenandoah River system, we were shocked and feared using the river. This event greatly decreased our use of the river while we watched what would develop over the next number of months. Being a fisherman, I also visited the South branch of the river system, finding that those parts of the river were dealing with the Algae Blooms on a much larger scale; this being the South Branch between Shenandoah and Luray, and Route 340 south to Elkton and Grottoes. Many attempts to use the river were thwarted, where I found that at access points where the water was low, it smelled foul, and there are large blooms on the edge and the water color was a thick dark green. Other rivers I have fished have not had this degree of impairment.

Our dog loved the water, but would not enter the water in some places, as she could smell the algae, I'm sure. Having experienced this several times, we were inconvenienced to load up and find another entry. Trips to the river became less frequent and our use and enjoyment were diminished. Fishing during the months of winter thru summer (Nov.-July) was difficult to navigate the blooms, especially when trying to land a fish through algae, with the mess and smell diminishing the enjoyment and health of the experience, especially with children watching and trying to fish themselves.

All of these issues and many more caused us to rethink our use of the Shenandoah River. Our beautiful natural resource and Virginia treasure needs to be listed as impaired waters and requires a plan to solve the problem or algae blooms.

Sincerely,

Craig Serrels
1341 Mount Clinton Pike
Harrisonburg, VA 22802
540-405-9283
Craig.serrels@gmail.com

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105 Richmond, Virginia 23218-1105

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2014 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the North Fork, South Fork and Main Stem of the Shenandoah extensively and I also use all three rivers to take my family canoeing and camping. However, for much of the year, large sections of each river seems to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event. Over the course of the year I use the river system and have seen this on the all the segments of the South Fork of the Shenandoah that I float and fish. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to swim or fish and I don't like to take my family swimming because they don't like the off-color murk water. When I do fish I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork from July through August because of the plankton green water. I have never seen these problems on other Virginia Rivers.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algaes are blooming I literally do not want to be anywhere near the river and I often choose to go somewhere else. The fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance. I found this problem in the following areas last year from July through August; Luray Dam, 211, Alma, Port Republic, Shenandoah, Route 50, Route7 among other places.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river. All three of these algaes make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Thank you for considering my comments,

Mr. Dietrich Maune
249 Monument Ave.
Harrisonburg, VA 22801
Email: maunedx@jmu.edu
Phone: 540-433-5364

P.O. Box 1036
Warrenton, VA 20188
Telephone: 540-270-1946
E-mail: douglaslees@comcast.net

August 31, 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

Dear Mr. Kennedy,

I am writing to voice my concerns about the Shenandoah River's water quality and request that the VA DEQ include the North Fork, South Fork, and Main Stem on its impaired waters 303D/305B list.

I fished the South Fork and Main Stem of the river this summer in July and August as follows:

- July 5, Andy Guest State Park on the South Fork near Bentonville, catching about 10 small-mouth bass only 8- to 10-inch in length and no red-breasted sunfish
- July 12, Watermelon Park on the Main Stem, catching no bass and only sunfish
- July 20, mile 18 to 19 on the South Fork near Luray, catching no bass and no sunfish and noticing numerous clumps of foul-smelling algae—this section of the river smelled like an outhouse
- Aug. 2, Andy Guest State Park on the South Fork near Bentonville, catching only 3 small-mouth bass and no sunfish

- Aug. 9, Main Stem upstream of Rt. 50 bridge, catching numerous red-breasted sunfish but only one 12-inch small-mouth bass and a smaller bass, which were both in good condition

I am an avid fly-fisherman who typically fishes the Shenandoah River in a float tube 6 to 8 times a year and have noticed a significant decline in water quality this summer. The algae is observably much worse this year than in previous years. The fish population has decreased significantly. The stench of the algae and reduced number of fish has greatly diminished my enjoyment of the river. I repeat, I would like the VA DEQ to include the North Fork, South Fork, and Main Stem on its impaired waters 303D/305B list.

Sincerely,

Douglas Lees III

Jeff Kelble <jeff@potomacriverkeeper.org>

Algae Letter

Hays Lantz <hlantz4446@gmail.com> Mon, Apr 16, 2012 at 4:55 PM
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>

Hi Jeff,

I just finished conducting a BMI study at Bergton and noted that algae was starting to grow in the North Fork near these headwaters.

I would like to continue our discussion regarding Lake Laura as it has to be a significant contributor to algae problems in Stony Creek and the North Fork.

[Quoted text hidden]
--
Dr. Hays B. (HB) Lantz, Jr.
236 Ellis Road, PO Box 744
Basye, VA 22810
540-856-2452 (Home)
540-624-9019 (Mobile)

"Success isn't how far you got, but the distance you traveled from where you started."

Dr. Hays Blaine Lantz, Jr.
P. O. Box 744, 236 Ellis Road
Telephone 540-856-2452
hlantz4446@gmail.com

April 14, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

The purpose of this correspondence is to communicate my concerns about the increasing nuisance algae problem in the North Fork of the Shenandoah River and the tributaries that are part of this watershed. I have lived on Stony Creek, a tributary to the North fork, and Lake Laura in Bryce Resort for almost thirty years. My background is in biology (three degrees) with much work in aquatic ecology, and I along with Friends of the North Fork have conducted water quality research on the Lake Laura watershed for many years. In addition, I am most familiar with past research on the Lake Laura watershed, including three scientific studies conducted by Dr. Thomas Grizzard of Virginia Tech and Aquatic Resources, Inc. Stony Creek and Lake Laura are part of my daily existence as I can look upon them from the deck of my home. I fish, boat, and walk around Lake Laura and the North Fork a minimum of 40-50 days per year.

The algae problem in Lake Laura and Stony Creek began to be noticeable in the early 1980's and has gotten increasingly worse with each successive year. Tremendous algal blooms now occur yearly in Lake Laura and cover about 1/3 of the surface. This growth is well documented by the Virginia Department of Game and Inland Fisheries, Dr. Grizzard, and me. The algal blooms create an environment in which boating, fishing, and swimming are next to impossible in Lake Laura and Stony Creek. .The recreational value and use of Lake Laura is decimated each year in the months of July through October. My use and enjoyment of Stony Creek and Lake Laura diminish significantly each year during this period of time. It is difficult if not impossible for me to take water samples during these months in Lake Laura for my studies Because of the algal blooms and subsequent decay, the odors emanating from Lake Laura are almost unbearable, to the point where my wife and I cannot sit on our deck and enjoy the vista.

Because of this situation with Stony Creek and Lake Laura I am requesting that the North Fork, South Fork and main stem of the Shenandoah River be added to Virginia's impaired waters 303D/305B list. This problem is as significant, if not more so, as many other impairments.

Respectfully submitted,

Dr. Hays B. Lantz, Jr.

C
Dr. John R. Cook
Dr
3254 Holly Hill Dr
Falls Church, VA 22042

3/29/12

John M Kennedy
DEQ Office of Water Monitoring & Assessment
P.O. Box 1105
Richmond, Va 23218 - 1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment
Integrated Report

Dear Mr. Kennedy,

I am writing to express my concern for the water quality of the Shenandoah River. The algae bloom on the Shenandoah made it almost impossible to fish from mid-summer on. It also affected the Potomac particularly the south bank. For the past 10 years I have averaged 40 days fishing mostly on Virginia series and the Potomac, I find the floating algae and the filamentous algae (snot grass) a serious detriment to my fishing

For the past 25 years I have been the record keeper for the Potomac River Smallmouth Club catch and release fishing contest, after the floods of 96 the fishing improved rapidly. The results of 03, 04 + 05 were our best and they have been in decline since, I believe the decline reflects declining river quality.

The purpose of this letter is to ask that the North Fork, South Fork + Main Stem Shenandoah be added to the list of impaired waters on the 2012 305(b)/303(d) Water Quality Assessment Integrated Report by the Va Dept of Environmental Quality.

I don't know if you are a fisherman or not but if the fishing is good that means that things are right in the river and that makes a lot of people happy!

Good fishing,

Jack

Patricia & John R. Cook
3254 Holly Hill Dr.
Falls Church, VA 22042-3660

4/2/12

John M Kennedy
DEQ office of Water Monitoring and assessment
PO Box 1105
Richmond, Va 23218-1105
RE Draft 2012 305(b) 303(d) Water Quality assessment
Intergrated Report

Dear Mr Kennedy,

I am writing an addendum to the letter I sent to you thru Jeff Kelble, the Shenandoah River Keeper. He requested that I be specific as to the timing of the algae bloom. I'm not exactly sure but I think it started late spring, May/June. It continued well thru the fall. As I said I didn't fish the Shenandoah much after early summer because of the algae. However I did fish the Potomac below Harper's Ferry and it had a problem particularly the South bank from the algae coming down from the Shenandoah.

Thank you for your consideration of matter.

Sincerely

Jack

April 8, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to you as a concerned Virginia resident, an avid fly fisherman, and an active member of Trout Unlimited and the Friends of the Shenandoah River (FOSR) organizations; as such, I would like to request that the Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish and float the North Fork, South Fork and Main Stem of the Shenandoah often, and as a former monitor for FOSR, I participated for over 5 years in twice-monthly water sampling at various sites on the Main Stem of the river in Clarke County. For much of the year, I have observed that large sections of each river experience a series of noxious algae blooms that seriously diminish my use and enjoyment of these waterways. The blooms turn the river a dark murky green color, like green paint, and make the water far less enjoyable to wade and fish in, as well as far less productive to fish in.

During summer months, there is often a large amount of "rock snot" covering the river bottom, which inhibits growth of the native grasses and also seems to make the fishing worse. There is also a lot of a floating bubbly-type algae on the surface of the water, which has a very foul rotting odor – needless to say, trying to wade-fish the river with that type of algae in abundance is unpleasant at best, and many times will lead me to choose other waters to fish instead.

Based on my fishing and canoeing experiences on other waterways in Virginia, West Virginia, and Maryland, I believe the algae problems are specific to the Shenandoah, which suggests to me that they are likely due to polluted run-off; if that's the case, the causes of the pollution should be identifiable and correctable.

PLEASE ensure that DEQ includes the three sections of the Shenandoah River on the 305B/303D list, so that it can be determined why we are having these algae problems, and so that a plan can be developed and implemented to make the Shenandoah River a pleasant place to fish, canoe/kayak, and swim. I appreciate in advance your consideration of this request.

Sincerely,

Dr. Mark Zimmerman
"Trout in the Classroom" and Communications Coordinator
Winchester Virginia Chapter of Trout Unlimited
309 Huntersridge Rd.
Winchester, VA 22602
E-mail: mbzimmerman@comcast.net
Phone: 540-722-4914

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2014 305(b)3/ 303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I enjoy spending my free time fishing and canoeing on the Shenandoah River. I don't swim in the river anymore due to the algae blooms and visible cow feces floating in the water. I fish solo in a two man Old Town Discovery 158 canoe. It allows me to stand up a majority of the time similar to a stand up paddle board or fishing from a jon boat. My friends do the same thing. We float different sections of the river fishing for big smallmouth and largemouth bass, and musky. I also do float trips with my dad. He likes to bass fish from his kayak while I musky fish from my canoe. We have caught some monster fish over the years. Last year, the Shenandoah seemed to be in the best shape it had been in years. The bass fishing was phenomenal. We could do a 6 to 10 mile float and catch over 200 fish. The smallmouth seemed to finally be at a mature size after the 2006 fish kill. 18" to 20" smallmouth could be caught on a regular basis. Then, 2014 came.

Standing up in a canoe the majority of a float trip gives us an advantage over the kayakers sitting low to the water. Also, wearing polarized glasses cuts out the glare and allows us to see in to the water. We can see everything! Especially when the water is clear. What we are seeing this year is EXTREMELY disturbing. There is simply no where near the amount of life in the river compared to last year. The bass fishing is awful this year. It is basically a waste of time to try and target big bass now.

I try to enjoy the river year around. However, for much of the year, large sections of the river seems to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the river. My girlfriend and her friends no longer join on us on float trips. She experienced a skin related infection after spending a weekend on the river. They liked to swim while my friends and I fish. Due to the the water quality, they no longer risk swimming in the river. The slow pools and eddy's are obviously filled with algae and cow feces. It is absolutely disgusting. The water quality has diminished our enjoyment of the river immensely.

The Shenandoah River seems to be in worse shape than other rivers I have fished in Virginia. Most likely due to the large amount of agriculture in the Shenandoah Valley.

When Spring finally sprung this year, the inevitable flooding ensued. What was noticeable at the headwaters of the South Fork Shenandoah in Port Republic, VA was the runoff and dirty water flowing. The water is either neon green or brown in late Winter/early Spring. This isn't normal. I believe the algae blooms this Spring 2014 killed off a majority of the aquatic life in the river.

I would like to ask that DEQ to include the three sections of the Shenandoah River on 305B/ 303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Thank you for considering my comments,

Drew Morrison
723 Old Furnace Rd.
Harrisonburg, VA 22802
(540)435-4670
morrisdmster@gmail.com

DRS. DONNA AND ZAHRL SCHOENY

Dear Jeff,

We are long time friends of the Browne's at 456 Misty Meadow Lane in Bentonville on the Shenendoah River. Since they have been property owners there we have spent many days and weeks on the river. When we first started going there, we fished and ate the fish we caught. We also were avid kayakers, keeping our kayak at their house. We were very excited when they also purchased the cabin on Misty Meadow Lane and spent almost every weekend or more there for years, getting to know the neighbors and feeling a part of the valley community. We have watched the decline of the river and are very saddened by the amount of algae, flotsam, and diseased fish that continues to worsen every year. Allowing one of the most beautiful rivers in this country (and we have travelled all 50 states} to decline at this rate is a travesty and embarrassment to the state of Virginia. We would implore the state to recognize that the Shenendoah should be on Virginia's Dirty Water's List. Our children and grandchildren love to come to the river to swim, fish, and float. One even worked for a summer for an Outfitter and lived with me at the cabin. We are concerned if it is still safe for them to continue enjoying the recreation available at this once idyllic spot. Please let us know if there is anything we can do to encourage the clean up our favorite river

Donna and Zahrl Schoeny

300 Lake Forest Lane

Charlottesville, Virginia 22901

DRS. DONNA AND ZAHRL SCHOENY

Dear Jeff,

We are long time friends of the Browne's at 456 Misty Meadow Lane in Bentonville on the Shenendoah River. Since they have been property owners there we have spent many days and weeks on the river. When we first started going there, we fished and ate the fish we caught. We also were avid kayakers, keeping our kayak at their house. We were very excited when they also purchased the cabin on Misty Meadow Lane and spent almost every weekend or more there for years, getting to know the neighbors and feeling a part of the valley community. We have watched the decline of the river and are very saddened by the amount of algae, flotsam, and diseased fish that continues to worsen every year. Allowing one of the most beautiful rivers in this country (and we have travelled all 50 states} to decline at this rate is a travesty and embarrassment to the state of Virginia. We would implore the state to recognize that the Shenendoah should be on Virginia's Dirty Water's List. Our children and grandchildren love to come to the river to swim, fish, and float. One even worked for a summer for an Outfitter and lived with me at the cabin. We are concerned if it is still safe for them to continue enjoying the recreation available at this once idyllic spot. Please let us know if there is anything we can do to encourage the clean up our favorite river

Donna and Zahrl Schoeny

300 Lake Forest Lane

Charlottesville, Virginia 22901

Jeff Kelble <jeff@potomacriverkeeper.org>

Jeff, here's my letter.

Dusty Wissmath <dwissmath@skiwhitetail.com> Wed, Sep 3, 2014 at 1:26 PM
To: "jeff@shenandoahriverkeeper.org" <jeff@shenandoahriverkeeper.org>

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, Virginia 23218-1105

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2014 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event, but not always. Over the course of the year I use the entire river system and have seen this on all three rivers from Port Republic down to Front Royal, from Broadway to Front Royal and from Front Royal to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to swim or fish and I don't like to take my family swimming because they don't like the off-color murk water. When I do fish I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire MainStem from July through August because of the plankton green water. I have never seen these problems on other Virginia Rivers.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algaes are blooming I literally

do not want to be anywhere near the river and I often choose to go somewhere else. When I have no other place to go then it just takes all the enjoyment out of my day. The fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance. I found this problem in the following areas last year from July through August; Broadway, Lupton, Deer Rapids, Edinburg, Woodstock, Strasburg, Front Royal, Goods Mill, Luray Dam, 211, Alma, Port Republic, Warren Dam, Shenandoah, Route 50, Route7 among other places.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river from Broadway to Strasburg. This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

As a professional fly fishing guide on the Shenandoah River, this issue affects me directly. I guide throughout the length of the drainage and I have had a number of clients who have commented on the algae. It makes their experience much less enjoyable both aesthetically and practically as it is almost impossible to fish during a heavy bloom. Some clients say that they will not fish again on this river and that affects me economically. Something must be done immediately. Something MUST be done immediately.

Thank you for considering my comments,

Dusty Wissmath

18116 Raven Rocks Road

Bluemont, VA 20135

540-220-9283

dwissmath@yahoo.com

Jeff Kelble <jeff@potomacriverkeeper.org>

shenandoah river algae

Dylan Cooper <deerslayer88@embarqmail.com> Sun, Apr 15, 2012 at 7:12 PM
To: jeff@shenandoahriverkeeper.org

Dear Mr. Kelble,

I am voicing to you my dear concern about the health of the Shenandoah River, particularly about the problem of algae.

I am a lifelong user of the Shenandoah River, mostly in the South Fork because I live in Luray, VA but I've covered almost all parts of the Shenandoah River system. During the spring and summer, I fish it, canoe it, and swim or wade in it, and I also hunt it in the fall and winter. For years I have noticed the increasing problem of algae in the Shenandoah and I would like something to be done about it.

My biggest problem is that with a fishing lure that goes anywhere near the bottom, and sometimes on top of the water as well, I must take green and/or brown algae off of the lure after almost every single cast. This not only becomes annoying, but it slows up fishing and decreases my chances of catching fish. No fish will think a lure covered in algae is something to eat. The algae gets tangled in my line and ends up fouling up my fishing reels or rod guides.

The algae also stains the water a dark green color. With water that is not clear, it's impossible to fish by sight.

I also like to catch live bait in the river to use for fishing. Brown-snot-covered, slippery rocks are not the best habitat for the macroinvertbrate I am looking for, nor is it any good for footing.

Overall the algae has diminished my enjoyment of fishing my favorite fishing water and summer paradise in very many ways.

Other problems I notice while boating is that the algae gets stuck on the anchor and then it gets carried into the boat to dry out and stink up my whole boating trip. Also, anything sticking out of the water has algae on it that has floated to the surface during certain times of the year and that stuff really stinks up my river trip.

Sometimes I will get out of the boat to wade or swim (when I slip off the brown-snot-covered, slippery rocks) and the algae gets stuck on my shoes or clothes. This algae can stay on my shoes even after I try to pick it off, especially if the shoes have Velcro or mesh on them. Then once my shoes dry, they stink for days! And don't try to sit down on the bottom of the river unless you want to ruin your bottoms with brown stains from algae.

I also experience problems with the algae when I waterfowl hunt on the river in fall or late winter. I wear chest waders to go out in the icy cold water and the brown-snot-covered, slippery rocks are once again dangerous to walk on. I've been in many situations where I barely kept my footing on the algae-covered bottom to prevent myself from falling in the hypothermia-inducing water. And now because of the algae being spread to different river systems (that's a whole different problem), felt bottomed shoes and waders are not allowed. So there goes any extra chance of holding one's footing.

I have a Labrador retriever that absolutely loves the water. He'll go in the river all year long to swim and drink. Many times, he'll stink afterwards from getting algae in his fur. I always have to give him a bath after taking him to the river. Sometimes he'll also throw up from drinking the river water.

As you can tell, I have many different problems with the algae in the Shenandoah River. The algae severely diminishes both my use and my enjoyment of the Shenandoah River system. As a result, I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to clean up our river and to make the algae go away!

Thank you very much for allowing my input and for sticking up for our river,

Dylan Cooper

Virginia Tech Biological Systems Engineering Major

306 Winkler Rd

Luray, VA 22835

Email: deerslayer88@embarqmail.com

Phone: 540-742-3273

April 10, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the South Fork of the Shenandoah extensively and I also use the river to kayak and take my family members fishing. However, for much of the year, large sections of the river seem to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

Over the course of the past five years I've fished the South Fork from Luray to Front Royal. The places I like to wade and fish are mile 13, 16 and up stream from Andy Guest Park. The past two years I've fished these locations less due to the fact that I not only find algae as mentioned above but I find fish that have abnormal growths on them. I typically fish these areas from May through September; however, I've begun seeking out other locations due to the poor conditions of the water. I've been in my kayak when I've had to paddle though thick pockets of algae.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the South Fork and the other sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Thank you for considering my comments,

Elwyn "Chip" Comstock
6822 Wemberly Way
Mclean, Va 22101
Email: comburns@aol.com
Phone: 703-356-9124
Potomac River Smallmouth Bass Club Librarian

April 17, 2012

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

By now, you know the story.

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the Shenandoah often, from Front Royal to Harper's ferry (which is where the confluence with the Potomac is). But it often looks like a pond (noxious algae blooms in the summer) and that seriously diminish my use and enjoyment of the river. Last year, the nasty, oppressive algae bloom spilled over into the Upper Potomac. That's where I fish too! So now, that impairs that fishery as well. And it stinks! No way would I want to get out of my kayak into that stuff….

These rivers deserve better!!

You must take some action to preserve our use of this river. It cannot die. It's that simple. Algae is a killer of life in the water but you already know that.

Thank you for considering my comments,

Mr. Ernesto J Rojas

21317 Comus Ct

Ashburn VA 20147-4864

Email: ernierojas@verizon.net

Phone: 703-729-0128

April 15, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I have fished the North Fork, South Fork and sometimes the Main Stem of the Shenandoah frequently over the last forty plus years. In recent years large sections of each river get nasty algae blooms that seriously diminish my use and enjoyment of the rivers.

It seems that the problems now begin early in the spring of the year with a dark green, murky color to the water that lasts several months. The fishing suffers during this time and it barely goes away around June when the snot grass version comes on and covers everything in the water including the rocks and the native grasses. I used to do a lot of wade fishing in the summer, but because of this snot grass overlay on the whole river bottom, I can't. The fishing isn't as good and the bottom is slippery and dangerous….and it smells bad. I've tried to get away from this by going to different stretches of the rivers, but it seems more and more to cover almost all of both branches and the main stem.

Although I don't live on the river, enjoying it has been a long and valuable part of my and my family's life. And I know that's true for so many of the people I've met there over the years.

The degradation that is happening to the Shenandoah system is a shame and affects a lot of people like me personally. Its worthy of some focused attention by the State of Virginia and its DEQ. Therefore I ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Thank you for considering these comments,

Francis C. Steinbauer
2501 Fowlers Lane
Reston, VA 20191

Jeff Kelble <jeff@potomacriverkeeper.org>

Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Frank Filipy <frank.filipy@gmail.com> Fri, Aug 29, 2014 at 9:31 AM
To: jeff@shenandoahriverkeeper.org

August 29, 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond VA 23218-1105

Dear Mr. Kennedy , I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah River as impaired waters on the 2014 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year for the past six or seven years.

I have been fishing, canoeing and birding of the South Fork of the Shenandoah all my life.

I am saddened by the deterioration of the water quality. Winter planktonic algal blooms, filamentous algae, blue green algae and fish with lesions have diminished the quality of my experience and use of the river. The worst of it occurs in the summer time, usually July and August. Many days in the summer I don't even go on the water due to algae. The strong offensive odor of the algae, globs of it floating down the river joining together in large stinky mats is disgusting. I have observed this on the South Fork of the Shenandoah from Newport to The White House Bridge (rte. 211), and from Bixlers Ferry Bridge to Take out # 18 below Compton Rapids. The excessive algae interferes with fishing and canoeing. I used to freely swim and wet wade in the river. Not any more.

This algae diminishes both my use and my enjoyment of the South Fork of Shenandoah River. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Thank you for considering my comments,

Frank Filipy

15034 Owls Nest Rd.

Nokesville VA 20181

703 753-3141

Jeff Kelble <jeff@potomacriverkeeper.org>

Algae letter

Frank Polito <thefrank.polito@gmail.com> Tue, Sep 2, 2014 at 5:53 PM
To: jeff@shenandoahriverkeeper.org

Jeff,

Below please find my letter about the algae. Please let me know if you need anything else.

Best,

Frank Polito

Dear Mr. Kennedy,

I am writing to complain about the algae problem on the south fork the Shenandoah river. I am an avid angler and have been a resident of Harrisonburg, VA for the past four years. This year, the quality of the water has been awful. The river is full of a murky algae that has severely affected my ability to enjoy the river.

I often spend my summer days floating from Island ford to Elkton or from Newport to Alma. I have noticed the algae in both sections that I constantly float. I personally believe there is a direct correlation between the algae blooms and the poor fishing conditions that I experienced this year. This year was the worst fishing that I have seen in my four years of fishing the river.

Initially one of the main draws of my relocation to the Shenandoah valley was the Shenandoah river and its abundant smallmouth bass population. It saddens me to see how far the quality of the river has degraded.

The algae problem has gotten so bad, that now I often drive much further away, to spend my money and recreation time elsewhere. It is time for a change. If the river quality improves, I have no doubt that the towns on the river will see in increase in economic success as a direct result of people enjoying the river. Please do something to save this river.

As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Best,

Frank Polito
770 Walnut Lane
Apt A
Harrisonburg, VA 22801

March 27, 2012

John M. Kennedy DEQ Office of Water Monitoring and Assessment P.O. Box 1105 Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the South Fork and Main Stem of the Shenandoah extensively and in the past, I have used all three rivers to take my family canoeing and camping. However, for much of the year, large sections of the river appears to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

In each of the past six years I have observed a late winter planktonic algae blooms. Over the course of the year I use the river system and have seen this on all three rivers from Lurey down to Front Royal on the South Fork, and from Route 50 to the Confluence with the Potomac River in Harpers Ferry. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to swim or fish and I don't take my family swimming because they don't like the off-color murk water. In fact, during the times the river gets real bad, I choose to go elsewhere to take my family. This represents an economic loss to the area, because I tend to spend money on gasoline, dinners, the occasional hotel, and do some of my home shopping in the boutique stores in the area.

By mid-May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. This algae rises up and floats on the water surface, and then accumulate on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When the algae blooms I literally do not want to be anywhere near the river and I often choose to go somewhere else. The fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance. I found this problem in the following areas last year from July through August; Front Royal, Luray, Alma, Port Republic, Bentonville, Millville, and from Route 50 down to Route7, then to Confluence with the Potomac River.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system but also the economy of the area. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae

problems, and to develop and implement a plan to make them go away. Thank you for considering my comments,

George “HookUp” Thurston
21079 Ethan Court
Sterling, VA 20164
Email: rd400d@hotmail.com
Phone: (202) 267-3545

April 12, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

Since my retirement in 2004, my principle recreational activity from April through October is fishing. I wade fish on all three sections of the Shenandoah about 40 times a year. A review of my fishing log for last year showed that last year I recorded the lowest average daily catch rates on the Shenandoah since I retired. I attribute this reduction to the condition of the river. In a normal year, the river water is fairly stained from the Spring rains until about the latter part of June. It then becomes clear enough to see the bottom when wading for the rest of the fishing season. Last year, the main stem and the South Fork stayed cloudy due to algae blooms until sometime in September. This condition reduced the number of fish I was catching and made the wading more dangerous. As a result, I basically stopped fishing on these two sections of the river until the water cleared and a normal fish catch rate resumed in September.

In June through September, I was forced to do most of my fishing on the North Fork. While the area in this section of the river where I fished didn't suffer the algae bloom that the other two sections did, it was loaded with rock snot. On just about every cast, I would have to clean the rock snot off my bait (even if the bait was rigged for weedless fishing) before making the next cast. I also had to be much more careful when wading to avoid slipping on or stumbling over coated rocks. As a result, my fishing experience was much less enjoyable and productive.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.
Thank you for considering my comments,

George Moran
4132 Point Hollow Ln
Fairfax, VA 22033
Email: morangm@yahoo.com
Phone: 703-818-2146

April 12, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

Dear Mr. Kennedy:

I have lead the Northern Virginia Chapter of Trout Unlimited (a TU chapter with over 800 members) on trips to local streams for over 10 years. Until the last two years, I had always taken groups to the N. Fork of the Shenandoah during the summer to fish for smallmouth bass. The current high nutrient content of the river creates oxygen depleting algae blooms, and causes fish kills and sickens bass. Consequently I have been cancelling trips pending improvement of the condition of the river.

I ask that VA DEQ include the North Fork, South Fork and/or Main Stem on its impaired waters 303D/305B list. Thank you.

Sincerely,
George Paine
Secretary
N. Virginia Chapter
Trout Unlimited

Jeff Kelble <jeff@potomacriverkeeper.org>

Shenandoah Riverkeeper - Help

Harry and Debbie Allan <cedarview_1@hotmail.com> Sat, Apr 14, 2012 at 2:53 PM
To: jeff@shenandoahriverkeeper.org

Jeff – Here is my letter to DEQ. Please feel free to edit as you see fit. Good luck and thanks again. – Harry Allan

---

April 14, 2012

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, VA 23218-1105

Dear Mr. Kennedy,

I fish the South Fork of the Shenandoah River extensively and have been doing so since the mid-1970's. The majority of my fishing time has taken place in the Bentonville area. In these past 35-40 years, I have personally witnessed the degradation of this once beautiful river and its fishery. I am writing to request that Virginia's Department of Environmental Quality add this river, along with the North Fork and mainstem as 'impaired' waters on the 2012 305(B)/303(D) Water Quality Assessment Report. Specifically, severe noxious algae blooms and massive springtime fish kills have seriously diminished my recreational use and enjoyment of the Shenandoah River.

From my extensive fishing experience on this river, I have found that July and August have historically been stellar months of the year for fishing quality. This is no longer the case due to the massive algae blooms early in the season. This problem, in addition to the well-publicized fish kills of the past few years, have forced me to all but abandon the Shenandoah River as a recreational destination.

I respectfully urge the Department of Environmental Quality to include the Shenandoah River on the 305 (B)/303(D) list and begin to take the steps necessary that would return this river to it's legendary status.

Thank you.

Harold Allan

P.O. Box 436

Aldie, VA 20105

703.327.4322

Date: Sat, 7 Apr 2012 14:01:35 -0400
Subject: Re: Shenandoah Riverkeeper - Help
From: jeff@shenandoahriverkeeper.org
To: cedarview_1@hotmail.com
[Quoted text hidden]

Henry J. Staudinger
2218 Riverview Dr
Toms Brook, VA 22660
April 15, 2012

Tel: 540-436-3491
Fax: 540-436-3099
E-Mail hjs@shentel.net

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105

Dear Mr. Kennedy:

My wife and I are landowners in Shenandoah County, with almost a mile frontage along the North Fork of the Shenandoah River . The river is an important feature of our property, enabling walks along the river to see wildlife, fishing, wading, canoeing, swimming as well as the view from our home.

Over the years the quality of the water deteriorated, adversely affecting our use and enjoyment as well as diminishing the value of our property. Some years ago we discovered that our cattle operation was contributing to the problem. As a result, we voluntarily put up fences along the river. We also converted a number of acres close to the river from crop land to hay ground and incorporate a number of best management practices to reduce erosion.

We are aware that DEQ monitors and at times has been active in reducing pollution sources. We look forward to DEQ's continued efforts to do. However, more needs to be done. One of the most obvious areas is to identify and address the cause of the algae that has become a more severe problem.

Although the source of the algae is not entirely clear to us, it is becoming an increasing problem. In particular, it discourages and at times makes it impossible to swim or fish. This not only adversely affects our use and enjoyment of the river, but the use and enjoyment of many others. It also diminishes the value of our property. It is not clear what action, if any, we might take to reduce the algae problem. However, it is clear that this is well beyond the ability of individual landowners to address. Please let me know what action DEQ is taking to address this particular problem.

Sincerely,

Henry J. Staudinger

September 5, 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

As a resident of Front Royal, Virginia since 1977 and longtime fisherman and outdoorsman, it pains me to see the shape that my beloved Shenandoah River has drifted into through neglect and political paralysis.

We have weathered floods and fish kills over the last several years, but our algae impairment has now become just one more nail in the coffin for our river. Over the past 5 years the amount of Algae has seemed to increase every year. It's gotten to the point where many of my friends and fellow smallmouth chasers almost refuse to come here to fish with me. The smell and the buildup on lures and baits make fishing a hassle at the least, and perhaps even health-threatening at the most. We're starting to see some of the Blue-Greens of the species along with the usual filamentous and this is just simply unacceptable. Not only am I losing fishing buddies but I have no doubt the local canoe and kayak outfitters and local restaurants and motels are beginning to see a drop in business as a result.

This has become an embarrassment to a town that touts itself as "The Canoe Capital of Virginia"

I stand foresquare behind Jeff Kelble, President of Potomac/Shenandoah Riverkeeper. org in pressing so hard to have the Shenandoah listed as impaired for Algae with both the VA DEQ and the EPA. Anything less is just ignoring the problem that we fishermen and outdoorsmen see getting worse and worse every year.

Thank you for time and attention.

Herschel L. Finch
310 Oakridge Ct.
Front Royal, Virginia 22630
540 270 1590

Conservation Chairman-Potomac River Smallmouth Club
Conservation Chairman-Izaak Walton League of America, Warren County Chapter
Pro Staff-Jackson Kayak Fishing Team

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105

Dear Mr Kennedy

I am writing to you to have you consider placing the Shenandoah River on the Virginia list of impaired waterways.

I am a frequent user of the river, an avid fisherman, outdoorsman, naturalist and photographer. I spend many days on our states rivers, but the beauty and proximity of the Shenandoah make it my favorite...until the last few years.

The last few years, starting about the end of June, I find long stretches of the river, choked with algae. This emerald green slime coats rocks in the river, making it dangerous to wade and then breaks off and becomes suspended in the water making it difficult to fish. This algae is not a 'normal' condition for the river. It's a direct result of excess nitrogen and phosphorus in the river. Cleaning a hookful of this stuff off every time you reel in a lure gets old very quickly. I do a lot of 'sight-fishing', looking for fish activity, or bait fish being chased, and bird activity that tells me larger fish are present in that area. When this algae moves in and coats the bottom, the fish move out because they can't survive in that area. There's no oxygen. Which also means the fish-eating birds I so enjoy photographing move out as well. I recall an area of the south fork near Andy Guest State Park that was almost completely coated in the emerald green goo. I did finally find a bare area2 miles downriver. This area was about the size of my dining room and there was at least 3 dozen large fish milling around it, trying to breath and find some food. But it's not just near the park. I have found it up beyond Luray near Newport, as well as Karo landing, Front Royal landing and on into the main stem. The North Fork gets almost completely clogged with this stuff due to its shallowness.

As the summer wears on, the blue-green algae begins to show up in the low-water eddies and backwaters of the river. This stuff not only smells horrendous, it is also toxic to people and animals. I can't imagine allowing my kids to come in contact with any water where blue-green algae is growing. I don't care to come in contact with it either. I find myself traveling further afield to other waterways, and other states even, to find water where I can fish and pursue my passions. But not before I spend a good deal of time scrubbing my kayaks and pontoons boat. I don't care to inflict this on anyone else's river.I would strongly request that DEQ place both forks and the main stem of the Shenandoah River on Virginia's impaired waters 303D/305B list so that a proper plan and funding for it's cleanup and improvement can be formulated. I think all Virginians would appreciate being able to point to such a nationally famous and historic river as a point of pride and not as a river with a pollution problem.

Herschel L. Finch
Conservation Chairman
Potomac River Smallmouth Club
310 Oakridge CT.
Front Royal , VA 22630
540-270-1590

Jeff Kelble <jeff@potomacriverkeeper.org>

Form submission from: Take Action!- Help the Shenandoah Riverkeeper Get Rid of Algae

Potomac Riverkeeper <sarah@potomacriverkeeper.org> Sat, Apr 14, 2012 at 6:32 PM
To: jeff@potomacriverkeeper.org

Submitted on Saturday, April 14, 2012 - 6:32pm
Submitted by anonymous user: [173.79.213.221]
Submitted values are:

Your Letter:
14 April 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

Dear Mr. Kennedy,

I'm writing this to let you know the Commonwealth *definitely* has a problem with algae growth in the Shenandoah River, caused by pollution that stimulates growth.

I used to fish the Shenandoah frequently, particularly the part around Luray but also downstream as far as the US Route 50 bridge. I greatly enjoyed Virginia's wonderful scenery combined with the opportunity to fish for smallmouth bass. I have essentially stopped fishing the Shenandoah because of the effects of pollution on both of these that the state government should have stopped. I'm grateful that some action is underway regarding chicken waste but much more remains to be done to return this river to the state where fishing is a rewarding activity for the citizens of the Commonwealth.

The Shenandoah used to be recognized as one of the great scenic rivers and smallmouth streams of the United States. Now I must drive to West Virginia to find water clean enough to support a healthy smallmouth sport fishing population.

Please do your job and enforce the water quality laws.

J. Mark Pullen
Name: J. Mark Pullen
Email: mpullen@netlab.gmu.edu

The results of this submission may be viewed at:
http://www.potomacriverkeeper.org/node/428/submission/822

August 29, 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2014 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I primarily fish and float the North Fork of the Shenandoah River, but also fish the South Fork and Main Stem of the Shenandoah occasionally. I live in Shenandoah County which has the majority of the North Fork flowing though it and use the river year round for fishing and floating in the summer and hunting in the fall and winter. However, for much of the year, segments of the North Fork are almost unusable due to the excessive algae blooms and it is taking away my enjoyment of the rivers, and what will hopefully be my kids' enjoyment. Having grown up in Shenandoah County I was on the North Fork every summer fishing and swimming and can remember much better times and much cleaner water.

In each of the past three summers my fishing or floating has been disrupted by algae blooms that either made paddling difficult or fishing impossible. When I have fished the fishing has been poor and I don't enjoy the experience as much. I don't see the same fish activity that I once did. Normally in the hot afternoons during the summer months (July-Sept) I'd wear myself out casting popping bugs into the shadows along the banks of the North Fork between Edinburg and Woodstock and Meems Bottom to Red Banks. That is no longer the case. Activity in the river drops to near zero and I constantly have to clean my line and fly of algae. It becomes more of a hassle than it is worth for the little enjoyment I receive under those conditions. I have never seen these problems on other Virginia Rivers. I went to college at Virginia Tech and fished the New River and never saw algae like I see on the North and South Forks. As my kids (2 and 4 years old) get older and want to be "in the water" I'm not inclined to that them to my home waters of the North Fork due to the algae. The algae has diminished my use and enjoyment of the North Fork of the Shenandoah River.

I want my kids to grow up to appreciate and enjoy the river but the algae cycles are a real threat to that opportunity. My kids won't be able to see beyond the algae, the murky water, and the smell like I can to really know the true potential of the North Fork and all it has to offer. For me this is about leaving something better than we found it for the next generation and future enjoyment As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Thank you for considering my comments.

Sincerely,

J Seth Coffman

4276 Stoney Creek Rd
Edinburg, VA 22824

Jeff Kelble <jeff@potomacriverkeeper.org>

North Fork river problems

Jack Chapman <chappyje@gmail.com> Fri, Apr 13, 2012 at 12:45 PM
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>, leslie.watson@fnfsr.org

April 13, 2012
Mr. John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-11-5

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am concerned about the quality of the Shenandoah River system and have particular knowledge of the North Fork, near Edinburg. I believe it should be considered "impaired".

We live on Swann Rd., outside of Edinburg. This road parallels the river. I began visiting this area in the mid 1980's due to the quality of the fishing. My wife and I bought a small place on the North Fork in 1999, with the intent to retire there. Which we did in 2007. Prior to retirement we were here most weekends and holidays. We are of modest means, which I state only to emphasize the importance of that purchase. The river and beauty of the area are what drew us here.

I want to outline the problems I've seen, in rough chronological order:

1. The rock-bass (redeye, goggleeye) have all but disapeared. This occurred possibly 15 years ago. They were formerly caught in abundance, sized up to 10" or so. Even though the smallmouth bass were the big draw, the redeye were a prized secondary catch. I talk to a lot of fisherpeople, and they are very aware of this.

2. The fish kills of bass and sunfish are well known. One weekend, I believe early April, the first year of the fishkill, I caught a number of nice bass/sunfish. The next weekend nothing. I caught one sunfish that entire season and quit fishing the area for several years. The sunfish continue to be scarce and small. The bass have had several good years, but are still uniformly small. I did some guiding in the area for several years and quit, partly because of the poor fishing.

3. Currently, the bass fishing is o.k. The fallfish are larger, more abundant and more fun than the bass. Very large carp are moving in to the area.
We have fishtanks we popluate with local critters and often look around and net them. It seems the crayfish & possbily sculpins are much less abundant.

4. We used to have grasses in the river. They grew up as the weather warmed, in the slower sections. I'm not sure if they were all native, but they seemed to house all kinds of aquatic insects and baitfish. These grasses all but disapeared, something like four or five years ago. We have instead, little bright green balls of some kind of algae attached to rocks.
And, the filamentous algae. It is disgusting. It strings out on any available surface, a genuine eight to ten feet. and literally chokes the river. Any significant rainfall causes some algae to detach and we have it floating down the river for days at a time. We hook way more algae that fish during those times.
I frequently wade fish upstream to the bridge (Rt. 698) and we often kayak downstream as far as the first rapids below the deteriorating low water bridge where Swann Road terminates, and paddle back. This algae certainly has diminished our enjoyment of the river. Lifted a paddle with eight feet of algae hanging from it lately? How about backcasting a popping bug with a half pound of gunk attached.

5. We see folks paddling and/or fishing early in the year. The river level us up and the algae has yet to bloom. It's a beautiful trip from Red Banks Rd. to Swann Rd. July through September, people rarely recreate on the river. I frequently go elsewhere.

I feel like we are two of many who live on or near the river and can't enjoy it, or invite friends and family to enjoy it, through large parts of the warm weather. This must be having a significant negative impact both enviromentally and economically.

I would hope these problems will be recognised and addressed by, at least, having this water declared impaired.

Thank You.

Sincerely,

Jack Chapman
P.O. Box 123
393 Swann Rd.
Edinburg, Va. 22824-123
Phone: 703-507-2226
Email: chappyje@gmail.com

August 15, 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105
RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing you today as a Shenandoah River fisherman, a land owner on the river, a member of the Shenandoah River Keepers and as a lifelong Virginia citizen who is deeply worried about the algae growth we experience each year on the Shenandoah River.

My wife and I own a small fishing cabin on the North Fork located near Maurertown, just up the road from Woodstock, VA on Route 11. For the past eight years, from late May till mid October, you'll find me wading up and down the Shenandoah just about every other week in search of smallmouth bass. A number of my fishing friends often accompany me on these expeditions. As I'm sure you can appreciate, I treasure my time on the river as do all the other fishermen I encounter. The beauty of the river and nearby mountains is inspiring. However, just about this time every year, something changes in the water which spoils our enjoyment of it. That's because with the late summer comes the algae blooms.

Are you a fisherman, Mr. Kennedy? Have you ever attempted to cast a lure either on or below the surface of a body of water chocked with algae? It can't be done. Every cast returns a slimed lure, and a fouled lure does not attract fish. Rather than casually wading up and down the river looking for the perfect "smally" hole, the algae forces me to seek out those ever diminishing parts of the river that are unaffected. More often than not, the fish appear to be smart enough to stay near the algae, so fishing success is rare. And one more thing, it smells bad. By way of comparison, I also fish on the Potomac River and Goose Creek in Loudoun County. There are no algae issues on those bodies of water.

When the algae blooms are bad, my associates and I stop coming to the river. Nearby families stop inviting guests for tubing trips. Perhaps less appreciated is the fact that after a day on the river, most of us jump in our cars and head to Woodstock for a meal. I imagine the tubers do the same. When we are not on the river, then we are not spending money in nearby towns. I'm sure area restaurant owners feel the pinch during those months when the algae blooms are particularly bad.

We need your help. Our river is being polluted with algae producing chemicals and those responsible must be identified and stopped. As such, I request that VA DEQ include the North Fork, South Fork and Main Stream on its impaired waters 303D/305B list. Please help us find the cause of the algae growth and create a plan to put an end to it.

Sincerely,

John Dalby
633 Meade Dr. SW
Leesburg, VA 20175
dalbyclan@verizon.net

Jeff Kelble <jeff@potomacriverkeeper.org>

Letter Writing.,.,. Please help Jeff out.

Jack McAllister <jackmcal@gmail.com> Thu, Apr 19, 2012 at 7:56 AM
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>

Jeff, please add my name to the petition of fishermen that have almost stopped using the South, North and Mainstem of the Shenandoah River due to the algae.....Jack McAllister, Stephenson, Va
[Quoted text hidden]

Jeff Kelble <jeff@potomacriverkeeper.org>

Algae Complaint Letter

Jacob Russo <russo2ja@gmail.com> Wed, Sep 3, 2014 at 12:17 PM
To: jeff@shenandoahriverkeeper.org

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2014 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish and guide on the North Fork, South Fork and Main Stem of the Shenandoah. However, for much of the year, large sections of each river seems to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event, but not always. Over the course of the year I use the entire river system and have seen this on all three rivers from Port Republic down to Front Royal, from Broadway to Front Royal and from Front Royal to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to swim or fish. When I do fish I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire MainStem from July through August because of the plankton green water. I have never seen these problems on other Virginia Rivers.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algaes are blooming I literally do not want to be anywhere near the river and I often choose to go somewhere else. When I have no other place to go then it just takes all the enjoyment out of my day. The fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance. I found this problem in the following areas last year from July through August; Broadway, Lupton, Deer Rapids, Edinburg, Woodstock, Strasburg, Front Royal, Goods

Mill, Luray Dam, 211, Alma, Port Republic, Warren Dam, Shenandoah, Route 50, Route7 among other places.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river from Broadway to Strasburg. All three of these algaes make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

I fish and guide on the river and am tired of sick fish, dead fish, and polluted water. This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. It has also effected my yearly income that I count on to support my family. If this continues I will seek out other rivers to guide and fish on. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.
Thank you for considering my comments,

Jacob Russo

Email: russo2ja@gmail.com

Phone: 540-272-9974

Jeff Kelble <jeff@potomacriverkeeper.org>

Letter writing

James Nashed <jamesnashed@hotmail.com> Tue, Apr 17, 2012 at 5:00 PM
To: Jeff Kelbe <jeff@shenandoahriverkeeper.org>

John M. Kennedy
DEQ Office of Water Monitering and Assessment
PO Box 405
Richmond, VA 22620

Dear Mr. Kennedy,

I am writing this letter as a concerned citizan of the state of Virginia and a person who enjoys the recreational use of the Shenandoah river. I use the river extensively throughout the spring, summer, and fall. mostly for fishing but also picnics and wading. I have been extremely alarmed at the amount of algae blooms over the past few years on differnet sections of the river. I have seen them extensively on the North Fork and South Fork, but also along the main stem where I fish a great deal.

Unfortunately this has impacted my ability to fish and certainly wade and swim safely in parts of the river. I find this especially bad in the summer on the North Fork south of Strasburg which I tend to avoid fully. But lately the parts of the South Fork from Luray northward and the main stem from Front Royal to the route 7 bridge also seem greatly affected. The algae and slime sometimes makes fishing a near impossibility due to the poor visibility and inability to keep lines and flies clear of algae. Myself and my family also are concerned enough to not swim or wade where large blooms are present due to health concerns. I have 2 small children who we also prohibit from swimming when blooms are present. This is a tragedy. The river and the surrounding land are some of the most beautiful in the state. I end up traveling to West Virginia or Maryland in search of fishable waters and recreation even with such an amazing river as the Shenandoah is so close to home.

The kinds of algae seem to be many. The floating algae seen in calmer waters in the late summer has a strong odor and colors the entire area a pudrid yellow-green color. The rocks which are already slick in the summer become impossible to walk on even with rubber and metal studded wading boots because on an immense amount of green "rock snot" that encompasses everything. Fishing under these circumstances is both impractical and unenjoyable. Of course no one in my family would swim or wade with this and instead we save our swimming for the beach trips in outer banks of North Carolina or the lakes and streams of Western Maryland. My enjoyment and use of the river the past few summers has been dimished significantly to be sure.

I love the Shenandoah river and wish the algae could be controlled. I urge the VA DEQ to include the North Fork, the South Fork, and the Main stem of the Shenandoah on the list of impared waters 303D/305B.

Sincerely

James Nashed MD
118 Woodfield Lane
Winchester, VA 22602
jamesnashed@hotmail.com

Date: Tue, 17 Apr 2012 11:57:38 -0400
Subject: Re: Letter writing
From: jeff@shenandoahriverkeeper.org
To: jamesnashed@hotmail.com
[Quoted text hidden]

August 28, 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

My name is Jamie Gold and I live in Northern Virginia. I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2014 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I am a board officer with the Potomac River Smallmouth Club, headquartered in Vienna, VA (www.prsc.org). We are a volunteer fishing and conservation organization with over 100 members. Members of the club and I fish and canoe on the Shenandoah River multiple times throughout the warm months of the year. We fish on the North Fork, South Fork, and Main Stem of the Shenandoah.

For the past several years we have noticed an increasing algae problem on the River. Each year there seems to be more algae than the prior year, which diminishes our canoeing and fishing experience. First, the smell from the algae is atrocious. For example, in July a group of us fished from the Rte. 50 Bridge to Locke's Landing. While fishing we came across a backwater area where a significant amount of algae had accumulated. The algae had started to rot and the odor was horrible. It smelled like a combination of untreated human waste and a decaying body. The smell carried 1/3 of the way across the river; it took a long time for the smell to get out of my nostrils. Second, fishing and canoeing have become frustrating because I am constantly cleaning algae off of my fishing lures and my paddle.

As a result of the algae problem, we have spent less time fishing the Shenandoah this year than we have in past years. We fish the Potomac River quite often, and it does not have anywhere near the algae problems of the Shenandoah. As a result, the trips we used to do on the Shenandoah we are now doing on the Pototmac River. This is sad and frustrating because the Shenandoah used to be one of my favorite rivers to fish and canoe.

I ask that the Virginia DEQ include the North Fork, South Fork and Main Stem of the Shenandoah on your impaired waters 2014 303D/305B list and address this growing problem. If you have any questions, or would like to discuss further, feel free to contact me at the phone number or email address below.

Sincerely,

Jamie K. Gold
192 Saint Johns Square
Sterling, VA 20164
571-213-4699
jkg116@gmail.com

1423 N. GREENBRIER ST. ARLINGTON, VA 22205 ▪ 703-534-4543 ▪ JAYEICHE@HOTMAIL.COM

April 18, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

Dear Mr. Kennedy,

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of severe algae problems the river experiences every year.

I own over 500 ft of riverfront property on the North Fork of the Shenandoah near Maurertown, Virginia. I frequently fish and float the river upstream and downstream of my property, but also have paddled most of the North Fork from Broadway to Front Royal, the South Forth from above Luray to Front Royal, and the Main stem Shenandoah River from Front Royal into West Virginia. I have fished most of the mid Atlantic rivers for the past 20 years, and unfortunately have seen a marked increase in noxious algae blooms and fish kills during that time period.

Specifically, in each of the past six years during late winter there are algae blooms which typically occur after high water events most likely caused excessive nutrient runoff. This bloom will turn parts of the river a dark murky green color with strands of gook the consistency of paint until about mid-summer. When the algae blooms, I generally don't swim or fish, nor do I recommend our guests and friends do the same as I have health concerns with the off-color murky water. When I do fish, I find the fishing is poor and take precautions upon returning home such as cleaning any scrapes or wounds with hydrogen peroxide and using antibiotic ointment. We also discourage pets and small children from entering the water at all.

By mid-summer each year, the river clears as the planktons seem to die back. But then a filamentous algae (rock snot) completely takes over and covers the river bottom which kills the native submerged aquatic vegetation trying to emerge. The native grasses turn brown, lose their leaves, and remain bare the rest of the year. Additionally, a floating bubbly type of algae (probably Cynabacteria or Blue Green Algae) comes at this time. Clumps of it often cover the water surface and accumulate on anything sticking out of the river, as well as in back eddies. This algae smells like sewage. When these algaes are blooming I literally do not want to be anywhere near the river. The fishing is frustrating because you cannot cast without fouling your line on the algae, the fish won't bite, and I find being around the river very unpleasant due to the odors and annoyance.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. We caution our guests regarding the use of the river during the summer months, and feel it is responsible in

part for a decrease in tourism in the Shenandoah Valley, and has negatively impacted property values. As a result I would like to ask that DEQ include all three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to improve water conditions and restore the river to a world class smallmouth bass fishery and national treasure.

Thank you for considering my comments, and please feel free to contact should you have additional questions.

Very Respectfully,

Jay Eiche

Trout Unlimited
Northern Virginia Chapter
PO Box 12182
Burke, VA 22009

April 6, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond, VA 23218-1105

Regarding: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

As the President of the Northern Virginia Chapter of Trout Unlimited, I represent approximately 850 dedicated anglers. Please disregard the word "trout" in my organization's name since our members actively pursue smallmouth bass during the summer.

Over the last several years, we have become increasingly concerned about the decline in the quality of fishing on the Shenandoah River. It started with fish kills several years ago and continues with the horrible algae bloom we experienced last year. The impact of the fish kill on recreational angling is obvious, but last year's persistent algae bloom diminished our enjoyment of the river almost as much. The cloudy, murky water made fishing a challenge and diminished our ability to use the river during the summer months. In addition, my members have begun to complain more and more about smell and wonder if contact with the water is dangerous. It seems as if the entire South Fork from Port Republic to Front Royal is equally impacted by this issue.

As a result of the algae, we are less likely to use the river for recreational angling. This will have an adverse economic impact on businesses in the valley as well as on my members as they drive farther to reach other locations in Maryland and Pennsylvania that offer better recreational angling opportunities.

I urge you to take action now and include the North Fork, South Fork and the Main Stem on your impaired waters 303D/305B list.

Sincerely,

Jay Lovering
President, NVTU
703 734-1558
jrlovering@aol.com

Jeff & Tomi Browne

456 Misty Meadow Lane, Bentonville VA 22610

(540) 631-9294

August 25, 2014

To: John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
From: Jeff & Tomi Browne
Re: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

We have owned property on the Shenandoah River for 15+ years and now are contemplating retiring here full-time from Northern Virginia. Things that attracted us most to this area were the water quality and the abundance of fishing. The water was sparkling clear, the river beds were clear of moss, and there was plenty of natural vegetation to provide hiding places for the smallmouth bass.

I have noticed that in years when the algae is bad, that the fishing is poor, footing is dangerously slippery, the water has an unhealthy tint to it, and the river smells. Last week I was paddling up-river from the Hazard Mill landing in Bentonville (Hazard Ford Road) and for the first quarter mile you can see the devastation caused by algae as the river grass has been killed over time. The largest smallmouth bass I've caught on the river have been in that stretch, but no more. Now I just paddle through it in order to get to faster waters that haven't been hit as hard.

I used to go wading in order to fly fish along the Hazard Mill stretch, but have drastically cut back on that due to the slippery conditions and the fact there are very few fish there anymore.

Reflecting on this summer while writing this letter, I realize that instead of fishing 4-5 times a week as I have in the past that I might try fishing once a week. The harm from the alga has cut my enjoyment of the river.

We want our grandchildren to enjoy the legacy of the Shenandoah River. But that can't happen if we don't track and control the things that hurt it.

We ask that you add the South Fork of the Shenandoah River to the impaired water 303D/305B list.

Jeff Little
2703 Rowe Road
New Windsor, MD 21776

410-635-3857 Home
443-244-2592 Cell

yakfish@verizon.net

April 9, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear John M Kennedy,

The last time I visited the Shenandoah River to fish was last September near Pugh's Run on the North Fork. I took my two sons to wade and fish where I have fond memories of catching feisty smallmouth bass. We spent about three hours wading downstream, catching a few small bass, a far cry from my memories of this previously impressive fishery.

The slippery filamentous algae that permeated the river bottom made wading treacherous for my sons. The "snot grass" coated their water sandals and when the decided to jump in further, the rest of their clothes. It also made for frustrating fishing, as each cast yielded a clump of algae that prevented our catching many fish. This was not the case several years ago. My tales of successful fishing were met by skepticism by two young anglers who would rather not return. Certainly the algae, symptomatic of the water quality issues that affect the fishery has diminished how often I have visited the river.

I would request that the Virginia Department of Environmental Quality include the North Fork, South Fork and Mainstem Shenandoah on its impaired waters 303D/305B list. Feel free to contact me if you have questions. Thank you for your time.

Sincerely yours,

Jeff Little

Jeff Kelble < jeff@potomacriverkeeper.org>

Form submission from: Take Action!- Help the Shenandoah Riverkeeper Get Rid of Algae

Potomac Riverkeeper < sarah@potomacriverkeeper.org> Wed, Dec 18, 2013 at 8:02 AM

To: jeff@potomacriverkeeper.org

Submitted on Wednesday, December 18, 2013 - 8:02am
Submitted by anonymous user: [155.178.5.12]
Submitted values are:

Your Letter:
TO:
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

From:
John Cabala
15019 Running Park Ct
Bowie, MD 20715

Dear Mr. Kennedy,

I am writing you to gain your support to add the N. Fork, S. Fork, and Main Stem of the Shenandoah River to Virginia's impaired waters 303D/305B list. First, let me start off by explaining to you what the river "used" to be like. The Shenandoah River used to be my favorite river in all of the VA/MD/PA area to fish for Smallmouth Bass. In its prime (prior to 2004), the river was clean enough to support a healthy population of fish. My friends, family and I "used" to fish and kayak the river every year (approximately 6 times per year), and it was always something we looked forward to. We fished many spots along the river, but our favorite location was the upper main stem. The fishing was incredible, and the fish were larger there than anywhere else I've ever fished. Unfortunately, the water quality diminished to the point where the fishing severely suffered, so we no longer fish the river. Now, we just support efforts to clean it up, and pray that those like you, who have influence to make improvements, will make the right choices.

Over the years, the river has been damaged by waste water treatment plant discharge and high concentrations of pesticide, herbicide, and fertilizer nutrient run-off from farms. I pray that one day the river will return to its healthy state once again so my friends and I can again enjoy fishing and kayaking trips on what used to be our favorite spot.

With proper laws in place that require safe limits for treatment plant discharge and farming practices, the river will have a chance to rebound. Your efforts to add the entire river to the 305D/305B list of impaired waters is just the beginning, and sure would make a lot of people like me happy.

Thank you for your time and consideration,

Name: John Cabala
Email: jrockjrock@comcast.net

The results of this submission may be viewed at:

http://www.potomacriverkeeper.org/node/428/submission/1268

Jeff Kelble <jeff@potomacriverkeeper.org>

Form submission from: Take Action!- Help the Shenandoah Riverkeeper Get Rid of Algae

Potomac Riverkeeper <sarah@potomacriverkeeper.org> Tue, Apr 17, 2012 at 1:56 PM
To: jeff@potomacriverkeeper.org

Submitted on Tuesday, April 17, 2012 - 1:56pm
Submitted by anonymous user: [76.21.150.216]
Submitted values are:

Your Letter:
Dear Mr. Kennedy,
I and many of my friends USED to travel to Virginia to fish the Shenandoa River. We did so for many years because the water used to be clean, and the fishing was great. That stopped over 5 years ago. I'm not sure what's happened, but I assume it's increased pollution entering the river, including pesticides, road salts, and lawn fertilizers. So, for the last 5 years, we've avoided your state, and none of our out of state money is being spent there either, for hotels, gas, restuarants, etc. I wish you could support cleaning up the river by including the three sections of the Shenandoah River on 305B/303D list, and identifying what's causing the terrible algae growths. Sincerely.
Name: John Cabala
Email: jrockjrock@comcast.net

The results of this submission may be viewed at:
http://www.potomacriverkeeper.org/node/428/submission/824

March 27, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I run a fishing camp for children every summer. In the past 3 years we have taken over 400 children to fish the North Fork, South Fork and Main Stem of the Shenandoah a whole lot and I also use all three rivers personally to take my family canoeing and rafting and have done so since 1982. However, for much of the past few years, large sections of each river seems to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event, but not always. Over the course of the year I use the entire river system and have seen this on all three rivers from Port Republic down to Front Royal, from Broadway to Front Royal and from Front Royal to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms, I opt to take the children to fish other bodies of water so they can catch more fish and swimming safely and comfortably. Last year was a particularly poor year for fishing for the children in my camps and they requested we spend more time elsewhere. Whenever the river is this murky color, it's disturbing to us and the animals that we encounter. The numbers of fish that we catch are lower and the fish are usually lethargic. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire MainStem from July through August because of the plankton green water.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algaes are blooming I literally do not want to be anywhere near the river and I often choose to go somewhere else. When I have no other place to go then it just takes all the enjoyment out of my day. The fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance. I found this problem in the following areas last year from July through August; Broadway, Lupton, Deer Rapids, Edinburg, Woodstock, Strasburg, Front Royal, Goods Mill, Luray Dam, 211, Alma, Port Republic, Warren Dam, Shenandoah, Route 50, Route7 among other places.

Finally, in the fall each year the native grasses die back and a bright green algae again covers the entire river. These algaes make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them

go away.
Thank you for considering my comments,

Mr. John D. Lipetz
2717 Pioneer Lane
Falls Church, VA 22043
Email: jdlipetz@fishandexplore.com
Phone: 703-609-8083

April 6, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Sir:

I am writing you as I am deeply concerned about the declining water quality and overall condition of the South Fork of the Shenandoah River, particularly the stretch between the low water bridge at Bentonville and the now closed campground at Hazard Mill. I used to fish this particular stretch of the Shenandoah many years ago and have always considered the fabled Shenandoah as one of the premiere waterways, not only in the state of Virginia, but also in the entire Mid Atlantic region.

During the 1960s, I fished this stretch of the river at least once a week and sometimes would camp for days at a time at the Hazard Mill recreation area some five miles upstream from the low water bridge at Bentonville. I have fond memories of my early days on the river as I recalled the crystal clear waters and rocky river bottom broken up by occasional rocky ridges which held great numbers of beautiful smallmouth bass. I continued to fish the river during the 1970s and 1980s and in dismay watched the quality of the river decline markedly. I first noticed the algae blooms , which were practically nonexistent during my early years on the river, become quite prevalent as time wore on. The last time I fished the Shenandoah was sometime during the 1990s. The algae had become a real pain as you had to clean your lure after every cast and the algae had taken on a real noxious odor which greatly diminished what I had always considered a memorable visit to what was then one of Virginia's best waterways. Considering the money and effort the state of Virginia has spent creating the Andy Guest State Park, which I presume was meant to replace the old Hazard Mill recreation area, one would think that you would want a pristine river which would attract plenty of fishermen and other outdoor enthusiasts. Unfortunately, this does not seem to be the case. As a matter of fact, the last time I fished the Shenandoah, it was in the Guest state park.

Finally in 2008, I decided to visit the low water bridge at Bentonville to see if the river quality had improved since I last fished there in the 1990s. It was during a particularly hot period in July. I was shocked to see the amount of algae both upstream and downstream from the bridge. The slime that clung to the rocks was clearly evident and the noxious odors that I first noticed in the 1990s had become worse. It is a sad epitaph for a river that so many considered one of the premier rivers in the Mid Atlantic.

It is for this reason that I implore the Virginia Department of Environmental Quality to include the three sections of the Shenandoah River on 305B/ 303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away. It is imperative

that the state of Virginia take the necessary steps to insure that this wonderful waterway is restored to its past pristine quality. Thank you for considering my comments.

Sincerely,

John F. Ehrlich

John F. Ehrlich
522 N. Norwood St.
Arlington, VA 22203
E-mail: bluesally@verizon.net
Phone: 703 527-5806

August 27, 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork of the Shenandoah River to the list of impaired waters in the 2014 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I have a cottage on the North Fork near Woodstock Virginia and my sole reason for choosing this location was to have access to the river and the ability to enjoy the river. I have had the cottage for 12 years and over the last six years I have observed a dramatic decline in water quality due to extensive growths of attached algae on rocks and grasses and profuse floating clumps of algae.

My primary use of the river is for fishing. I wade fish in front of the cottage near the Headley Bridge off of Ridgeley Road and I canoe and fish on stretches of the North Fork between 10 miles above the Burnshire Dam and 15 miles below the Burnshire Dam. For the last few years, I have not known whether the river would be usable in the summer and early autumn or instead that it would be choked with smelly algae that make it unusable and offensive to the senses. If we get a heavy rain and a flush of the river, some of the algae gets carried downstream and use of the river returns for a short while. If not, the river simply not usable.

For years, I looked forward to staying at the cottage and using the river and sharing it with my family and friends. Now I warn them not to come unless they are prepared to not use the river. I am spending less time at the cottage and enjoying the river less because of the algae.

So that the impact of the algae in the North Fork of the Shenandoah River on my personal use and enjoyment of the river is clear, let me share four points:

- When the river is full of algae, and that does happen every year to varying degrees, it is impossible to fish because the fishing lures and baits get coated with algae before a fish can be caught.
- Second, when I wade fish, my pants and shirt get coated with algae, which stinks and stains. I have to wash them immediately or store them in plastic bags, due to the odor, until they are washed.
- Third, my family and friends are not able to tube float or swim when the algae is throughout the river.
- Lastly, the algae presents a foul odor (somewhere between sewage and a dead animal) such that you do not want to be on the river in a canoe or along the banks.

I want to share a specific problem that occurred this year. In early July we had some heavy rain that washed out some of the algae that had been accumulating. I was at the cottage on the weekend of July 12th and was able to wade and fish. With that good experience I invited my partner's family of five...two

adults and three boys...to come out the following weekend of July 19th and 20th. We and they had planned to canoe, fish, and swim. When we got to the cottage, we found the algae was back with vengeance. The swimming and tube floating were cancelled. We fished a little from the low water bridge but could not wade fish. Clumps of algae in the slow water near the bridge made it a stinky environment. The older boys asked what was wrong with the river, when they smelled the algae and saw the slimy floating clumps during a brief canoe trip.

The on and off presence of algae in noxious abundance over the summer and fall months has had a dramatic negative impact on the river, my personal enjoyment of the river and its use and enjoyment by my family and friends.

How can there be any doubt that the North Fork is impaired due to presence of algae? As a result, I must insist that the Department of Environmental Quality include the Shenandoah River on the 305B/303D list, determine why we are having these algae problems, and develop and implement measures to control the algae and make the North Fork suitable for my enjoyment and the enjoyment of all the citizens of Virginia.

Thank you for considering my comments,

John Holmes

286 Ridgeley Road
Woodstock VA 22664
and
2443 N. Quantico Street
Arlington VA 22207

cookscharm@aol.com
703-963-1665

April 15, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Mr. Kennedy
I own a cottage on the banks of the North Fork of the Shenandoah River at 286 Ridgeley Road in Woodstock, Virginia. The reason I have a place on the river is that I am an avid canoeist and fisherman. I regularly wade and fish the half mile of the river in front of my property and float and fish the 5 miles that are up river, from the Pugh's Run intersection with the North Fork at the Artz Road Bridge to my house, between March and October. I use the river for my enjoyment several times a week on average during these periods. Excessive algae in this part of the river is diminishing my use and enjoyment of the river in a significant way and I ask that this area be designated impaired, and that the state take action to reduce the nutrient discharges that are causing this impairment.

For a number of years now, I have noticed excessive and increasing algae growth and accumulation in the river in the summer and early fall. The algae is a major impediment to my enjoyment of the river. In particular, as the algae accumulates, it makes fishing difficult and then impossible. The algae floating on the top interferes with casting and the algae on the bottom and on the grasses, fouls the lure, which renders it ineffective. As a result, I have to give up fishing much of the river during the months of August and September.

In addition, as this algae accumulates, it appears to rot and gives off a nauseating odor…similar to sewage. When this happens I simply cannot enjoy canoeing or fishing this stretch of the river.
A big part of my enjoyment of the river, is having friends and family out for the weekend. When the river is clogged with algae, I end up cancelling family visits and cannot share the river with my friends. I have tried using other parts of the river when this condition exists and find the same thing from my house twenty miles up river and five down river

This algae impairment of the river is significant. I have expected the state and local authorities to remedy it but nothing has happened. I understand that one step in the process is to have this part of the river designated as impaired under 303D and 305B of the Clean Water Act. Please complete the designation as soon as possible.

Sincerely,

John Holmes
286 Ridgeley Road
Woodstock VA 22664

John M Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond VA 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

My name is John Politz. I am a native and resident of Louisiana. Awhile back, I had the pleasure to live in the Shenandoah Valley for a few years and to have the opportunity to enjoy my favorite activities of fishing and hunting.

During the years of 2000 - 2003, I fished all over the Shenandoah River, the South Fork and the Main Stem. However, the North Fork was where I spent most of my time fishing. I saw problems with algae at all areas of the river.

Because of the problems with the algae, fishing became a hassle with having to avoid it for it would get tangled up on the line. There isn't much worse than having an enjoyable day of fishing ruined by some algae. I still return to the valley and visit old friends every few years. I like to take the opportunity while here to get some fishing in. I spend money at the local businesses because of fishing but if the algae continues to worsen, then fishing might not be on my list of things to do while here.

The problems of the river have made me decide to write the VA DEQ to say that the Shenandoah River needs to be included on the impaired waters 303D/305B list. The Shenandoah River is a great river and I tell people wherever I go that it has some of the best fishing in the country. Please don't make a liar out of me and take care of your river.

Respectfully,
John Politz
535 Broadmoor Blvd
Shreveport, LA 71105

April 17, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I live in Northern Virginia and am an avid angler. I fish most rivers and lakes in the state. In the past I have had some great fishing experiences on the Shenandoah River. I have fished mostly the North Fork and Main Stem sections by myself and with my family. Over the last few years I have avoided this river due to heavy algae blooms and filamentous algae (rock snot) that seem to cycle yearly. The bad smell of the algae blooms, lack of fish and slime have made any river outing unpleasant. This is not to mention the fish kills and lesions on fish I have seen.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Thank you for considering my comments,

John R. Durbin
21841 Maywood Terrace
Sterling, VA 20164
E-mail: john_d_20164@yahoo.com
Phone: 703-406-9096

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, Virginia 23218-1105

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I would like to write to you to encourage you to add the Shenandoah River, including the North and South Forks, to the list of impaired waterways due to excessive annual algal blooms. These blooms significantly reduce the recreational quality of the river for several months each year and demand immediate action by the Virginia Department of Environmental Quality.

I fish a few times a year on the Shenandoah River, primarily on the South Fork near the town of Shenandoah, and have noticed that the water is particularly stained. This dark green water occurs regardless of water level and seems to be the result of algae rather than simply off-color water caused by rainwater runoff. This reduces the quality of fishing in the river as it is difficult to find fish, and often the fish that can be found are unwilling to feed. It also makes wade fishing difficult, or even dangerous, as underwater obstacles cannot be seen in the murky water. Additionally, in the summer specifically, I have notice a brown clumpy algae growing on the bottom in sufficient density to obscure most of the rocks and plant life. This algae is even worse as it fouls fishing lures and line with blobs of algae, which makes it nearly impossible to fish successfully.

As an avid fisherman and college student this problem is particularly frustrating, as I have limited opportunities to fish the Shenandoah. My spring and summer breaks seem to be during peak algae season and my father and I have taken to fishing other rivers, like the James and the Rivanna, to avoid having to deal with the algae. It is extremely frustrating having to deal with the aftereffects of significant pollution from

agricultural, industrial, and residential sources. Action from the Virginia Department of Environmental Quality is the only appropriate response to the damage to the Shenandoah River. Adding the river to the 2014 305B/303D Water Quality Assessment Integrated Report would be an important step towards restoring the river to its historic quality.

Thank you for your time,

Jonathan Hashisaki

1046 Dry Bridge Rd

Charlottesville, VA 22903

Email: jdhashisaki@email.wm.edu

April 10, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I own property on Main Stem of the Shenandoah River near Rt. 7. My family and I swim, paddle, float and fish the river mostly in our vicinity (from Rt. 50 to the WV line). During the summer one unfortunately has to check first for the presence of green algae clumps to determine if the river experience will be worth pursuing. These clumps smell terrible and are a strong indicator for my family and me to avoid recreating on or in the river.

The seemingly annual growth of algae is a disappointment and diminishes both my use and my enjoyment of the Shenandoah River. As a result I would like to ask that DEQ include the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a remediation plan.

Thank you for your consideration,

Jonathan M. Turkel
96 Kent Farm Lane
Bluemont, VA 20135
Email: jmturkel@gmail.com
Phone: (540) 955-0305

Shenandoah River

I am writing a letter requesting that the North fork, South fork, and main stem of the Shenadoah river are added to the impaired waters list.

I like to go to the Shenadoah river with my mommy and my daddy. Some things I do in the river are fishing, swimming, and [illegible]. My enjoyment has been diminished because of big, slimy, mushy, icky clumps of algae floating around, causing me to try to avoid those areas, therefore decreasing my enjoyment by limiting the areas that I can go in.

Sincerely,

Kaitlyn Potts, age 8

April 14, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the South Fork and Main Stem of the Shenandoah River as impaired waters on the 2012 305(b)/303(d) Water Quality Assessment Integrated Report due to the severe algae problems that the river is experiencing every year.

Every year, as many others in this wonderful watershed, my thoughts--post winter--turn to the river. In my backyard, so to speak, this refers to the Shenandoah. I have spent the past several seasons kayaking various sections of the south branch and middle segment, from as far south as Port Republic to Island Ford (October, 2011) to the more northern sections of the South Branch, including Foster's Landing to Burners Ford (July 2010), Alma to Rt. 211 (September 2010 river cleanup day), and the middle stem, Myerstown WV, to Millville Mills Landing (Nov 2011).

Sadly, I have witnessed the increasing degradation of water conditions from algae blooms and growth, visible in increasing intensity every year. It chokes out the normal native grasses that usually are seen, gently wafting in waters offering protection for young fish and various aquatic life. Later in the season, some areas become so thickened by growth, that areas paddled in early spring are almost unrecognizable. The areas that are affected by the algae blooms suffer such a lack of oxygen that the fish population suffers. This in turn affects where the birds nest, and the vibrancy of their young. I have seen fish in areas with large sores on their sides, and hear stories of others from fellow paddlers. The connection of it all is becoming harder to miss, even for those visiting on a more occasional basis.

I am writing at the commencement of the season in hopes that what I have witnessed while kayaking these waters can be properly addressed and dealt with in a serious fashion by those in governmental positions. As I have been involved in a small way on occasion to join in organized cleanups on the river, I realize that the effort to eradicate or more effectively control the pollutants that are responsible for the explosion of algae growth requires action well above the efforts of small groups of volunteers. We can, and will, continue to attend to the removal of trash that ends up in the river, but this issue is out of our league.

Please help us with the ecology of this lovely watershed by attending to the protection of this vibrant and important area of Virginia. Many people travel from all over the state, around the country--and various parts of the world-- to enjoy watersports, fishing and camping on the Shenandoah.

Being the daughter of an Air Force Colonel, I have lived all over the country, and halfway around the world. I have been in every state of the Union, except Alaska. I can state without equivocation that the Shenandoah Valley is unsurpassed in her raw, native beauty. That is one main reason why I chose to live here.

The Shenandoah River is a crown jewel of the state, and is threatened to be a yearly eyesore if this is not vigorously addressed. We cannot afford to let this happen; economically from a tourism standpoint, or ethically, as entrusted stewards of these lands. Thank you.

Sincerely,

Kara Rice
37 Wellspring Road
Browntown, VA 22610

Shenandoah Riverkeeper
P.O. Box 405
Boyce, VA 22620
540.837.1479
keeper@shenandoahriverkeeper.org
www.shenandoahriverkeeper.org

March 27, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish multiple sections of the South Fork of the Shenandoah River from the Town of Shenandoah to the Town of Luray. The algae and grass problems continue to rise more and more each year. I do not know the reason but I do know that it is frustrating to fish because my fishing lures are constantly getting slime or grass on them. Wading the river during the summer used to be a favorite pastime of mine. I now feel it is unsafe due to the slime on the rocks making footing unstable and I really don't feel safe walking around in the foamy water that is often in the river. I have began heading away from my favorite hometown river in favor of lakes that require me to drive much longer distances.

I also fish in the lower Potomac River several times a year because my family owns a house there. The stuff I see floating in that river and the fact that fishing has really gotten bad there makes me wonder if the algae I am seeing in the Shenandoah River is also having an impact on the Potomac River. If the fishing continues to get worse on the Potomac the house will go up for sell there.

My brother recently got his Captains License and can now begin his own charter guide service but the two areas (South Fork of the Shenandoah and Lower Potomac) are not looking to be a option due to water quality issues. I have also looked into starting a bait and tackle shop in my hometown but now I am having second thoughts on this as well.

I have volunteered my time to help with water cleanup in my hometown. I have been involved in rain garden installations, river bank erosion reduction projects, Save the Bay clean up days, runoff water control, tree plantings, willow spiling projects, attended TMDL meetings, participated in the Grasses for the Masses program, organized youth and adult fishing tournaments, etc. I personally feel regulations regarding water quality from industry, farm land and local government need to be firmly put in place so our waterways can begin to heal. I also feel that tax breaks should be given to any landowner that creates properly designed riparian buffers along their stream/river banks. For that matter anyone that can properly control water runoff from their property should somehow be rewarded.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.
Thank you for considering my comments,

Mr. Kirk Comer
917 Junior Avenue
Shenandoah, VA 22849
Email: kirkcomer@gmail.com
Phone: 540-742-1323

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, Virginia 23218-1105

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Mr. Kennedy,

I'm concerned about the health of the Shenandoah River. Both the North Fork South Fork and Main stem. Over the years I have enjoyed spending time with family and friends as well as customers on the river. The algae problem has gotten bad enough that I am hesitant to take trips during the time of the algae blooms. It has a musky smell that takes the pleasure out of what would have been a great day on the water. Plus when fishing it is forever fouling in your hooks. I refuse to allow anyone to get in the water to wade or swim. The Shenandoah is a special place to me. I would like for you to

a) Recognize that the river has this problem

b) Add it to their 303D/305B list of "impaired" or unhealthy waters

c) Develop a plan to solve it, and then solve it

Thanks

L.E. Rhodes

7456 Blenheim Road

Scottsville VA 24590

April 2, 2012

John M. Kennedy
DEQ Office of Water Monitoring Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d)Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to you to request the Virginia Department Of Environmental Quality add the North and South Forks as well as the main stem of the Shenandoah River as impaired waters on the 2012 305(b)/303(d) Water Quality Assessment Integrated Report. The reason is the severe algae problems the river experiences every year.

I and friends of mine as well as our families visit the river a number of times a year to enjoy fishing, wading and picnics. We have found that often the algae problems render the river practically unusable not to mention the disgusting odor at certain times. I am aware that this has been approached before in the past and the state has turned a blind eye to it. I find that to be a poor way of treating one of Virginia's great rivers that has been such an important part of the history of the state as well as this part of the country. I fear that we as a people have fallen short of being good stewards of the many wonders of nature that we are blessed with. It is said that the native American Indians often spoke of how the white man would eventually mess up his environment. Indians even today look upon nature as gifts from the creator and as such are to be taken care of. I feel that if the situation is not addressed many people who use and love this beautiful river will find this algae so unpleasant as to be deprived of its use and enjoyment. I love to fish the river but after reeling in your line covered with smelly slime time after time really discourages me from fishing there. You are never sure when these algae blooms are taking place so instead of traveling to the Shenandoah River we would reluctantly choose some other water. Many people enjoy kayaking including myself because it affords you the opportunity to fish as well as do some wading. Of course this type of activity is not possible during the algae blooms. To sum up I find the algae bloom situation renders the Shenandoah River less than an ideal place to enjoy my summer and fall favorite past times.

I would like to ask the DEQ to investigate this situation and if possible find and put into action a plan to remedy this problem. Thank you for considering my remarks.

Lawrence DiJoseph
6715 Moly Drive
Falls Church, Va 22046
Email: dijosephjr@gmail.com
Phone: (703) 241-2393

April 12, 2012

Leslie D. Mitchell
P. O. Box 675
Woodstock, VA 22664

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I am a volunteer water monitor for Friends of the North Fork of the Shenandoah River, so I observe the portions of the North Fork near Strasburg on a bimonthly basis. As director of Friends of the North Fork of the Shenandoah River I am also alerted by the public to algae blooms that occur on an annual basis. I paddle the river and also simply enjoy viewing its splendor as well. However, for much of the year, the North Fork seems to be experiencing a series of noxious algae blooms that impair my use and enjoyment of the river.

In recent years, I have noticed or been alerted to numerous and different types of algae blooms and observed thick algae growth on the river's surface and below the surface. The blooms I have observed have been in the summer and early fall of the year. Please see photos below of an algae blooms: 1) June 22, 2010 between about a mile downstream of Deer Rapids Bridge; 2) Same location and date; 3) and 4) Bloom that occurred in the North Fork between Deer Rapids south of Strasburg and the Rt. 55 Bridge across the North Fork, northeast of Strasburg in July of 2011. The algae smells bad, is difficult to paddle through and creates an unpleasant recreational experience in general, especially as it causes one to wonder what it is that is causing these imbalances in the water, allowing this unusual algae growth to occur

The presence of these algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to avoid these blooms in the future.

Thank you for considering my comments,

Ms. Leslie D. Mitchell

P. O. Box 675
Woodstock VA 22664
Email: ldmwtaa@yahoo.com
Phone: 540-459-8457

Photos of Algae:

Mark J. Frondorf
4114 N. 21st Street
Arlington, VA 22207
Phone: 571-969-0746

13 April 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

The purpose of this letter is to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and the Main Stem of the Shenandoah as impaired waters on the 2012 305(b)/303(d) Water Quality Assessment Integrated Report.

I have lived and fished in Virginia since 1984. My favorite river is the Shenandoah and I fish multiple stretches of the river every year. Over the course of the last five years I have noticed a real and persistent problem with algae blooms on the Shenandoah River. I have encountered these blooms on the North Folk, the South Fork as well as the Main Stem of the Shenandoah River. As a result of the algae blooms, I no longer fish the Shenandoah from July – September. The river smells, it turns a putrid green, the fishing drops off dramatically, as well as fouling your line on almost every cast.

This past summer, I donated my time as a fishing guide to the Shenandoah River Rodeo that took place in Front Royal, VA. I was embarrassed to take major contributors out on the supposed crown jewel of Virginia rivers as rock snot coated every rock and eddy pocket on the river. If not addressed, this algae will ultimately result in the loss of jobs and businesses as anglers and tourists take their hard-earned dollars elsewhere to enjoy the outdoors.

I understand you are in a difficult position with a tight budget and stretched staff. I don't envy your duties and responsibilities. But these responsibilities require you to add the Shenandoah River to the impaired waters on the Water Quality Assessment Report.

Respectfully,

Mark J Frondorf

Mark J. Frondorf

April 5, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I kayak, canoe, fish and hike at various locations on the Shenandoah North Fork, South Fork, and Main Stem, as well as the Potomac rivers. Over the past several years I have observed a decline in both rivers clearly linked to the algae problem in the Shenandoah. As a result my use and enjoyment of all three of the above-referenced portions of the Shenandoah has diminished drastically, and my use and enjoyment of the Potomac below Harper's Ferry has also been seriously diminished. Last year my family canceled an annual June canoe outing on the South Fork of the Shenandoah due to unpleasant conditions and poor fishing resulting from the annual algae bloom. The number of trips that I make to the Shenandoah has dropped off from approximately six per year 10 years ago to one or two per year over the past three years. While I return occasionally myself to see how the river is doing, it is no longer a place I am proud to take out-of-town guests for an outing.

When my wife and I were dating in the 1980s we found several locations along the North Fork of the Shenandoah in the Strasburg area where we would picnic, wade and fish. For a number of years we occasionally returned to these spots, such as the VA Rt. 55 crossing. When we visited that area in the summer of 2011, the river bottom and water conditions were drastically changed from a decade earlier. There was abundant "snot weed" and a lack of the grasses that were previously in the river. The river bottom was not visible and what was previously an attractive river for wading was not at all inviting. What should have been a pleasant outing remembering good times from earlier years turned out to be very disappointing and left us concerned that future generations will not realize what a lovely resource the Shenandoah once was.

Of course the Shenandoah flows into the Potomac and the problems of the Shenandoah have seriously impacted the Potomac below Harper's Ferry. A couple of years ago a number of us who fish and paddle the Potomac from Harper's Ferry to Little Falls began noticing cloudy water and extremely slow fishing from early in the season into July. In 2011, it seemed that the Potomac never attained its usual midsummer clarity. This aerial photo, taken by a member of the Shenandoah Riverkeeper organization on August 30, 2011, explains why. It shows the green, algae-stained water of the Shenandoah flowing into the relatively clear water of the Potomac at Harper's Ferry. I know from paddling and fishing the river at this location and all the way downstream to Little Falls that the algae-stained water basically "takes over" not far below the Rt. 340 bridge and affects use of the Potomac by thousands, if not tens of thousands, of people, as well as billions of organisms that live in the river. This photo confirms that the problems of the Shenandoah are having a dramatic impact on the Potomac and eventually on the Chesapeake.

I could cite many more specific instances, but clearly the algae blooms of the Shenandoah have diminished my use and enjoyment of the Shenandoah River system and the Potomac. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and develop and implement a plan to eliminate them.

Thank you for considering my comments,

Mark R. Myers
12128 Quorn Lane
Reston, VA 20191
Email: markrobertmyers@yahoo.com
Phone: 703-328-5843

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2014 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I am an avid fly fisherman, a Life Member of Trout Unlimited, and an active leader of our TU chapter in Winchester, VA. I fish the South Fork and Main Stem of the Shenandoah regularly and extensively, and the North Fork less frequently. In the last three weeks, I've done several all-day float-fishing trips for smallmouth bass and sun fish on the South Fork and Main Stem of the river. It has been painfully obvious to those of us who enjoy the Shenandoah that for much of the year, large portions of each section of the river seem to experience a series of noxious algae blooms that seriously diminish our fishing experience on the rivers.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event, but not always. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms, I often find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero.

By mid-May or June each year, as the river clears, the planktons seem to die back. But then a filamentous algae (rock snot) takes hold, covering the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface, and then accumulate on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli.

When these algae are blooming, it completely ruins our fishing experience. The fishing is frustrating because you cannot fish without fouling your line on the algae, and the fish won't bite a fly, streamer, or popper with algae sticking to it. This condition is greatly diminishing the attractiveness of our beautiful Shenandoah River system.

Finally, in the fall each year the native grasses die back and a bright green, filamentous algae again covers the entire bottom of the river from Broadway to Strasburg. All three of these algae make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on the 305B/303D list, determine why we are having these algae problems, and develop and implement a plan to eliminate the problems.

Thank you for considering my comments,

Mark Zimmerman, Ph.D.
309 Huntersridge Rd.
Winchester, VA 22602
Email: mbzimmerman@comcast.net
Phone: 540-722-4914

130 Mosby Circle
Warrenton, VA 20186-3240
Telephone: 540-878-5368
E-mail: hellomarybeth@yahoo.com

August 31, 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

Dear Mr. Kennedy,

I want to voice my concerns about the Shenandoah River's water quality and request that the VA DEQ include the North Fork, South Fork, and Main Stem on its impaired waters 303D/305B list.

I fished the South Fork and Main Stem of the river this summer in July and August as follows:

- July 20, mile 18 to 19 on the South Fork near Luray, catching no bass and no sunfish and noticing numerous clumps of foul-smelling algae and several dead fish—it smelled like raw sewage
- Aug. 2, Andy Guest State Park on the South Fork near Bentonville, catching no fish
- Aug. 9, Main Stem upstream of Rt. 50 bridge, catching numerous red-breasted sunfish but only one 12-inch small-mouth bass

I am an avid fly-fisher who typically fishes the Shenandoah River in a float tube 6 to 8 times a year and have noticed a significant decline in water quality this summer. Despite wearing waders, I contracted a rash on my arms when fishing the South Fork of the river near Luray and again when fishing at the Andy Guest State Park. I believe the river water is unhealthy and unsafe. The algae is observably much worse this year than in previous years, and the fish population has decreased significantly. The stench of the algae and reduced number of fish has greatly diminished my enjoyment of the river. I am not fishing it again until the water quality improves. I repeat, I would like the VA DEQ to include the North Fork, South Fork, and Main Stem on its impaired waters 303D/305B list.

Sincerely,

Mary Beth Martin

April 20, 2012

Mr. John M. Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2012 30(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

I am writing to request that the Virginia Department of Environmental Quality add the Shenandoah River system as impaired waters to the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of severe and growing algae growth and blooms.

My husband and I have had a riverfront home on the North Fork outside of Woodstock since 1991. Over the years, I have made considerable use of the North Fork for fishing, canoeing and kayaking. I have also collected water samples and benthic data to support the Citizen's Water Quality Monitoring program through the Friends of the North Fork of the Shenandoah River. I have been saddened to observed considerable changes in water quality over the years.

The visible issue is primarily an increase in attached aquatic vegetation. During the dry summer months, algae also increases dramatically to the point that it covers and clogs grass beds, sometimes making it difficult to even kayak or canoe over it. In recent summers, we have consistently observed rooted filamentous brown algae coating rocks on the river bottom. By late summer, large masses of stinky brown slime accumulate on the surface, detracting considerably from our enjoyment of the river. There seems to be little doubt that the health of the North Fork of the Shenandoah River is deteriorating.

I encourage DEQ to include the Shenandoah River system in its 2012 Water Quality Assessment Report.

Sincerely,
Mary Gessner
829 Hickory lane
Woodstock, VA 22664

Jeff Kelble <jeff@potomacriverkeeper.org>

Thanks for your help with the Shenandoah algae issue

Frondorf, Matthew <matthew.frondorf@cinbell.com> Thu, Apr 19, 2012 at 2:30 PM
To: "jeff@shenandoahriverkeeper.org" <jeff@shenandoahriverkeeper.org>

April 19, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the North Fork, South Fork and Main Stem of the Shenandoah occasionally and I also use all three rivers to take my family canoeing and camping. However, for much of the year, large sections of each river seems to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers. Driving from Cincinnati Ohio to the Commonwealth takes time and money and I am afraid this will cease if the present conditions continue. My family spends money on gas, lodging, food, souvenirs, licenses, and camping gear totally several hundred dollars per trip – we will be forced to find another destination for our leisure time that is algae free. I am certain you and your family would make the same decision.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event, but not always. Over the course of the year I use the entire river system and have seen this on all three rivers from Port Republic down to Front Royal, from Broadway to Front Royal and from Front Royal to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to swim or fish and I don't like to take my family swimming because they don't like the off-color murk water. When I do fish I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire Main Stem from July through August because of the plankton green water.

Beginning in mid-May or early June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algae are blooming I literally do not want to be anywhere near the river and I often choose to go somewhere else.

When I have no other place to go then it just takes all the enjoyment out of my day. The fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance. I found this problem in the following areas last year from July through August; Broadway, Lupton, Deer Rapids, Edinburg, Woodstock, Strasburg, Front Royal, Goods Mill, Luray Dam, 211, Alma, Port Republic, Warren Dam, Shenandoah, Route 50, Route7 among other places.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river from Broadway to Strasburg. All three of these algae make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.
Thank you for considering my comments,

Mr. Matt Frondorf

2715 Eugenie Lane

Cincinnati OH 45211

513-251-0700

Email: matthew.frondorf@cinbell.com

Matt Frondorf

Cincinnati OH

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Jeff Kelble <jeff@potomacriverkeeper.org>

Form submission from: Take Action!- Help the Shenandoah Riverkeeper Get Rid of Algae

Potomac Riverkeeper <sarah@potomacriverkeeper.org> Sun, Apr 15, 2012 at 11:56 AM

To: jeff@potomacriverkeeper.org

Submitted on Sunday, April 15, 2012 - 11:56am
Submitted by anonymous user: [76.120.244.178]
Submitted values are:

Your Letter:
Mr. Kennedy,
As an outdoorsman and father who recreates in the South Fork and main stem of the Shenandoah River 4 to 5 times a year, I urge you to add the Shenandoah River system (NF, SF, and main stem) to Virginia's impaired waters 303D/305B list due to reduced water clarity and increased amount of algae.

For over 20 years, I have camped, canoed and fished on the SF of the Shenandoah near Newport, VA every April and September. I float and fish the SF of the Shenandoah from Luray to Front Royal at least once a year. I also wade fish the main stem of the Shenandoah River near Harpers Ferry multiple times every summer for over 30 years. During that time, I have noticed a gradual reduction in water clarity and increases in algae in the river. During the most recent 10 or so years, these clarity and algae issues have increased dramatically to the point where these issues have diminished my enjoyment considerably on every outing on the river. Even the smell of the river in the summer makes me question wading in the Shenandoah. As a result, I now consider not going to the river when the opportunity arises. I also second guess recommending the Shenandoah River to new acquaintances, as their experience is likely to be marred by the algae and water clarity issues.

I have always been proud to be a Virginian and of the Shenandoah River. She is still visually beautiful from a distance and the valleys she flows through are still a delight to travel through. I still catch some fish when I do go, but not like in year's past. Her resiliency is great, characterized by the increasing, but lagging recovery from the fish kills a few years ago. Only diligent efforts by the citizens' of the Old Dominion's with the help of our great State, can we bring the Shenandoah River back to her former glory.

Thanks in advance for your consideration. I can be reached at:

Michael E. Dunn
21370 Ashburn Run Place
Ashburn, VA 20147

(703) 723-4443

Name: Michael E. Dunn
Email: med5987@comcast.net

The results of this submission may be viewed at:
http://www.potomacriverkeeper.org/node/428/submission/823

March 27, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the North Fork, South Fork and Main Stem of the Shenandoah extensively and I also use all three rivers to take my family canoeing and camping. However, for much of the year, large sections of each river seems to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event, but not always. Over the course of the year I use the entire river system and have seen this on all three rivers from Port Republic down to Front Royal, from Broadway to Front Royal and from Front Royal to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to swim or fish and I don't like to take my family swimming because they don't like the off-color murk water. When I do fish I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire MainStem from July through August because of the plankton green water.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algaes are blooming I literally do not want to be anywhere near the river and I often choose to go somewhere else. When I have no other place to go then it just takes all the enjoyment out of my day. The fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance. I found this problem in the following areas last year from July through August; Broadway, Lupton, Deer Rapids, Edinburg, Woodstock, Strasburg, Front Royal, Goods Mill, Luray Dam, 211, Alma, Port Republic, Warren Dam, Shenandoah, Route 50, Route7 among other places.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river from Broadway to Strasburg. All three of these algaes make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.
Thank you for considering my comments,

Nick Elgas
13211 Sapphire ridge pl
Bristow VA, 20136
703-498-9723

WATERKEEPER® ALLIANCE

Jeff Kelble <jeff@potomacriverkeeper.org>

algae

coolmtnman@verizon.net <coolmtnman@verizon.net> Wed, Apr 11, 2012 at 9:07 PM
To: jeff@shenandoahriverkeeper.org

1. I like to use the Shenandoah River for kayaking nearly every weekend. I have kayaked nearly every section of the Shenandoah river in the past 3 or 4 years. I'm also an avid bird watcher and take my binoculars with me on every trip.

2 In areas where ducks congregate which tends to be still water areas, I've noticed algae underwater and floating. It seems to be more prevalent during the very warm months when the ducks are raising their young.

3 I remember one day when I became ill after floating past the sewage treatment plant in Mt Crawford. I'm very healthy and it's extremely rare that I would become ill during the summer months.

4. Becoming ill has diminished my use of the river in this area.

5. I request that the VA DEQ include the North Fork,South Fork and Main Stem on its imparied waters 303D/305B list

6. Orv Lehman 5947 Harpine Hwy Linville Va 22834 540-560-4847

April 10, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year. As a frequent fisherman and paddler on the water, I see the huge impacts currently impeding the fishing on the Shenandoah system when I fish there. The contrast with other rivers like the Susquehanna, Rappahannock, Potomac, North Anna, James, and New is all the more striking, as those other flows do not show the same levels of algae that I find every year on the Shenandoah watershed. Even when weather and water flow conditions are similarly low, only the waters of the Shenandoah system show the severe different algal impacts at different times of the year that combine to make enjoying the use of this beautiful river impossible anymore.

I used to fish the North Fork, South Fork and Main Stem of the Shenandoah extensively. For much of the year, however, large sections of each river seems to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers. I now spend my time on less polluted rivers in other areas, both in Virginia and other states such as Pennsylvania, Maryland, and West Virginia, rather than spend my valuable time on an unenjoyable body of water.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event, but not always. Over the course of the year I use the entire river system and have seen this on all three rivers from their most upper reaches (Port Republic and Cootes Store) to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. The impact is so severe that even when fishing the Potomac below Harpers Ferry, the Virginia bank is consistently heavily stained and unfishable, while the Maryland shore had noticeably clearer water with better fishing. Above the mouth of the Shenandoah, this problem does not exist on the Potomac, and the difference in water color and clarity is clearly visible from elevated areas around Harpers Ferry if you want to go take a look. I'm sure it will be back again this year, probably sooner rather than later given the recent weather. Something needs to be done.

When the algae blooms I cannot swim or fish on these rivers, and I will not travel to the Shenandoah Valley to fish the any of the parts of the Shenandoah because the trip is not worth the time or cost. Local merchants lose my business because of the water quality issues. When I do fish with friends, I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish. They are usually lethargic and often they don't feed at all. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire MainStem from July through August because of the plankton green water.

By mid May or June each year as the upper river clears the planktons seem to die back. But then a filamentous algae (I call it snot grass because of the consistency and color. You get the picture.) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algaes are blooming I literally do not want to be

anywhere near the river and I often choose to fish the Rappahannock, which does not have the severe water quality issues above the fall line than the Shenandoah basin does. Unfortunately, it often is too low to float in summer because of a lack of rain when the Shenandoah is still kayakable. In that case, I travel to better fishing destinations that are often out of our state.

The fishing on the Shenandoah is frustrating because I cannot fish without fouling my line on the algae, and the fish won't bite lures or bait with algae sticking to it. Imagine wiping Ghostbusters-like slime from your lure and line after every cast. Sound enjoyable? It smells, literally. In fact, just being around the river is an assault on the olfactories due to the very unpleasant odors. I found this problem in the following areas last year from July through August; Broadway, Lupton, Deer Rapids, Edinburg, Woodstock, Strasburg, Front Royal, Goods Mill, Luray Dam, 211, Alma, Port Republic, Warren Dam, Shenandoah, Route 50, and Route7 and at places in between while floating between accesss points. Clearly pollution is having an impact when such large stretches of river are all impacted by the same poor water quality.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river from Broadway to Strasburg. When fishing this time of year the water is usually clear from the green paint algal blooms, so I can see the bottom. The fish avoid areas of the river with the dead and dying native grasses. They should be thriving here, feeding on baitfish hiding in the cover the aquatic vegetation provides, but they are clumped and clustered into small pockets where the current manages to keep the green algae from collecting, which are very few. On a six mile float from State Park to Karo in early November, the majority of the river bottom was covered in this gunk. All three of these algaes make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away. Thank you for considering my comments.

Sincerely,

Mr. Peter Pfotenhauer
10604 Wakeman Drive
Fredericksburg, VA 22407
Email: ppfoten211@aol.com
Phone: 540 498 0966

Jeff Kelble <jeff@potomacriverkeeper.org>

Algae in the Shenandoah letter

Preston Lazer <lazerpj@hotmail.com> Wed, Aug 27, 2014 at 10:49 PM
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>

Jeff, I've corrected the year to 2014 as requested; please pass my letter along to Mr. Kennedy. I will indeed mention this issue to fellow fishermen. Thanks again for your advocacy on this important issue.

TO: John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

My name is Preston Lazer and I have historically been an avid recreational user of the Shenandoah river, fishing and canoeing several times a month during the warmer months. Now, with increasing regularity, I am beset by the disgusting stench of rotting algae in my nostrils and wads of revolting algae on my fishing flies and lures. I was both embarassed and disgusted back in mid July when I took a guest for a first trip on the section of the South Fork from Karo Rapids to Front Royal landing. I thought this would be a great chance to show what a gem Virginia has! Instead, what I had talked up as "one of the top things to do in Virginia" turned into bewilderment at what had happened to our river. For much of the trip, it was just an exercise in frustration to fish because every time we retrieved a fly, it was covered in algae snot. Also, the stench was overpowering at times. Beyond the obvious asthetic damage, I also have to wonder what this is doing to the fish population? For someone who carries fond childhood memories of church picnics along the Shenandoah's headwaters and many fine fishing trips throughout my life on both forks and the main stem, this is like an assault on a member of my family.

I have primarily noticed this increase in algae on the South Fork between Bixlers Ferry and Front Royal over the past few years (probably because that's where I used to like to go), but the incident on the Karo to Front Royal stretch has caused me not to return there anymore this year. I noticed a little algae just upstream of Elkton during the first week of August, but it wasn't nearly as bad as that just upstream of Front Royal in mid July; the flat area just above the Front Royal landing was so bad that it was like a dead zone. I am sorry to say that when one of my friends called to ask for advice on unique things to do in Virginia with his visitors from Denmark, I told them to go visit the New River in West Virginia rather than to float the

Shenandoah so they don't embarass themselves like I did. I feel bad also for the outfitters on the South Fork whose businesses are not being helped by the status quo. I really feel bad for my two young sons who may need to develop an interest in video games now that the frontier for excellent fishing in Virginia appears to be closing.

To say the least, the algae has "diminished my enjoyment" of the Shenandoah. It is now also true that it has "diminished my use" of the river. It is now starting to "diminish use" by people who ask my advice. I tell them to go to the Upper Rappahanock! I don't see this algae there, I guess because it's not so full of cow and chicken dung and it has a better riparian buffer.

Because of historical abuses, folks in the Shenandoah drainage are already drinking heavy metals, PCB's, and enough discarded medication and other chemicals to cause intersex fish. Now we have this algae. When do we say, "Enough?" I have a legal and moral responsibility to properly maintain the septic drainfield on my own rural property and I do so, even though a family of four people can only make just so much solid waste. Why then is it OK for commercial operations to allow tons of raw animal feces and chemical fertilizers to run off into the water that thousands of people use for drinking and recreation? I don't dump my sewage in the local creek on the grounds that it would cost too much to maintain a working septic drainfield at my house, but that's essentially the only argument that the polluters are offering. I realize that farming is a tough business; If cost is the barrier, it might be reasonable to request that the thousands of stakeholders who drink the water help pay to remediate the properties, pay for riparian buffers taken through eminent domain, or whatever that it would take to make it feasible for landowners to fix the problem...but the time for excuses is over.

It is long past time for us to quit treating the Shenandoah like a sewer. I request that the VA DEQ include the Shenandoah on its impaired waters 303D/305B list since those who are causing the problem will not (and perhaps cannot) fix it unless compelled or helped to do so.

Sincerely,

Preston Lazer
2501 Gore Drive
Haymarket, VA 20169
lazerpj@hotmail.com

Date: Sun, 24 Aug 2014 09:14:37 -0400
Subject: Re: Algae in the Shenandoah letter
From: jeff@shenandoahriverkeeper.org
To: lazerpj@hotmail.com

Preston! Fantastic letter.

WHile I'm terribly sorry that the things you wrote about are true, I am pleased to see you make such a personal effort.

I have two favors to ask. First, I mistakenly put "2012 305b/303d" in my request. It's 2014 and I need you to make that change for me if you don't mind and resend. My apology for the mistake. I have since corrected it.

Second. In the letter you mention other fishermen that you fish with. Would you be able to personally ask them to write a letter if they have had a similar experience. Other family members can write, friends, whoever. The ball is starting to roll and my goal was 500 letters in a month. I'm learning this is an incredibly high goal.

Thank you again.
Jeff

On Sat, Aug 23, 2014 at 9:28 PM, Preston Lazer <lazerpj@hotmail.com> wrote:

> Jeff,
> Thanks for keeping the pressure on for this algae issue. Here's my letter:
>
> TO: John M. Kennedy
> DEQ Office of Water Monitoring and Assessment
> P.O. Box 1105
> Richmond, Virginia 23218-1105
> RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report
>
> Dear Mr. Kennedy,
>
> My name is Preston Lazer and I have historically been an avid recreational user of the Shenandoah river, fishing and canoeing several times a month during the warmer months. Now, with increasing regularity, I am beset by the disgusting stench of rotting algae in my nostrils and wads of revolting algae on my fishing flies and lures. I was both embarassed and disgusted back in mid July when I took a guest for a first trip on the section of the South Fork from Karo Rapids to Front Royal landing. I thought this would be a great chance to show what a gem Virginia has! Instead, what I had talked up as "one of the top things to do in Virginia" turned into bewilderment at what had happened to our river. For much of the trip, it was just an exercise in frustration to fish because every time we retrieved a fly, it was covered in algae snot. Also, the stench was overpowering at times. Beyond the obvious asthetic damage, I also have to wonder what this is doing to the fish population? For someone who carries fond childhood memories of church picnics along the Shenandoah's headwaters and many fine fishing trips throughout my life on both forks and the main stem, this is like an assault on a member of my family.
> I have primarily noticed this increase in algae on the South Fork between Bixlers Ferry and Front Royal over the past few years (probably because that's where I used to like to go), but the incident on the Karo to Front Royal stretch has caused me not to return there anymore this year. I noticed a little algae just upstream of Elkton during the first week of August, but it wasn't nearly as bad as that just upstream of Front Royal in mid July; the flat area just above the Front Royal landing was so bad that it was like a dead zone. I am sorry to say that when one of my friends called to ask for advice on unique things to do in Virginia with his visitors from Denmark, I told them to go visit the New River in West Virginia rather than to float the Shenandoah so they don't embarass themselves like I did. I feel bad also for the outfitters on the South Fork whose businesses are not being helped by the status

quo. I really feel bad for my two young sons who may need to develop an interest in video games now that the frontier for excellent fishing in Virginia appears to be closing.

To say the least, the algae has "diminished my enjoyment" of the Shenandoah. It is now also true that it has "diminished my use" of the river. It is now starting to "diminish use" by people who ask my advice. I tell them to go to the Upper Rappahanock! I don't see this algae there, I guess because it's not so full of cow and chicken dung and it has a better riparian buffer.

Because of historical abuses, folks in the Shenandoah drainage are already drinking heavy metals, PCB's, and enough discarded medication and other chemicals to cause intersex fish. Now we have this algae. When do we say, "Enough?" I have a legal and moral responsibility to properly maintain the septic drainfield on my own rural property and I do so, even though a family of four people can only make just so much solid waste. Why then is it OK for commercial operations to allow tons of raw animal feces and chemical fertilizers to run off into the water that thousands of people use for drinking and recreation? I don't dump my sewage in the local creek on the grounds that it would cost too much to maintain a working septic drainfield at my house, but that's essentially the only argument that the polluters are offering. I realize that farming is a tough business; If cost is the barrier, it might be reasonable to request that the thousands of stakeholders who drink the water help pay to remediate the properties, pay for riparian buffers taken through eminent domain, or whatever that it would take to make it feasible for landowners to fix the problem...but the time for excuses is over.

It is long past time for us to quit treating the Shenandoah like a sewer. I request that the VA DEQ include the Shenandoah on its impaired waters 303D/305B list since those who are causing the problem will not (and perhaps cannot) fix it unless compelled or helped to do so.

Sincerely,

Preston Lazer
2501 Gore Drive
Haymarket, VA 20169
lazerpj@hotmail.com

--

Jeff Kelble
President, Potomac Riverkeeper Inc.
Phone: 540-837-1479
Cell: 540-533-6465
Email: jeff@shenandoahriverkeeper.org
Website: www.shenandoahriverkeeper.org

Potomac Riverkeeper Inc.
1615 M Street N.W. 2nd Floor
Washington, DC 20036

Shenandoah Riverkeeper
P.O. Box 405
Boyce, VA 22620

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Shenandoah Riverkeeper uses citizen action and enforcement to protect and restore water quality in the Shenandoah River Valley for people, fish and aquatic life

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Mr. Kennedy,

It is with sincere urgency and the utmost importance that I request Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2014 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I have lived in Virginia my entire life and have spent several days (and money) fishing, swimming, kayaking and canoeing, the South Fork and Main Stem. I was introduced to the river when I attended JMU in 1991 and have loved it ever since. My settling (and staying) in Harrisonburg in 2002 had very much to do with the river's proximity and surrounding spring creeks. I do not need to tell you how blessed we are to have such a valuable recreational resource here, and the score of river users, both local and visitors, attests to that fact. Indeed most of that activity is in the summer, but I know of quite a few anglers (local and paying clients) hitting the water throughout the year.

Over the years I've noticed the algae and accepted it as a fact of nature. However, especially in recent years, I can't help but notice how intolerable it has become. Odd colorations, odors and the unsightliness of it diminish the entire experience. My activity on the river starts around May and runs through November; fishing anywhere from Port Republic to Front Royal. That is a long stretch of time and water to deal with the ill effects of the algae problems. In time, if let to persist I'll stop visiting the Shenandoah altogether. I do not want that to happen.

I request that the DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Thank you in advance for your diligence and time in getting this matter the attention and corrective measures it deserves.

Best Regards,

Ricardo Lianez
4350 Wiltshire St
Harrisonburg VA 22802

4270 Brown Roane Ln.
Harrisonburg Virginia, 22801
September 28, 2014

Re: draft 2014 305(b)/303(d) water quality assessment integrated report

John M Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond, VA 23218 – 1105

Dear Mr. Kennedy:

I am writing this letter to express my serious concern about the algae buildup I have experienced over the past year in the Shenandoah River. I have lived in the Shenandoah Valley for six years and I use the South Fork of the Shenandoah River and the North Fork of the Shenandoah River recreationally. I have done canoeing trips on the South Fork, and this past year I did three all day float trips on the South Fork of the Shenandoah River. I've occasionally gone fishing on both the South Fork and the North Fork, and it is always a big treat for my grandchildren to spend the day on the South Fork when they visit from New Mexico.

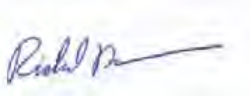
Before moving to the Shenandoah Valley I lived in Arizona for 35 years, and although Arizona has considerably less water than Virginia, the streams that I visited for recreational activities in Arizona did not have the algae problems that I have seen on the South Fork of the Shenandoah. This summer I purchased an inflatable raft to use on float trips down the Shenandoah and the inaugural voyage was a one-day trip, July 31, that I took with my son and grandson. We experience a considerable amount of algae on the float trip, but I was shocked when I saw the bottom of the white boat when we were taking it out of the truck, back at the house-- it was stained green from algae!

I realize that algae is a food source for many insects and microorganisms, but I am sure that you are aware that the real problem comes when the algae die, depleting the water of oxygen. There were two reports in the Washington Post within the last month, the first was about the Chesapeake Bay and a dead zone that had a volume of approximately 1 $mi.^3$, and the second was about algae problems in states in the Midwest where oxygen depleted water has killed fish and insects. I don't believe the South Fork of the Shenandoah is as severely impacted as the Chesapeake Bay or some of the streams that were mentioned in the Washington Post article, but if something isn't done soon, it will be ruined as a recreational resource.

I sincerely hope you will use your powers to implement policies that will protect the North Fork and the South Fork of the Shenandoah River. Algal growth and buildup is a serious problem, one that I have observed personnaly, that will only get worse if action is not taken.

Thank you for the opportunity to express my concerns and please feel free to contact me if I can provide any additional information

Sincerely,



Richard D Foust Jr.

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

This letter requests that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2014 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river continues to experience.

Each year I spend many days during the spring, summer and fall months kayaking the Shenandoah from Port Republic to Front Royal to Castleman's Ferry. The algae blooms impair the water quality of the river in many locations, making it undesirable to swim or wade in the water. This adversely impacts and diminishes my use and enjoyment of the Shenandoah River.

I request that DEQ include the above named sections of the Shenandoah River on the 305B/303D list, determine the cause of these algae problems, and develop and implement a plan to mitigate the environmental impact.

Thank you for considering my concern.

Richard F. Fox

616 Timberland Manor Drive

Bentonville, VA 22610

Richard Mrstik
220 Silverleaf Drive
Sterling, VA 20164
Stikmann@aol.com

14 September 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to you to express my concern about water quality issues on the Shenandoah River. I enjoy canoe, kayak, and wade fishing on the Shenandoah and Potomac Rivers several times a year.

During the 4th of July weekend this year, my wife and I floated the section of the Shenandoah that runs from Karo Landing to Front Royal, stopping to fish at several riffles along the way. At the lower end of this float I stopped to wade fish and found the water choked with floating dark green algae. These algae fouled every cast I made, got into my swimming trunks, stained my tee shirt, and generally smelled awful. My wife would not enter the water because of the smell and appearance of the algae. The presence of the algae definitely diminished my enjoyment of the river this year.

I have discussed this problem with other friends who fish the Shenandoah more frequently and found that it is not an isolated incident; others have had the same experience. I fish the Potomac River near my home more frequently, and although I have encountered some algae there, it has been far less and didn't have the pungent, foul smell of the algae I've encountered on the Shenandoah.

I request that the Virginia Department of Environmental Quality include the South Fork of the Shenandoah River on its impaired waters 303D/305B list and look for ways to clean up the algae and improve water quality on the river.

Very respectfully,

Richard Mrstik

Jeff Kelble <jeff@potomacriverkeeper.org>

my letter

icepeep@aol.com <icepeep@aol.com> Thu, Apr 5, 2012 at 9:30 AM
To: jeff@shenandoahriverkeeper.org

April 5, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the North Fork, South Fork and Main Stem of the Shenandoah extensively and I also use all three rivers for canoeing, nature watching and nature photography. However, for much of the year, large sections of each river seems to experience a series of noxious algae blooms that completely destroy my use and enjoyment of the rivers.

In each of the past six years during late winter a planktonic algae blooms, usually after a high water event, but not always. Over the course of the year I use the entire river system and have seen this on all three rivers from Port Republic down to Front Royal, from Broadway to Front Royal and from Front Royal to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to fish because fishing is smelly and slimy. When I do fish I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire MainStem from July through August because of the plankton green water.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage. When these algaes are blooming I literally do not want to be anywhere near the river and I often choose to go somewhere else. When I have no other place to go then it just takes all the enjoyment out of my day. The fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance.This happens throughout the river system.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river from Broadway to Strasburg. All three of these algaes make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.
Thank you for considering my comments,

Robert Abrams
6504 Byrnes Drive
McLean, Virginia 22101-5225

703 734 1238
icepeep@aol.com

Robert E. Forbes
2316 Concert Court
Vienna,VA 22181
703 281-5283

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105

September 17, 2014

Re: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

I am writing to urge you to add the North Fork, South Fork and Main Stem of the Shenandoah River to the 0303D/305B list of impaired/unhealthy waters.

I have fished the Shenandoah River System for many years--about 12-15 times during the late spring, summer and early fall each year. Most of the time, I wade in the river as I fish, which gives me an intimate connection with the water column from top to bottom. Algae growth, a constant warm weather problem, is much more prolific this year and has greatly diminished my enjoyment of the river

The algae has been especially notable on the North Fork of the river near Edinburg and in the South Fork downstream from Burner's Bottom during the late spring and summer months of 2014. Associated with the algae is a strong smell. In all of my 14 years of wade fishing in the Potomac and Shenandoah river systems, I have never before noted such a nasty smell, especially in the South Fork and Main Stems of the river. After one South Fork wade fishing trip in July, 2014, when I got in my car, I noticed an overpowering odor of rotting material and thought the odor must have come from something decaying in my car. Then I realized the odor came from my shorts that had been immersed in the Shenandoah River while I was fishing!

Algae growth is promoted by excessive nutrients entering the river. The levels of nitrogen and phosphorus are obviously more than the river can handle without becoming impaired. As a retired civil/sanitary engineer, I know that the point sources of nutrient pollution, *i.e.,* municipal and industrial discharges, can be controlled with adequate facilities that are properly designed, constructed, operated and maintained to meet the strict requirements of their NPDES discharge permits. Although non-point pollutant sources, such as agriculture, storm water runoff and septic tank overflows, are more difficult to control, success is possible when all parties are engaged with adequate direction and funding. Clearly, Virginia must make a much stronger commitment to reducing these nutrients to prevent further impairment of the Shenandoah River.

The first step to restoring this once-beautiful river is for the DEQ to recognize the extent of the problem. Please add the Shenandoah River to the 303D/305B list of "impaired" waters and develop and implement a plan to solve its algae problem.

Sincerely,

Robert E Forbes

Robert E. Forbes
Professional Civil/Sanitary Engineer (retired)

April 16, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105

Re: Adding Shenandoah River to 2012 305(b)/303(d)Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

I am writing to request that Virginia' Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah River as impaired waters on the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I have fished all sections of the Shenandoah River for many years and, since my retirement as a civil engineer in 2003, I have kept a log of each fishing trip. I have made 121 catch-and-release fishing trips on various Shenandoah River locations since 2003. The majority of the trips were on the Main Stem between Route 50 and Locke's landing. The fishing consisted of wading and canoeing, with smallmouth bass the most desired quarry. I was usually accompanied by my fishing buddies, my sons or my grandson on these river expeditions.

In general, I have observed increased weed growth over the years, often to the point where it is not possible to fish some locations by the middle of summer. There is no question that this excessive growth is due to over-fertilization by nitrogen and phosphorous. Based on more than 30 years' experience in the study and design of wastewater treatment facilities, I know more must be done to up upgrade these facilities to meet the requirements of the Chesapeake Bay cleanup initiative. In addition, the non-point sources of storm water and agricultural waste must be controlled to reduce this problem growth in the Shenandoah River.

Algae growth was a major problem on the Main Stem last year and the murkiness of the water was aesthetically very unpleasant. It severely diminished the fishing until it cleared up in early fall. This algae pollution was very evident when one looked on Google Maps at the confluence of the Potomac and Shenandoah Rivers at Harpers Ferry. The dirty Shenandoah water was in stark contrast with the clear Potomac flow.

In addition, the slimy film that accumulates on the river bottom fouls up fishing line and lures and is particularly a problem for wade fishing. The growth is slippery and as I move around, I have occasionally fallen even when using a stout wading staff and studded fishing boots with felt soles.

The Main Stem smallmouth bass population has declined as reflected in my 9 years of fishing logs. Fish do not thrive in polluted water and the long-term consequences are not good for this section of the Shenandoah watershed. Most important, the water from the Shenandoah River merges with the Potomac River and eventually becomes the source of drinking water for Loudoun County, most of Fairfax County, and the Washington D.C. metropolitan area. The pollution of the Shenandoah requires additional treatment and accompanying expense so that the drinking water in these areas will comply with the Safe Drinking Water Act.

As our nation's population expands, the use and enjoyment of outdoor recreation is more and more important for the spiritual rejuvenation and physical wellbeing of our people. The proximity of the Shenandoah River to major Virginia population centers makes it imperative that we preserve its beauty and wildness. Please consider placing these waters on the impaired list under the 305(b)/303(d) Water Quality Assessment to determine the various sources causing the weed growth and algae problems, and to develop, implement and finance a plan to solve them.

Thank you for your consideration of this request.

Sincerely,

Robert E. Forbes
2316 Concert Court
Vienna, VA 22181
703 281-5283
Bforbes32@verizon.net

August 27, 2014

Mr. John M. Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond, VA 23218-1105
RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I have lived in the Shenandoah Valley for 15 years. When I first moved here I really enjoyed canoeing and fishing the South Fork of the Shenandoah River. That has changed the past 7 or 8 years as it is very noticeable to a non-scientific person such as me that the river is suffering. I've seen many dead fish on the river and much more algae than when I first moved here. I've never enjoyed seeing cattle wading in the river and I don't understand how that is allowed by our state officials. The stretch of river I am most familiar with is from Port Republic all the way down to Luray. My most recent outing was July 12th when I floated and fished from Island Ford to Elkton. My friend and I saw lots of algae and the fishing was absolutely terrible. We saw dead fish lying on the bottom of the river and caught very few fish which is very unusual on this stretch of river. I caught two smallmouth bass with lesions on their sides. I know there is a problem when the fish have lesions on them.

I had planned to float the river numerous times this summer but, when one sees these conditions you have to wonder how healthy it is to be in water when you see high levels of algae and dead fish. It does not take an expert to understand we have a huge issue with toxic levels of chemicals flowing into the Shenandoah River. I am urging you to take the appropriate actions to begin reducing the amount of algae that is building up in the Shenandoah River. It is a treasure of this state and should be treated as such.

Best Regards,

Rodney Miner
2906 Brookstone Dr.
Harrisonburg, VA 22801
540-705-6784

Jeff Kelble <jeff@potomacriverkeeper.org>

Alge blooms on Shenandoah

Evans Ron <riverbound29@yahoo.com> Thu, Apr 12, 2012 at 1:49 PM
Reply-To: Evans Ron <riverbound29@yahoo.com>
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>

----- Forwarded Message -----
From: Evans Ron <riverbound29@yahoo.com>
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>
Sent: Wednesday, April 11, 2012 10:33 AM
Subject: Alge blooms on Shenandoah

Dear Shenandoah Riverkeeper,
I am writing you this letter in concern for the water quality of the Shenandoah River. Seems like "here we go again" is my thought as I float down the river. I lived waterfront on the mainstem just downriver from Berryville,VA for 2 years, and moved when all this started.
I am a full-time firefighter who has guided for smallmouth in the past for years. I currently do not guide however I have friends and family out on the river at least once a week. Guiding ceased for me with the fishkills earlier this decade.
Seems like once it starts warming up about this time every spring, we are plagued by a blue-green alge that smells HORRIBLE. We also notice dead fish prodominatly through the march-sept months. My concerns are for the health of the river and the fish that live in it...however my concern is evern greater for those that spend time in and around the river, and for those that get water from the river. Its discouraging to be out there, and it worries me are we getting exposed to fertilizers and chemicals from farms and chicken operations that might harm us our our families. It makes us decide to go to other rivers in other states sometimes. I certianly don't feel comfortable wading, or throwing the ball for my dog to swim in it like she always has. This also hurts the economy and businesses we spend money with to be there in the first place.
I fish many different areas of the Shenandoah...North Fork, South Fork, and theMain Stem. I used to enjoy the runs from Luray down, and from Bentonville down. On the North fork I liked to spend time from Edinburg to Woodstock. The mainstem I spent time from Route 50 down to WV. However I see the problem on all stretches. I am contacting you because I see on your website that you are politicing for the health of the Shenandoah. Please forward my letter with any others you recieve through the appropriate channels. I know with the economy and budget things are tough for all agencies...however the Shenandoah is a true gem and I would call it arguably the prime resource for the state of Virginia....and it would be an absolute shame to see the health of the river get ANY worse. The DEQ and VDGIF need to do all they can possibly do to restore and protect this precious landmark of Virginia.
This expected low water year could be the death of this river. Nows the time to act.
I would strongly urge the people in the right positions to put the Shenandoah on the 303D list as soon as possible. Help protect and restore VA's prime resource.
Contact me if I can help in any way.
Thank-You!
Ron Evans
(240)421-8198

Jeff Kelble <jeff@potomacriverkeeper.org>

Form submission from: Take Action!- Help the Shenandoah Riverkeeper Get Rid of Algae

Potomac Riverkeeper <sarah@potomacriverkeeper.org> Fri, Apr 13, 2012 at 6:06 PM
To: jeff@potomacriverkeeper.org

Submitted on Friday, April 13, 2012 - 6:06pm
Submitted by anonymous user: [107.4.147.5]
Submitted values are:

Your Letter:
April 13, 2012

John M. Kennedy DEQ Office of Water Monitoring and Assessment P.O. Box 1105 Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.
I am an avid fly fishing angler and have worked with the VDGIF and Jeff Kelble closely on various projects involving the health of the Shenandoah River. When I first moved the Harrisonburg, VA from Philadelphia and instantly noticed the quality difference in the water of the Shenandoah compared to the Delaware River where I grew up fishing.

The noxious algae blooms I have observed and come in contact with worry my deeply. As a graduate of EMU with a B.A in Environmental Sustainability I know the fact that pollution run off is harming our beautiful river. I have come to love the Shenandoah River even with all its problems and would love it even more if it was cleaner.

I spend the better part of a year working as an intern with The Shenandoah Riverkeeper trying to pin point the worst polluters of the fresh water systems in Rockingham Co. After working with the Riverkeeper I was more aware of all the problems we have with the river. Also, as a fanatic of small mouth bass fishing on a fly rod, it was hard to not blame the depletion of their species on the health of the river.

The algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Thank you for considering my comments,

Sam Berenstain
531 E. Wolfe Street
Harrisonburg, VA 22801

Name: Sam Berenstain
Email: samberenstain05@gmail.com

The results of this submission may be viewed at:
http://www.potomacriverkeeper.org/node/428/submission/821

Jeff Kelble <jeff@potomacriverkeeper.org>

Shenandoah call to action

Scott Osborne <scottosborne3@gmail.com> Tue, Sep 2, 2014 at 6:00 PM
To: jeff@shenandoahriverkeeper.org

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2014 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I use the river extensively throughout the spring, summer and fall months for recreational fishing as well as professionally guided fly fishing services. Typically, I use the river 2-4 times a week during these months as flows allow for successful navigation of the river.

I have seen a very steep decline in fish numbers and size, especially smallmouth bass from port republic down to white house. I have heard of decreased populations as well as size further down river, but I do not float those sections. There have been numerous days that my clients were relatively disgusted by the incredible amounts of algae in the river and all of us knew it was the culprit for the slow day of fishing as well as the terrible smell. They did not even want to get in the river to cool off on the hottest of days.

The proliferation of algae in the river will not only keep me from using it for my own recreation, but also from guiding and providing the local area with revenue because fish populations have been severely hurt. The algae has absolutely both diminished my use and diminished my enjoyment of some of my most favorite stretches of river in the WORLD. I have fished all over the world, and the Shenandoah is one of my favorite, but only when it is not choked by algae.

I Request that VA DEQ include the North Fork, South Fork and/or Main Stem on its impaired waters 303D/305B list.

Thank you for your time and consideration,

Scott Osborne

1214 Reservoir Rd

Charlottesville, VA 22903

434-962-9781

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, VA 23818-1105

RE: Draft 2014 305(b)303(d) Water Quality Assessment Integrated Report

8/23/14

Mr. Kennedy,

I find it a sad state of affairs that I once again have to request that the Virginia Department of Environmental Quality add the entire Shenandoah River system as impaired waters on the 2014 Water Quality Assessment Integrated Report.

I was tempted to resend the letter that I sent to you in 2012 making the same request. In that letter I talked about the miserable July weekend my group of 5 seasoned river rats had on the South Fork of the Shenandoah during a two-day float trip from Bentonville to Front Royal. We encountered the worst algae bloom any of us had ever experienced. We endured the stench and the slime for those two days. We all longed to get out of the river and clean our bodies. Two members of my group have used the river for several decades. I have been frequenting the Shenandoah and Potomac since 2001 on a very regular basis. We are tough guys but we don't like the floating algae.

Unfortunately I can add new experiences from this year. I drive along the main stem of the Shenandoah between Shepherds Ford and the bridge at Rt 7 most every day. This summer I have seen several groups of people carrying tubes walking down the River Road (Rt 606 in Clarke County) downstream toward the bridge. I was curious one day and stopped to talk to a group of teenagers. I asked them if they were walking down to the bridge takeout because the current was too slow. They replied that the river was nasty and stunk. One young lady replied that the "green globs of crud" floating in the river "creeped her out." Another youngster said they would rather walk barefoot on the paved road than stay in the river.

My group of two canoeists and one kayaker encountered a fairly significant bloom just last Sunday, 8/17, on the same stretch of the Shenandoah. It was nasty enough that our teenaged female guest asked that we get out of the river as she was not comfortable with the floating algae. We stopped about halfway through our float. I hitched a ride to the takeout point to retrieve my truck and a nice day was ruined as the result of the algae in the water.

I have encountered blooms on other sections of the main stem and the South as well this summer. I fish for smallmouth at least once every two weeks on the Shenandoah system. Even if I were told the algae would not harm me, I would not want the water to get into my mouth, nose or ears. I will not swim in it on purpose. Nor will I even wade in the water if I have a cut or open wound. In essence, the algae causes my experience on the river to be less enjoyable and makes me (and my guests) not want to use the river when the bloom is on water.

There could be an economic impact caused by these conditions if they continue to prevail during the summer months. Outfitters, guides, retailers, innkeepers and other businesses up and down the Shenandoah system rely on tourists for a large portion, if not all, of their annual income. Failure to take action to remedy this problem could very well impact their bottom line. If these businesses suffer, everyone in the state suffers.

In summary, I think it is high time that the state of Virginia take some action to stop the pollution. Your agency could start by including the Shenandoah River system on its list of impaired waters. Us older generation citizens have allowed the pollution to go unchecked for too long. We owe it to our younger citizens to start the process of making the river clean. I do hope you will consider my comments.

Stanley E. Ikonen

2479 Morgans Mill Road

Bluemont, VA 20135

texfinn@hughes.net

540-955-2747

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, Va 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Mr, Kennedy,

My wife and I live on the west slope of the Blueridge equidistant between Rt. 7 and Rt. 50. We literally can coast down to the Shenandoah River once we hit the paved road (Va #649) that heads downhill to Va #605 aka The River Road. When I retired in 2001 and decided to bring my wife back home to the D.C. area we chose our home based for a large part on the nearness of the Shenandoah River. We both love canoeing, recreational kayaking, and I am an avid smallmouth bass fisherman. At the time of our move in 2001, the Shenandoah was rated as one of the top 10 smallmouth bass fisheries in the United States. We were very excited about renovating the old farmhouse we had bought and living near one of the prettiest rivers in America.

Since we have lived here the river has undergone some pretty "nasty" events. First were the fish kills on both forks and the main stem in the past ten years. In spring of 2002 I caught my first bragging size smallmouth, a beautiful 19" fish. Beautiful that is except for the lesions on its tail that looked like cigar burns. But that's another story.

Algae bloom. That's the big story now. For much of the year the river is turned a murky, nasty looking green from the algae. The water doesn't smell all that good as well. Last June I canoed the South Fork of the Shenandoah from Bentonville to Front Royal with a group of friends. It was one of the most unpleasant experiences of the year. Worse than the record-breaking heat was the appearance, smell, and an almost slimy feeling of the water. It was disgusting. We stayed overnight on a sand bar. I usually take a swim before I bed down to remove the day's dirt and sweat. Not that night. I choose not to expose myself to the water anymore than needed. We reveled the next day when a thunderstorm hit. We all dashed for cover on the bank but would run out into the rain risking being lightning rods to rinse our bodies. Another group of friends out on the main stem that same weekend said they experienced the same conditions i.e. nasty looking water, lethargic fish, and a general feeling of "yuk."

As a result of my experience my wife canceled a float trip for the next weekend. A group of her girl friends had planned to come out to our home, do a day trip on the main stem of the Shenandoah, and then wine and dine at a restaurant in Upperville. Instead my wife drove to D.C. Her and her friends spent a lot of money in the District rather than out in country where the small business owners are hurting. I suspect that other family and friend groups have canceled trips thus denying needed income for fishing guides, canoe outfitters, and other businesses in the Shenandoah River valley.

Please find a solution for this condition. Recreation and tourism is an important cog in the economic engine that runs this fair state. Let's not let green water stop that flow of needed income into the valley. Perhaps a good start would be to include all three sections of the Shenandoah River on the 2012 303B/303D list. I believe our beautiful river is endangered and impaired. Let's stop whatever is causing the bloom and start getting the water clean. No one wants to expose themselves or their children to unclean water. Think about it.

Sincerely,

Stan Ikonen

2479 Morgans Mill Road

Bluemont, Va 20135

Email:texfinn@hughes.net

Phone:540-550-3555

September 1, 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

Dear Mr. Kennedy,

My name is Steve Kimm and I am President of the Potomac River Small Mouth Club. We are an organization of over 100 fishermen who regularly fish the Shenandoah.

I personally fish the Shenandoah on a regular basis. However, when using the river, I have noticed that large sections experience severe algae problems. This seriously diminishes my use and enjoyment of the rivers.

When these blooms happen, fishing is bad. It's hard to fish when your line is full of algae. The fish won't bite lures or bait with algae stuck to it. I prefer not to be on the river due to the unpleasant odor. I found this problem last year starting in the Spring and ending in the Fall. It absolutely takes away the enjoyment of being on the river.

I would like to ask that DEQ to do three things:

- Include the three sections of the Shenandoah River on 305B/303D list
- Determine why we are having these algae problems
- Develop and implement a plan to make them go away

Thank you for considering my comments.

Regards,

Steve Kimm

President, Potomac River Small Mouth Club

Steve Kimm
1933 Wilson Lane #T2
McLean, VA 22102
sjkfishes@yahoo.com

Steve Kimm
1933 Wilson Lane #T2
McLean, VA 22102

April 11, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the South Fork and Main Stem of the Shenandoah and I also use these rivers to take club fishing trips. However, for much of the year, large sections of each river seems to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

In each of the past five years during late winter a algae blooms, usually after a high water event, but not always. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I may still fish but I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero. For the past two years I simply could not fish the lower south fork or the entire Main Stem from July through August because of the plankton green water.

By mid May or June each year as the river clears the planktons seem to die back. But then a another algae (rock snot) takes hold and covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae often comes about this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algaes are blooming I literally do not want to be anywhere near the river and I am forced go somewhere else.

I personally found this problem in the following areas: Front Royal, Goods Mill, Luray Dam, 211, Alma, Port Republic, Warren Dam, Shenandoah, Route 50, Route7 among other places…

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a Virginian and a taxpayer I encourage DEQ to include the all sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and more importantly to develop and implement a plan to make them go away.

Respectfully,

Steve Kimm

September 7, 2014

Steven R. Adams
5504 Talon Court
Fairfax, VA 22032

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2014 305(b)/303 (d) Water Quality
Assessment Integrated Report

Mr. Kennedy:

I have spent several weekends every summer for the past 27 years floating and fishing the North and South forks, and the Main Stem of the Shenandoah River.

However, in the past few years my enjoyment of this river has become increasingly unpleasant. Just this past July 2014, on a float trip on the South Fork of the Shenandoah River, from Alma to White House near Luray, I encountered numerous stretches of the south fork with large amounts of algae. The algae smelled like something was rotting, it was slimy, and stuck to everything on my kayak and fishing gear. The algae also made the bottom very slippery and dangerous in places. Just trying to get in and out of my kayak was problematic.

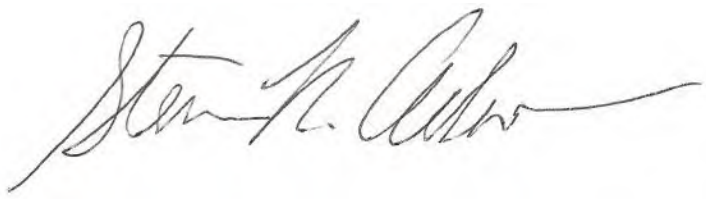
The problem has been getting worse every year. I float the Shenandoah River every summer, and the algae is making it less and less desirable to fish and use the river for recreational canoeing and kayaking.

Please help protect and preserve one of Virginia's most precious resources by including the North Fork, South Fork, and Main Stem of the Shenandoah River on the impaired waters 303D/305B list.

I believe the algae problem is worse in the Shenandoah River than other area rivers. Only a few years ago the algae bloom from the Shenandoah River was so bad, it affected parts of the Potomac River which the Shenandoah flows into at Harpers Ferry and below the confluence.

This serious problem cannot be ignored any longer.

Sincerely,



Steven R. Adams

April 14, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I own property on the South Fork of the Shenandoah near Front Royal. My family and I enjoy the river upstream and downstream of my property, most frequently in areas between the Guest Shenandoah River State Park and the Front Royal Luray Avenue boat landings. For each of the past eight years, we have used the river approximately 4 to 8 days per month between May and September to kayak, tube, swim or fish. We also picnic near the river and exercise our dogs along our riverfront.

While we hope to enjoy the peace and serenity of my land, for much of the year the South Fork seems to be experiencing a series of noxious algae blooms. In each of the past six years we have seen the river turn a dark murky green color, like green paint, from late winter or early spring until about mid-summer. These planktonic algae blooms negatively impact our enjoyment and use of the river, because we do not like to swim or fish in the murky water, and do so less frequently when we see them. These blooms seem to follow high water events, but not always.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year.

Additionally, a floating bubbly type of algae (Cynabacteria or Blue Green Algae) comes at this time. Brown-green clumps fill the water surface and accumulate on anything sticking out of the river. This algae smells like sewage or rotting broccoli. When these algaes are blooming we do not want to be anywhere near the river. An experience we had with one of these blooms in late June 2011 left such a negative impression on us that we did not tube or kayak for months afterward.

The cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to keep the river system clear of them.

Thank you for considering my comments,

Stuart C. Harding
1325 Stoney Bottom Rd.
Front Royal, VA 22630
Email: stuart_c_harding@yahoo.com
Home: 540-636-9721
Cell: 540-683-1801

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

26 August 2014

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I have some very bad news for you; our Shenandoah River, the Daughter of the Stars, a Commonwealth natural wonder, and an income source from recreation, ***stinks*** – literally.

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2014 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the North Fork, South Fork and Main Stem of the Shenandoah extensively, and I also take my family to vacation river properties. Last week I spent close to $2,000 for a South Fork cabin for my wife and I, my daughter and son-in-law, and two grandsons in Elkton. My oldest son is a fisherman. He wanted to know what "the green junk" was in the water. We couldn't find a picnic stop for the two canoes because cows had the best spots! You need to read the Berenstain Bears, "*The Bears Picnic*." wherein they find the world's best picnic spot. The Doah doesn't have one anymore.

Last month my son visited from NYC where he kayaks on the Hudson off the West Side piers. We fished the South Fork. Guess which river came out best to him? NYC was better! The Hudson in NYC better than our Doah. *Damn Yankees!*

My most recent experiences, in the past two months, with the slime have been between Routes 7 and 50, from Island Ford to Elkton, and from Burners Bottom to Bentonville. But I could name you many more stretches. In fact, I can't name you a stretch where I go that doesn't have what we fisherman call "river snott."

These algae blooms are ruining one of the Commonwealth's greatest natural resources. As a Virginian and a taxpayer:

I am tired of the stink.
I am tired of the poor fishing.
I m tired of the river color.
I am tired of cows using the river for an outhouse.

For goodness sake, get out there and smell the bloom. It is not roses and neither you nor I, nor our neighbors or visitors like the smell, the color, the algae clumps, and the poor fishing.

Please, have DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to fix the problem.

Very Respectfully Yours Sir,

LTC (RET) Terrence Cooney, US Army
7514 Rambling Ridge Drive
Fairfax Station, VA 22039

Email: tcooneyva@aol.com
Phone: 703-455-7304

LTC Terrence E. Cooney, USA (RET)
7514 Rambling Ridge Drive
Fairfax Station, VA 22039

30 March 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105

Subject: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

My wife and I retired from the military in the Old Dominion over 20 years ago. I used to be a very frequent visitor to the Shenandoah River, both the forks and the main stem. I have enjoyed Andy Guest State Park. We enjoyed romantic stays at the local valley B&Bs, while canoeing the river and picnicking on its banks. I now fish and float elsewhere, and we enjoy B&Bs elsewhere. The Shenandoah, the pride of the valley, has become a *cesspool.* The Shenandoah River featured in numerous "Virginia is for Lovers" ads, and books and movies, synonymous with tales of the Civil War, is now a filthy breeding ground for algae – the green slime.

This is not merely an issue of "green think" or "tree hugging;" the Commonwealth is *seriously* in danger of losing one of its premier natural beauties … and sources of tax revenue. That loss translates to more taxes for me to offset loss of tourism dollars. *Enough.* Please take the appropriate steps to include the three sections of the Shenandoah River on the 305B/303D list. Find out what the algae problem is and fix it.

It is unconscionable that citizens of the Commonwealth should even have to write such a request to our Government. All our native Virginia American President's have known and loved this river. Will our generation be the one to abandon it?

I am Very Respectfully Sir,

LTC Terrence Eugene Cooney, USA (RET)

Copy: Governor Bob McDonnell, Office of the Governor
Patrick Henry Building, 3rd Floor, 1111 East Broad Street, Richmond, Virginia 23219

April 21, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I own property adjacent to the South Fork of the Shenandoah River in the Shenandoah/Shipwreck area about 1 mile downstream from the dam in Shenandoah. My family and I have enjoyed the fact that we live in such a beautiful area and have been able to enjoy the numerous opportunities the river has to offer. There has been a significant change in the river in the past years and the time we spend there has been far less enjoyable. In fact, we spend less and less time there and have been traveling to other areas to enjoy similar activities on other waters.

The main issue is an algae that has been dominating the river waters in recent years. When we first moved to the area, the river was prolific with numerous fish species of great size and the river bottom was dominated by native grasses and aquatic invertebrates. In addition, the river valley was well populated by a plethora of migratory and resident bird species and ducks. What we have noticed is a significant decline in the number, size and variety of all species mentioned above. After a significant fish kill several years ago, the smallmouth bass never recovered. There are still smaller fish around, but as they grow, sores develop and I find dead fish about 12-14 inches in length along the shore later in the summer. Rarely do I ever find them bigger than that. The numbers of minnows, darters, chubs, bluegill and suckers have also declined significantly. The native grasses that would form huge underwater forests in the shallows have all but disappeared. These areas were very productive feeding grounds for the fish as well as excellent retreats for breeding fish and a nursery for the juveniles to feed and hide. The area would also support numerous birds such as kingfishers, ducks of all kinds, tanangers, orioles, green herons, etc., that thrived on the grasses, fish or invertebrates produced in these vital areas. These birds are rarely seen here anymore and the diversity and enjoyment of seeing and studying them is all but gone.

What we have noticed is that late in the winter, very early spring, a green, slimy algae will form on the rocks and the water will turn a greenish color. It is variable based on the water flow in the river and tends to occur after huge rains when the water levels starts to fall. It will last for months and will be replaced later in the late spring and early summer with long green filaments of what has been called "rock snot" by my neighbors. The native grasses try to take hold but they are soon overwhelmed by this algae which sticks to the blades and smothers it out. Even in faster flowing waters, this algae is very sticky it will weigh the grass down and eventually it dies out. What is left behind is a slimy rock bed that is all but devoid of life except for the small black snails come to graze on the algae is unbelievable numbers. When you wade the shores and start flipping rocks to see what is left, there is not much at all. A few hearty inverts, but no crayfish, very few if any helgramites or large stonefly larvae, and just a small sample of tiny minnows. I have also noticed a decline in the number of frogs and snakes that frequent the shores, and as for

the beavers, we have not seen one in three years. It's no wonder why the diversity of life has moved or died off.

By late summer, when the water levels are low, we really don't even spend time at our section of the river. There is a funky green foam that occurs in the shallow, still waters and in the eddies. It has a rancid smell at times and to wade or swim is out of the question for my family. Even to paddle, it is not very enjoyable and when friends or family come to visit, they are shocked to see the water quality. We have been going to other waters far further upstream in the national forest or across the mountain into West Virginia to enjoy the experience we once had right here at home.

There is little doubt in my mind that the cause for this is the inorganic and organic chemical surplus that gets added to the water each year. The amount of nitrogen and phosphorous added to our beautiful watershed must be excessive but little if any monitoring is done to control it. There is also a huge trend in "no till' farming in our immediate area that is based on tremendous amounts of "round-up" or other herbicides being sprayed and ending up in our river. I do not have the facts to prove this which is why I am requesting that the DEQ include the three sections of the Shenandoah River on 305B/303D list to determine why we are having these algae problems and diversity loss and come up with a plan to protect this section of the river.

Sincerely,

Thomas A. Bahleda, M.D.
449 Shipyard Rd.
Shenandoah, VA 22849

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Mr Kennedy,

My name is Tim England and I have lived here in Winchester VA since 1998. Before moving here, I was in the military and lived in TN, TX, AR, MT, MS and CA. I have fished and hunted in what I would consider some of the the finest areas of our country. I can say without hesitation that the best river that I ever fished was the Shenandoah River. That includes the Mississippi River, Tom Bigbee Waterway and the Missouri River in Montana. Some of my best memories were floating down the South Fork in 1999- 2001. That all came to a halt. SInce then, the combination of the fish kills and the algae on the river have taken this great resource and ruined it. The fishing has become so unproductive, that I have essentially stopped river fishing.

Usually April brings solid rainfall and great fishing. By June, due to low water and hot temperatures, the river becomes an eyesore and stops becoming an asset to the state. I don't know what is causing this, but this I do know. Other states like Montana (with Whirling Disease) become very proactive when evasive infestations threaten the waters. Even Maryland has become very forward thinking. They are aggressively battling the Snakehead, require watercraft exclusivity on lakes like Liberty and Prettyboy and have enacted a new law requiring rubber wading boots on rivers instead of felt to prevent invasive species.

The effects of weeds and algae have certainly gotten out of control in VA. Every year, the weed growth in Lake Frederick becomes thicker and deeper in the lake. Since I don't fish the Doah anymore, all I have to go by is the sight of algae on the river as I drive on route 7 on my way to work. I can tell it is getting worse, thicker and deeper each year.

Hopefully, the tide will turn for this great river which has become the example for all other VA rivers NOT to follow. Hopefully, it will return the glory years of not so long ago.

Thanks,
Tim England
92 Abbey Rd
Winchester VA 22602

Jeff Kelble <jeff@potomacriverkeeper.org>

Shenandoah River Algae

Tommy Everett <jteiii13@gmail.com> Wed, Sep 3, 2014 at 7:16 PM
To: jeff@shenandoahriverkeeper.org

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2014 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I fish the North Fork, South Fork and Main Stem of the Shenandoah. However, for much of the year, large sections of each river seems to experience a series of noxious algae blooms that seriously diminish my use and enjoyment of the rivers.

In each of the past six years during late winter a planktonic algae blooms. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. When the algae blooms I often choose not to swim or fish. When I do fish I find the fishing is poor and I don't enjoy the experience as much. Whenever the river is this murky color, it's disturbing to fish and the fish are usually lethargic and often they don't feed at all. Activity in the river drops to near zero.

By mid May or June each year as the river clears the planktons seem to die back. But then a filamentous algae (rock snot) takes hold covers the river bottom and the native grasses which are trying to emerge. The native grasses turn brown and lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of algae (I'm told its Cynabacteria or Blue Green Algae) comes at this time. Clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. This algae smells like sewage or rotting broccoli. When these algaes are blooming I literally do not want to be anywhere near the river and I often choose to go somewhere else. When I have no other place to go then it just takes all the enjoyment out of my day. The fishing is frustrating because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae sticking to it. I find being around the river very unpleasant due to the odors and annoyance.

Finally, in the fall each year the native grasses die back and a bright green filamentous algae again covers the entire bottom of the river. All three of these algaes make it nearly impossible to fish and again diminishes and sometimes eliminates my ability to enjoy the river.

This cycle of algae diminishes both my use and my enjoyment of the Shenandoah River system. As a result I would like to ask that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Thank you for considering my comments,

Tommy Everett

626 Flordon Drive
Charlottesville, VA 22901

jteiii13@gmail.com
434-996-5334

Jeff Kelble <jeff@potomacriverkeeper.org>

A letter

Trace Noel <noeltrace@gmail.com> Thu, Apr 19, 2012 at 7:23 PM
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>

April 19, 2012

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Sir,

I write to loudly protest the lack of action of your agency and the Commonwealth with regard to the decades of algal growth that continues to plague the South and North Fork as well as the Main Stem of the Shenandoah River. Further I request that this internationally recognized water source be placed on Virginia's impaired waters 303/305B list. To do otherwise is a breach of public trust.

Amongst the many problems the Shenandoah River(s) has is crippling algal blooms. This stuff stinks, ruins camping trips and makes a travesty of the fabled shanty "Oh, Shenandoah". I do not believe even the second or third verses contain the words stink or algae. Might want to 'google' that!

Currently, I personally use the river fishing, boating, wild life/bird photography and canoe camping more than 50 days a year. Recently, the impact of the poor health of the Shenandoah(s) has seriously diminished my river use. I no longer invite friends to enjoy this flowing cesspool. Assuring them that the algae is, in fact, not human waste somewhat diminishes the experience and knocks the wind out of my life long enjoyment of sharing Virginia's great outdoor tradition.

I am a fly rod angler and the algae - in both forms - are ruinous to fishing flies and have reduced my participation on and use of this watershed dramatically. In the area I most often use - Page and Warren Counties, the slack water stench of the floating algal lubs and the spun green algal silk - often measured in meters - foul my boat, PFD, fishing gear, clothing, shoes and a great deal of my angling equipment. I rarely swim in the river other than a quick 'cool off' dunk.

The rivers' condition actually breaks my heart.

As a retired outfitter on the South Fork of the Shenandoah with more than 20 years of daily and first hand experience I can speak directly to the impact that both phases of the algal bloom has on the river.

During the Spring and Summer large clots of algae break loose and head downstream. Resembling tumbling and floating human waste, these algal turds gross out urban guests, exasperate anglers, collect in slow moving water and leave a vomitus stench that diminish the experience by both private landowner and thousands recreational users. One simply has to visit the Guest/Shenandoah River State Park to view the septic floaters, fecal looking river bottom or the 20 yard long ribbons of bright green fibrous strings.

The impact to the watersport recreation industry in the Shenandoah Valley – read economic loss to struggling rural communities - is substantial From float tubers to anglers with tangled lines our operation suffered diminished participation from urban guests who chose other ways to spent discretionary income. The

Shenandoah River is the economic engine that drives valley growth and tourism. Without protection it will continue its long slow decline.

No one will sing songs lovely songs about river. The river is now under your watch.

Respectfully,

Trace Noel

352 Shenandoah Hts. Road
Front Royal, VA 22630
noeltrace@gmail.com

2012 AlgaeLetter.docx
21K

March 27, 2012
John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,
I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on thee 2012 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae problems the river experiences every year.

I am a retired professional sanitary engineer with a keen interest in water quality and the health of our rivers. For the past 50 years I have been an avid bass fisherman. I grew up fishing the James River. As a result of being retired and having more free time I routinely fish most of the major smallmouth bass rivers in Virginia. I have fished the North Fork, South Fork and Main Stem of the Shenandoah for decades and for all that time observed noxious algae blooms that seriously diminish my use and enjoyment of the rivers. In fact, due to the very poor water quality I see much of the year and the serious fish kills and resulting steep decline in the quality of the angling I use the river much less. Of all the many rivers I recreate on in Virginia the water quality in the Shenandoah system is by far the worst to the point that it seriously diminishes the recreational experience.

The most extensive and troublesome problems are the planktonic algae blooms that I often see after a high water events. I have seen this on all three rivers from Port Republic down to Front Royal, from Broadway to Front Royal and from Front Royal to the Confluence with the Potomac River. This bloom turns the river a dark murky green color, like green paint, from late winter until about July. I do not fish the river when this condition occurs because fishing is generally poor. Activity in the river drops to near zero. At this same time water quality on other bass rivers is fine so I fish other streams. I am presently very concerned about recent the fact that for the past two years the algae blooms were so bad I could not fish the lower South Fork or the entire Main Stem from July through August.

It is almost impossible to escape the impacts of poor water quality and over enrichment of the Shenandoah River system on recreational angling. In May and June blue green and filamentous algae begins to appear and impact our native grasses as well as the ability to fish. They turn brown, lose their leaves and remain bare the rest of the year. Additionally, a floating bubbly type of blue green algae clumps rise up to and float on the water surface and then accumulates on anything sticking out of the river and in back eddies. The fishing is impossible because you cannot fish without fouling your line on the algae, the fish won't bite lures or bait with algae. I find this problem everywhere I fish in the river.

I would like to see improvements in water quality and be able to recreate more often on the Shenandoah river system. I request that DEQ include the three sections of the Shenandoah River on 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to make them go away.

Urbie Nash , P.E.
1232 Frederick Street
Waynesboro, Virginia 22980

Jeff Kelble <jeff@potomacriverkeeper.org>

Shenandoah River

Vernon Mann <vomann@hughes.net> Mon, Apr 9, 2012 at 2:01 PM
Reply-To: vomann@hughes.net
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>
Cc: Michael Hobert <lawyers2@verizon.net>

Dear Mr. Kennedy, I am writing this letter to you to request that Virginia Departmental of Enviormental Quality add the North Fork,South Fork and Main Stream of the Shenandoah River as Impaired Waters on the 2012 305(b)3/303(d) Water Quality Assessment Intergrated Report because of the severe algae and other polution problems.

We live along the Shenandoah River, 3.3 miles south of the Route #7 Bridge and have 247 feet of River Frontage which we keep well groomed. In the summer time we love to sit down on the river front and just watch the river run by. I am 87 yrs old and years ago I used to be an ardent fisherman and did a lot of wade fishing on the Shenandoah all the way from Luray area down to the Rt. 7 bridge. Back is those days the Shenandoah was something to be proud of pure clean spring fed water and if you needed a drink of water you just dipped you hand in and took a drink which I did many times - but not anymore.

I personally don't fish anymore but when my three sons and our grand kids come they like to fish and get into the water but I no longer permit this due to the algae and other polution problems -

In addition to the Algae problem we have another problem which is getting worse by the years - Every morning from about 7am till about 8:30am there are mounds of white foam (my guess is Phosphates) floating down the river. This is apparently controlled up stream somewhere as during the warm weather on week ends the foam does not run on Sat and Sunday but on Monday it's turned loose again - this has been going on for quite a few years 10 or 15 years and is gradually getting worse by the year. I have reported this before but to no avail - just can't figure out why you can't find the source .

W e used to have friends out to fish and swim or float down the river in iner tubes but since the river is in such bad shape most of them have given up .

I have even seen some fish that were caught that had sores on them- Who wants to eat fish like that?

Sure hope you can help clear up this problem on the Shenandoah so that our up coming people can enjoy the river as much as we did. Thanks for Your Help and May God Bless You !! Vernon & Eileen Mann

3295 River Rd.

Bluemont,Va.20135

Phone:540 955-3851

202 Sylvan Lane
Madison, VA 22727
September 11, 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond, VA 23218-1105

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy:

As a member of the Potomac River Smallmouth Club in Vienna, VA, I am highly concerned about the increasing amount of algae found in the entire Shenandoah River and Potomac River. Over the years I have seen the amount of algae and grass increase greatly. It makes the rivers hard to fish and has hurt fishing for all recreational fishermen. I have been an active member of the Potomac River Smallmouth Club for about 10 years. I have steadily noticed that the quantity and quality of Smallmouth bass has gone down. We use to have a lot of 20 plus inch fish. Now we are lucky to see 10 that size for the entire club. I know because I keep the records for our fishing contest.

One of the things that really irritates me about the current river conditions is that after each float down the river, I have to remove algae from my boat. I expect to wash the boat after each trip, but the algae requires I "scrub" the boat bottom to be sure it is clean. I don't want to take this mess to any other body of water. I am also aware that the water quality is not as pristine as possible. I have to be sure that I have no open wounds prior to going fishing. I have heard of more than one that ended up in the hospital due to an open wound that got infected. I expect to take normal precautions, but to not go fishing due to a small cut, is not what I have in mind.

The algae and grass I have seen on the river is primarily noted by mid summer to early fall. Early fall is prime fishing time and the over abundance of grass makes it very difficult to fish. All I catch is grass, not fish. I most often fish the South Shenandoah River and the Potomac River. Both rivers have been affected by this problem. The Shenandoah River has had the greatest problem.

I request that the VA DEQ include the North Fork, Sough Fork and the Main Stem of the Potomac be on its impaired 303D/305B list. Thank you for your consideration.

Sincerely,

W. W. Harvey

4 April 2012

ATTN: John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to ask that the Virginia Department of Environmental Quality include the North Fork, the South Fork, and the Main Stem of the Shenandoah River as impaired waters in the 2012 305(b)3/303(d) Water Quality Assessment Integrated Report. The entire Shenandoah watershed has been experiencing severe algae problems for several years.

When I started fishing the North Fork and Big Stoney Creek in the mid-1970s, the Shenandoah and Stoney were crystal-clear watersheds. I used to take my Sons fishing out there with me, and took our Boy Scout Troop out there quite a number of times. But then I was assigned overseas for several years, and when I came back, Stoney and the North Fork were mere shadows of themselves by the mid- to late 1980s. Many fish had sores on them and there definitely weren't the numbers of earlier years.

I still fish the North Fork, South Fork and Main Stem of the Shenandoah. However, for much of the year, large sections of each river experiences a series of algae blooms that seriously diminish water conditions and my use and enjoyment of the rivers. In each of the past 6 years during late winter there have been algae blooms. Over the course of the year I have seen this on all three rivers near Edinburgh, Shenandoah, and below the Front Royal Dam. This bloom turns the river a dark murky green well into summer. When the algae blooms I often stop wet wading, fearing an infection. In fact, a couple of years back a couple of members of my fishing club scratched themselves in some of the rivers in the Potomac watershed, not sure if it was the Shenandoah system, but they almost died. When I do fish, I find the fishing is poor.

By mid-May or June "rock snot" takes over the river bottoms, and the native grasses, which are trying to emerge, turn brown and lose their leaves and remain bare the rest of the year. There is also a floating bubbly type of algae that comes at this time. Clumps rise up and float on the surface.

The Shenandoah watershed is a mess, and I urge you to ask that DEQ include the three sections of the Shenandoah River on the 305B/303D list, determine why we are having these algae problems, and to develop and implement a plan to clean up the Shenandoah. I don't know if you are a fisherman, but when I first started fishing it, the 'Doah was pristine. Now we've had several major fish kills and the algae problems. The river used to attract fishermen from all over Virginia and all along the East Coast. Old friends of mine who live in New Hampshire used to come down every year to fly fish for smallmouth bass. They haven't come down in several years. Heck, why would anyone drive 500 miles to get skunked by the fish and the algae.

I am the activity chairman of the Potomac River Smallmouth Club, with over 100 members. If you would ever like to hear our comments on the state of the Shenandoah, we would be proud to host you. We meet most months on the last Wednesday of the month from 1930 to 2100 at the Vienna Fire House. Please check out our Website at www.prsc.org.

Respectfully,

William J. "Bill" Amshey, Jr.
3007 Rayjohn Lane
Oak Hill, Virginia 20171
703-716-0838

William M. Prokopchak
1714 Lewis Street
Winchester, VA 22601
23 August 2014

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
PO Box 1105
Richmond, VA 23218-1105
RE: Draft 2014 303(d)/305(b) Water Quality Assessment Integrated Report

Mr. Kennedy:

I'm writing you today to describe to you the problems that I see with the Main Stem, North Fork, and South Fork of the Shenandoah River and why the Virginia Department of Environmental Quality (VA DEQ) should consider the **Shenandoah River to be a 303D/305B impaired river** and develop a plan to solve the problem.

I have known the Shenandoah River for more than 25 years. My family and I have swum, fished, and paddled its waters from Elkton to the Rt. 7 bridge. I recall those early days when the water was (except during high water) clear and there was a Smallmouth Bass behind every rock. Fish of all kinds lived in its waters, and the fish were healthy looking and strong. During those early years, I probably averaged being on or in the river twice a month from May through October.

As a father of three young children in the 1980s we thought of the Shenandoah River as both prolific and historic. But things began to change, and change dramatically in the late 1990s. I first started to notice the changes when, after paddling the river, my clothes smelled awful and when river water dripped inside our mini-van made the car smell, too.

As the years passed, the fish in the river became sick. Awful-looking lesions started to appear on the fish and by the early 2000s nearly every fish I caught had the same problem. Some of them had multiple red, bleeding, and very sore looking lesions. I became afraid to be in the water. I certainly would not allow my children to eat any fish that came out of such water.

In the summer on the main stem especially, the water was often so green with floating algae that I don't think the fish could see well enough to find my streamers and poppers. Large floating pieces of the green/black gunk broke loose from the bottom and decayed, making the river a stinking mess. There were times that I could not fish because the algae collected on my line and lures and seriously interfered with my enjoyment of the river. This algae problem continues today.

Problems on the Shenandoah River reminded me of the decline and fall of Lake Erie. As a child I remembered, the waters of Erie were clear and cold. Restaurants proudly served the Walleye and Perch from the lake. But by the time I was in high school, Erie was a foul, green, stinking mess. I moved to Virginia with the US Air Force and stayed on as a student at both The College of William and Mary and Virginia Tech and moved to Winchester with my wife and kids. The same thing I saw happen to Lake Erie was now happening to the formerly beautiful Shenandoah River.

Then came the fish kills starting in 2003. That's when I stopped using the river for a number of years. If the water was killing the fish, it surely couldn't be healthy for my family. My canoe and kayaks sat idle. To be sure, the condition of the water did more than just diminished my enjoyment of the Shenandoah, it completely stopped me from using the river. I stayed away from the river but my concerns about it remained.

It was about 2008 when I ventured back into the Shenandoah River. The fish were again reproducing, but it was obvious that the river was still impaired. The green algae still appeared in summer and at times the river smelled like dung. The algae inhibited the growth of the healthy natural river grasses.

During the past four summers, I have been on the Shenandoah nearly weekly from May through September. The river is still impaired. During July and August, the Main Stem still looks like green pea soup and smells awful. When it does, I stay away.

When the Main Stem is at its worst, I try the South Fork, the water has not been so clouded by the floating algae in recent summers up there, but in places, it quite literally stinks. It is hard to enjoy the river or a picnic on it with the smell.

The area immediately upstream from the village of Shenandoah is probably the worst section of the South Fork, but there are some bad spots between Alma and White House that I paddle past as quickly as I can because of the smell. The cattle in the South Fork certainly contribute to the algae growth and river-bank erosion.

I regularly fish in and picnic near the beautiful waters of other Virginia Rivers. The Rapidan, The Conway, The Piney, because they are largely protected by Shenandoah National Park, are clear and healthy. The Shenandoah could be clean again, too. I ask that the VA DEQ include all sections of the Shenandoah River on the 303D/305B list of impaired rivers and develop a plan to correct the algae problems.

Sincerely,

William M. Prokopchak
540-722-2620
wilpro@comcast.net

Kelble, Jeff <jeff@potomacriverkeeper.org>

algae

Chris Fordney <chrisfordney@gmail.com> Tue, Jan 27, 2015 at 2:33 PM
To: jeff@shenandoahriverkeeper.org

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, VA 23218-1105

Dear Mr. Kennedy:

I am a frequent kayaker on the Shenandoah River, and I would like to register my concern with the problem of algae blooms on the river.

I have seen algae blooms on both forks of the river, from Harrisonburg and Luray to Clarke County, but I have seen particularly bad spots just upriver and downriver from Strasburg in Shenandoah County. Various forms of algae are present all year but seem to get worse in the summer, when it adheres to rocks on the bottom and makes footing difficult. The presence of algae has affected my enjoyment of the river, especially my willingness to swim when unsightly, unhealthy and bad-smelling blooms are present. The problem appears to be getting worse over the past decade I have been kayaking.

I ask that DEQ place the Shenandoah River on the 305/B/303D list to investigate the algae problem and begin steps to curtail or eliminate the problem.

Sincerely,
Chris Fordney
104 Rebel Circle
Winchester, VA 22602

Kelble, Jeff <jeff@potomacriverkeeper.org>

Algae Complaint Letter (incorrect date): John Mark Pullen

Madelaine Pierce <madelaine@potomacriverkeeper.org> Fri, Jan 16, 2015 at 10:53 AM
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>

Form: Take Action!- Help the Shenandoah Riverkeeper Get Rid of Algae
Submitted by Anonymous
Wednesday, January 14, 2015 - 10:59am
192.5.215.220

Your Letter:
January 14, 2015

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105

RE: Draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

The EPA is not doing its job. That is clear from all the noxious algae in the rivers. Greenbriar RIver in WV is BAD. But Shenandoah in VA (and a little in WV) is worse because so many people rely on it for recreation, or they used to before it got to be so bad.

Please increase activity to restore the once-pristine Shenandoah. In particular, VA DEQ should include the North Fork, South Fork and/or Main Stem on its impaired waters 303D/305B list.

John Mark Pullen
PO Box 1608
Shepherdstown, WV 25443
email: mpullen@netlab.gmu.edu
phone: 304-876-8142

Name:
John Mark Pullen

Email:
mpullen@netlab.gmu.edu

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

January 26, 2015

Dear Mr. Kennedy,

I am writing to add my voice to the chorus of concern for the water quality on the Shenandoah River. Specifically, my concern centers around the amount of algae in the river, and the correlation it appears to have on the amount of fish that I am seeing.

I am an avid kayaker, and have kayaked the waters of the North Fork, the South Fork, and Main Stem.. as well as occasional trips to the confluence of the Shenandoah and Potomac at Harper's Ferry, and the South River's confluence with the combined Middle/North Rivers. I have noticed an increase in the amount of algae in most parts of the river, with a noted decrease only when reaching the Potomac, at Harper's Ferry. The increase is prevalent in most areas that I kayak, with very noticeable areas of concern being the North Fork around Strasburg and the South Fork between Andy Guest State Park and Karo Landing. The algae builds during the summer, and seems to choke out the fish. I was actually shocked this past year, in the very noticable decrease in the amount of fish in the river. Very few large or smallmouth bass; the main large species noted were carp.

I have heard an alarm in the voices of various visitors to the river, that I come in contact with, as well. Those who come to fish, or float… many seem to have similar concerns. The large masses of algae that break free and float to the surface are unsightly, and sometimes have a foul odor.

I really hope that some attention can be paid to this increasing problem of algae. It is an impediment to full enjoyment of the river, and the concerns I have are deeper than just the beautification aspect. It is the overall health of the river, and decreasing fish population that is of major concern. This beautiful river, our lovely Shenandoah, is a cornerstone of tourism for our region of Virginia. An important one. Please help us keep it that way.

Sincerely,

Kara Rice
37 Wellspring Rd.
Browntown, VA 22610

Mark J. Frondorf
4643 20th Rd., #4
Arlington, VA 22207
571-969-0746
mjfrondorf@gmail.com

25 January 2015

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report
[delivered via email]

Dear Mr. Kennedy,

The purpose of this letter is to request that the North Fork, South Fork and Main Stem of the Shenandoah River be added to the Impaired Waters List on the 2014 305(b)3/303(d) Water Quality Assessment Integrated Report due to the heavy nutrient load stressing the river, resulting in severe algae blooms that negatively impact the Shenandoah's recreational use.

I am a professional fishing guide and have taken many clients on the North Fork, South Fork and Main Stem of the Shenandoah River. Unfortunately, for the last three years, especially during July/August, I have dissuaded clients from fishing the river due to the noxious algae blooms that turn the fishing off, make paddling/rowing exceptionally difficult and prevent anyone from wanting to get into the river for swimming or wading for fear that the toxic blue-green algae could affect their health. These tourism and recreation dollars have gone north to Maryland and Pennsylvania

These heavy algae blooms occur on all three sections of the river. The Clean Water Act requires that designated uses for each water body must include recreation and aquatic life, otherwise known as the "fishable/swimmable" goals. The CWA goals are not being met on the Shenandoah and I respectfully request the DEQ to include these three sections on the 305B/303D list, determine why heavy algae blooms have become so pervasive, and to develop and implement a plan to restore the river to its natural, healthy state.

Rarely, do any of us get an opportunity to make a decision that will bring about real, lasting, and positive change in our Commonwealth for generations to come. This is your chance. This is your opportunity. Please do the right thing and add the three sections of the Shenandoah River to the List of Impaired Waters.

Respectfully,

//signed//

Mark J. Frondorf

Kelble, Jeff <jeff@potomacriverkeeper.org>

Fw: tired of algae

coolmtnman@verizon.net <coolmtnman@verizon.net> Sun, Jan 25, 2015 at 3:02 PM
To: jeff@shenandoahriverkeeper.org

From: coolmtnman@verizon.net
Sent: Friday, January 23, 2015 9:14 PM
To: Reallyreallytiredofalgae@itstinks.com
Subject: tired of algae

John M. Kennedy
DEQ Office of Water Monitoring and Assessment
P.O. Box 1105
Richmond, Virginia 23218-1105
RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy

I have encountered algae when I go kayaking with my family and friends in the Shenandoah river.
Sometimes it's so bad I can hardly paddle through it.
It smells bad and is unpleasant to be around.

Sometimes I see cattle in the river and places where there's lots of runoff.
The job of the EPA is to allow us the freedom to have clean air and water.

Sincerely Orv Lehman 5947 Harpine Hwy Linville VA 22834
540 560 4847

January 15, 2015

John M. Kennedy

DEQ Office of Water Monitoring and Assessment

P.O. Box 1105

Richmond, Virginia 23218-1105

RE: Draft 2014 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

I am writing to request that Virginia's Department of Environmental Quality add the North Fork, South Fork and Main Stem of the Shenandoah as impaired waters on the 2014 305(b)3/303(d) Water Quality Assessment Integrated Report because of the severe algae and pollution problems the river experiences every year.

My familiy owns property on the South Fork of the Shenandoah just north and downriver of the Town of Shendoah. For over four decades and three generations our family and friends have swam, floated and fished these waters from the dam in Town of Shenandoah to the take out at Grove Hill River River Road (off of Rt 340). We have also hiked the state and national parks in the surrounding area and have vested a lot of ourselves in preserving this beautiful area. However, over the years the algae appears to be increasing in the waters along this stretch. This summer and fall it was apparent that the algae was once again a possible culprit responsible for a degradation of the river in the noted areas we frequent.

In September, I floated the river with several friends by kayak. The water was amazing for a large portion of the float. You could see the aquatic life was active and abundant. One of the guys likened it to fishing in an aquarium. The exception was in a few areas that stuck out to us. The initail area of concern was a small area where we put in with our kayaks at the family property. The algae can overwhelm the slow moving or stagnant sides that have created

pools for the aquatic life. One such pool at the edge of the property will get rancid with a dark green floating algae by late summer. Because of this, we tend not to come down to the area in late summer anymore. It really takes away our direct access to the river. This has always been so good for wading and play areas for the kids and family dogs. Next, we came along the area that parallels the Shenandoah Speedway (due west of the of the track and at the base of the Washington National Forest), there was significant tire dumping and standing algae in the shallows. As a side note, the tire dumping is also occurring along with runoff from the dirt track. This is occurring on the southeast corner of the property on the hillside that is a part of the dirt track (along the feeder creek where power lines easement borders the southeast property line). It is likely the track never received permission to build the dirt track. This was added after original construction and is clearly outside of permissible areas for noise, drainage and runoff. Several times during the summer I have noticed dead fish in the creek. Mosquitoes were prevalent around the tire dumping areas in both locations.

As we came to the final portion of the float, it was as if we were on a different river. The river bottom, west of the Rt 340 bridge, was covered in a brown slime. The aquatic life was almost nonexistent for a very long stretch of the river. This was very not at all representative of the rest of the river in the area. That last portion was the last impression we had of an amazing float.

Algae and pollution are an apparent detriment to the South Fork of the Shenandoah River. This has been a challenge for many years. My fear is that it will worsen if not addressed before a terminal turning point is encountered for this river. It has already taken away enjoyment to many of my friends and family that have enjoyed coming here over the years. Standing water with rancid algae is not pleasant to wade through. You can forget trying to catch a fish amongst the goo that is floating amongst the pools. It was really disheartening to experience such a long stretch river that has been devastated by the algae. The remnants could only be described as “a barren lifeless stretch of underwater covered in coat of brown silty slimy matter.” This really takes away our enjoyment of an amazing river in our Commonwealth.

I would like to formally request that Virginia Department of Environmental Quality include the North Fork, South Fork and/or Main Stem of the Shenandoah River on its impaired waters 303D/305B list. The algae and pollution have diminished our enjoyment of the river. I find it ironic the issue is within the area of it's namesake, Town of Shenandoah. More sad than ironic. I have been on other rivers in Virginia and have not seen this issue as prevalent as it is today on the Shenandoah River. Help this area determine a course of action to heal and improve these waters from pollution and degradation.

I offer any assistance necessary to facilitate your services. I would be happy to coordinate access to the river from our property if that would prove to be of any help. I am willing to accompany or provide any other assistance to further an investigation or research. Please feel free to call me if there are any questions.

Regards,

Steve DiLoreto

8400 Gatwick Terrace

Glen Allen, VA 23060

804-306-1384

steven.diloreto@yahoo.com

Specific locations while fishing the river on September 20, 2014:

1) Family cabin on S. Fork Shenandoah River located on Mill Run Drive north of the Town of Shenandoah. (38.507916, -78.636596)

2) S. Fork Shenandoah River at end of Precision Road behind race track.

(38.514487, -78.642135)

3) Stretch of S. Fork Shenandoah River west of Rt 340 near Grove Hill River Road (650).

(38.533061, -78.617889) General area below rapids and along the area up river from the Rt 340 bridge.

Aug 25, 2014

SEP 02 2014

Dear Mr. Kennedy,

I have fished and recreated on the Shenandoah River for over 50 years. I have spent many wonderful days with my four children floating all parts of this beautiful River, But not any more!

The summer algae is so bad that now I just stay off the river.

What a shame that one of Virginias most beautiful rivers is so polluted with algae that it is no longer a place to play.

I know you are considering designating the Shenandoah as an impaired river. I heartily hope you will act on this matter because the healing process needs desperately to begin.

I thank you for all you are doing and will do to preserve this wonderful natural resource for all Virginia residents.

Sincerely,

Cabell Williams

CabellWilliams@Gmail.com

Cabell Williams III
5422 Albia Rd
Bethesda, MD 20816

DEQ's Response to Earthjustice, the Potomac Riverkeeper, and Citizen Comments Regarding Algae in the Shenandoah River

In response to citizen comments to the draft 2014 IR, additional photographic evidence, and recent information provided by citizen groups, DEQ has determined that there is uncertainty about the attainment status of the recreation designated use for 7 assessment units in the Shenandoah River basin. These waters (listed in the table below) comprise a total of 25 stream miles and are classified in the Assessment Database as Category 3C for the recreation use, meaning that DEQ has received information suggesting water quality problems may exist, but this information is not sufficient for making a determination of impairment at this time. The potential cause of impairment has been identified as algae. These waters will be prioritized for monitoring so that their attainment status can be resolved with additional data. DEQ intends to develop a scientifically valid field method for evaluating algae cover that can be applied consistently throughout the state, with an initial study area focused on the Shenandoah River.

Working with EPA, DEQ has developed a plan for monitoring algae in the Shenandoah River. This plan—which will be implemented starting the summer of 2016—is detailed in EPA's final approval letter of Virginia's 2014 303(d) List. The letter is enclosed with this document.

Assessment units in the Shenandoah River that have been classified as having an indeterminate recreation use status, with algae identified as a potential cause of impairment.

Assessment Unit ID	Stream Name	Location Description	Length (mi)
VAV-B40R_SSF01B14	South Fork Shenandoah River	South Fork Shenandoah River from the Bentonville Landing Bridge downstream to the Andy Guest State Park STP outfall.	2.2
VAV-B40R_SSF03A14	South Fork Shenandoah River	South Fork Shenandoah River from the Foster's Landing Rapids downstream to Seekford's Ford.	5.4
VAV-B37R_SSF02B14	South Fork Shenandoah River	South Fork Shenandoah River from Naked Creek downstream to the Shenandoah STP outfall.	2.0
VAV-B35R_SSF01A00	South Fork Shenandoah River	South Fork Shenandoah River from its confluence with Dry Run downstream to its confluence with Naked Creek.	3.6
VAV-B51R_NFS05A00	North Fork Shenandoah River	North Fork Shenandoah River from the Strasburg Public Water Intake downstream to the 5 mile upper limit of the PWS designation for the Winchester Public Water Intake.	1.6
VAV-B51R_NFS06A00	North Fork Shenandoah River	North Fork Shenandoah River from the 5 mile upper limit of the PWS designation for the Strasburg Public Water Intake downstream to the Strasburg Public Water Intake.	5.9
VAV-B45R_NFS02A00	North Fork Shenandoah River	North Fork Shenandoah River from its confluence with Fort Run downstream to its confluence with Plains Mill Spring Run.	4.5

ATTACHMENT A

VA. Coastal Zone Management Program

te Water Control Board

Water Quality Standards

COASTAL ZONE
INFORMATION CENTER

Commonwealth of Virginia

TD224
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V58
1982

Publication No. RB-1-80

Revised Edition: April 1982

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WATER QUALITY STANDARDS

ADOPTED PURSUANT TO
SECTION 62.1-44.15(3)
OF THE CODE OF VIRGINIA (1950),
AS AMENDED

COMMONWEALTH OF VIRGINIA

STATE WATER CONTROL BOARD
RICHMOND, VIRGINIA

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INTRODUCTION

Basis and Purpose

The State Water Control Law mandates the protection of existing high quality State waters and provides for the restoration of all other State waters to such condition of quality that any such waters will permit all reasonable public uses and will support the propagation and growth of all aquatic life that might reasonably be expected to inhabit them (Section 62.1-44.2). The adoption of water quality standards under Section 62.1-44.15(3) of the Law is one of the Board's methods of accomplishing the Law's purpose.

Water quality standards consist of narrative statements that describe water quality requirements in general terms, and of numeric limits for specific physical, chemical or biological characteristics of water. These narrative statements and numeric limits describe water quality necessary to meet and maintain reasonable and beneficial uses such as swimming, public water supply and the propagation and growth of aquatic life. Standards include general, as well as specific, descriptions since not all requirements for water quality protection can be numerically defined. In those cases where numeric standards have not been formulated for scientific or practical reasons, the general standards establish broad requirements to protect and maintain beneficial uses of State waters. Standards are not static. They will change and be constantly adjusted to reflect changes in law, technology and information available to the Board and its staff.

Standards are used in the administration of several Board programs. One is the issuance of permits for the discharge of treated wastewater under requirements of both Federal and State law. Federal law (the Clean Water Act) mandates that certain minimum treatment levels be achieved by all dischargers. Before the Board issues a discharge permit, it must first determine if those minimum levels are sufficient to maintain water quality standards in the receiving stream. If not, the Agency formulates and enforces more-stringent permit requirements which will allow water quality standards to be met. The Board's 401 Certificate program also uses water quality standards. Under Section 401 of the Clean Water Act, if a Federal permit is needed by any project which will or may result in a discharge to State waters, the project must first receive certification from the appropriate State that the project will not violate water quality standards. The State Water Control Board is the 401 certifying agency in Virginia, and it judges whether or not water quality standards will be contravened by proposed projects in administering this certificate program.

Water quality standards are intended to protect the beneficial uses of State waters. Virginia's standards do not assign specific uses to all streams, although they do specifically designate and protect trout streams and public water supplies. The standards are intended to protect all State waters for recreational use and for the propagation and growth of a balanced population of fish and wildlife. By protecting these two uses, which usually require the most stringent standards and the highest degree of protection, other usually less restrictive uses like industrial water supply, irrigation and navigation are usually also protected. Should additional standards be needed to protect other uses as dictated by changing circumstances or improved knowledge, they can be formulated and adopted.

Background

From its beginning in 1946 until the mid 1960's, the State Water Control Board generally approached control of water quality problems on a case-by-case basis, frequently through the adoption of wastewater treatment requirements for individual watersheds. By the mid 1960's, however, the Board was beginning to recognize the need for a program of water quality control with a broader, more extensive base — one that would deal with actual in-stream water quality throughout the entire state.

The Federal "Water Quality Act of 1965" (PL-89-234), provided the incentive and framework for such a program by requiring the establishment of certain water quality standards on a local, state and national scale. States which did not, or could not, adopt standards would have such standards of water quality established by the Federal government. As a result of the Federal Act amendments, and under State law, the Board first developed and adopted water quality standards in 1966 through 1968. They were substantially amended in 1970.

The 1972 amendments to the Federal Water Pollution Control Act (PL-92-500), restated the concept of water quality standards, and set certain water quality goals such as the attainment, by July 1, 1983, of water quality which provides for protection of fish, shellfish, and wildlife and for recreation in and on the water. In 1973 Virginia's water quality standards were slightly amended to ensure compliance with these aims.

This Act further required that a state's water quality standards be reviewed at least once every three years and, as appropriate, modified or amended. Virginia has completed one review period through public hearings in 1976 and formal adoption of amendments in 1977. The present standards have received Federal (Environmental Protection Agency) approvals required by the Act.

Development and Adoption

In order for water quality standards to be realistic while at the same time serving the stated purpose of maintaining or improving water quality, there first must be established a relationship between the standard itself and the benefit to be derived from adoption and enforcement of the standard. This relationship is generally determined by making certain studies, or evaluating already completed studies that define or attempt to define which standards and limits are required to provide needed levels of protection for certain water uses. A decision is then made regarding what levels of protection are desired. Finally, the appropriate standards and limits are chosen in order to provide the desired level of protection.

There are many sources of information or studies useful in the development of standards, and from time to time studies are conducted as needed by the Board or by consultants under contract to the Board. A major source of information is the publication *Quality Criteria for Water* published by the United States Environmental Protection Agency in 1976, pursuant to the requirements of the 1972 amendments to the Federal Water Pollution Control Act.

Since standards are designed to protect specific uses, the need for new or amended standards often comes about as a result of an identified new use of a body of water. For example, the Commission of Game and Inland Fisheries may identify a stream which was previously not known to support trout, as a natural trout stream. In this case the standards may be modified to protect this particular use. Also, a locality might want to use a stream as a water supply, and if the stream was not already classified as a public water supply, new standards would have to be applied to protect this use.

Information from every segment of the population is helpful in the development of standards, and their adoption can have wide-ranging effects. Public participation at all levels of standards development is therefore important to the adoption and implementation of standards which reflect the desire of the general public. The Law requires opportunity for public participation in the adoption of standards. Accordingly a public hearing with adequate public notice is mandatory before any standard can be adopted, amended, or cancelled. Following public notice of the hearing, the public has an opportunity to study the proposed standard and determine its effects. At the public hearing any person who is either for or against the proposed Board action on the standard may state his interest in the matter and enter relevant evidence into the hearing record.

Recent amendments to State Law added another mandatory consideration relative to standards — that the Board must give ample consideration to social and economic costs and benefits which will result from any action on the standard, whether by adoption, modification or cancellation.

Following the public hearing the Board's staff examines the hearing record, makes recommendations, and submits both the record and the recommendations to the Board for its consideration and action. Board action on a standard becomes effective thirty (30) days after filing with the Registrar of Regulations who keeps all standards and regulations of State agencies on file.

Both Federal and State legislators recognized the need for periodic review of all standards, and now both Federal and State law require that standards be reviewed at least every three years for possible changes and that the Board hold sufficient public hearings for public input. The Board is required to modify or cancel these standards, or to adopt new ones, as needed.

Summary and Explanation of Contents

This booklet contains the following seven sections:

1. Standards with General State-wide Application (Pages 5-11)

   These standards apply to waters on a State-wide basis and include such things as the General Standard, dissolved oxygen and temperature standards for surface waters, and groundwater standards.

2. Standards with More Specific Application (Pages 12-14)

   These standards have a specific application; they include, for example standards for public water supplies.

3. Procedural Requirements (Pages 14-15)

   This section includes rules that are a part of the standards such as a rule specifying acceptable analytical procedures for water testing.

4. River Basin Section Tables (Pages 15-65)

   This section divides the State's nine river basins into sections and indicates what specific standards apply to these specific sections.

5. Key to Special Standards (Pages 67-75)

   This section contains the entire printing of special standards that were too long to include in the tables of Section 4.

6. Water Quality Criteria (Pages 76-78)

   This section includes information about levels of pollutants to protect surface water and groundwater quality; they are not standards.

7. River Basin Maps (Plates 1-13)

   Maps in this part of the booklet delineate the river basin sections described in Section 4.

Additional more specific information can be found in the individual parts of the booklet.

In order to find applicable standards for a surface stream, first one must determine what River Basin Section the stream is in. This may be determined directly from the River Basin Section Tables or it may be necessary to determine the section by using the maps in Section 7.

The River Basin Section Tables indicate each section's Classification (Roman numeral I through VI). Paragraph 1.04 contains the standards that apply to the particular class and paragraphs 1.05 - 1.07 indicate other standards. The River Basin Section Tables also indicate whether any Special Standards apply to the stream. The entire special standard is printed in the special standards column if short; otherwise, a letter in the Special Standards Column refers the reader to the Special Standard which can be found in the Key to Special Standards Section, Section 5, beginning on page 67.

To determine applicable groundwater standards for an area, one can determine which physiographic province the area is in by checking Figure 1 on page 11, then by referring to the standards in Paragraph 1.09 on pages 9-10 that apply to that physiographic province.

In the sections that follow, all standards are shown in boldface type both in text and in tables. The text in lightface type preceding such standards is generally specific explanatory information relating to the standards that follow.

ATTACHMENT B

Information Concerning 2014 Clean Water Act Sections 303(d), 305(b) and 314 Integrated Reporting and Listing Decisions

- MEMORANDUM
- Enclosure
  1. Timeliness of State Integrated Report (IR) submissions and EPA approval
  2. Recommendations for the appropriate consideration of Natural Conditions to support removing a water from or not including a water on the 303(d) list
  3. Potential approaches for identifying nutrient-related impaired waters for the 303(d) list based on narrative nutrient water quality criteria and/or direct evidence of failure to support designated uses
  4. Assessment and Total Maximum Daily Load (TMDL) Tracking and Implementation System (ATTAINS)
     A. Information update on using and reporting Statewide Statistical Survey Data in ATTAINS, and the National Water Quality Inventory Report to Congress
     B. Information on the data systems EPA will support for tracking assessment decisions for inclusion in ATTAINS
  5. Antidegradation and Listing Guidance

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C.20460

OFFICE OF WATER

September 3, 2013

MEMORANDUM

SUBJECT: Information Concerning 2014 Clean Water Act Sections 303(d), 305(b), and 314 Integrated Reporting and Listing Decisions

FROM:

Denise Keehner, Director /s/
Office of Wetlands, Oceans, and Watersheds

TO:

Water Division Directors, Regions 1 – 10
Robert Maxfield, Director, Office of Environmental Measurement and Evaluation, Region 1

I am pleased to provide you with information to assist you and your States as you prepare and review the 2014 Integrated Reports (IR), in accordance with Clean Water Action (CWA) Sections 303(d), 305(b), and 314. This memorandum focuses on: 1) timeliness of State IR submissions and EPA approvals, 2) recommendations for the appropriate consideration of Natural Conditions in listing decisions, 3) potential approaches for the identification of nutrient-related impaired waters for the 303(d) list based on narrative nutrient water quality criteria and/or direct evidence of failure to support designated uses, 4) an update on tools and formats for submitting IR data to EPA – Assessment and Total Maximum Daily Load Tracking and Implementation System (ATTAINS), and 5) an update on EPA's development of guidance on how States can address antidegradation policies and procedures in their Section 303(d) programs. In addition, EPA will continue to work with the States and Regions in the coming months to identify any issues that may necessitate further clarification in future reporting cycles.

Over the past year, EPA and the States have developed a Long-Term Vision and Goals for assessment, restoration, and protection under the CWA Section 303(d) Program, which was endorsed by the Association of Clean Water Administrators. The development of a new long-term vision was an important element of the program's evolution and will better prepare and align efforts under the program to address current and future challenges and opportunities for protecting and restoring water quality. As part of this effort, EPA and the States developed six goal statements with milestones for completion: prioritization (2016), assessment (2020), protection (2016), alternatives (2018), engagement (2014), and integration (2016). EPA and the States intend to carry out this Vision and associated goals consistent with a more detailed implementation plan.

In addition, EPA and the States continue to make progress on the Integrated Reporting Georeferencing Pilot. As geospatial data and technology have evolved, EPA continues to seek efficiencies and improvements in the georeferencing of State water quality assessment and impairment decisions at the federal level. It is anticipated that this effort will be finalized by the end of 2013.

This memorandum is not regulation and does not impose legally binding requirements on EPA or the States. EPA recommends that the States prepare their 2014 IRs consistent with previous IR guidance including EPA's 2006 IR Guidance, which is supplemented by EPA's 2008, 2010, and 2012 IR memos and this memorandum available at EPA Guidance.

I would like to thank our State partners, interstate commissions, and Regions for their input on the information in this enclosure. I particularly appreciate the continued hard work and dedication in developing the IRs so that we can report to the public on the status of the nation's waters. If you

have any questions or comments concerning this memorandum, please contact me or have your staff contact Shera Reems at 202-566-1264 or reems.shera@epa.gov.

Enclosure

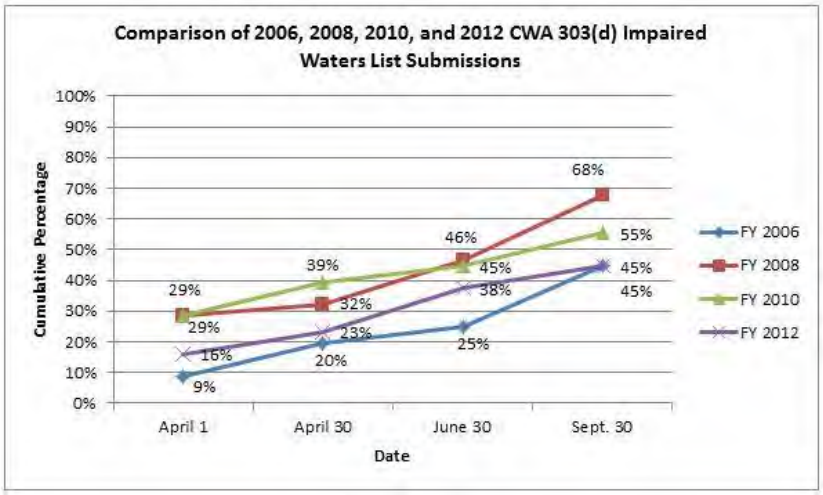
cc: Regional Section 303(d) Coordinators
Regional Monitoring Coordinators
Regional Water Quality Standards Coordinators
Regional NPDES Coordinators
Alexandra Dunn, Association of Clean Water Administrators

INFORMATION CONCERNING 2014 CLEAN WATER ACT SECTIONS 303(d), 305(b), AND 314 INTEGRATED REPORTING AND LISTING DECISIONS

1. Timeliness of State Integrated Report (IR) submissions and EPA action on State Section 303(d) lists.

The U.S. Environmental Protection Agency (EPA) and States need to continue best efforts to provide on-time State Integrated Report (IR) submittals (all 56 States and Territories by April 1, 2014) and EPA action on the States' Clean Water Act (CWA) Section 303(d) lists (within 30 days of their submission). While EPA and the States made progress from the 2006 to the 2008 reporting cycle, this progress did not continue with the 2010 and 2012 reporting cycles. For the 2008 reporting cycle, 38 States submitted their IRs by the end of the fiscal year compared to 31 for the 2010 reporting cycle and 25 for the 2012 reporting cycle (see Figure 1). Also, EPA action on States' Section 303(d) lists continues to fall short of the progress made in the 2008 reporting cycle. For the 2012 reporting cycle, of the 25 lists submitted to EPA by the end of FY 2012, EPA had taken action on only 15 lists as of the end of the calendar year, and these lists took an average of 53 days to approve. This average will be significantly higher once all 303(d) lists are approved. Timely State submittal of IRs and EPA review and approval or disapproval of lists is central to meet EPA and State responsibilities under the CWA and to evaluate EPA and State success in accomplishing our strategic plan goals to restore and maintain the nation's waters.

Figure 1: Timeliness of State 2006, 2008, 2010, and 2012 IR Submissions



EPA recognizes that State resources to complete these actions are limited. Hence, both EPA and the States need to continue best practices to provide timely information on the status of the nation's waters, including the State identification of waters under Section 303(d)(1)(A) of the CWA. Such Section 303(d) lists consist of "water quality limited" waters (i.e., waters that fail to meet one or more applicable water quality standard). In the 2008 IR Memo,[1] "Information Concerning 2008 Clean Water Act Sections 303(d), 305(b), and 314 Integrated Reporting and Listing Decisions," EPA outlined several best practices used by States and Regions to help facilitate improved timeliness of submission and review of water quality reports and Section 303(d) lists. We recommend that States and Regions refer back to these best practices and identify areas in which it is feasible to make improvements.

As an outcome of the EPA and State effort to identify opportunities to reduce State reporting burden under CWA Sections 303(d) and 305(b)[2], EPA will soon provide additional recommendations to move toward more timely IR submittals and EPA review and approval or disapproval of 303(d) lists. One driver for this effort was a request by a number of States for EPA to evaluate whether a change in reporting frequency from two years to four or five years would reduce State burden. EPA commenced a series of meetings with State partners that first identified key steps in the IR process, followed by discussions focused on those steps requiring the highest level of effort by States. These steps included: 1) State review and use of available data to make assessment decisions, 2) State preparation of data and associated geospatial information and entry into an assessment database, 3) State preparation and submission of final Section 303(d) lists and 305(b) reports to EPA, and other relevant documentation, 4) State preparation or refinement of its assessment and listing methodology, and 5) State response to public comments. During each discussion, EPA

requested State input on a series of questions, including how a change in reporting frequency would help or not help alleviate State burden. At the conclusion of these meetings, while a few States indicated that EPA should lengthen the reporting cycle, the majority of States recommended that EPA not change the length of the reporting cycle. EPA and the States did identify several areas within the existing framework as good candidates for streamlining to improve the efficiency of assessment, listing, and reporting. In addition to this effort described above, EPA is currently working to identify opportunities to maximize efficiencies and streamline EPA's Section 303(d) list review process.

1. Information Concerning 2008 Clean Water Act Sections 303(d), 305(b), and 314 Integrated Reporting and Listing Decisions
2. Undertaken pursuant to Executive Order 13563 "Improving Regulations and Regulatory Review"

2. Recommendations for the appropriate consideration of Natural Conditions to support removing a water from or not including a water on the Section 303(d) list

In the 2006 IR Guidance, "Guidance for 2006 Assessment, Listing, and Reporting Requirements Pursuant to Sections 303(d), 305(b), and 314 of the Clean Water Act," and the 2008 IR Memo, "Information Concerning 2008 Clean Water Act Sections 303(d), 305(b), and 314 Integrated Reporting and Listing Decisions," EPA provided information on the role of natural conditions concentrations of pollutants in 303(d) listing decisions.

EPA stated that applicable water quality standards are the basis for determining whether a waterbody must be included on a State's Section 303(d) list. States may have approved natural conditions provisions in EPA approved water quality standards that specify the applicable aquatic life water quality criterion will be equal to the natural conditions level of a pollutant if it is determined that the natural conditions does not meet the otherwise applicable criteria. In the absence of an EPA-approved natural conditions provision or an EPA-approved site-specific criterion based on natural conditions, the otherwise applicable criterion is the basis for determining whether a waterbody belongs on the State's Section 303(d) list.

EPA's guidance on the appropriate use of natural conditions provisions for making 303(d) listing decisions remains unchanged for the 2014 reporting cycle. For example, as discussed in the EPA IR guidance for the 2006 and 2008 reporting cycles, if a State has an EPA-approved natural conditions provision or site-specific criterion based on natural conditions, it may use these as the basis for determining if a water should be included on a State's Section 303(d) list. When a State evaluates whether a potential designated use impairment is the result of natural conditions, the State should consider all sources of the pollutant being evaluated. If the pollutant concentrations do not meet the EPA-approved water quality standards, and anthropogenic sources of the pollutant are present, the water is considered impaired and should be included on the State's Section 303(d) list even if natural sources of the pollutant are present. In the 2008 IR Memo, EPA provided several theoretical examples to illustrate these recommended approaches; however, note that these examples do not address all possible scenarios or variations in EPA-approved water quality standards. In addition, EPA continues to support its statement that natural conditions provisions are not appropriate for human health criteria. For more information see, "Establishing Site Specific Aquatic Life Criteria Equal to Natural Background (PDF) (3 pp, 125K, About PDF)".

If a State determines that a water fails to meet an applicable water quality standard solely due to naturally occurring levels of a pollutant, and it has an approved applicable natural conditions provision, the State should include in its IR submission for the 2014 and future reporting cycles a rationale for either removing or not including the water/pollutant combination on the State's Section 303(d) list. The rationale should identify the geologic or other conditions that cause the natural loading of the pollutant to exceed otherwise applicable water quality standards. In addition, the rationale should document why anthropogenic sources of pollutant loading, such as municipal, industrial, agricultural, contaminated groundwater, or anthropogenic airborne deposition, were determined not to be sources of pollutant loading. The rationale should also cite the approved, applicable natural conditions provision upon which the State is relying. Including this rationale will provide interested stakeholders with a more complete understanding of the State's use of its natural conditions provision and help expedite EPA's review of the State's IR submission.

More information on EPA's existing guidance on the use of natural conditions provisions for making Section 303(d) listing decisions is available at Guidance for 2006 Assessment, Listing, and Reporting Requirements Pursuant to Sections 303(d), 305(b), and 314 of the Clean Water Act and Information Concerning 2008 Clean Water Act Sections 303(d), 305(b), and 314 Integrated Reporting and Listing Decisions.

3. Identifying nutrient-impacted waters for the Section 303(d) list for States without numeric nutrient water quality criteria

Addressing nutrient pollution in our nation's waters is one of EPA's top priorities. Over the past decade EPA has called upon the States and others to increase their efforts to address nutrient pollution. In a March 2011 memorandum to the States, tribes and territories, EPA reiterated the need for action by stating, *"States, EPA, and stakeholders, working in partnership, must make greater progress in accelerating the reduction of nitrogen and phosphorus loadings to our nation's waters."*

National monitoring efforts, such as USGS reports on surface water quality[3] and EPA's National Aquatic Resource Surveys,[4] document the widespread impacts of nutrients on our nation's waters. A USGS report examining changes in nutrient concentrations at selected sites monitored between 1993 and 2003 indicates increasing levels of nutrients at about one-third of the sampled sites. EPA's National Aquatic Resource Surveys (NARS) report that nutrients are a widespread problem. NARS, often referred to as probability-based surveys, provide nationally consistent and scientifically-defensible assessments of our nation's waters and can be used to track changes in condition over time. Each survey uses standardized field and laboratory methods and is designed to yield unbiased estimates of the condition of the whole water resource being studied (i.e., rivers and streams, lakes, wetlands, or coastal waters). Based on completed survey results, about 50% of the nation's streams and 45% of the nation's lakes are in fair to poor condition for nitrogen or phosphorus levels relative to reference condition waters. This translates to about 300,000 miles of

perennial streams and seven million acres of lakes across the U.S. The analyses show that when streams and lakes rate poor for excess levels of nitrogen and phosphorus they are twice as likely to have poor biological health based on benthic macroinvertebrate condition.

While the NARS probability-based results are not directly comparable to the national tally of segment-specific waters included on States' Section 303 (d) lists, it is helpful to consider the magnitude of the differences between the two regarding the effects of nutrients. Based on information submitted by the States, about 155,000 miles of rivers and streams and about four million acres of lakes are included on States' Section 303(d) lists for nutrients or nutrient-related impairment causes.[5,6] EPA expects States will consider the NARS findings to increase efforts to identify and manage nutrient pollution. The NARS raw data as well as data in EPA's STORET database are available at the Water Quality Portal. These data can be used, along with other existing and readily available data and information, to evaluate whether waters are meeting applicable water quality standards including designated uses, narrative and numeric criteria, and antidegradation policies. These findings could also be used to set monitoring priorities to generate sufficient data and analyses to identify and manage nutrient pollution. As States gain experience implementing State scale statistical surveys to complement the targeted monitoring, they will be in a position to use such surveys as a feedback mechanism to gauge completeness of the Section 303(d) list and effectiveness of overall efforts to reduce nutrient pollution.

The CWA and EPA's implementing regulations require States to identify water-quality limited segments still requiring TMDLs where pollution controls are not stringent enough to meet any applicable water quality standard. Applicable water quality standards include designated uses and the criteria that must be met to support the uses as well as antidegradation requirements.[7] Furthermore, if a designated use is not supported and the segment currently fails to meet an applicable water quality standard or is "threatened," it must be included on the State's Section 303(d) list even if the specific pollutant causing the water quality standard exceedance is not known at the time.

Lack of numeric criteria for nitrogen and phosphorus adopted into State water quality standards and/or an inability to readily apply narrative criteria are sometimes cited as reasons for not assessing or including waters on States' Section 303(d) lists for nutrient-related impairments of designated uses. A number of States have listed waterbodies for nutrients and nutrient-related impacts based on a range of methods starting from simple visual assessments to straightforward decision matrices to more complicated stressor ID analyses.

Listing Approaches

A State can determine whether a waterbody is attaining its applicable narrative nutrient or other relevant narrative criteria and designated uses by using results of visual assessments. For example, field observations of excessive algal growth, macrophyte proliferation, adverse impacts on native vegetation (e.g., eelgrass), presence or duration of harmful algal blooms, unsightly green slimes or water column color, and/or objectionable odors may be a basis to include a waterbody on the State's Section 303(d) list for failing to meet one or more applicable narrative criteria and designated uses.[8]

A State can also place a waterbody on its Section 303(d) list by using other existing and readily available water quality-related information from local, State, or federal agencies, members of the general public, or academic institutions.[9] Evidence of narrative criteria and/or designated use impairment can include documentation of fish kills (aquatic life use) and beach closures or outbreaks of waterborne illness among swimmers (recreational use). A particular case of the latter related to nutrients is illness resulting from blooms of toxic blue-green algae (cyanobacteria). States should also consider feedback from the general public and waterbody users about the condition of the waterbody such as photographs or testimonials of abundant algal mats that impede recreation or create unsightly aesthetics in the waterbody.

It is important to note, however, that lack of plant growth or other visual pieces of evidence is not a reason to exclude or delist a waterbody for nutrient pollution as a cause of impairment where nutrient levels are elevated because other factors may be masking the nutrient response.

Another approach to assessing waterbodies is to evaluate nitrogen and phosphorus data sets derived from water column samples. For instance, some States have developed numeric water quality targets or thresholds for nitrogen and/or phosphorus that are used as quantitative "translations" of their narrative criteria. Unlike EPA approved water quality standards containing numeric nutrient criteria, the nitrogen and phosphorus target values are often described in State guidance or methodology documents. A State can also use the numeric target values in combination with measurements, such as dissolved oxygen, pH and/or chlorophyll-*a* (or other nutrient pollution response parameters), to reach a nutrient-related cause of impairment when implementing their narrative criteria.

Some States complete stressor identification analysis aimed at determining whether nutrients caused or contributed to the failure to meet the narrative criteria. Often, the stressor ID methods involve using multiple lines of evidence, including information on the causal variables (e.g., total nitrogen or total phosphorus) and response indicators (e.g., chlorophyll-*a*, dissolved oxygen, pH, macroinvertebrates, periphyton). States may set benchmarks, weigh particular indicators, or invoke statistical analyses using confidence levels. Some States use a step-wise or tiered approach. States employing the tiered approach often identify a set of core parameters on which to base their evaluation. Depending on the number of indicators that exceed threshold values (weighted or unweighted), the final assessment invokes secondary and sometimes tertiary parameters.

Because nutrients increase the overall productivity of a waterbody, biological information can provide an important clue into nutrient impairment, and a number of States include some kind of biological assessment as one of the lines of evidence when assessing whether a waterbody is meeting its narrative criterion. Data on macrophyte cover, chlorophyll-*a*, algae assemblages, including diatoms, are used to gauge the biological condition of the water. Biological condition is often measured using an Index of Biological Integrity (IBI), Observed/Expected (O/E) models, and other analytical techniques. When making decisions regarding impairments related to nutrient pollution, it is important to use information on biological endpoints that

are sensitive to increased concentrations of nutrients. Linking nutrients to the biological response can be done via stressor identification, multiple lines of evidence, or other means. Additionally, some States have used biological information independently and will list the source of the biological impairment in the "unknown" category until the stressor-ID or TMDL analysis determines the pollutant of concern.

To assist States, EPA is providing a number of examples of approaches that can be used for assessing whether waters are attaining nutrient-related narrative criteria and/or supporting designated uses. These examples are presented to inform States that have not yet established nutrient assessment methods for applicable narrative criteria and to illustrate how some States assess their waters pending the adoption of numeric nutrient criteria.

EPA does not endorse one method over another, nor does it limit potentially acceptable methods to those provided here. Likewise, the appropriateness of a particular method will depend on the variety of fact-specific circumstances that may be present.

3. USGS Circular 1350: Nutrients in the Nation's Streams and Groundwater
4. National Aquatic Resource Surveys
5. For purposes of presenting State information in ATTAINS, EPA compiles State reported impairments into national category groups, and as such, EPA defines "nutrient-related" as impairments that fall under the following parent category groups: nutrients, organic enrichment/oxygen depletion, ammonia, algal growth, and noxious aquatic plants. See Water Quality Assessment and Total Maximum Daily Loads Information
6. Based on the most recent data available in ATTAINS for each State, the sum of the size reported for unique assessment units that are identified as impaired (i.e., either on a State's Section 303(d) list or have an approved TMDL for rivers and streams and lakes). Information was pulled for only nutrient-related national categories, defined as impairments that fall under the following parent category groups: nutrients, organic enrichment/oxygen depletion, ammonia, algal growth, and noxious aquatic plants. See Water Quality Assessment and Total Maximum Daily Loads Information. For 303(d) data, size is an optional field, and several waters did not have size information. Therefore, this information likely underestimates the extent of the nutrient problem across the nation. (accessed: January 14, 2013)
7. EPA's 303(d) listing regulations at 40 CFR 130.7(b)(3) define a "water quality standard applicable to such waters" and "applicable water quality standards" as "those water quality standards established under 303 of the Act, including numeric criteria, narrative criteria, waterbody uses, and antidegradation requirements."
8. Specific listing decisions depend on the particular language provided in a State's narrative criteria or designated use description.
9. 40 C.F.R Section 130.7(b)(5) requires that each State assemble and evaluate "all existing and readily available" water quality-related data and information, which "at a minimum," includes water quality problems that have been reported by local, state, or federal agencies, members of the public, or academic institutions.

State Examples of Section 303(d) Listing Approaches for Nutrient-related Narrative Criteria

Oregon

Narrative Criterion: *For all surface waters, the development of fungi or other growths having deleterious effect on stream bottoms, fish or other aquatic life, or that are injurious to health, recreation, or industry may not be allowed.*

- Oregon has placed waters on the Section 303(d) list based on health advisories. Specifically, these health advisories are issued by the Oregon Department of Human Services, in conjunction with other agencies, warning that potentially harmful levels of cyanotoxins produced by algae are present in the water. The health advisories are based on visible scum, with supporting photographs and water analysis, cell counts or toxicity levels, or a combination of two or more options. The advisories apply to several designated uses, including domestic and industrial water supply, irrigation livestock watering, fish and aquatic life, fishing, boating, contact recreation, and aesthetic quality. Additional details on the State's assessment method and the health advisory protocol are available at: Methodology for Oregon's 2010 Water Quality Report And List of Water Quality Limited Waters (PDF) (88 pp, 1.2MB, About PDF) and Harmful Algae Blooms (PDF) (88 pp, 1.2MB, About PDF).

Vermont

Narrative Criterion: *In all waters, total phosphorus loadings shall be limited so that they will not contribute to the acceleration of eutrophication or the stimulation of the growth of aquatic biota in a manner that prevents the full support of uses. In all waters nitrates shall be limited so that they will not contribute to the acceleration of eutrophication, or the stimulation of the growth of aquatic biota, in a manner that prevents the full support of uses.*

- Vermont uses public feedback and complaints in addition to field surveys of algae blooms to assess waters for attainment of the above water quality standard. For the swimming/contact recreation use in lakes, waters are considered impaired if an ongoing record of public complaint concerning the algal conditions in the water has been established. For cyanobacteria (blue-green algae), waters displaying ongoing summer blooms of toxin producing cyanobacteria and having microcystin concentrations at elevated levels (i.e., World Health Organization (WHO) guideline of 1 µg/l) are considered impaired. For the drinking water supply use, waters are considered impaired if they display ongoing summer blooms of toxin producing cyanobacteria and have microcystin concentrations in excess of the same WHO guideline above. Additional details on the State's assessment method are available at: Vermont Surface Water Assessment and Listing Methodology (PDF) (34 pp, 675K, About PDF).

Montana

Narrative Criterion: *State surface waters must be free from substances attributable to municipal, industrial, agricultural practices or other discharges that will…create conditions which produce undesirable aquatic life.*

- Montana's assessment method to address nitrogen and phosphorus pollution for wadeable streams includes an "overwhelming evidence of nutrient impairment" provision for which photo documentation is adequate to make an impairment determination for aquatic life use. The State defines overwhelming evidence of nutrient impairment as either fish kills that involve massive growths of senescing algae mats (bottom attached or floating) or stream beds covered with filamentous algal growth for a substantial distance. Sample photos and more details can be found in Montana's Assessment Methodology for Determining Wadeable Stream Impairment Due to Excess Nitrogen and Phosphorus [redacted].

Delaware

Narrative Criterion: *Waters shall be free from…any pollutants that may impart undesirable…colors to the water or to aquatic life found therein, may endanger public health, or may result in dominance of nuisance species.*

- The State's 2010 assessment methodology includes numeric water quality targets for nitrogen and phosphorus in guidance that can be used for the majority of waterbody types in the State as a basis for Section 303(d) listing for aquatic life use. Additional details are available at: State of Delaware 2010 Combined Watershed Assessment Report (305(b)) and Determination for the Clean Water Act Section 303(d) List of Waters Needing TMDLs (PDF) (165 pp, 4.4MB, About PDF) [redacted].

Iowa

Narrative Criterion: *Waters shall be free from materials attributable to wastewater discharges or agricultural practices producing objectionable color, odor, or other aesthetically objectionable conditions.*

- Iowa uses Trophic State Index (TSI) values for chlorophyll-*a* and Secchi depth as a basis for Section 303(d) listing (i.e., "aesthetically objectionable condition") for primary contact recreation for lakes. Under a different narrative criterion, the State also uses TSI values for chlorophyll-*a* or total suspended solids concentrations to assess aquatic life use support in shallow lakes. Additional details on both assessment methods are available at: Methodology for Iowa's 2012 Water Quality Assessment, Listing, and Reporting Pursuant to Sections 305(b) and 303(d) of the Federal Clean Water Act (PDF) (155 pp, 4.4MB, About PDF) [redacted].

New Mexico

Narrative Criterion: *Plant nutrients from other than natural causes[10] shall not be present in concentrations that will produce undesirable aquatic life or result in a dominance of nuisance species in surface waters of the state.*

- New Mexico uses a two-tiered approach to assess whether wadeable, perennial streams are attaining the State's narrative nutrient criterion and support aquatic life use. Both stressor and response variables are used in two sequential levels of assessment to determine if the State's narrative criterion is attained.

  The Level I assessment is a screening evaluation and based on a review of available data, including on-site observation (i.e., percent algal cover, periphyton growth, and presence of anoxic layer) and in-stream measurement (i.e., total nitrogen, total phosphorus, dissolved oxygen, and pH) indicators. Except for pH, all of the thresholds for these indicators are provided in the State's listingguidance. The threshold for pH is a separate State water quality standard. If two or more Level I indicators exceed their Level 1 thresholds, a Level II assessment is subsequently used.

  The Level II assessment uses a multiple lines of evidence approach to take into account diverse lotic systems. This level of the assessment uses a more robust set of measurements for both stressor (percent total nitrogen and total phosphorus concentrations above threshold concentrations) and response (diel fluctuations of dissolved oxygen and pH, and chlorophyll-*a* ($\mu g/cm^2$) variables.

  A waterbody is considered not attaining the narrative criterion if at least one causal variable and one response variable exceed thresholds in the Level II assessment. More information is available at: Nutrient Criteria Development [redacted].

10. New Mexico has an additional stand-alone provision for natural conditions in their water quality regulations.

Moving Forward to Improve Section 303(d) Listing Programs for Nutrients

EPA strongly encourages States to evaluate the status of their waters with respect to nutrient pollution and to add to their Section 303(d) list waters failing to meet applicable nutrient-related narrative criteria or waters with evidence of unsupported designated uses. For those States that have developed nutrient-related assessment methodologies, EPA anticipates that they will continue to improve their efforts and enhance their nutrient assessment programs. For States without nutrient-related assessment methodologies, EPA is providing the above examples to demonstrate the flexibility States have to develop nutrient-related assessment methodologies based on applicable narrative criteria pending the completion of numeric nutrient criteria.

To facilitate stakeholder input and EPA review of States' Section 303(d) lists, States are encouraged to describe or reference in their assessment methods the rationale for selecting the approach and associated threshold levels for the stressor and/or response parameters used to translate the narrative criteria. In addition, States may need to consider updating their monitoring protocols to address any new or modified stressor and/or response parameter used in the methodology.

As discussed in the 2006 IRG, States should also include in their assessment methods their data quality, quantity, and representativeness expectations and protocols for making water quality attainment determinations. Such expectations are particularly important when information from stakeholders can be used to assess whether applicable water quality standards are being met. For example, to facilitate a timely submittal of States' Section 303(d) lists and EPA review, States should consider including expectations that stakeholder data and information (e.g., waterbody user testimonials and photographs of waterbody conditions) include supporting information such as the date, specific location, and period of time that the waterbody condition was observed. Regarding location, States should also consider making available to the public information about their waterbody segmentation approach to facilitate stakeholders' ability to associate the observations with specific waterbody segments if more specific geographic measurement tools (e.g., hand held geographic positioning systems) are not available. The protocols should be published along with any solicitation for data and information. Ideally, such QA/QC protocols should be made available to the public in advance of any such solicitation for any given IR cycle. As a general matter, the protocols should strike a balance between employing only the very highest quality data and information and employing the most useful information about the conditions of as many segments as possible. Additional details on EPA's previous guidance regarding data quality, quantity, and representativeness considerations for making Section 303(d) listing decisions are available at: 2006 Integrated Report Guidance.

When States do not evaluate all existing and readily available data and information relevant to applicable narrative criteria and designated uses or fail to provide a rationale for not using certain existing and readily available data or information, EPA will take appropriate actions consistent with the CWA.[11] For example, in 2010, one State modified its assessment methods to make attainment decisions based on numeric criteria only and removed from the list a number of lakes originally listed for not attaining the State's narrative nutrient criterion based on trophic conditions. EPA conducted an independent analysis of available data for each lake removed from the State's Section 303(d) list and concluded that 12 of the lakes should be added to the State's Section 303(d) list based on not meeting the narrative nutrient criterion.

Together, EPA and its State partners are responsible for achieving the goals of the CWA to restore and maintain the chemical, physical, and biological integrity of the nation's waters. Thus, EPA encourages States to renew their efforts to identify those waters impaired by nutrient pollution that are not meeting applicable water quality standards.

11. See 40 CFR 130.7(d)(2)

4. Assessment and Total Maximum Daily Load (TMDL) Tracking and Implementation System (ATTAINS)

As discussed in the 2012 IR Memo,[12] IR data include State water quality assessment decisions, attribute data, and the geospatial data representing the geographic locations of those assessed waters. This information is needed in order for EPA and the public to better understand the status of the nation's waters. EPA's ATTAINS database[13] is the repository for State IR attribute data, and the Reach Address Database[14] contains State IR geospatial data. EPA compiles State-submitted IR data to develop and publish the National Water Quality Inventory Report to Congress (CWA Section 305(b)), determine States' variable portion of the Section 106 grant allocation formula, inform water quality decisions, and to conduct national analyses with various stakeholders to help restore the nation's waters.

12. Information Concerning 2012 Clean Water Act Sections 303(d), 305(b), and 314 Integrated Reporting and Listing Decisions
13. Water Quality Assessment and Total Maximum Daily Loads Information
14. Geospatial Data Downloads

A. Information update on using and reporting Statewide Statistical Survey Data in ATTAINS, and the National Water Quality Inventory Report to Congress

In the 2010 IR Memo,[15] EPA discussed how States can use CWA Section 106 grant funds to improve monitoring programs and to implement statistically-valid surveys. EPA continues to support both Statewide statistical surveys and site-specific targeted monitoring to cost-effectively meet the reporting requirements under CWA Sections 303(d) and 305(b). EPA's discussion in the 2010 IR memo remains unchanged.

For the 2014 reporting cycle, EPA will again seek to incorporate Statewide statistical survey findings reported in ATTAINS into its national water quality summary. To assist States with reporting Statewide statistical survey data results to EPA, a new web entry tool is available. This tool replaces all Statewide statistical survey data submission tools used in prior cycles, including the Excel spreadsheet template provided for the 2010 and 2012 reporting cycles, and the probability survey module in the Assessment Database (ADB). For the 2014 reporting cycle, the Statewide statistical survey web data entry tool is the only mechanism for reporting Statewide statistical survey results to EPA. States may request access to the Statewide statistical survey web data entry tool available at: EPA Web Application Access.

B. Information on the data systems EPA will support for tracking assessment decisions for inclusion in ATTAINS

As discussed in the 2012 IR Memo,[16] EPA reports on the status of the nation's waters, shares this information with the public and other interested parties, and prepares a biennial National Water Quality Inventory Report to Congress. Data management of water quality assessment decisions is key to analyzing and sharing data across water programs and measuring progress in EPA's Strategic Plan.

In addition to the timely submission of IR data, States should also ensure consistency between their IR report and the associated electronic data submitted to EPA. States and EPA Regions should work together during the review of the IR and ensure that corrections to the report are also made to the associated electronic data. EPA expects that the States' associated electronic data, including geospatial data, should be submitted immediately following EPA's final action on States' Section 303(d) lists.

EPA recognizes that States need flexibility in the tools they use to collect and report IR data. As such, for the 2014 reporting cycle, EPA will continue to support the existing tools for reporting site-specific targeted monitoring data: EPA Assessment Database (ADB), State compatible assessment database, and the Exchange Network (EN) Office of Water Integrated Reporting (OWIR) data flow. As mentioned in the previous section, States that conduct Statewide statistical surveys should report this information in their IR report and use the Statewide statistical survey web data entry tool to submit the associated electronic data. For information on these tools, please visit the following Web sites:

- Site-specific targeted monitoring results
  - EPA Assessment Database
  - ADB Compatible Database
  - EN OWIR data flow (IR attribute data)
- Statistical survey results
  - Statewide statistical survey web data entry tool

As part of EPA's effort to streamline 303(d) and 305(b) reporting (described in Section 1), EPA is considering revisions to the processes that EPA and States use to manage and report 303(d) and 305(b) data, including the ATTAINS data system and the ADB.[17] Working with State partners, EPA expects to make significant progress on these efforts in 2013 and 2014. The first effort has been completing the Integrated Reporting Georeferencing Pilot, which was first discussed in the 2012 IR Memo.

For geospatial data, EPA recommends that States use the Hydrography Event Management (HEM) Tool and HEM EPA Add-On Tools, based in ArcGIS 9.x or ArcGIS 10, to manage assessed and impaired water events. For the 2014 reporting cycle, EPA will continue to support geodatabase or shapefile geospatial data formats, or files sent via the EN utilizing the NHDEvent data flow. For States that are interested in using the EN for submitting their geospatial data and are also using the HEM Tool to manage their geospatial events locally, there is a tool called "HEM to NHDEvent XML Conversion Tool" (HEM2XML) that converts geospatial events into the EN NHDEvent format. For more information on these tools and associated documentation, please visit the following Web sites:

- HEM Tool
- HEM EPA Add-on Tools
- EN NHDEvent dataflow (IR geospatial data)
- HEM2XML tool

15. Memorandum: Information Concerning 2010 Clean Water Act Sections 303(d), 305(b), and 314 Integrated Reporting and Listing Decisions
16. Information Concerning 2012 Clean Water Act Sections 303(d), 305(b), and 314 Integrated Reporting and Listing Decisions
17. In the interim, for States upgrading their computers to the Windows 7 operating system, please see the installation instructions

5. Antidegradation and Listing Guidance

Antidegradation is a component of a State's water quality standards (i.e., designated uses, criteria to meet those uses, and antidegradation requirements) that focuses on maintaining and protecting the chemical, physical, and biological integrity of the nation's waters, consistent with the CWA and its implementing regulations. CWA Section 303(d) and EPA's implementing regulations require States to identify waters not meeting any applicable water quality standard (CWA §303(d)(1)(A), 40 C.F.R. 130.7(b)(3)). EPA's listing regulations specify that "applicable water quality standards" refer to criteria, designated uses, and antidegradation requirements (40 CFR 130.7(b)(3)).

Most State water quality assessments have focused on whether numeric and narrative water quality criteria are being attained, and typically, these assessments capture where waters are most in need of restoration. However, it is possible that some waters are not meeting the antidegradation portion of water quality standards. For example, it is possible that available data and information for a water identified by a State as an Outstanding National Resource Water (ONRW) indicates degradation in water quality. If those data and information indicate that the water is not meeting the State's requirement for maintenance and protection of the water quality of the ONRW under the antidegradation portion of its water quality standards, in accordance with CWA and EPA regulations, the waters would be listed on the State's Section 303(d) list even if pollutant concentrations do not exceed water quality criteria levels.

EPA is working to develop additional guidance to address how antidegradation requirements should be considered when assessing waters under CWA Section 303(d).

ATTACHMENT C

Vermont Surface Water Assessment and Listing Methodology

In accordance with
USEPA 2006 Guidance

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Chapter Three. Assessment Use Support Determinations

The following pages provide specific criteria, principles for making decisions, and other information that DEC applies when making an assessment of water quality conditions and determining whether individual designated and existing uses are fully supported, stressed, altered, impaired or unassessed (described above generally). Information below is presented by each of the seven designated uses to show how relevant, representative and reliable water quality monitoring data and other information relates directly to the degree of use support for assessment reporting purposes. Additional considerations for lakes are included under aquatic life use where the assessment methodology differs from riverine environments.

Aquatic Biota/Habitat (Aquatic Life) Use

In assessing Aquatic Life Use, the DEC Watershed Management Division uses several types of water quality and water quantity data and information to determine use support. The specific data types are biological monitoring, habitat assessment, conventional pollutants, toxicants, and invasive aquatic species. For lakes, additional assessment guidelines are used to directly or indirectly assess uses using conventional pollutants, nutrients, and information regarding water-level impacts. Where there is biological (aquatic community) data then use support is determined by the assessment of that data even if conventional pollutant measures or habitat indicators are indicating otherwise. Specific decision-making criteria are as follows:

Biological Monitoring

Full Support: Biological assessments for fish and/or macroinvertebrate communities demonstrate compliance with appropriate threshold criteria as described in DEC biocriteria implementation methodologies. In the absence of applicable biocriteria, all available information and data are used to make scientifically defensible weight-of-evidence findings that designated aquatic life uses are fully supported. In most cases, biological condition ratings of *excellent, very good,* and *good* will indicate full support status for Class A(1), Class B(1), and Classes A(2) B, B(2) and B(3) respectively.

Stressed: Biological assessments for fish and/or macroinvertebrate communities and/or habitat assessments indicate that impacts have occurred but are inconclusive with regard to support status determination or demonstrate that the biological condition is at risk of making a transition between support and non-support. In the absence of applicable biocriteria, all available information and data are used to make scientifically defensible weight-of-evidence findings that designated aquatic life uses are stressed. Additional biological assessment may be needed. In most cases, biological condition ratings of *"excellent-to-very good"* will indicate stressed status for Class A(1) waters, *"very good-to-good"* will indicate stressed status for Class B(1) waters; "good" or "good to fair" will indicate stressed status for Class B waters and *"good-to-fair"* will indicate stressed status for Class A(2), B(2) and B(3) waters.

Altered: Biological assessments for fish and/or macroinvertebrate communities demonstrate non-compliance with appropriate threshold criteria as described in DEC biocriteria implementation methodologies and the cause is not a pollutant (e.g. flow regulation or non-native species). In the

absence of applicable biocriteria, all available information and data are used to make scientifically defensible weight-of-evidence findings that designated aquatic life uses are not fully supported. In most cases, biological condition ratings of *very good or lower, good or lower,* and *fair or lower* will indicate altered status for Class A(1), Class B(1), and Classes A(2), B, B(2) and B(3) respectively. Generally, biological data indicating non-attainment from the previous two or more successive samples are necessary in order to determine this condition.

Impaired: Biological assessments for fish and/or macroinvertebrate communities demonstrate non-compliance with appropriate threshold criteria as described in DEC biocriteria implementation methodologies and the cause is due to a pollutant of human origin. In the absence of applicable biocriteria, all available information and data are used to make scientifically defensible weight-of-evidence findings that designated aquatic life uses are not fully supported. In most cases, biological condition ratings of *very good or lower, good or lower,* and *fair or lower* will indicate impaired status for Class A(1), Class B(1), and Classes A(2), B, B(2) and B(3) respectively. Generally, biological data indicating non-attainment from the previous two or more successive samples are necessary in order to determine this condition.

Habitat Assessment

Full Support: Depending on the water's classification and typing {A(1), A(2), B, B(1), B(2), B(3)}, very high or high quality habitat with up to a moderate change from natural or reference condition exists "consistent with the full support of all aquatic biota and wildlife uses."

Stressed: Stream or river physically under stress – in adjustment with stresses greater than as naturally occurs to a "fair" condition derived from a geomorphic assessment completed using recognized protocols.

Altered: Changes to the habitat are greater than minimal to a moderate change from reference, depending on the water's classification and typing. There is an undue adverse effect on the physical nature of the substrate. Aquatic habitat surveys show significant deviation from the reference condition due to human-caused changes and/or geomorphic assessment indicated "fair" to "poor" conditions. All life cycle functions, including over-wintering and reproductive requirements, are not adequately maintained and protected due to the physical habitat changes.

Impaired: A pollutant of human origin is shown to cause more than the allowable change to aquatic habitat as defined by Vermont Water Quality Standards.

Conventional Pollutants (defined by USEPA as: temperature, pH, D.O., turbidity, nitrate-nitrogen, phosphorus)

Full Support: Waters that are not stressed or impaired due to conventional pollutants, assessed using the Vermont Water Quality Standards. For example, the total increase from the ambient temperature due to all discharges and activities is not known to exceed 1.0 degree F for a coldwater fishery and the total increase from ambient temperature due to all discharges and activities shall not exceed the temperature criteria derived from tables 1 or 2 in Section 3-01.B.1.c. except as provided for in Section 3-01 B.1.d. of the Vermont Water Quality Standards (pertaining to both a coldwater and warmwater fishery).

Stressed: Waters where the level of a conventional pollutant or a combination of conventional pollutants of human origin may be resulting in some disturbance. For example, temperatures are such that in coldwater fishery waters, one or more trout species are reduced in number or biomass as compared to reference condition. Waters with alkalinities between 2.5 and 5.0 mg/l (as CaCO3), and pH values may occasionally drop below 6.5. Coldwater fishery waters where dissolved oxygen may be between 6 and 7 mg/l and 75 to 85% saturation.

Altered: This assessment category is not used in this context.

Impaired: Temperatures are too high as a result of human activities to fully support coldwater fish species in waters designated as a coldwater fishery OR the total increase from the ambient temperature due to all discharges and activities exceeds 1.0 F for a coldwater fishery and the total increase from ambient temperature due to all discharges and activities exceeds the temperature criteria derived from tables 1 or 2 in Section 3-01.B.1.c. except as provided for in Section 3-01 B.1.d. of the Vermont Water Quality Standards (pertaining to both a coldwater and warmwater fishery).
Reliable, representative monitoring indicates that pH values repeatedly fall below 6.5 standard units or exceed 8.5 standard units across a range of weather conditions, and values are not due to natural sources.
Reliable, representative monitoring indicates D.O. values or percent saturation repeatedly fall below the standard for the water's classification and type except as noted below.
Reliable, representative monitoring shows that turbidity values are more than occasionally above the standard for a the water's classification and type as measured across a range of weather conditions and values are not due to natural sources.
Reliable, representative monitoring shows that nitrate-nitrogen and/or phosphorus repeatedly and/or consistently exceeds the standard for the water's classification, type, and elevation except as noted below.

Toxicants (priority pollutants, metals, chlorine & ammonia)

Full Support: Waters that are not stressed or impaired due to toxicants, as described below.

Stressed: Water quality monitoring or sediment samples reveal the presence of toxics below criteria or there are no relevant criteria and the source of the pollutants has not been remediated. Groundwater data in wells adjacent to the stream shows levels of pollutants above the Vermont Groundwater Enforcement Standards but no in-stream data exists or no sediment samples have been taken.

Altered: Toxicants are considered pollutants, therefore, the category "altered" is not applicable.

Impaired: In most cases, the following exposure presumptions are applicable to compliance determinations: for any one pollutant, an acute aquatic biota criterion is exceeded more than once within a 3-year period, for longer than one hour, above ten-year, seven-day flow minimum (7Q10) flows; or a chronic aquatic biota criterion is exceeded for more than four consecutive days in a three year period, above 7Q10 flows.

(DEC recognizes that the literal interpretation of the exposure scenario cited would be difficult to replicate in a field situation. The language cited reflects the exposure conditions used to develop the numerical criterion that is the water quality standard. It is likely that available monitoring data would be collected under a variety of temporal and spatial formats. In evaluating data, DEC uses the exposure assumptions of the criterion development as guidelines in the interpretation of data and uses empirical and judgmental means to assess whether or not there is reasonable potential for those exposure assumptions to be violated. Given the variable nature of available information, evaluations will vary on a case-by-case basis. DEC takes into consideration guidance provided by EPA when evaluating toxicants in surface waters (see "Technical Support Document for Water Quality-based Toxics Control." EPA/505/2-90-001).

Invasive Non-native Species

Invasive non-native species such as Eurasian watermilfoil (*Myriophyllum spicatum*), water chestnut (*Trapa natans*), alewives (*Alosa pseudoharengus*) or zebra and quagga mussels (*Dreissena spp*.) have significant impacts on existing aquatic plant and animal communities. Information on the extent and distribution of these species is used to assess aquatic life use support.

Full Support: No established population of an invasive non-native species.

Stressed: Invasive non-native species are present but in low densities (e.g. scattered areas of plant growth in limited areas of the littoral zone). In the case of Eurasian milfoil, lakes within a 10-mile radius of an infested lake are considered stressed, unless access to the lake is remote or inaccessible by conventional means.

Altered: Invasive non-native species present in densities sufficient to alter native biological communities. For example, overall plant density is classified as "moderate," indicating locally abundant (50% or greater coverage) growth, or "heavy," (75% or greater littoral cover overall) indicating growth in most shoreline areas.

Impaired: Invasive non-native species are not considered pollutants. Therefore, this category is not applicable.

Additional Aquatic Life Use Considerations for Lakes

Lakes - Conventionals (alkalinity, dissolved oxygen, nitrate-nitrogen)

Full Support: Waters that are not stressed or impaired.

Stressed: Reliable long-term monitoring data indicates that a lake's alkalinity routinely drops below 12.5 mg/l (as CaCO3) during the spring runoff period.
Reliable long-term monitoring data indicates that a lake's hypolimnetic dissolved oxygen concentration periodically falls to (or near) 0 mg/l or 0% saturation during peak summer stratification, but macroinvertebrates are present. The area designated as stressed, as a result of human disturbance, is limited to the lake acreage underlain by the hypolimnetic oxygen-deficient area.

Altered: This assessment category is not used in this context.

Impaired: Reliable monitoring data indicates that alkalinity routinely drops below 2.5 mg/l (as acid neutralizing capacity) during the spring runoff period.
Reliable monitoring data indicates that a lake's hypolimnetic dissolved oxygen concentration falls to (or near) 0 mg/l or 0% saturation for a period of greater than 50% of the summer stratification period, **and** the hypolimnetic sediments are devoid of a macroinvertebrate community. The area designated as impaired, as a result of human disturbance, is limited to the lake acreage underlain by the hypolimnetic oxygen-deficient area. However, if in the best professional judgement of DEC scientists, the dissolved oxygen deficit is due to natural causes, aquatic life uses will be considered instead as fully supported.
The epi- and metalimnetic lake waters will be considered impaired if dissolved oxygen concentrations fall below Water Quality Standards in greater than or equal to 10% of samples, and the anoxia is not a natural phenomenon.
Reliable monitoring data indicates nitrates in excess of 5.0 mg/l in 10% or more of samples collected.
A minimum of four evenly-spaced sampling events across the summer stratification period are commonly used to make a determination regarding conventional pollutants in lakes, except for alkalinity, which is most commonly measured in spring, which corresponds to peak acidity loading for lakes.

Lakes - Conventionals (phosphorus)

Vermont is working under a cooperative funding agreement with the New England regional office of USEPA to develop scientifically-based nutrient criteria that are relevant to Vermont waters, for inclusion in Vermont's Water Quality Standards. Pending development of these new criteria, the following is used to assess use support for lakes using phosphorus data.

Full Support: Vermont's Water Quality Standards provide that full support lakes have experienced no acceleration of eutrophication or stimulation of the growth of aquatic biota in a manner that prevents the full support of uses.

Stressed: Photic-zone and/or whole column total phosphorus concentrations are elevated in relation to statewide norms, resulting in stimulation of growth of aquatic plant species that results in no more than a minor to moderate change in aquatic biota, depending on water management type.

Altered: Phosphorus is a pollutant; therefore this category is not applicable.

Impaired: Photic-zone or whole column total phosphorus concentrations, as determined by the DEC Spring Phosphorus Monitoring Program, the Vermont Lay Monitoring Program, or other special studies, have increased significantly, or are significantly elevated relative to statewide norms, and resultant algal blooms produce more than a moderate change in the aquatic biota. For Lake Champlain, Lake Memphremagog and South Bay of Lake Memphremagog, summer average phosphorus concentrations exceed criteria expressed in §3-01(A)(2)(c) of the Water Quality Standards.

Lakes - Fluctuated Reservoirs and Lakes

Reservoirs present special cases in regards to assessment of aquatic life use support (ALUS). In the absence of direct biological measurements beyond routine aquatic plant survey data, ALUS can be assessed using the following decision-making 'tree.' In order to use this decision tree, several pieces of information regarding the reservoir are useful. These include bathymetry, maximum and mean waterbody depth, the limnological shoreline development index, and the magnitude and timing of the drawdown. These data can be used collectively to estimate the proportion of the littoral zone likely to be affected by the drawdown regimen. Where available, biological data (in particular the presence and distribution of aquatic macrophytes within the littoral zone) are also useful.

1) Can the level of the waterbody be regulated by an artificial structure (e.g. dam, sluice, weir)?
   Answer is NO: no alteration or stress to ALUS due to water level fluctuation. ***Full Support.***
   Answer is YES: go to 2.
2) Is the waterbody connected to a licensed or unlicensed hydroelectric generating system, a flood control system, or subject to promulgated Vermont Water Resources Board rules regulating the fluctuation?
   Answer is NO: a stress or alteration to ALUS could potentially exist, but must be verified by direct assessment before the waterbody can be correctly assessed; go to 4.
   Answer is YES: go to 3.
3) Is the waterbody regulated by a federal Clean Water Act Section 401 water quality certification issued by VTDEC after January 1, 1990?
   Answer is NO: go to 4.
   Answer is YES: ***no alteration or stress to ALUS due to water level fluctuation if operated in accordance with the license.***
4) Is the waterbody in fact subject to periodic fluctuations that are attributable to operation or manipulation of the outflow structure?
   Answer is NO: ***a stress to ALUS is presumed to exist***, due to the ability of the outflow operators to fluctuate water levels if the need arises, which can negatively impact littoral zone communities. Such littoral zone impacts have the potential to cause cascading changes within the trophic web of the waterbody but cause no more than a minor change in habitat or moderate change in aquatic biota from the reference condition. The entire waterbody acreage will be assessed as stressed for ALUS.
   Answer is YES: Go to 5.
5) Does there exist a sufficient area of littoral habitat below the drawdown zone to enable establishment of a viable and stable aquatic community, with all expected functional groups, while accommodating the drawdown regimen, **or**, does available biological data suggest that such a community exists within the drawdown zone?
   Answer is NO: ***ALUS is altered.*** These alterations create more than a moderate change to aquatic habitat. Littoral zone impacts of this magnitude will have cascading impacts throughout the trophic web, resulting in more than a moderate change in aquatic biota from the reference expectation. Aquatic macroinvertebrate and fish assemblages exhibit more than moderate changes in the relative proportions of tolerant, intolerant, taxonomic and functional components. Accordingly, the entire acreage is assessed as altered.

   Answer is YES: ***ALUS is stressed.*** These stresses cause no more than a moderate change to aquatic habitat. Littoral zone impacts of this magnitude could have cascading effects within

the trophic web of the waterbody, but these are presumed to create no more than a moderate change to aquatic biota from the reference expectation based on the relative proportions of tolerant, intolerant, taxonomic and functional groups. The waterbody's entire acerage is presumed to be stressed for ALUS.

Fish Consumption Use

Vermont interprets the U.S. EPA guidance on fish consumption use attainment to indicate that no waters fully support fish consumption. This is due to well-documented contamination of varying levels of lakes by mercury in waters, sediments, and aquatic biota arising from atmospheric deposition. In the tissues of fish inhabiting Lake Champlain (and elsewhere), other contaminants including polychlorinated biphenyls, polyaromated hydrocarbons, and "DDT" derivatives, have been identified.

DEC does not, however, subscribe to the notion that fish tissue consumption is impaired on a statewide basis. This is because most fish species can, indeed, be consumed from most Vermont waters, albeit at a reduced rate. Fish consumption use is considered impaired only in the event that the fish species subject to the consumption advisory is documented to exist in the waterbody and contaminant data exist for that species from the particular waterbody. This approach is consistent with current EPA guidance.

Full Support: No fish consumption advisory in effect.

Stressed: "Restricted consumption" of fish is in effect (restricted consumption is defined as limits on the number of meals or size of meals consumed per unit time for one or more fish species).

Altered: Tissue contaminants are derived from the deposition or release of pollutants into the aquatic environment. Accordingly, this assessment category is not relevant.

Impaired: Fish consumption use is considered impaired only in the event that the fish species subject to the consumption advisory is documented to exist in the waterbody and contaminant data exist for the species from the particular waterbody. For a given fish species present in a waterbody, a 'no-consumption' advisory is in place for a designated sub-population (e.g., children or women of childbearing age) or for the general population.

Swimming/Contact Recreation Use

For assessment of Swimming/Contact Recreation Use, the DEC Watershed Management Division uses one or more types of data to determine whether this use is supported. The specific data types are bacterial monitoring, invasive aquatic species growth, and on rare occasion, the presence of chemical contaminants. Decision-making criteria are as follows:

Indicator Bacteria

E. coli (an abbreviation for the scientific name of the bacterium *Escherichia coli*) concentrations are known to vary considerably over space and time in response to natural and human-related factors. In order to assess waters for support of swimming and contact recreation using *E. coli* monitoring data, a minimum number of data points are necessary, and supporting contextual data such as antecedent weather and flow conditions must be considered. DEC considers at least five (5) reliable and quality assured sample results over a swimming season and gathered across a range of weather/flow conditions to be the minimum practical number of samples necessary to document representative conditions and to assess attainment of contact recreational uses. In a practical sense, weekly or more frequent *E. coli* data across the swimming season is most useful to determine impairment and observe weather-related patterns in bacterial concentrations. If there are questions regarding the representativeness of the data, the water is identified as needing monitoring and is recommended for follow-up *E. coli* sampling in the next season.

Very few strains of *E. coli* are themselves pathogenic. Rather, they are indicators of the presence of fecal material of warm-blooded animal origin. This fecal material may contain harmful pathogens. *E. coli*-based criteria are expressed either as geometric mean values, or as one-time, instantaneous single-sample values. These values equate to a likelihood of developing gastrointestinal illness from exposure to waterborne pathogens associated with *E. coli*. EPA originally (1986) derived its freshwater criterion recommendations using a set of statistical relationships relating geometric mean *E. coli* levels to observed gastrointestinal illness rates directly attributable to the *E. coli* exposure. Using these relationships, EPA has recommended that the most conservative *E. coli*-based criterion be a geometric mean of 126 *E. coli* /100ml. At highly populated freshwater beaches (defined as greater than or equal to 2,427 swimmers/day on average) that are subject to direct sewage effluent contamination, exceedance of this criterion means that on a season-wide average basis, 8 in 1,000 swimmers will develop gastrointestinal illness due to *E. coli* exposure. In 2012, EPA reaffirmed its 126 *E. coli* /100ml geometric mean recommendation considering the most available data and studies.

Vermont's standards have criteria for bacteria that reflect a high level of protection for swimmers and other forms of contact recreation use. The current criteria are far more conservative than those recommended by EPA. Vermont's current criteria are not to exceed a three-sample geometric mean of 18 *E. coli* /100ml (or a single sample maximum of 33) for Class A(1) and A(2) waters, and not to exceed 77 *E. coli* /100ml for Class B waters in all management types. Interpreted using EPA's statistical relationships, a single instantaneous concentration of 77 *E. coli* /100ml equates to a 75% likelihood that a beach closure will prevent swimmers from incurring a 3.4 in 1,000 risk of developing gastrointestinal illness. Such an interpretation must be treated cautiously as any illness rate attributed to *E. coli* exposure less than 8 in 1,000 is below the level quantifiable using EPA's statistical relationships.

Recent research conducted within Vermont indicates that the present Vermont Class B criterion can be exceeded in low to moderate streamflows issuing from forested watersheds due to natural background sources. Based on calculations using EPA's statistical relationships, 77 *E. coli* /100ml, expressed as a geometric mean of several samples, results in a projected illness rate of 6 in 1,000 swimmers. While this level of risk approaches the EPA minimum recommendation, it is consistent with the intent of current and prior Vermont water quality criteria for bacteria, beginning in 1985.

In addition, new EPA guidance (USEPA, 2012) on the application of water quality criteria for pathogens allows that impairment determinations can be based on geometric seasonal means or some percentage of samples from the dataset not to exceed a specific value. EPA expresses preference for use of a longer-term indicator (geometric mean) for reporting use attainment. Given these considerations, a common-sense approach must be applied when assessing waters using *E. coli* monitoring data. The following guidelines are applied during the assessment process:

Full Support: Waters are suitable for swimming.

Stressed: Individual samples only occasionally exceed the class-specific single-sample criteria values. The geometric mean does not exceed the criterion value.

Altered: *E. coli* indicator bacteria are considered a pollutant. This assessment category is not applicable.

Impaired: For class B waters in all water management types, the geometric mean of 77 *E. coli* /100 ml is exceeded in a given segment or area and the contamination can be attributed to sources other than natural background. DEC accepts a weight-of-evidence approach to confirm that *E. coli* values are or are not of natural origin. A minimum of five samples collected regularly over the swimming season is needed, and flow and antecedent precipitation are accounted for in this determination. For class A(1) and A(2) waters, the geometric mean of a minimum 3 samples exceeds 18 *E. coli* /100ml, and the contamination can be attributed to sources other than natural background (i.e. human, livestock, domestic animal sources). Generally, data from at least two swimming seasons are needed to assess waters as impaired for swimming.

Alternatively, waters with CSOs present that do not meet DEC's 1990 CSO Control Policy are considered impaired for swimming without the direct water E. coli sampling numbers.

Nutrients and Invasive Aquatic Species

Full Support: Waters have native plant species and communities as would be expected and in good ecological balance. Waters are not stressed or altered by invasive non-native aquatic species.

Stressed: Invasive non-native species are present but not at levels where a nuisance has been documented or in "light" densities (scattered areas of growth in limited areas of the littoral zone). In the case of Eurasian milfoil, lakes within a 10-mile radius of an infested lake are considered stressed, unless access to the lake is remote or inaccessible by conventional means.

Altered: Invasive non-native species present in densities such that swimming uses are not met. For aquatic macrophytes, typically these conditions are characterized by greater than 75% cover of the non-native macrophyte and designated as "moderate" or "heavy" infestations. For species other than aquatic macrophytes such as zebra mussels, colonies would be present in such densities and at such depths as to impact swimming uses due to potential for injury to bare feet. Nutrients are not applicable in this category.

Impaired: An on-going record of public complaint concerning the algal conditions in the water has been established. For cyanobacteria (blue-green algae), waters display on-going summer blooms of

toxin-producing cyanobacteria and have microcystin concentrations at elevated levels in excess of the World Health Organization guideline of 1 ug/l. Invasive non-native aquatic species are not applicable in this category.

Chemical Contamination

Water quality criteria do not address incidental/accidental ingestion of water or dermal exposure to recreational users where there is chemical contamination present. Chemical contamination can enter surface waters or be deposited on beaches from both natural and anthropogenic sources. These may be point sources, such as municipal and industrial outfalls, or nonpoint sources such as runoff from land or leaching from old hazardous waste sites. In most cases there will be significant dilution or attenuation of contaminants.

Drinking water guidelines can provide a starting point for deriving values that could be used to make a screening level risk assessment. It has been suggested (WHO Guidelines for Safe Recreational Waters 2009) that water quality standards for chemicals in recreational waters should be based on the assumption that recreational water makes only a minor contribution to intake.

It is assumed that contribution of swimming is equivalent of 10% of drinking-water consumption. Based on drinking water consumption value of 2 liters a day, this would result in an intake of 200ml per day from recreational contact with water. A simple screening approach therefore would be that a substance occurring in recreational water at a concentration of ten times the drinking water guidelines (VDOH Drinking Water Guidance) is considered stressed and needs further assessment.

Organic contaminants can be present in surface waters from industrial and agricultural activity. EPA studies have shown that dermal contact and inhalation can contribute as much as water ingestion. Many of these are associated with sediments and particulate matter. Consideration should be given to the possibility of sediment being disturbed and ingested by infants and young children. EPA Regional Screening Levels (RSL) for Residential Soil can be used to screen sediment chemistry data from a site. If the screening value is exceeded, it suggests the need for specific evaluation of the contaminant taking local circumstances into consideration.

Full Support: No chemical contamination present in sediments or surface waters at any level of concern.

Stressed: A chemical is present in surface water samples at a concentration that is ten times the Vermont Department of Health Drinking Water Guidance. Or, for dermal exposure to the contaminants in sediments, the EPA Regional Screening Levels for Residential Soils are exceeded. Further assessment is needed following exceedance of screening levels.

Altered: This category is not used under these situations.

Impaired: A water is part of a Superfund site or other Hazardous waste site where special health and safety training and precautions are required to access the site or the public is restricted access from all activities including swimming, fishing and trespassing for health and safety reasons by an entity such as the Vermont Department of Health.

Secondary Contact/Non-Contact Recreation Use

For assessment of Secondary Contact/Non-Contact Recreation Use, the DEC Watershed Management Division uses information regarding water quantity and water quality, data and other information regarding the game fishery and records of public feedback and complaint to determine levels of support.

Full Support: Water quantity and quality sufficient for boating and fishing.

Stressed: Odor, color, plant growth, low water conditions occasionally discourage boating or fishing.

Altered: Fishing and/or boating are limited due to insufficient or diminished or lack of water, aquatic nuisance species or channel alterations. Boating is not feasible to the degree deemed achievable for the water's Water Management Type.

Impaired: Fishing and/or boating are limited due to water quality or aquatic habitat impairment(s) caused by pollutants from human sources.

Drinking Water Supply Use

Drinking water supply use is assessed using data on toxicants and bacteria; information on water treatment plant operation and operating costs; and, data describing cyanobacterial (blue-green algae) toxin concentrations.

Full Support: Water quality suitable as a source of public water supply with disinfection and filtration.

Stressed: This category is not applicable.

Altered: A well-established zebra mussel infestation is known to increase cost or effort to produce water that is suitable for drinking.

Impaired*:* In rivers, streams, brooks and riverine impoundments the exceedance, due to human sources, of any one human health-based toxic pollutant criteria listed in Appendix C of the Water Quality Standards (or as otherwise determined by the Natural Resources Agency Secretary in accordance with the Toxic Discharge Control Strategy) at flows equal to or exceeding the median annual flow for toxic substances that are classified as "non-threshold toxicants" or at flows meeting or exceeding the 7Q10 flow for toxic substances that are classified as "threshold toxicants." In all other waters, the exceedance, due to human sources, of any one human health-based toxic pollutant criteria listed in Appendix C (or as otherwise determined by the Secretary in accordance with the Toxic Discharge Control Strategy) at any time. (Note: "non-threshold toxicants" are probable or possible human carcinogens and "threshold toxicants" are not known or probable human carcinogens).

Criteria established by the Federal Safe Drinking Water Act can be met only by employing treatment practices that operationally or financially supercede customary practices that include filtration and disinfection.
Finally, waters display on-going summer blooms of toxin-producing cyanobacteria and have microcystin concentrations in excess of the World Health Organization guideline of 1 μg/l.

Aesthetics Use

For assessment of Aesthetic Use, the DEC Watershed Management Division uses water quality and water quantity information from field surveys for rivers and streams and public feedback and complaints as well as field surveys for lakes and ponds to determine levels of support.

Full Support: Water character, flows, water level, riparian and channel characteristics, all exhibit good to excellent aesthetic value consistent with the waters classification. Water clarity and substrate condition is good. No floating solids, oil, grease, scum, or algae blooms. Limited or no record of public concern.

Stressed: Aesthetic quality is compromised somewhat. Water unnaturally turbid at times. Moderate levels of invasive, non-native plant growth. Small or disturbed riparian zone. Some record of public concern or complaint.

Altered: Aesthetic quality is poor due to a diminished amount of water to no water in the channel or lake resulting from human activities or due to moderate or heavy densities of invasive, non-native species. Streambanks are severely slumping, stream is braided, channel is highly straightened and rip-rapped, and channel bed material is severely jumbled and unsorted.

Impaired: Aesthetic quality of water is poor. Water is frequently and unnaturally turbid. Substrate is unnaturally silt-covered, mucky, or otherwise changed so as to adversely affect the aesthetics in an undue manner. Presence of solid waste, floating solids, scum, oil or grease occurs frequently and persistently. Rocks are unnaturally colored by metal contamination.

Agricultural Water Supply Use

There are no EPA definitions for agricultural water supply nor any state definitions and criteria. Consequently, this use is unassessed and the four assessment categories are not used.

Chapter Four. Listing and De-Listing Methodology

For the purposes of identifying and tracking important water quality problems where the VTWQS are not met, VTDEC has developed the Vermont Priority Waters List. This list is composed of several parts each identifying a group of waters with unique water quality concerns. Development of each part is guided by various regulations and/or management considerations including federal Clean Water Act requirements, EPA guidance or Vermont-specific management objectives. This list is produced biannually on even numbered years. Table 2 outlines the composition of the Priority Waters List while specific details of each list's composition are given below.

Table 2. Summary of Vermont Priority Waters List

List Section	Assessment status	Description
Part A (303d List)	Impaired	Also known as the §303(d) Impaired Waters List. This federally mandated list identifies impaired waters scheduled for TMDL development
Part B	Impaired	Waters assessed as impaired for which TMDLs are not required
Part D	Impaired	Impaired waters that have completed and EPA approved TMDLs
Part E	Altered	Waters assessed as altered due to the presence of invasive species
Part F	Altered	Waters assessed as altered due to flow regulation

Impaired Waters

All waters determined to be impaired are placed on Part A (303d List), Part B or Part D.

Part A - 303d List

Part A of the Priority Waters List identifies impaired surface waters that are scheduled for total maximum daily load (TMDL) development. Part A of the List is prepared in accordance with current EPA guidance and federal regulations 40CFR 130.7 ("Total maximum daily loads (TMDL) and individual water quality-based effluent limitations"). A TMDL is required for these waters in order to establish the maximum amount of a pollutant that may be introduced into the water after the application of required pollution controls and to ensure the Water Quality Standards are attained and maintained.

In addition to identifying the waterbody, Part A identifies the pollutant(s) causing the impairment, the priority ranking for TMDL development, which water use(s) are impaired and a brief description of the specific water quality problem.

Identification of Pollutant

The federal regulation governing 303(d) List development, 40CFR §130.7(b)(4), requires states to include the "pollutants causing or expected to cause violations of the applicable water quality standards". This pollutant then becomes the basis for TMDL loading allocations or for the control measures necessary to bring about compliance.

Where there is monitoring data that identifies a violation of numeric criteria, identification of the pollutant is evident. For example, long-term monitoring data may identify a segment of Lake Champlain as exceeding the numeric criterion for total phosphorus. Other numeric criteria are less indicative of the specific pollutant as in the instance of a dissolved oxygen criteria. The numeric criterion in this instance can be measured (low dissolved oxygen) but the pollutant causing that condition is not directly identified. Where there is monitoring data that identifies a violation of a narrative standard, the identification of the causal pollutant becomes more complex. An example is where biomonitoring data indicates a violation of the biocriteria for aquatic life use support.

In the instance of a biocriteria violation, VTDEC attempts to be as accurate as possible in identifying the causal pollutant. Where appropriate, VTDEC subscribes to EPA's Stressor Identification Methodology (USEPA, 2000b) or similar process. These assess site specific stressors and indicators such as biological and habitat indicators, land use information, proximity of known pollutant sources or other relevant information to identify by inference the most probable causal pollutants or stressors. This process can provide a defensible list of pollutant stressors or suite of stressors of common origin as in the case of runoff from impervious surfaces (i.e. stormwater).

At times, however, it may be necessary to identify a water as impaired without providing a specific causal pollutant. In these instances the pollutant is identified as "undefined".

TMDL Scheduling

Priority ranking for TMDL development is done with consideration of many factors. These include but are not limited to: (1) health issues, (2) the nature, extent, and severity of the pollutant(s), (3) the use or uses that are impaired, (4) the availability of resources and methods to develop a TMDL, (5) the degree of public interest, and (6) the utility of TMDL development to the elimination of the impairment.

Public Comment Opportunity, Submittal to EPA and EPA Approval

Upon compilation of the draft Part A-303d List, it is made available to the public for review and comment. Notification of availability is at a level sufficient to allow broad coverage of the general public and may include notices in newspapers, web sites and direct notification through email or mailing lists. In addition to notification, public meetings may be conducted to further the public's understanding. Following receipt of public comments, a response summary is developed that describes how the comments were addressed. Appropriate changes are made to the list and a final version of the Part A-303d List is then sent to the New England regional office of EPA for review and approval.

De-listing - Interim List

During development of new Part A-303d Lists, there may arise the need to propose for de-listing water(s) identified on previous lists. In this instance, waters proposed for de-listing are presented on the Interim List. This list is termed "interim" because it only exists during the period of Part A-303d List development in order to notify the public and EPA of de-listing proposals and to provide the rationale and justification for such proposals.

On the Interim List, each entry contains specific information for that particular waterbody as to why it is being proposed for de-listing. The waterbody-specific rationale is intended to provide "good cause" for de-listing and may be based on the following determinations:

- Assessment and interpretation of more recent or more accurate data demonstrate that the applicable WQS(s) is being met.

  The absence of impairment can be substantiated by data of a comparable quantity and quality as the data that was required to assess the water as impaired (for example, 2 years of biological or chemical data needed to establish impairment generally means 2 years of data needed to establish attainment).

- Flaws in the original analysis of data and information led to the segment being incorrectly listed.

- Documentation that a water included on a previous Part A-303(d) List was not required to be listed by EPA regulations, e.g. segments where there is no pollutant associated with the non-compliance

- A determination pursuant to 40 CFR 130.7(b)(1)(iii) that there are other pollution control requirements required by state, local or federal authority that will result in attainment of WQSs for a specific pollutant(s) within a reasonable time.

  In order to de-list these impaired waters from Part A, VTDEC must be convinced that other pollution control requirements, such as best management practices, will result in the attainment of Vermont Water Quality Standards. Specifically, DEC needs to show that (1) there are legal requirements in place (e.g. regulations, permits implementing regulations) that apply to the source(s) causing the water quality impairment and (2) that such legally required pollution control practices are specifically applicable to the impairment in question **and** are sufficient to cause the water to meet water quality standards within a reasonable time. These waters are then listed on Part B of the Vermont Priority Waters List.

- Approval or establishment by EPA of a TMDL since the last Part A-303(d) List
  These waters are then listed in Part D of the Vermont Priority Waters List.

- Other relevant information that supports the decision not to include the segment on the Part A-303(d) List

Part B List

All waters listed in Part B are assessed as impaired and do not require development of a TMDL as described in 40 CFR 130.7. Impaired waters that do not need a TMDL are those where other pollution control requirements (such as best management practices) required by local, state or federal authority are expected to address all water-pollutant combinations and the Water Quality Standards are expected to be attained in a reasonable period of time. DEC will provide information to show that (1) there are legal requirements in place (e.g. regulations or permits implementing regulations) that apply to the source(s) causing the water quality impairment and (2) that such legally required pollution control practices are specifically applicable to the impairment in question **and** are sufficient to cause the water to meet water quality standards within a reasonable time. Additional discussion of the Part B requirements are given in the EPA Integrated Report guidance document (USEPA 2005).

Part D List
All waters identified on Part D are assessed as impaired and have completed and approved TMDLs. If future assessments show the impairment has been eliminated, the waters will be removed from the Part D List. A comprehensive list of completed TMDLs is maintained on the Watershed Management Division's website.

Altered Waters

All waters determined to be altered are placed on one of several lists that track altered waters. These lists include: Part E List (water altered by invasive non-native species), and Part F (waters altered by flow regulation). The listing methodology for each list is given below.

Part E List
Waters appearing in Part E are assessed as "altered." They represent situations to be given priority for management where aquatic habitat and/or other designated uses have been altered to the extent that one or more designated uses are not supported due to the presence of aquatic invasive species. Waters will be removed from the Part E List when the population of the aquatic invasive species declines or is eliminated and the water is assessed as either "stressed" or in "full support" of the designated uses.

Part F List
Waters appearing in this part of the Vermont Priority Waters List are assessed as "altered." They represent priority management situations where aquatic habitat and/or other designated uses have been altered by flow regulation to the extent that one or more designated uses are not supported. Alterations arise from flow fluctuation, obstructions, or other manipulations of water levels that originate from hydroelectric facilities or other dam operations or from water withdrawals for industrial or municipal water supply or snowmaking purposes.

Waters will be removed from the Part F List as corrective actions are implemented.

Stressed Waters

Stressed Waters List
The Stressed Waters List identifies waters that have been assessed as "stressed". In the event a future assessment indicates non-compliance with the VTWQS, DEC will assess the water as "impaired" or "altered," depending on whether or not the cause of the violation is a pollutant, and place it on the appropriate part of the Priority Waters List.

Full Support Waters

Waters that fully support designated uses are not tracked on the Vermont Priority Waters List.

Comparison to EPA's Listing Categories

In 2005, the USEPA issued guidance ("*Guidance for 2006 Assessment , Listing and Reporting Requirements Pursuant to Sections 303(d), 305(b) and 314 of the Clean Water Act*") to provide

states a recommended reporting format and suggested content to develop a single document that integrates the reporting requirements of Clean Water Act section 303(d) and 305(b). Known as the "Integrated Report", it is EPA's strategy to report on water quality standards attainment of assessed waters, document availability of data and information for each segment, identify trends in water quality conditions and provide information to managers for priority setting. This comprehensive report is broken down into five parts into which all water segments within a state can be categorized. These categories are described in Table 3.

Table 3. USEPA Integrated Report listing categories

Category 1	All designated uses are supported, no use is threatened	
Category 2	Available data and /or information indicate that some but not all of the designated uses are supported	
Category 3	There is insufficient available data and/or information to make a use support determination	
Category 4	Available data and/or information indicate that at least one designated use is not being supported or is threatened, but a TMDL is not needed. This category is further divided into sub categories a-c;	
	4a	Segments with completed TMDLs
	4b	Segments for which control measures other than a TMDL are expected to bring about WQS compliance
	4c	Segments demonstrating failure to meet WQS but not by a pollutant
Category 5	Available data and/or information indicate that at least one designated use is not being supported and a TMDL is needed – 303(d) List	

As guidance, Vermont is not required to follow the USEPA suggested listing format as outlined in the guidance document and has instead opted to present the state's Priority Waters List as described above. It should be noted however that VTDEC does submit Vermont's water quality status to EPA electronically which is compatible with the five category format. Table 4 compares the parts of the Priority Waters List to EPA's five categories.

Table 4. EPA Categories compared to Vermont's Priority Waters Lists

EPA Category	Vermont listing component	notes
Category 1	NA	Waters in full support are not tracked on the Priority Waters List[1]
Category 2	NA	Waters where some but not all of the uses are supported are not tracked on the Priority Waters List
Category 3	NA	Unassessed waters are not tracked on the Priority Waters List[2]
Category 4a	Part D	The waters in Part D are assessed as impaired. Waters coming back into compliance after a TMDL is complete will be removed from Part D.
Category 4b	Part B	
Category 4c	Parts E & F	
Category 5	Part A	EPA approved 303(d) list as well as proposed delistings

1 Waterbodies or river miles in full support can be identified from Vermont's database through queries
2 Waterbodies or river miles that are not assessed can be identified from Vermont's database through queries

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ATTACHMENT D

Assessment Methodology for Determining Wadeable Stream Impairment Due to Excess Nitrogen and Phosphorus Levels

DECEMBER 2011

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ACRONYMS

Acronym	Definition
ADB	Assessment database
AFDW	Ash Free Dry Weight
ARM	Administrative Rules of Montana
BACI	Before-After-Control-Impact
BACIP	Before After Control Impact Paired
BOD	Biochemical Oxygen Demand
BPJ	Best Professional Judgment
CFR	Clark Fork River
CV	Coefficient of Variation
DEQ	Department of Environmental Quality (Montana)
DO	Dissolved Oxygen
DPHHS	Department of Health and Human Services
EPA	Environmental Protection Agency (US)
HBI	Hilsenhoff Biotic Index
LOWESS	locally-weighted regression line
QAPP	Quality Assurance Project Plan
SAP	Sampling and Analysis Plan
SOD	sediment oxygen demand
SOP	Standard Operating Procedures
SRP	Soluble Reactive Phosphate
TKN	Total Kjeldahl Nitrogen
TMDL	Total Maximum Daily Load
TN	Total Nitrogen
TP	Total Phosphorus
WQPB	Water Quality Planning Bureau (DEQ)

NITROGEN AND PHOSPHORUS IMPAIRMENT ASSESSMENT: METHOD SUMMARY

The following Method Summary should provide sufficient detail for an assessor to undertake an assessment of nitrogen and phosphorus impacts in a wadeable stream. *You will probably still need to refer to details provided later in the document.* Large rivers are not addressed by this methodology; a list of large rivers to which these methods do not apply is shown below in **Table S-1**.

Table S-1. Non-wadeable river segments within the state of Montana

River Name	Segment Description
Big Horn River	Yellowtail Dam to mouth
Clark Fork River	Bitterroot River to state-line
Flathead River	Origin to mouth
Kootenai River	Libby Dam to state-line
Madison River	Ennis Lake to mouth
Missouri River	Origin to state-line
South Fork Flathead River	Hungry Horse Dam to mouth
Yellowstone River	State-line to state-line

Part 1: Defining the assessment reach

1. Compliance determinations described in this document are carried out on an assessment reach. Here we define an assessment reach as: *a wadeable stream segment listed in the Assessment Data Base (ADB;(Montana Department of Environmental Quality, Planning, Prevention and Assistance Division, 2009) and updates), or a sub-segment of an ADB stream segment.* A sampling unit within an assessment reach is defined *as: a sample collected from the assessment reach that is largely independent of other samples collected within the assessment reach and collected during the time when the numeric nutrient criteria apply.* Please consider the following:

    a. The aggregate of samples collected from an assessment reach should provide good overall representation of the assessment reach. Individual sites within the assessment reach that have known or suspected pollution problems should be sampled equitably along with sites where pollution problems are not suspected or are minimal or less pronounced. Do not just target the hotspots.

    b. Given the guidelines in 1a above, the assessor will have to judge if further stratification of the stream reach (i.e., create two or more sub-reaches) is warranted. If, for example, a relatively un-impacted upstream reach of an assessment reach can be isolated and its condition is substantially different from other downstream parts of the assessment reach, sub-segmenting may likely be justified. As a rule of thumb, it is better to lump than split reaches to avoid excessive sub-segmentation of streams and the consequential administrative and sampling requirements.

    c. Each sub-reach will have the same general data requirements (dataset minimums, tests, etc.) as the parent assessment reach would have had if it hadn't been divided.

    d. Samples should be collected when the criteria apply, during the ecoregion-specific Growing Season (**Table S-2**). However, a ten day window (plus/minus) on the Growing Season start

and end dates is acceptable in order to accommodate year-specific conditions (e.g., an early-ending spring runoff). Samples collected outside the Growing Season may be useful for other purposes (e.g., isolating load sources), but should not to be used for compliance determination for the Growing Season.

Table S-2. Start and Ending Dates for Three Seasons (Winter, Runoff and Growing), by Level III Ecoregion

Ecoregion Name	Start of Winter	End of Winter	Start of Runoff	End of Runoff	Start of Growing Season	End of Growing Season
Canadian Rockies	Oct.1	April 14	April 15	June 30	July 1	Sept. 30
Northern Rockies	Oct.1	March 31	April 1	June 30	July 1	Sept. 30
Idaho Batholith	Oct.1	April 14	April 15	June 30	July 1	Sept. 30
Middle Rockies	Oct.1	April 14	April 15	June 30	July 1	Sept. 30
Northwestern Glaciated Plains	Oct.1	March 14	March 15	June 15	June 16	Sept. 30
Northwestern Great Plains	Oct.1	Feb. 29	March 1	June 30	July 1	Sept. 30
Wyoming Basin	Oct.1	April 14	April 15	June 30	July 1	Sept. 30

2. Samples from within an assessment reach may generally be considered independent of other samples from the assessment reach if they meet or if you do the following:
   - Sites (or very short reaches functionally equivalent to sites) should be located at least 1 stream mile apart.
   - Sites may be placed < 1 mile apart on an assessment reach **if** there is a flowing tributary confluencing with the reach between the two sites.
   - Along an assessment reach, try to sample sites moving from downstream to upstream to avoid potentially re-sampling the same stream water.
   - Land use changes and land form changes should be considered and can be used to help define (1) breaks between assessment reaches and/or (2) additional sampling sites within an assessment reach
   - Samples collected at the same site should be collected about 30 days apart. <u>This does not apply to long-term or instantaneous measurement of dissolved oxygen.</u>

Part 2: Assessment Methodology

The following are recommended to determine nitrogen and phosphorus impacts in wadeable streams.

<u>For Mountainous and Transitional[1] Streams:</u> Assessment is carried out as a level I, level II process. If the level I results are inconclusive, move to the 2nd level[2].

<u>Level I:</u>

a. Collect, within the assessment reach, benthic algal Chl *a* and AFDW (Ash Free Dry Weight) from one or more sites[3] (following DEQ SOPs, including approved low-chlorophyll visual

[1] See **Table 4-1** later in this document for the list of ecoregions (levels III and IV) where this methodology applies.
[2] Nothing precludes the assessor from collecting, in a single sampling season, all data needed to carry out a level II assessment. Cases may arise (e.g.., land access issues) that may make this approach preferable.
[3] Treat each Chl *a* sampling event as an independent evaluation of use support Do not average together results from different sites within the assessment reach If Chl *a* is measured more than once at the same site, treat each sampling event as unique (NOT as temporal repeat measures)

estimation methods) for a minimum of three sampling events. A minimum of twelve or thirteen[4] independent nutrient samples should be collected within the same assessment reach. Use of diatom samples at level I are optional, but if the data exist (n ≥ 2 samples), they must be used in the assessment. Disperse sampling effort across sites as much as possible. The nutrient data are evaluated using the "Exact Binomial Test" and the "One-Sample Student's t-test for the Mean" which are housed in one of two Excel spreadsheets. If the assessment reach is a new, un-listed segment, use "MT-NoncomplianceTool.xls". If the assessment reach is already listed for a nutrient, use MT-ComplianceTool.xls". However, if a stream is currently listed for nitrogen but not for phosphorus, use the "MT-NoncomplianceTool.xls" to assess the phosphorus data.

b. In both spreadsheets, for either test, set alpha to 0.25 (25%) and the critical exceedance rate (p) to 0.2 (20%) in cells B5, B6. In the Binomial test the effect size (p2; gray zone) should be 0.15 (15%) and is set as a function of the exceedance rate. So, in "MT-NoncomplianceTool.xls" this means p2 should be set to 0.35 (cell B7), and in "MT-ComplianceTool.xls" p2 should be set to 0.05 (cell B7). If in the future DEQ decides that a lower exceedance rate (e.g., 10%) is needed, the gray zone will need to be adjusted accordingly.

c. Compliance with the nutrient criteria is determined via decision rules, which consider the Chl *a* and AFDW averages calculated for each sampling event, the results from the two nutrient statistical tests, and diatom metric results (if available). Go to the first tab of the Excel spreadsheet named "NtrntAssessFramework.xlsx". If the result is clear (assessment reach <u>is</u> or <u>is not</u> nutrient impaired), you are finished. If not, follow the instructions in the spreadsheet for level II assessment.

d. Most often, you will be assessing both N and P in an assessment reach. Consider the N and P results side-by-side; does is appear that one nutrient or the other is giving a clear signal (e.g., Binomial and T-test are both FAIL for Total Phosphorus (TP), but both PASS for Total Nitrogen (TN)? In this case, the best nutrient to list would be TP. Mixed results for both nutrients often will require a move to a level II assessment, and may lead to listing both N and P.

<u>Level II</u>:

a. Moving to level II often (not always) involves additional data collection, including more nutrient samples and benthic algal Chl *a*/AFDW samples. Level II data include both diatom and macroinvertebrate samples (at least two sampling events for each). **The exception to this is the Middle Rockies ecoregion, for which there are no validated diatom increaser metrics. In this ecoregion collect at least three macroinvertebrate samples.** As for level I, each sampling event for diatoms should be considered on its own merits (do not average results across sites, or across time at a site). In contrast, macroinvertebrate samples collected across time <u>at a site</u> should be averaged together; however, keep and assess data from <u>different</u> sites separate. When your dataset is ready, first pass data again through the

[4] Twelve independent nutrient samples for new, unlisted streams, and thirteen for streams already listed for nutrients on the 303(d) list. A nutrient sample is a type of nutrient (e.g., TP or TN) Sample minimums apply to *each* nutrient type. Smaller sample sizes may be justified; see **Section 3.2.2.1**, this document

Level I process using NtrntAssessFramework.xlsx. If results are clear, you are finished; if not, go to the 2nd tab.

b. Some data combinations at level II (2nd tab of the spreadsheet) will still lead to an unclear result. If this occurs, consult with your manager about how to proceed.

For Warm Water Plains Streams[5]*:* Assessment is carried out as a level I, level II process. If the level I results are inconclusive, move to the 2nd level[2].

Level I:

a. Determine, for at least 3 sampling events, the dissolved oxygen (DO) delta (i.e., the daily DO maximum minus the daily DO minimum). The daily minimum can be measured pre-dawn to 8:00 am, while the daily maximum usually occurs between 2:30 pm to 5:00 pm. Alternatively, collect a long-term DO dataset by deploying a YSI 6600 (or similar instrument) in at least one site; measure DO for at least 1 full day, with a 15-min time step. Even if you collect DO data with a deployed instrument, you still need a total of three sampling events (three days). **However, DO delta values need not be collected 30 days apart.** Also, collect within the assessment reach at least two diatom samples and a minimum of twelve or thirteen[4] nutrient samples. Disperse sampling effort across sites as much as possible. The nutrient data are evaluated using the tests "Exact Binomial Test" and the "One-Sample Student's t-test for the Mean" found in one of two Excel spreadsheets. If the assessment reach is a new, un-listed segment, use "MT'NoncomplianceTool.xls". If the assessment reach is already listed for a nutrient, use MT-ComplianceTool.xls". However, if a stream is currently listed for nitrogen but not for phosphorus, use the "MT-NoncomplianceTool.xls" to assess the phosphorus data.

b. See 1b above (in the Mountainous and transitional streams section) for instructions on setting test conditions in each Excel spreadsheet.

c. Compliance with the nutrient criteria is determined via decision rules, which consider together the results from the diatom metrics, the DO delta values, and the two statistical tests for nutrients. Go to the 3rd tab (plains level I) of the Excel spreadsheet "NtrntAssessFramework.xlsx". Long-term DO datasets require special consideration; see **Section 3.2.4**, scenario 2, and **Section 5.0** for details. If the result is clear (assessment reach is or is not nutrient impaired), you are finished. If not, follow the instructions in the spreadsheet for level II assessment.

d. Most often, you will be assessing both N and P in an assessment reach. Consider the N and P results side-by-side; does is appear that one nutrient or the other is giving a clear signal (e.g., Binomial and T-test are both FAIL for TN, but both PASS for TP)? In this case, the best nutrient to list would be TN. Mixed results for both nutrients often will require a move to a level II assessment, and may lead to listing both N and P.

[5] See **Table 5-1** later in this document for the list of ecoregions where this methodology applies.

Level II:

a. A level II assessment will often require additional data collection, including more nutrient and DO data, and (in some cases) biochemical oxygen demand (BOD_5) data. As for level I, each DO delta value should be considered on its own merits (do not average results across sites or across time), as is also the case for BOD_5 samples. When the data are ready, first pass the now-larger dataset back through the Level I assessment process. If results are clear, you are finished; if not, go to the appropriate scenario in the 4th tab (plains level II).

b. Some level II data combinations still lead to an unclear result. If this occurs, consult with your manager about how to proceed.

1.0 INTRODUCTION

The purpose of this document is to describe a framework for making decisions. Specifically, it defines a process by which one can determine if a wadeable stream is or is not impaired by nitrogen and phosphorus pollution (i.e., excess nutrients). The document covers a number of subjects including how to determine an appropriate sampling frame, which parameters are most useful for assessing nitrogen and phosphorus problems, how many samples are needed, how data are to be treated statistically, and how disparate data types are to be assembled in a final decision matrix.

In this document we have attempted to organize the information in a manner such that the users can locate what they needs quickly, and then read further for details only if they want to. The “why” discussions (i.e., why did we select a particular assessment parameter, why did we pick the impact threshold, etc.) are found in the appendices. We did this because we know that stream assessments are time-consuming undertakings, and therefore users will want to access the critical information easily.

1.1 SCOPE OF THE ASSESSMENT METHODOLOGY

Different assessment methods are recommended for different regions of the state (**Figure 1-1**). The assessment parameters that have been recommended for each region are the ones we believe are the most accurate and sensitive for determining nitrogen and phosphorus impacts for wadeable streams in those areas. For example, we recommend measuring dissolved oxygen in eastern Montana plains streams, but we have not recommended this approach for western Montana salmonid streams. This should not be construed to mean that DO concentrations in salmonid streams are never affected by nitrogen and phosphorus pollution, or that in any way this recommendation overrides existing DO standards for those state waters. Rather, we believe that in western Montana salmonid streams there are assessment tools other than DO that are more sensitive and will more readily detect nitrogen and phosphorous problems.

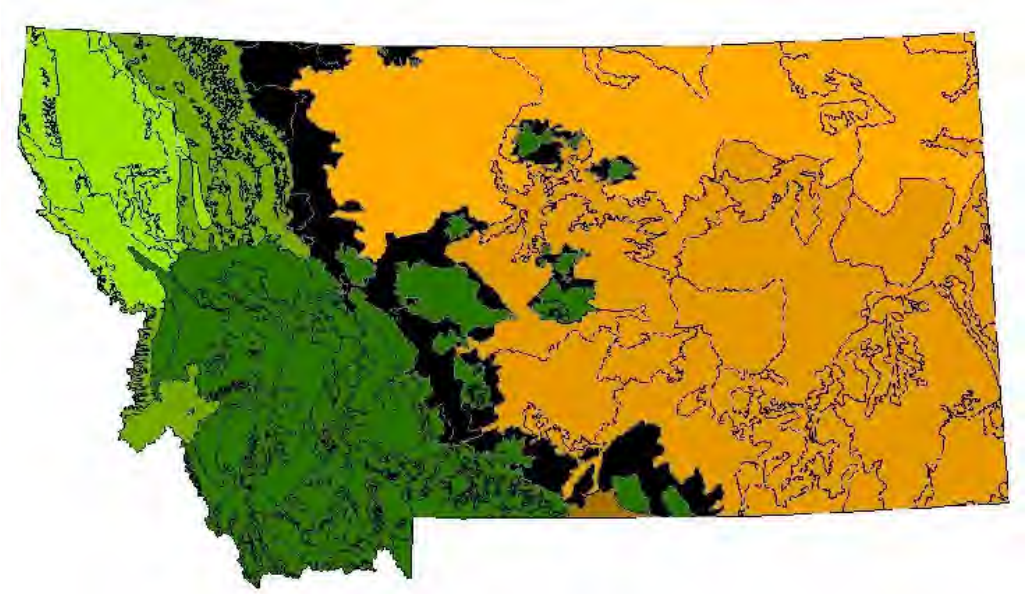


Figure 1-1. Map Showing Different Regions in which Different Assessment Methodologies Apply. The areas shown in shades of green and black comprise the mountain and transitional streams region. The areas shown in shades of brown comprise the eastern Montana plains region.

As mentioned above, methods in this document apply to wadeable streams. A DEQ workgroup spent considerable time working to define the break between wadeable streams and rivers and non-wadeable rivers, the results of which are presented in Flynn and Suplee (2010). The waterbodies that are not considered wadeable are provided below in **Table 1-1.**

Table 1-1. Non-wadeable river segments within the state of Montana

River Name	Segment Description
Big Horn River	Yellowtail Dam to mouth
Clark Fork River	Bitterroot River to state-line
Flathead River	Origin to mouth
Kootenai River	Libby Dam to state-line
Madison River	Ennis Lake to mouth
Missouri River	Origin to state-line
South Fork Flathead River	Hungry Horse Dam to mouth
Yellowstone River	State-line to state-line

2.0 Recommendations for Isolating a Stream Reach for Assessment (the Sample Frame)

Identifying and isolating an appropriate stream reach (sample frame) is the first required task. The following definitions are presented:

- **Sample Frame**: A wadeable[6] stream segment listed in the Assessment Data Base (ADB) (DEQ 2009, and updates) OR a sub-segment of an ADB stream segment. A segment such as this is referred to in this document as an "assessment reach".
- **Population**: All the water flowing through the assessment reach during the time period when the numeric nutrient criteria apply, and the surface area of the stream bottom over which the water flows.
- **Sampling Unit**: A sample collected from the assessment reach that is largely independent of other samples collected within the assessment reach and collected during the time when the numeric nutrient criteria apply.

A sampling frame must be representative of the population and, in stream assessment, this demands good judgment in the particular subject matter being studied. **Sections A.1** and **A.2** of **Appendix A** of this document, which is an updated and shortened version of an earlier Appendix H (Varghese and Cleland, 2008), contain a discussion on approaches to identifying assessment reaches.

The key idea presented in **Appendix A** is that each assessment reach that is assessed should be sufficiently homogenous that data collected from sites along the reach can be considered to represent the entire reach. To determine compliance with numeric nutrient criteria using statistical methods, it is important that (1) pollution sources generally be evenly dispersed along the reach, and (2) each sample is independent of the others. Following up on this idea, if an assessment reach appears to need further subdivision (e.g., into a reach above and a reach below a pollution point source), then each new assessment reach should generally be sampled with the same intensity (i.e., minimum sample size) as the parent reach would have been if it had not been subdivided. This will assure that the statistical rigor associated with specified sample-size minima (discussed below) is maintained. At the same time, as a general rule, it is better to lump than split to avoid unnecessary sampling and administrative work.

The need to create reasonably uniform assessment reaches is inherently in conflict with the need to "lump", the purpose of which is to keep stream reaches from being excessively subdivided (and all the additional work that entails). Judgment is needed on the part of the assessor to balance these two opposing factors and come up with an optimal sampling strategy for any given stream.

This process should be compatible with a randomized study of stream reaches as well as targeted, risk-assessment based approaches; again, the key point is that each assessment reach is sufficiently defined.

3.0 Assembling the Nutrient Assessment Data into a Decision Framework

Section 2.0 above discussed approaches used to identify appropriate assessment reaches. This section discusses how data that will have been collected from the assessment reach are to be assembled into a decision-making framework. The parameters and methods apply to wadeable streams. Non-wadeable waterbodies were listed back in **Table 1-1.**

[6] Wadeable streams are perennial as well as intermittent (ARM 17.30.602 [15]) streams in which large portions of the channel are wadeable during baseflow conditions. For the list of waterbody segments not considered wadeable (i.e., the large rivers), see **Table 1-1** above Derivation of the **Table 1-1** list is found in Flynn and Suplee (2010).

3.1 Overview of Useful Parameters for Carrying out Nutrient Impairment Assessments

Among the vast array of parameters that can be measured in a stream, we narrowed the list to those we believe are the best, readily-measured indicators of stream nitrogen and phosphorus enrichment (**Table 3-1**). Many of these parameters are discussed in Suplee *et al.* (2008), and are also discussed in detail in the appendices of this document.

Table 3-1. Parameters in Streams that are Considered Useful in Assessing Nutrient Enrichment

Parameter	How collected	Linkage to nutrient enrichment	Primary or secondary indicator*
Total nitrogen	Water sample	Total instream concentrations are indicative of the level of nutrients that are ultimately biologically available for autotrophic or heterotrophic uptake.	Primary
Total phosphorus	Water sample	Total instream concentrations are indicative of the level of nutrients that are ultimately biologically available for autotrophic or heterotrophic uptake.	Primary
Benthic algal biomass	Benthic samplings of stream bottom	Nutrients stimulate benthic algal growth in wadeable streams Benthic algal growth can develop to nuisance levels; nuisance algae level is known. Excess algal growth affects on DO have been documented.	Primary
Dissolved oxygen *delta* (daily max value minus the daily min value)	Instantaneous: By hand-held instrument, at dawn and in the late pm. Continuous monitoring: by deployed instrument	Nutrient enrichment stimulates autotrophic primary productivity and heterotrophic decomposition of organic material. Both of these in turn affect dissolved oxygen patterns in streams.	Primary
Diatom biometric (nutrient increaser taxa metric)	Benthic sampling of stream bottom	As primary producers, diatoms can be directly stimulated by increased availability of N and P. Diatom population structure has been found to vary in predictable ways with increasing nutrient enrichment.	Primary and Secondary
Macroinvertebrate biometric (Hilsenhoff Biotic Index, or HBI)	Kicknet sampling of stream bottom	A large number of macroinvertebrate taxa have been assigned a numeric value which represents each organism's tolerance to low dissolved oxygen/organic pollution. Resulting metric (HBI) found to significantly correlate to total nutrient concentrations in Montana streams.	Secondary
Biochemical oxygen demand (BOD_5)	Water sample; must be at laboratory within 48 hrs	High BOD can indicate presence of large quantities of dissolved and suspended organic matter, whose decomposition can produce a large DO demand. Can help determine if DO sags are caused by high primary productivity, high BOD, or both.	Secondary

Table 3-1. Parameters in Streams that are Considered Useful in Assessing Nutrient Enrichment

Parameter	How collected	Linkage to nutrient enrichment	Primary or secondary indicator*
Stream macrophyte species	By hand; field identification	Observed shift in dominance to a single macrophyte species in highly enriched prairie streams; loss of *Chara*.	Secondary

*Primary means the parameters is considered to be a very good indicator of nutrient enrichment. Secondary mean the parameter is considered a good indicator of nutrient enrichment, or helpful in identifying other factors affecting DO (e.g., BOD).

Note that **Table 3-1** contains physical and biological measurements. We support the long-held view in the WQPB that stream assessment is best carried out by looking at both data types together. The famous water-pollution biologist H.B Hynes said it best: “When the chemist and the biologist both work on the assessment of pollution they can discover much more together than either can alone” (Hynes, 1966).

The parameters in **Table 3-1** need to be arranged in a decision making framework in order to produce consistent decision outcomes (i.e., stream *is* impaired by nutrients, stream *is not* impaired by nutrients). **Figure 3-1** below outlines the process we recommend for this purpose.

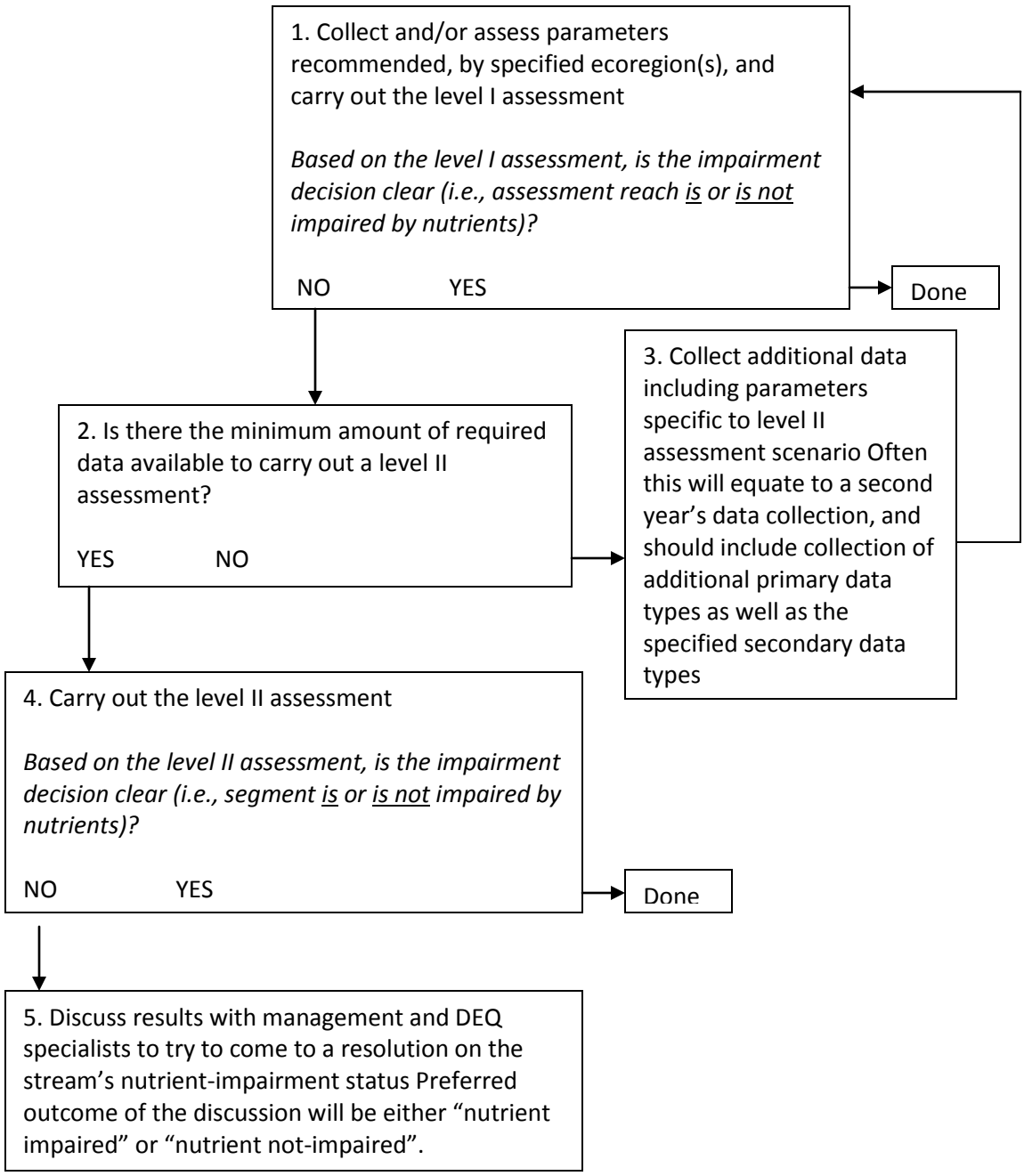


Figure 3-1. Flow-path for decision making using data parameters in Table 3-1.

If the level I assessment leads to an unclear decision, the assessor should then use the data (primary and secondary, if data sufficiency met) to carry out the level II assessment[7]. If a level I assessment is inconclusive and leads to a 2nd year of data collection, always pass the now-larger dataset back through the level I assessment matrix first. It may result that that the conclusion is now clear, without having to go to level II. **NOTE:** Nothing in the approach shown in **Figure 3-1** precludes an assessor from collecting,

[7] The approach shown in **Figure3-1** closely parallels the decision framework of EPA's CALM guidance (U.S. Environmental Protection Agency, 2002); see **Figure 3.2**, page 3-10 of that document.

in a single field season, all data needed to complete a level I and a level II assessment. Situations may arise (e.g., land access issues) where this approach is preferable.

As can be seen, one notable aspect of the approach in **Figure 3-1** is that the data we have labeled "secondary" (**Table 3-1**) are brought into the decision framework only after the primary data have lead to an unclear conclusion. In effect, secondary data are being held to the side until the primary data have been played out to their fullest. The approach attempts to keep data combination scenarios to a minimum and decision making as simple as is reasonable (Occam's razor; "plurality should not be posited without necessity")[8].

The different combinations of results that can occur have been assembled in an Excel spreadsheet (**NtrntAssessFramework.xlsx).** In the spreadsheet, the user identifies the unique combination of results from their assessment reach, and then derives a conclusion. For each combination of results, the spreadsheet provides an outcome (i.e., nutrient impaired, not-nutrient impaired, unclear), and an explanation as to what is likely going on in the stream's ecology. **Different parameter sets are used in different geographic regions, therefore the user must use the tabs for the region applicable to their stream.** Regional tabs are further subdivided to correspond to a level I or level II assessment, per the approach shown in **Figure 3-1**. As an example, three result combinations for the mountain and transitional region are given in **Table 3-2**.

Table 3-2. Three combinations of results, and the conclusions that can be drawn from them, using the parameters listed in Table 3-1.
All three examples apply to streams of the mountain and transitional region of the state, and are from a level I assessment.

Scenario	Nutrient Binomial Test	Nutrient T-test	Benthic Algae	Diatom Increaser Taxa-Probability of Impairment (OPTIONAL)*	Resulting Decision	Further Sampling?
1	PASS	PASS	≤120 mg Chl *a*/m^2 or ≤35 g AFDW/m^2	<51%	Waterbody <u>is not</u> nutrient impaired. All indications show that the stream is in compliance.	No

[8] We did this for two reasons. First, we believe that in most cases some types of data are inherently better for nutrient-enrichment assessment than others, and if the decision can be made using those data alone, the assessment will be simpler and less expensive. Second, it reduces the total number of data-combination outcomes and, in turn, the number of scenario-by-scenario conclusions about impairment that have to be made. To illustrate, for any given data type for which there is a dichotomous outcome (i.e., result is <u>above</u> or <u>below</u> some threshold), the number of possible permutations is 2 raised to the number of data types. Three data types result in (2^3) = 8 possible data-combinations, and one must consider what each unique combination of results is saying about nutrient impairment. Five data types considered together already results in 32 unique combinations, and so on If not all of the additional data are as useful as the previous, it becomes questionable whether the additional work, cost, and complexity are warranted.

Table 3-2. Three combinations of results, and the conclusions that can be drawn from them, using the parameters listed in Table 3-1.
All three examples apply to streams of the mountain and transitional region of the state, and are from a level I assessment.

Scenario	Nutrient Binomial Test	Nutrient T-test	Benthic Algae	Diatom Increaser Taxa-Probability of Impairment (OPTIONAL)*	Resulting Decision	Further Sampling?
12	PASS	FAIL	>120 mg Chl *a*/m^2 or >35 g AFDW/m^2	>51%	Waterbody is nutrient impaired. Non-compliance with the T-test suggests that pulsed nutrient loads are allowing high algae biomass to be maintained via luxury uptake. Diatoms confirm enrichment finding.	No
16	FAIL	FAIL	>120 mg Chl *a*/m^2 or >35 g AFDW/m^2	>51%	Waterbody is nutrient impaired. All indicators show that the stream is not in compliance.	No

*However, if the data minima are available for diatom metric category, they must be used in the decision framework

Subsequent sections will provide detail on which assessment parameters apply where, which statistical tools are to be applied to which parameters, etc. The important point to note here is that the combinations of results you will encounter have been accounted for in the spreadsheet tool (**NtrntAssessFramework.xlsx)**.

Returning to **Figure 3-1**, the "Discuss results with management and DEQ specialists" outcome occurs when the level II assessment has still not resulted in a clear conclusion. This resolution step was suggested by Mark Bostrom (DEQ Bureau Chief) as a way to come to a conclusion without ending up in an endless do-loop. Details of this process remain to be worked out; a potentially useful framework for carrying out the determination has been developed by EPA (Cormier, et al., 2000; Cormier and Suter, II, 2008).

3.2 Details on the Use of Nitrogen and Phosphorus Concentration Data, and Other Measured Parameters, to Support Nutrient-Impairment Assessments

As noted above, different groups of parameters best apply to particular regions of the state. The applicable list of parameters, their impact thresholds, and the delineation of the regions are provided in **Section 4.0** (mountain and transitional streams) and **Section 5.0** (plains streams). In order to maintain temporal independence to the best degree possible, samples collected sequential at a site should be collected about **30 days** after the previous sampling. (There are exceptions to this; see individual parameter list in **Section 3.2.2** below.) Spatial independence of sites within an assessment reach can generally be established by following these guidelines:

- Sites (or short reaches equivalent to sites) should be located a minimum of 1 stream mile apart.

- Sites may be placed < 1 mile apart along the assessment reach if there is a flowing tributary confluencing with the segment between the two sites.
- Try to collect water samples starting at the downstream end of the assessment reach moving upstream, to avoid re-sampling the same water.
- Land use changes and land form changes should be considered and can be used to help define additional sampling sites within an assessment reach.

See **Section A.3** in **Appendix A** for the derivation of these guidelines.

Numeric nutrient criteria apply during summer baseflow[9], also referred to as the growing season. Start and end dates for the growing season vary by ecoregion (Suplee, et al., 2008); see **Table 3-3** below. These dates should be adhered to for collection of the other parameters in **Table 3-1** as well. However, a ten day window (plus/minus) on the Growing Season start and end dates is acceptable, in order to accommodate year-specific conditions (e.g., an early-ending spring runoff). The assessor should use their best professional judgment when deciding if early or later sampling is warranted.

Table 3-3. Start and Ending Dates for Three Seasons (Winter, Runoff and Growing), by Level III Ecoregion

Ecoregion Name	Start of Winter	End of Winter	Start of Runoff	End of Runoff	Start of Growing Season	End of Growing Season
Canadian Rockies	Oct.1	April 14	April 15	June 30	July 1	Sept. 30
Northern Rockies	Oct.1	March 31	April 1	June 30	July 1	Sept. 30
Idaho Batholith	Oct.1	April 14	April 15	June 30	July 1	Sept. 30
Middle Rockies	Oct.1	April 14	April 15	June 30	July 1	Sept. 30
Northwestern Glaciated Plains	Oct.1	March 14	March 15	June 15	June 16	Sept. 30
Northwestern Great Plains	Oct.1	Feb. 29	March 1	June 30	July 1	Sept. 30
Wyoming Basin	Oct.1	April 14	April 15	June 30	July 1	Sept. 30

3.2.1. Nitrogen and Phosphorus Data

The nitrogen and phosphorus criteria are <u>not</u> presented in this document. Readers should refer to Suplee et al. (2008) and its addendums to locate the nutrient concentration values. DEQ anticipates that the nutrient criteria will be adopted by the Board of Environmental Review. After adoption they will be referred to as "base numeric nutrient criteria", and will be housed in a new DEQ circular (DEQ-12). Please use the most updated versions of the criteria in all assessments. Check with Standards on status.

Nutrient (TN, TP) concentration data from an assessment reach are to be assessed collectively, i.e., <u>all</u> nutrient data collected along the reach are to be assessed together, using statistical tests. We recommend two statistical testing procedures to evaluate the nutrient dataset; the Exact Binomial Test and the One-Sample Student's T-test for the Mean. The rationale for using two statistical tests is in **Appendix A**. The tests are in two Excel spreadsheets and their use is described below.

To use the statistical tests, do the following:

[9] Lakes generally require year-round nitrogen and phosphorus criteria if they are to be protected from cultural eutrophication This may in turn affect the time-of-application of nutrient standards in the near-field tributaries of those lakes.

- For new, un-listed stream segments, use the Excel spreadsheet tool named "MT-NoncomplianceTool.xls".
- For already-listed stream segments, use the Excel spreadsheet tool named "MT-ComplianceTool.xls"
- In both tools, for either test, set alpha to 0.25 (25%)[10]. For the Binomial set the critical exceedance rate to 0.2 (20%) in cell B6. The effect size (gray zone) should be 0.15 (15%) and is set as a function of the exceedance rate. So, in the "MT-NoncomplianceTool.xls" this means p2 should be set to 0.35 (i.e., enter 0.35 into cell B7), and in "MT-ComplianceTool.xls" p2 should be set to 0.05 (enter 0.05 in cell B7).

Both tests (Binomial, T-test) will produce a result (PASS, FAIL). For the Binomial, you need to compare the allowable number of exceedances shown by the test ("**e**s", found in column D) to the actual number of exceedances manifested by your dataset. For the T-test, you will need to enter the dataset into the spreadsheet along with the criterion concentration against which the data are being compared. If the assessment reach complies with a test, the result is PASS; if the assessment reach does not comply with a test, the result it FAIL.

Note: If a non 303(d)-listed nutrient species is the same element as a listed one (e.g., stream is listed for nitrate, but you are also assessing TN, and TN is not currently listed), use the "MT-ComplianceTool.xls" for the non-listed nutrient species as well.

3.2.1.1 Minimum Sample Size for Nitrogen and Phosphorus Data

In the vast majority of cases the assessor will be making the nutrient-impairment decision with a fairly small nutrient-concentration dataset (probably < 13 samples). Statistics derived from small datasets such as these are subject to a fair amount of uncertainty. For example, outcomes from the Binomial Test (compliant, non-compliant) will, for nutrient sample sizes around 13, have confidence levels of about 75% (i.e., alpha and beta error of about 25% each).

For assessment reaches, the target number of nutrient samples is 12 (new, un-listed stream segments) or 13 (already-listed stream segments). The rationale for these sample sizes is presented in **Appendix A**.

HOWEVER: Cases exist where a dataset smaller than 12 or 13 will provide a sufficiently clear result that further nutrient sampling is not warranted. At about 6 samples or less, beta error in the Binomial test can become unacceptably high (> 65%) and increasingly worse with smaller ***n.*** At 7-8 samples, however, there are cases where a certain number of exceedances would be extremely unlikely unless the stream's true exceedance rate was much in excess of 20%. Therefore, for sample sizes of 7, ≥ 4 exceedances can be considered FAIL for the Binomial test. If <4 exceedances are found, sampling should be resumed until the minimum of 12 or 13 is achieved. The T-test can also be used with 7 samples but its power is lower at this sample size. Please see the bullets in **Appendix A**, **Section A.5**.

Also, circumstances may arise where nutrient sampling that is planned over two field seasons may lead to a reduction in the necessary number of samples. For example, if at the end of year one ten (10) TN and TP samples have been collected from an assessment reach on an unlisted stream, and the number of exceedances in each dataset is one (1), it would not be necessary to collect the addition two samples

[10] Alpha, exceedance rate, and the gray zone can be changed via the input cells in the upper left hand corner of the spreadsheet.

(to achieve 12) the following year. This is because even if both of the subsequent samples were above the criteria, the decision outcome (assessment reach "attains") would not be altered. Assessors should consider these types of situations at the end of each field season in order to best optimize work and cost.

Important Caveat: When the nutrient-concentration dataset is large (as defined below), the nutrient impairment decision should be made using nutrient concentrations alone.

- Large nutrient dataset for already-listed segments: 90 samples in the assessment reach
- Large nutrient dataset for unlisted segments: 50 samples in the assessment reach.

The large sample sizes were determined using the Binomial distribution with an alpha of 0.05 (95% confidence level) and a balance between alpha and beta error (i.e., beta is also about 0.05).

If the large sample sizes listed above are available, the assessor should generally forgo the use of parameters other than total nitrogen and phosphorus (i.e., those in **Table 3-1**) in nutrient-assessment decision making. Nutrient concentrations alone can be used to assess standards compliance, via the Binomial test only.

3.2.2 Minimum Sample Sizes for Other Parameters

The remaining parameters in **Table 3-1** (with the exception of BOD_5; more on it below) are effect variables, i.e. they are affected by changes in nutrient concentrations. Sample size requirements for each parameter are summarized below. Each result, from a sampling event and for a parameter, should normally be considered on its own merits when using the decision spreadsheet (NtrntAssessFramework.xls) and completing the assessment. Exceptions to this exist; see below. The parameters applicable to specific regions (mountain and transitional streams vs. plains streams) and the impact thresholds associated with those parameters are given **Section 4.0** and **Section 5.0**. ***Important Note*: Within their region of application, parameters shown below are required in order to carry out a level I assessment (see Figure 3-1). If a parameter is only required for a level II assessment, this will be indicated.**

Benthic Algal Biomass Samples (Chl *a* and AFDW): At least three (3) sampling events for benthic algal biomass are to be carried out in the assessment reach. These may include approved visual estimation methods. If more than one site is established in the assessment reach, disperse sampling effort across the different sites. Otherwise, assure that about 30 days have passed before sampling again at the same site.

Diatom Samples (Nutrient Increaser Taxa Metric): At least two (2) diatom sampling events are to be carried out in the assessment reach. If more than one site is established in the assessment reach, disperse sampling effort across the different sites. Otherwise, assure that about 30 days have passed before sampling again at the same site. **Note:** Diatom samples are required at level I in the plains, but are only required for a **level II** assessment in the **mountain and transitional region.** However, since there is no validated diatom increaser metrics for the Middle Rockies ecoregion, they are not required collection there.

Macroinvertebrate Samples (HBI Metric): At least two (2) macroinvertebrate sampling events are to be carried out in the assessment reach, unless the site is in the Middle Rockies ecoregion, in which case at least three (3) sampling events are to be carried out. If more than one site is established in the assessment reach, disperse sampling effort across the different sites. **If only one site is present is the assessment reach**, **or if you decide to collect across-time samples at several sites**, collect the across-time samples at the site(s) approximately 30 days apart. The across-time HBI scores from a site should then be averaged prior to comparison to the threshold. If you only have one site, you will only have one HBI value for the assessment reach to compare to the threshold. **Note:** Macroinvertebrate samples are only required for a **level II** assessment, and only in the **mountain and transitional region**.

Sampling to Determine Dissolved Oxygen Delta: At least three (3) DO sampling events (i.e., days) are to be carried out in the assessment reach. If more than one site is established in the assessment reach, disperse sampling effort across the different sites. **DO deltas fluctuate rapidly, and therefore you do not need to wait 30 days to collect subsequent DO data at a site.** For example, collection at a site over 3 contiguous days is acceptable. The more DO deltas that can be collected in the assessment reach, the better.

BOD_5: At least three (3) BOD sampling events are to be carried out in the assessment reach. If more than one site is established in the assessment reach, disperse sampling effort across the different sites. Samples for the standard 5-day biochemical oxygen demand (BOD_5) test are similar to nutrient samples, in that they are a stream-water measurement that can change rapidly, and BOD's affect on DO varies according to other factors (e.g., wind mixing). **Note:** BOD_5 samples are only required for a **level II** assessment, and only in the **plains region**.

Observation Data for Macrophytes and Benthic Algae: The Fish Cover/Other form, i.e. the component of the form pertaining to macrophytes and benthic algal growth, is to be filled out in accompaniment with each benthic algal biomass and/or diatom sampling event. It should also be filled out at each site in the assessment reach at least once each summer. If across a summer growth conditions have notably changed at the site, fill it out again.

3.2.3 Determine the Nutrient Most Likely to be Harming Use(s)

Normally both N and P, and potentially different species of N (e.g., nitrate, TN), will be simultaneously evaluated within an assessment reach. Cases will arise where the harm-to-use signal from one element or the other is clearly stronger, which will help streamline the assessment determination and subsequent work (e.g., TMDL development). An example is provided below.

Table 3-4. Simultaneous Review of Multiple Nutrients and Effect Variables

Assessment Reach	Nutrient	n	Binomial	T-test	Diatom Increaser Taxa	Benthic Chl *a*
Fred Cr reach 1	NO_3+NO_2	14	PASS	PASS	Exceeds criteria	Exceeds criteria
Fred Cr reach 1	TN	14	PASS	PASS	Exceeds criteria	Exceeds criteria
Fred Cr reach 1	TP	14	FAIL	FAIL	Exceeds criteria	Exceeds criteria

Total P results in **Table 3-4**, when run through the assessment process in the "NtrntAssessFramework.xlsx" spreadsheet, result in a clear "nutrient impaired" decision, at level I. This is largely driven by the two FAILS for the statistical tests (i.e., TP concentrations were very elevated). But because each nutrient type is assessed separately and TN is PASS for both statistical tests, the TN outcome is considered unclear and would, as a result, move to level II. As a result, the TN-impairment determination would be driven only by the biotic response variables and not by TN (see scenario 10,

'Mountain and Transitional' tab, in NtrntAssessFramework.xlsx). However, the most succinct conclusion (again, applying Occam's razor) is that the problem in this assessment reach is related to P, not N, as can be seen in **Table 3-4**, and only P should be listed. Arranging and reviewing the data as shown in **Table 3-4** should prevent unnecessary chasing of vague results for a nutrient when clearly the alternate nutrient is the issue.

Cases will arise where both nutrients will give mixed results within the two statistical tests, and therefore neither nutrient is clearly the culprit. In such cases, in accordance with the final outcome of the weight-of-evidence assessment, N and P should probably both be listed as probable causes.

3.2.4 Examples of Nutrient-impairment Decisions

Below are 3 assessment reach examples and their outcomes, to demonstrate the process.

(**1**) The assessment reach is in western MT and has 6 sampling sites. Each site has been sampled 2 times for nutrient concentrations (TN, TP) and once for benthic Chl *a.* **Action*:*** The nutrient samples (n = 12) are assessed, by type (TN or TP), using the two statistical tools, which will result in PASS or FAIL for each test. Both TP tests are FAIL, but the TN tests are both PASS. Each of the six Chl *a* sampling events (each comprised of 11 replicates which have been reduced to a sampling-event average) are independently compared to the criteria. One of them exceeds 120 mg Chl *a*/m^2, so declare Chl *a* as 'Exceeds Criteria' for the assessment reach. The data suggest a TP problem but not a TN problem, per methods in **Section 3.2.3** above. TP is listed as the cause, and further data collection and assessment for TN is not necessary.

(**2**) The assessment reach is on an eastern MT plains stream. There are 3 sampling sites where nutrients have been sampled 4 times and DO has been continuously monitored by deployed instrument for two summer months, at one site. **Action*:*** The nutrient samples (n = 12) are assessed, by type (TN or TP), using the two statistical tools, resulting in PASS or FAIL for each test. Both TN tests are FAIL, TP tests are mixed (1 PASS, 1 FAIL). Daily DO deltas from the long-term DO dataset should be calculated and compared to the DO delta threshold of 5.3 mg/L. Because this is a long-term dataset, close attention should be paid to the percent of DO deltas exceeding the threshold; if >10%, DO would be declared 'Exceeds Criteria' for the assessment reach[11]. Both TN and TP are suspected (N much more strongly) and should both be listed.

(**3**) The assessment reach is in western MT and the assessment has gone to a 2nd year of data collection. There are four sites. Nutrients have been collected at the 4 sites three times, benthic Chl *a*/AFDW once at each site, macroinvertebrate samples have been collected at 3 sites once each, and twice at the 4th site, and diatom samples have been collected at all 4 sites two times each. **Action*:*** All data from both years are first routed through the level I decision framework. The nutrient samples (n = 12) are assessed, by type (TN or TP), using the two statistical tools, which will result in PASS or FAIL for each test. Both TP are FAIL, and both TN tests are PASS Each of the four Chl *a* and AFDW sampling events (each comprised of 11 replicates which have been reduced to a sampling-event average) are independently compared to the criteria. One of them exceeds 35 g/m^2 AFDW, which is sufficient to declare Chl *a*/AFDW as 'Exceeds Criteria' for the assessment reach. Each macroinvertebrate HBI metric score where there is only one observation per site (three sites) is considered independently. For the 4th site, the two temporally-

[11] If DO deltas >5.3 mg/L comprise < 10% of the dataset, consider if the site has a strong presence of macrophytes or not. If macrophytes are very common, the site could be declared as 'Meets Criteria'. If macrophytes are not common, it could be declared 'Does Not Meet Criteria'. Consult Standards Section for further assistance.

collected HBI scores are averaged. One of the preceding macroinvertebrate HBI scores is >4, thus 'Exceeds Criteria' would be declared for the macroinvertebrate category for the assessment reach If the diatom increaser taxa scores (all 8) were each <50% probability of impairment, then the diatom metric score would be declared as 'Meets Criteria' for the assessment reach. The assessment reach is found to be impaired for TP at level I without having to use the macroinvertebrate results. For TN, the assessment could move to level II assessment and this would show nutrient impairment; but that outcome is driven purely by biometrics, which are sensitive to both nutrients. The overall dataset, per methods in **Section 3.2.3** above, suggests a TP problem but not a TN problem. TP is listed, and further data collection and assessment for TN is not needed.

3.2.5 Overwhelming Evidence of Nutrient Impairment-All Regions

Some circumstances related to excess nutrient pollution are severe enough that a rigorous data collection effort is not required. Photo documentation will suffice. Below are listed conditions that can be considered overwhelming evidence; these apply equally to wadeable streams across the state. These conditions are likely to be intertwined with organic pollution problems.

- Fish kills involving massive growths of senescing algae mats. These mats may be attached to the bottom or floating. Dissolved oxygen levels at dawn will likely be less than 1 mg/L.

- Filamentous algal growth covering the entire bottom from bank to bank and extending continuously for a substantial longitudinal distance (> 150m). Use the photographs below (**Figure 3-2** and **3-3**) as a guide. Don't confuse these conditions with sporadic, longitudinally-patchy growths of heavy filamentous growth, in between which there is lighter algal growth. The latter are not extreme enough to warrant overwhelming evidence, and should be sampled/assessed per the method earlier described.

Figure 3-2. Photographs of heavy, bank-to-bank and longitudinally continuous Cladophora growth
Left photo is from (Sandgren, et al., 2004).

Photo courtesy Dr. Vicki Watson.

Figure 3-3. Massive *Cladophora* growth in the Clark Fork River, MT, 1984.
This nuisance alga is aptly named "blanket weed".

4.0 Nutrient Impairment Assessment Methodologies: Wadeable Streams in the Mountain and Transitional Region

The following subsections describe assessment methods best suited for use in mountainous streams and streams that transition between mountains and plains. Analysis shows that level IV and level III ecoregions are the most useful classification tool for defining nutrient zones (Varghese and Cleland, 2005), and nutrient criteria have been developed using ecoregions as the base zoning system (Suplee *et al.*, 2008). Consideration has also been given to the legal classification system for streams (B-1, C-3, etc.) which defines the streams' designated beneficial uses. There is a very high degree of correspondence between streams with salmonid fish among their beneficial uses (A-closed, A-1, B-1, B-2, C-1, C-2) and certain groups of ecoregions. Specifically, the mountainous level-III ecoregions (15, 16, 17, and 41) plus specified level-IV ecoregions along the Rocky Mountain front — Level IVs that are subunits of the level-III Northwestern Glaciated Plains (42) and Northwestern Great Plains (43) ecoregions — comprise a group well suited for assessment methodologies presented in this section. Four (4) additional level IV ecoregions (42l, 42n, 43o, 43t) that were not presented in Suplee *et al.* (2008) have been added to the group. These four level IV ecoregions are also transitional along the Rocky Mountain Front and comprise regions in which all or virtually all waterbodies are classified as supporting salmonid fishes among their beneficial uses. The regions are shown in **Table 4-1**.

Table 4-1. Ecoregions (levels III and IV) in which Assessment Methods in this Section Best Apply

Ecoregion Scale	Ecoregion Name	Ecoregion Number
Level III	Northern Rockies	15
Level III	Idaho Batholith	16
Level III	Middle Rockies	17
Level III	Canadian Rockies	41
Level IV	Sweetgrass Uplands	42l
Level IV	Milk River Pothole Upland	42n

Table 4-1. Ecoregions (levels III and IV) in which Assessment Methods in this Section Best Apply

Ecoregion Scale	Ecoregion Name	Ecoregion Number
Level IV	Rocky Mountain Front Foothill Potholes	42q
Level IV	Foothill Grassland	42r
Level IV	Unglaciated Montana High Plains	43o
Level IV	Non-calcareous Foothill Grassland	43s
Level IV	Shields-Smith Valleys	43t
Level IV	Limy Foothill Grassland	43u
Level IV	Pryor-Bighorn Foothills	43v

Note: The level IV ecoregion "Unglaciated Montana High Plains" (43o) has more than one polygon in Montana. Only the polygon located just south of Great Falls, MT should be considered part of the transitional streams group. Also, the level IV ecoregion "Foothill Grassland" (42r) has polygons associated with both the Middle Rockies *and* Canadian Rockies level III ecoregions. 42r polygons are associated with the level III ecoregion (either Middle Rockies or Canadian Rockies) against which they abut.

4.1. Assessment of Benthic Algal Growth

For wadeable streams, we recommend that site-average benthic algae densities of 120 mg Chl *a*/m^2 and 35 g AFDW/m^2 be used as thresholds (i.e., maximum allowable levels) to prevent impact to the fish and associated aquatic life uses (i.e., to maintain DO standards in DEQ-7), and the recreation use (ARM 17.30.637(1)(e)). Details on how these values were derived are in **Appendix B**.

Note: AFDW results from core samples should never be included in determining a site's average AFDW. The method measures organic material from the entire core sample, not just the surface where the algae are growing, and will therefore over-report AFDW.

Each sampling event result should be considered on its own merits when using the decision spreadsheet (NtrntAssessFramework.xlsx) and completing the assessment. That is, if 3 sampling events for benthic alga growth were undertaken and 1 of the Chl *a* averages exceeds the recommended threshold, then the conclusion for the assessment reach for the benthic algae category would be "exceeds 120 mg Chl *a*/m^2".

4.2 Assessment Using Biometrics

Biometrics based on diatom algae are stressor-specific (e.g., address nutrient pollution) and apply to specific regions. A diatom sample that indicates >51% probability of impairment by nutrients indicates the sample is from a site manifesting an excess nutrient problem. Details on how the diatom biometrics were developed and the thresholds derived are presented in the periphyton SOP (Montana Department of Environmental Quality, 2011b).

Always consider cautiously the results from samples collected very early and very late in the sampling season. Algae are a successional community, and if you sample too early, you will sample fewer 'pioneer' species and too late, you will start seeing the community as a whole die off - some taxa sooner than others. These changes can affect metric results.

Various biometrics based on macroinvertebrates were reviewed. We selected the Hilsenhoff Biotic Index (HBI) as the best tool for assessing nitrogen and phosphorus pollution problems. An HBI score of 4.0 should be used as the threshold (i.e., maximum allowable value) to prevent impact to fish and associated aquatic life uses. Details on how the biometrics were selected and the thresholds derived are presented in **Appendix B**.

Each sampling event result for a biometric should be considered on its own merits when using the decision spreadsheet (NtrntAssessFramework.xlsx) and completing the assessment. That is, if 2 sampling events for macroinvertebrates were undertaken and 1 of the results was an HBI score of 5.0, then the conclusion for the assessment reach for the macroinvertebrate category would be “>4.0” (i.e., exceeds).

5.0 Nutrient Impairment Assessment Methodologies: Wadeable Streams in the Plains Region

Table 5.1 below shows areas of the state in which the methods of this section best apply. Essentially, the methods apply to all of ecoregion 42 (Northwestern Glaciated Plains) and ecoregion 43 (Northwestern Great Plains) *except for* the level IV ecoregions along the Rocky Mountain Front which are being lumped with the mountainous ecoregions (see **Table 4-1**).

Table 5-1. Ecoregions (level III) in which Assessment Methods in this Section Best Apply
Some level IV ecoregions associated with the level IIIs shown are excluded; these are listed below each level III.

Ecoregion Scale	Ecoregion Name	Ecoregion Number
Level III	Northwestern Glaciated Plains	42
Level IV ecoregions of the Northwestern Glaciated Plains not in the Warm Water Fishery Class:		
Level IV	Sweetgrass Uplands	42l
Level IV	Milk River Pothole Upland	42n
Level IV	Rocky Mountain Front Foothill Potholes	42q
Level IV	Foothill Grassland	42r
Level III	Northwestern Great Plains	43
Level IV ecoregions of the Northwestern Great Plains not in the Warm Water Fishery Class:		
Level IV	Unglaciated Montana High Plains	43o
Level IV	Non-calcareous Foothill Grassland	43s
Level IV	Shields-Smith Valleys	43t
Level IV	Limy Foothill Grassland	43u
Level IV	Pryor-Bighorn Foothills	43v

Note: The level IV ecoregion “Unglaciated Montana High Plains” (43o) has more than one polygon in Montana. Only the polygon located just south of Great Falls, MT is excluded from the Warm Water Fishery Class.

5.1 Assessment Using Biometrics

Biometrics based on diatom algae are stressor-specific (e.g., address nutrient pollution) and apply to specific regions. A diatom sample that indicates **>51%** probability of impairment by nutrients indicates the sample is from a site manifesting a nutrient problem. Details on how the diatom biometrics were developed are presented in the periphyton SOP (Montana Department of Environmental Quality, 2011b).

Each biometric sampling event result should be considered on its own merits when using the decision spreadsheet (NtrntAssessFramework.xlsx) and completing the assessment. That is, if 2 sampling events for diatoms were undertaken and 1 of the results was "65% probability of impairment by nutrients", then the conclusion for the assessment reach for the diatom category would be ">51%," (i.e., exceeds).

Note: Diatom biometrics for the plains region have an inherently high false negative rate (62%; i.e., the chance that the metric declares a truly nutrient-impacted site as having no nutrient impact). This fact is given consideration, in that the resulting decisions in the spreadsheet (NtrntAssessFramework.xlsx) lean somewhat to the protective side.

Always consider cautiously the results from samples collected very early and very late in the sampling season. Algae are a successional community, and if you sample too early, you will sample fewer 'pioneer' species and too late, you will start seeing the community as a whole die off - some taxa sooner than others. These changes can affect metric results.

5.2 Assessment Using the Difference Between the Daily Maximum Dissolved Oxygen Concentration and the Daily Minimum Dissolved Oxygen Concentration (Delta)

We recommend that the magnitude of the daily DO concentration change (daily maximum minus the daily minimum, or *delta*) be used to assess plains streams. Elevated daily DO delta values indicate high productivity and the potential for DO standards exceedances (per DEQ-7) that would impact fish and aquatic life. We suggest that a DO delta of 5.3 mg/L be used as the threshold. **Assessors need not wait 30 days to take subsequent DO measurements at a site; each DO sampling event may be considered on its own merits.** Details on how the DO threshold was identified are provided in **Appendix C**.

Each DO sampling event result should be considered on its own merits when using the decision spreadsheet (NtrntAssessFramework.xlsx) and completing the assessment. That is, if 5 sampling events for DO delta were undertaken and 1 of the DO deltas exceeds the recommended threshold, then the conclusion for the assessment reach for the DO delta category would be "exceeds 5.3 mg/L". Further consideration may be needed if the data were collected long-term[12]. **Note:** Do deltas in the plains region have an inherently high false negative rate (63%; i.e., the chance that the DO deltas indicate that a truly

[12] If DO deltas >5.3 mg/L comprise < 10% of the dataset collected using an instrument deployed at least 14 days, consider if the site has a strong presence of macrophytes or not. If macrophytes are very common, the site could be declared as 'Meets Criteria'. If macrophytes are not common, it could be declared 'Does Not Meet Criteria'. Consult Standards Section for further assistance.

nutrient-impacted site has no nutrient impact). This fact is given consideration in that the resulting decisions in the spreadsheet (NtrntAssessFramework.xlsx) lean somewhat to the protective side.

5.2.1 Deploying a Continuous DO Monitoring Device in Wadeable Plains Streams

If a continuous monitoring device is to be deployed (e.g., YSI 6600 sonde), we recommend that at least one (1) full day of data be collected to properly calculate a daily DO delta. The day of deployment and the day of retrieval are usually truncated, and so at least one full day in between assures the necessary data are collected. We recommend a fifteen minute time step for monitoring, as that has worked well in our experience and provides good data resolution. Initial calibration, as well as drift from calibration— which is determined at the time the unit is retrieved— should be documented per the project's QAPP and or SAP.

5.2.2 Instantaneous DO Monitoring in Wadeable Plains Streams

Daily DO minimum and maximum concentrations each need to be obtained, and can be collected using a hand-held instrument. The daily DO minimum needs to be collected starting in the pre-dawn hours, up to as late as 8:00 am. The daily DO maximum will *usually* occur between 2:30 pm and 5:00 pm; observations can be collected every 15-30 minutes during this time to identify the highest value. Continue monitoring after 5:00 pm if observations are still climbing. Further details on how these time frames were identified are provided in **Appendix C**.

The YSI 85 instrument has a 50 reading, manual-entry memory which can be used for collecting DO maximums. With the unit on and the sensor properly deployed, depress the ENTER button for two seconds to record an instantaneous observation. Data may be downloaded later.

For the purpose of calculating DO delta, at least 3 DO sampling events (i.e., 3 different days) should be taken in each assessment reach; if collected at the same site, they do <u>not</u> need to be collected 30 days apart (e.g., 3 days in a row is OK).

5.2.3 BOD_5

We recommend that biochemical oxygen demand, or BOD_5 (also called just BOD), be used to assess plains streams at level II. We recommend a threshold of 8.0 mg/L be used. Each BOD sampling event result should be considered on its own merits when using the decision spreadsheet (NtrntAssessFramework.xlsx) and completing the assessment. That is, if 3 sampling events for BOD were undertaken and 1 of the BODs exceeds the recommended threshold, then the conclusion for the assessment reach for the BOD category would be ">8.0 mg/L" (exceeds).

5.2.4 Algal and Macrophyte Indicators of Nutrient Enrichment in Wadeable Plains Streams

The Fish Cover/other form (see periphyton SOP, (Montana Department of Environmental Quality, 2011b)) should be filled out when assessing plains streams. Although not required to fill out the form, we recommend that the dominant macrophytes be identified, which will help with your assessment back in the office using the information below.

In the Northwester Glaciated Plains ecoregion, streambed cover by filamentous algae should generally be less than 30% for a single sampling event and less than 25% for the summertime average (Suplee, 2004). These data can be collected visually using the Fish Cover/Other form, which is provided in the

periphyton SOP (Montana Department of Environmental Quality, 2011b). (Although a somewhat tangential issue, the presence of a healthy and widely distributed macrophyte community should be taken as indicative that the stream has a reasonable level of morphologic stability; stream instability has been found to be a major factor in controlling algae and macrophyte dynamics in prairie streams [Suplee, 2004]).

Throughout the plains region, attention should be paid to the types of macrophyte species present. We have observed that northern watermilfoil (simultaneously known as *Myriophyllum exalbescens, Myriophyllum sibiricum, and Myriophyllum spicatum L. var exalbescens* (Muenscher, 1944). DiTomaso and Healy (2003) is extremely common throughout the Northwestern Glaciated Plains and Northwestern Great Plains ecoregions, as has been observed by others (Klarich, 1982). However, in stream sites where high nutrient enrichment is occurring, we have observed northern watermilfoil's (and other macrophyte's) near-complete replacement by coontail (aka hornwort), *Ceratophyllum demersum*[13] Coontail is a rootless, free floating macrophyte—though it can anchor itself to bottom substrates via specialized buried stems—that can proliferate in streams which are being heavily loaded with nutrients (DiTomaso and Healy, 2003). In this it is similar to floating and benthic algae in that it relies on water-column nutrients for growth, because it does not take up nutrients from the sediment via roots, as other macrophytes do. Choking mats of coontail, or its presence to the exclusion of other macrophytes, should be taken as a strong indicator of nutrient over enrichment. Close-up and panoramic photos should be taken to record the extent of the problem, and aide identification of the plants in-office.

Finally, we documented during the Box Elder Creek dosing study that *Chara* spp. (commonly called stonewort or muskgrass) were greatly depressed in number in the nutrient-dosed reaches compared to the Control reach, and also compared to the pre-dosing period. *Chara* spp. are a branched form of algae, are an important component of natural aquatic ecosystems (DiTomaso and Healy, 2003), and are often associated with clean water.

6.0 ACKNOWLEDGEMENTS

We would like to thank Dr. Vicki Watson and her students for the many years of work she has carried out in support of DEQ projects. These projects (e.g., the reference streams project) have provided the basic data required to develop many of the guidelines in this document. We would also like to thank all of the DEQ staff and field crews who, over the years, have collected mounds of data that also support this work. Thanks to the Carter County Conservation District for the cooperation and support on the nutrient dosing study. Finally, we want to thank the contractors whose excellent work helped build the foundation on which this document stands: Arun Varghese and Josh Cleland (ICF International); Dr. Loren Bahls (*Hannaea*); Mark Teply (Cramer Fish Sciences); Wease Bollman (*Rhithron* Associates); and Dr. Jeroen Gerritsen and Dr. Lei Zheng (Tetra Tech).

[13] Coontail and watermilfoil can readily be distinguished in the field with a good macrophyte guidebook and a hand-held magnifying glass.

APPENDIX A. STATISTICAL CONSIDERATIONS

A.1.0 INTRODUCTION

The numeric nutrient criteria addressed in this appendix are not intended to be ideal standards, i.e., "no sample shall exceed" values. As such, appropriate inferential statistical tests, assumptions about stream sampling frames, etc. must be developed so that the criteria can be correctly applied. This appendix outlines these statistical considerations and provides rationales for the various approaches used. It also provides precautionary points where certain assumptions depart from more conservative statistical thinking, and discusses how improper sampling design has the potential to mislead a conclusion made about a stream's condition. The key issues addressed herein are:

- Sampling frames, populations, and sampling units for streams, and associated assumptions and precautions
- Consideration of what constitutes our best description of sample independence in streams (spatial and temporal), and associated assumptions and precautions
- Determination of appropriate critical exceedance rates for nutrients (nitrogen and phosphorus)
- Statistical testing procedures and accompanying decision rules
- Minimum sample sizes

A.2.0 SAMPLE FRAME, POPULATION, AND SAMPLING UNITS

All studies involving statistical evaluations of data require that a sample frame, population, and sampling unit be defined. Streams are particularly poor entities for establishing these parameters because streams are an interconnected network rather than discreet entities. Nevertheless, streams are the entities to be sampled so some effort must be made to segregate them into definable units. For the purposes of determining compliance with numeric nutrient criteria, we define the following:

- **Sample Frame:** A wadeable[14] stream segment listed in the Assessment Data Base (ADB) (DEQ 2009, and updates) OR a sub-segment of an ADB stream segment. These segments are referred to here as an "assessment reach".
- **Population:** All the water flowing through the assessment reach during the time period when the numeric nutrient criteria apply, and the surface area of the stream bottom over which the water flows.

[14] Wadeable streams are perennial and intermittent streams in which large portions of the channel are wadeable during baseflow conditions. For the list of waterbody segments not considered wadeable (i.e., the large rivers), see Flynn and Suplee (2010).

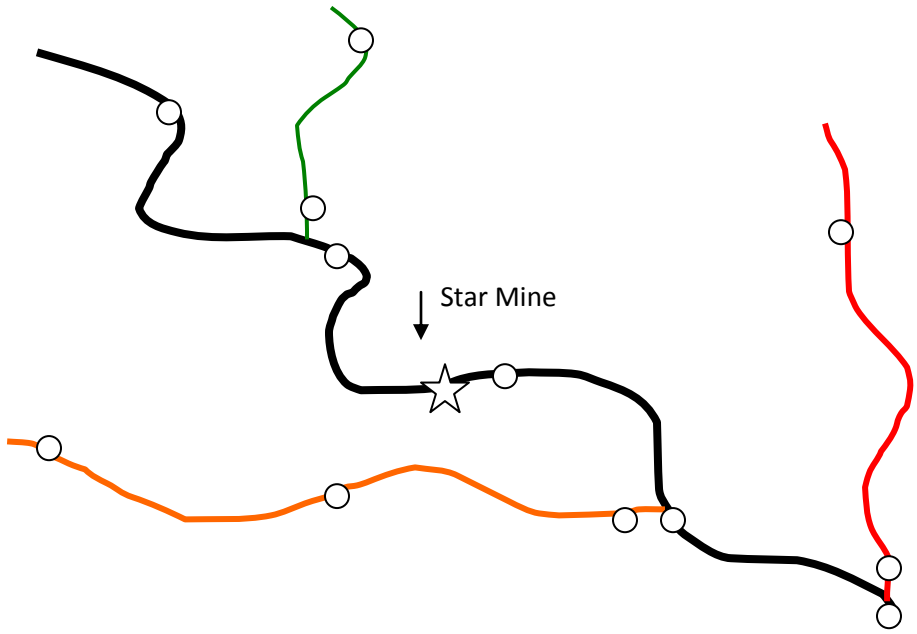
- **Sampling Unit:** A sample collected from the assessment reach that is largely independent of other samples collected within the assessment reach and collected during the time when the numeric nutrient criteria apply.

Assumptions: Each assessment reach (ADB segment or sub-segment) will be made up of a series of sampling sites, or a series of very short study reaches that are essentially sites (**Figure A2-1**). The minimum number of sites on an assessment reach is provided in DEQ SOPs (Montana Department of Environmental Quality, 2005). **Figure A2-1** illustrates the variety of ADB segments that may be found; segment lengths can vary tremendously. For purposes of determining compliance with numeric nutrient standards using statistical methods, it is usually assumed that (1) pollution sources are evenly dispersed along the reach, (2) sampling sites are randomly located along the reach, and (3) each sample is independent of the others (Spatial and temporal independence guidelines for sites are addressed in **Section A.3** below).

It some cases, ADB segments may have pollution problems (hotspots) concentrated only in a particular part of the stream, say, the last 5 stream miles. In such cases, it may not make sense to view the original ADB segment as the best possible sampling frame. That is, it would be better to further stratify the sample frame and, thus, the population of interest .This will prevent distortion of results caused by mixing together, for common analysis, data from the relatively un-impacted sub-segment with data from the impacted sub-segment. For example, in **Figure A2-1** it might be prudent to consider the sub-segment upstream of the Star Mine as a sampling frame apart from the sub-segment below the mine. Stratification is common in studies employing purely random sampling, where it is referred to as stratified random sampling (Cochran, 1977). Stratification allows maximal precision of estimates for minimal sampling effort (Norris, et al., 1992). **The assessor carrying out the analysis on an ADB segment will have to judge if further stratification is warranted.** If it is warranted, then sampling requirements, described above and further detailed below, would apply to *each* of the new sub-segments (aka assessment reaches), individually.

Precautionary Considerations: Pollution sources are rarely evenly-dispersed along stream segments, violating assumption 1 above. And purely random sampling is usually not practical due to stream access issues, etc. Targeting only the known or potential pollution "hotspots" — even within an assessment reach that has been broken out from a larger ADB segment — could over represent the hotspots and distort the statistical tests. Sampling and analysis plans (SAPs) should proceed with goal-oriented sampling (U.S.Environmental Protection Agency, 2000) that works towards striking an equitable balance between the number of hotspot sites and the number of un- or minimally impacted sites *within the defined assessment reach.* That is, the aggregate of collected samples should be representative (U.S. Environmental Protection Agency, 2002) of the assessment reach as a whole. Advanced knowledge and expertise of the field will be needed to accomplish this (Norris, et al., 1992), and modifications to the assessment reach boundaries can be made on-the-fly during field work, if deemed necessary. It is possible to sub-segment a stream reach to the point where, for a particular assessment reach, there really is little left but hotspots; if this is the case, then the hotspots *are* representative of the assessment reach. As a general rule, it is better to lump than split to avoid unnecessary sampling and administrative work. **The requirement to create reasonably uniform assessment reaches is inherently in conflict with the need to "lump" for the purpose of keeping assessments as simple as possible. Judgment is needed to balance these two opposed factors and come up with an optimal sampling strategy.**

Although this quasi-systematic approach is not a substitute for truly random sampling it will, if carried out properly, achieve good sample interspersion and representativeness. For further discussion of randomization *vs.* interspersion approaches, see page 196 of Hurlbert (1984).



Example sampling sites (hollow dots) are shown along each segment.

Figure A2-1. Four different stream reaches (shown by different colors), each representing 1 sampling frame (ADB stream segment)

A.3.0 Determining Sample Independence

According to definitions in Hurlbert (1984), much sampling carried out by DEQ on individual streams tends to violate spatial and temporal independence assumptions and results in pseudoreplication. For example, samples collected over time at a site can be serially correlated, which precludes temporal independence (Hurlbert, 1984). However, the statistical views advocated by Hurlbert are not universally supported; contrary opinions on the matter can be found in the literature (Stewart-Oaten and Murdock, 1986; Stewart-Oaten, et al., 1992; Osenberg, et al., 1994) and have led to what one journal referred to as a "healthy debate" (*Ecological Applications*, volume 4, No. 1, 1994). In general, more needs to be known about detection of non-independence and the frequency with which temporally independent samples can be collected (Underwood, 1994).

Time-series collected samples from a site may be used in inferential statistical testing, if used cautiously; this requires that one assumes that actual trends in time are identical in magnitude and direction for all the sites across the study (Norris and Georges, 1993). Osenberg *et al.* (1994) examine time-series serial correlation of physical and biological measurements in a BACI (Before-After-Control-Impact) study and conclude that, in the marine environment they study, sampling can occur at a site every 60 days without yielding substantial serial correlation.

DEQ recognizes the issue of temporal pseudoreplication, but also needs to be practical about the reality of sampling streams which, by their very nature, make collection of independent samples difficult In DEQ's reference project (Suplee, et al., 2005), 30 days has generally been used as a minimum time span between sampling events at a site to infer temporal independence of water samples. This time span was based on the experiential observation that, during the brief Montana summer, substantial changes in flow, temperature, and vegetation (both riparian and instream) occur from month to month, changes that would likely effect water quality. But Stewart-Oaten *et al.* (1986) recommend that the assumption of temporal independence be tested, rather than assumed. The Durbin-Watson test statistic is widely used to check for time-series serial correlation. Stream sites with monthly nutrient sampling during the summer were available in Montana, and some of these sites were tested using the Durbin-Watson statistic Results are shown in **Table A3-1** below.

Table A3-1. Durbin-Watson Values for Time-series Collected Nutrient Samples at Selected Sites

All Samples were Collected Approximately 30 Days apart Nutrients Showing Probable Time-series Serial Correlation (95% Confidence Level) are Highlighted.

					Nutrient	
Stream Site	**Months Sampled**	**Time Range**	**n**	**Total N**	**Total P**	**NO_{2+3}**
Rock Creek Site 2	June, July, Aug, Sept	2001-2004	12	1.18	1.43	2.3
Clark Fork R. at Deer Lodge (site 9)	July, Aug, Sept	1998-2006	25	1.81	1.78	1.68
Clark Fork R. above Little Blackfoot R. (site 10)	July, Aug, Sept	1998-2006	26	2.01	1.57	1.46
Clark Fork R. above Flathead R. (site 25)	July, Aug, Sept	1998-2006	26	1.76	1.21	2.08

In general, Durbin-Watson values around 2 mean there is no serial correlation, whereas values greater than approximately 2.5 or less than about 1.5 lead one to suspect negative or positive serial correlation, respectively (Neter, et al., 1989; Ott, 1993). What can be concluded from this limited analysis? Most nutrients did not show serial correlation, and one of the three that did is borderline cases (statistic =1.43, but power of test very low). Overall, it appears that serial correlation is present in nutrient samples collected a month apart, but the effect is very weak It is evident that 30-day separated water samples can provide a fairly high degree of independence for nutrients.

DEQ is aware that spatial independence is also a concern. Water flows from upstream to downstream, consequently influencing the spatial independence of downstream sampling sites. No generally applicable spatial minimums were found as of this writing. U.S.EPA guidance (USEPA, 2002) generally glosses over the topic of spatial independence in streams.

To address spatial independence, we tested a Montana dataset. We used the pre-dosing baseline data collected as part of the Box Elder Creek nutrient dosing study (Montana Department of Environmental Quality, 2010a). We found that total nutrient samples collected within hours of one another at two sites located 0.73 stream miles apart were not spatially correlated. We compared nutrient samples collected from the Low Dose site to those collected on the same day at the High Dose site which is 0.73 miles downstream. Box Elder Creek is perennial and was flowing during all sampling events. No tributary intervenes between the sites. Samples were collected within 1-2 hours of one another, during the summer index period. We only considered samples collected *prior to* nutrient dosing, as these are comparable to what one would encounter during routine stream sampling/assessment. Using the Rank von Neumann test (U.S.Environmental Protection Agency, 2006), we found that there was no serial correlation for total N or total P (i.e., we could not reject the null hypothesis "no serial correlation"), at an alpha of 0.05. There was serial correlation for Soluble Reactive Phosphate (SRP). We were unable to

assess soluble N as there were too many non-detects in the datasets, which led to too many rank-ties; too many rank-ties precludes proper statistical evaluation (Gilbert, 1987).

Spatial independence can therefore be established (albeit as rules of thumb) for total nutrients as a minimum of about 1 mile between two sites. Other factors leading to spatial independence include a tributary confluencing on a stream between two sampling sites, or if major land form or land use changes occur along the reach (Montana Department of Environmental Quality, 2007; Montana Department of Environmental Quality, 2011a).

Giving consideration to our findings, below are guidelines for establishing independence of samples collected within an assessment reach:

- Sites (or short reaches equivalent to sites) should be located a minimum of 1 stream mile apart.
- Sites may be placed < 1 mile apart along the assessment reach if there is a flowing tributary confluencing with the segment between the two sites.
- Try to collect water samples starting at the downstream end of the assessment reach moving upstream, to avoid re-sampling the same water.
- Land use changes and land form changes should be considered and can be used to help define (1) breaks between assessment reaches and/or (2) additional sampling sites within an assessment reach.
- Samples collected at the same site (or short reach) should be collected 30 days apart.

Total nutrient samples that meet the above conditions may generally be considered spatially and temporally independent for the purposes of determining compliance with the nutrient criteria. As such, they may be used in inferential statistical analyses and to make conclusions about the assessment reach in question.

Precautionary Considerations: The last bullet above (temporal independence resulting from approximate 30-day time spans) is <u>not</u> applicable for some bioassemblage samples (e.g., macroinvertebrates, fish). These organism populations operate on different (longer) time scales from water samples and diatoms and may show considerable year-to-year stability. Please see **Section 9.0** of Suplee (2004)) and Bramblett *et al.* (2005) for more details on temporal patterns of these biological assemblages. Diatom populations tend to shift quickly, within 1-5 weeks, in response to environmental changes (LaVoie, et al., 2008). Thus, this rate of change is sufficient to be able to consider diatom sampling events spaced 30 days apart as being largely independent of one another.

A.4.0 Selection of Inferential Statistical Tests, Confidence Levels, and Associated Decision Rules

A.4.1 Rationale for Using Two Inferential Statistical Tests to Help Determine Compliance with Nutrient Standards

Exhaustive reviews of the pros and cons of statistical tests available for determining compliance with numeric standards have already been published (U.S. Environmental Protection Agency, 2002). For brevity, rather than revisit all the detailed considerations put forward in those documents, recommendations are provided herein concerning what where judged to be the most applicable tests. These recommendations are then followed by a series of decision rules that allow the user to apply the tests in tandem. For purposes of compliance with numeric nutrient criteria, two tests should be used; the Exact Binomial Test and the One-Sample Student's t-test for the Mean.

- Exact Binomial Test: This test assumes data are dichotomous in nature (i.e., only two possible outcomes). For compliance with a criterion this reduces to (1) samples that exceed the criterion and (2) samples that do not exceed the criterion. If confidence levels, power, and exceedance rates (more on these below) are established upfront, minimum sample sizes can also be determined. The main disadvantage of the test is that it is blind to exceedance magnitude; that is, it takes no account of whether a sample exceeds the criterion by 1% or 1,000%.

- One-Sample Student's t-test for the Mean: This test does not assume the data take on a dichotomous relationship relative to the criterion. The test compares the mean of the samples in question to the criterion. The desired confidence levels in the test are established upfront. But unlike the Exact Binomial Test, it *is* greatly influenced by high values and outliers which can skew the dataset mean relative to the bulk of the other samples in the dataset. It is also influenced by the proportion of non-detects in the dataset[15].

The Exact Binomial Test is useful for determining sample sizes, and is not influenced by large numbers of non-detects in the dataset. In fact, if the magnitude of nutrient criterion exceedances was irrelevant, then the Exact Binomial Test could probably be used by itself. But this is not the case; one must consider the issue of luxury nutrient uptake by algae.

One of the main purposes of establishing nutrient criteria is to control excess algae growth and its effects on water quality. Many benthic and water-column algae have the ability to take up the non-limiting nutrient, be that N or P, in excess of immediate need and utilize it for growth later (luxury nutrient uptake; (Elrifi and Turpin, 1985; Portielje and Lijklema, 1994; Stevenson and Stoermer, 1982). If extracellular nutrient concentrations then decline in the water, growth can still be maintained on intracellular stores (Droop, 1973; Rhee, 1973). Therefore, pulsed loading events of nutrients to streams may allow algae to carry out luxury nutrient uptake which can sustain growth for several cell generations well after the pulse has ended.

[15] For the purposes of using the t-test, users should initially convert all non-detects to 50% of the reported detection limit (USEPA, 2006). If >> than 15% of the dataset will be affected, consult Standards Section.

Luxury nutrients uptake is a kinetics phenomenon dependent on the physiological condition of the algae, duration and magnitude of the nutrient pulse, etc.; complex factors not easily addressed by a simple t-test. But the t-test can help assess the *potential* for luxury nutrient uptake because pulsed loads of elevated nutrient concentrations, if captured during sampling, would increase the dataset mean and would show that mean water quality has exceeded the criterion; this is useful information not provided by the Exact Binomial Test.

Each test possesses strengths the other does not. Therefore, we recommended that the t-test be used in tandem with the Exact Binomial Test via a series of decision rules (**Section 3.0,** main document).

A.4.2 Form of the Null Hypothesis, Alpha, Beta, Effect Size, and Critical Exceedance Rate

All of the factors listed in **Section A.4.2's** title are interrelated and influence one another in statistical hypothesis testing. Again, rather than reiterate here the mass of discussion devoted to these topics already covered elsewhere ((U.S. Environmental Protection Agency, 2002; California Environmental Protection Agency State Water Resources Control Board, 2004), we will simply state what we concluded to be the best statistical parameters (form of null hypothesis, alpha, beta, etc.) associated with the two tests (Exact Binomial and One-Sample Student's t-test for the Mean), and provide further explanation where warranted.

A.4.2.1 Form of the Null Hypothesis for the Statistical Tests

<u>For Streams Already on the 303(d) List</u>:

- Null Hypothesis: Waterbody is not in compliance with numeric nutrient standards
- Alternative Hypothesis: Waterbody is in compliance with numeric nutrient standards

<u>For Streams Not on the 303(d) List</u>:

- Null Hypothesis: Waterbody is in compliance with numeric nutrient standards
- Alternative Hypothesis: Waterbody is not in compliance with numeric nutrient standards

In effect, this is a "guilty until proven innocent" approach for streams already considered to have water quality problems, and an "innocent until proven guilty" approach for newly assessed streams California uses the same approach (California Environmental Protection Agency State Water Resources Control Board, 2004).

A.4.2.2 Alpha, Beta, Effect Size

In statistical testing alpha, beta, effect size, and critical exceedance rate interact, and changes in one affect changes in the others. In environmental compliance work, there are strong arguments for attempting to balance type I (alpha) and type II (beta) errors; in doing so, it is important to consider the form of the null hypothesis and the implications for making one error or the other. Basically, each type of error has ramifications; one type of error leads to degradation of the environment, the other type of error leads to unnecessary expenditures on the part of the regulated entity. Working towards balancing type I and II errors is a process which inherently recognizes the consequences of each error type (California Environmental Protection Agency State Water Resources Control Board, 2004, Page 52, Appendix C; Mapstone, 1995, Page 178; Schroeter, et al., 1993; U.S. Environmental Protection Agency,

2002). *Given that* working towards balancing type I and type II errors is a valuable endeavor, here are general recommendations for the parameters to be input into statistical tests for nutrients in wadeable streams:

- Alpha should be about 0.25 or less (equates to ≥ 75% confidence level), depending on the form of the null hypothesis and its implications.

- Beta should be about 0.3 or less (equates to ≥ 70% power), and will vary according to the samples size (more on sample size minimum in **Section 5.0**).

- Effect size (gray zone) should be set at 0.15, per USEPA (2002).

In the statistical spreadsheet tools that accompany this technical appendix ("MT-NoncomplianceTool.xls" and "MT-ComplianceTool.xls"), one or the other file is used depending upon whether you are dealing with a new, unlisted stream (use "MT-NoncomplianceTool.xls") *vs.* a 303(d) listed stream (use "MT-ComplianceTool.xls"). You will be able to set alpha, critical exceedance rate (p), and effect size (p2) in the Exact Binomial Test in both of the files. The program will then return various sample sizes, their associated beta values, and the maximum number of exceedances allowed while still remaining in compliance with the criterion.

For the One-Sample Student's T-test, you must input alpha and the nutrient criterion in mg/L. The One-Sample Student's T-tests will then provide a result indicating if the statistical test can or cannot confirm the alternative hypothesis. (The alternative hypothesis will reverse, according to whether you are using the tool for a listed or for a new, unlisted stream).

A.4.2.3 Critical Exceedance Rate

Critical Exceedance Rate: *An estimate of the actual proportion of samples that exceed an applicable water quality criterion. When more than this proportion exceeds the criterion, the standard is not attained (i.e., stream is not in compliance with standard).*

Among the four statistical parameters critical to the Exact Binomial Test—alpha, beta, effect size, and exceedance rate—exceedance rate needs some kind of empirical ground-truthing to assure its validity. The implications of different alpha and beta errors can be understood relative to the form of the null hypothesis, while the effect size (gray zone) is not knowable *a priori*, and is therefore assumed; we recommend an effect size of 0.15 per EPA (U.S. Environmental Protection Agency, 2002). In contrast, an exceedance rate can be estimated using lines of reasoning, empirical evidence and literature values. The considerations used to estimate an exceedance rate for numeric nutrient standards were (**1**) recommended exceedance rates from EPA (U.S. Environmental Protection Agency, 2002) and (**2**) long-term benthic algae and nutrient relationships on the Clark Fork River, MT (Consideration (**1**) and (**2**) are further detailed below.). We recommend:

- A critical exceedance rate for compliance with numeric nutrient standards be set at 0.2 (20%)

Below are our two major considerations leading to the selection of the 20% exceedance rate.

(1) EPA recommends that, for a number of different polluting substances (e.g., fecal bacteria, conventional pollutants, toxic trace metals, etc.), criteria exceedance rates be set between 0.1 and 0.25

(10 to 25%) to protect beneficial uses (Environmental Research Laboratory-Duluth, 1997; U.S. Environmental Protection Agency, 2002).

(2) The analytical approach described in **2.1** below was undertaken in June 2008, and only considered Clark Fork River data through 2006. Subsequent data collection (through 2009) and a somewhat different approach to ascertaining an acceptable exceedance rate allowed us to update this analysis, as provided in **2.2.** Both analyses (that from 2008 [**2.1**], and the work done in 2011[**2.2**]) arrive at the same basic conclusion, and both are presented here. If readers are already familiar with the work in **2.1,** we recommend you skip to **2.2**.

(2.1)*The following analysis was completed in June 2008*.

Introduction: Numeric nutrient (TN and TP) and benthic algae (mg Chl *a*/m^2) standards have been in place on most of the Clark Fork River in Montana for about 6 years. A systematic collection of nutrient and algae data has been ongoing since 1998. At a number of sites both algae and nutrient data have been collected multiple times each year for nearly 10 years. These data lent themselves well to empirically deriving a numeric nutrient exceedance rate because some river sites almost always exceed the algae standards, while others do not. The question became:

> *Do sites on the Clark Fork River that routinely exceed the numeric algae standards exceed the river's established numeric nutrient (TN and TP) standards more frequently than sites that do not exceed the numeric algae standards?*

Benthic algae levels in excess of 150 mg Chl *a*/m^2 (maximum) are not to be exceeded during the summer (ARM 17.30.631). Maximum in this case does not refer to a single high repeat measure from a Clark Fork River site; it refers to the mean value of a series of repeat measures (n = 15 to 20) that are collected at a site *during a particular sampling event.* Clark Fork River sites are usually sampled several times throughout the summer. It has been noted for some years that, during the summer, some sites are usually above the algae standards, while others are not. TN and TP standards were established on the Clark Fork River (ARM 17.30.631) and, if ultimately met, should keep benthic algae below the nuisance threshold described above. However, an exceedance rate was never explicitly established in the regulations In carrying out the exceedance rate determination described herein, it is assumed that the magnitude of the TN and TP criteria on the Clark Fork River were accurately determined, and therefore any exceedance rate drawn from this analysis is meaningful.

Methods: Benthic algae and TN and TP concentration data where concurrently available for seven Clark Fork River sites from 1998-2006. Data were restricted to the time period June 30th to October 1st to generally comply with the summer growing season for this ecoregion (Suplee, et al., 2007)and the regulatory timeframe in ARM 17.30.631. Every benthic Chl *a* measurement from a site (n = 15-20 per sampling event) collected over time was treated as a repeat measure. This resulted in a grand total of 285 to 333 repeat measures of Chl *a* at each site for the period 1998-2006. A grand benthic Chl *a* mean was calculated for a site by averaging all the repeat measures collected between June 30th and Oct 1st for all available years. Nutrient data collected at the corresponding sites during the same time frames where similarly compiled. At each site nutrients were collected as a single grab sample and, as a consequence, there were fewer data (43 to 78 N or P samples per site). Total N data were not collected; however, Total Kjeldahl Nitrogen (TKN) and NO_{2+3} were. Therefore, for each site, individual Total N concentrations were calculated by summing the TKN and NO_{2+3} sample results collected simultaneously during a sampling event.

Next, the Clark Fork River TN and TP criteria concentrations were matched to their corresponding values in the nutrient cumulative frequency distributions for each site, and the associated percentile was recorded. For example, the TN criterion for the Clark Fork River is 0.3 mg/L, and it resulted that at site 9.0 (Clark Fork at Deer Lodge) 0.3 mg TN/L corresponded to the 23rd percentile of site 9.0's cumulative TN frequency distribution. This process was carried out for all 7 sites for both TN and TP. There is a break at the Blackfoot River confluence where the Clark Fork's upstream TP criterion (0.02 mg/L) differs from that below (0.039 mg/L); each TP criterion was applied as appropriate for a site's location along the river.

Results: Table A4-1 shows the results for 3 sites that, over the 1998-2006 time period, did not exceed the Clark Fork River's benthic algal biomass criteria. For this group of sites the nutrient criteria exceedance rate (both TN and TP) was, on average, about 8%. That is, nutrient samples whose concentrations exceed the standards occur only about 8% of the time at these sites. **Table A4-2** shows three sites that *did* exceed the benthic algae standard; for this group of sites, the nutrient criteria exceedance rate was, on average, about 58%. Sites in **Table A4-1** (did not exceed algae standard) had a range of exceedance rates (TN and TP) from 0.1%-24%, and sites in **Table A4-2** (exceed algae standard) had a range of exceedance rates from 27.7% to 88%. The remaining site examined (Site 12; Clark Fork River at Bonita), which is not presented in **Tables A4.1** or A**4.2**, had a mean algae density (144 mg Chl *a*/m^2) so close to the algae standard it was considered borderline. Site 12's exceedance rate was 30.8% for TN, 68% for TP.

Table A4-1 Sites on the Clark Fork River (CFR) Not Exceeding the Maximum Benthic Algae Standard (Growing Season, 1998-2006)

			Percentile in Site's Nutrient Frequency Distribution Matching CFR Standard		**Criteria Exceedance Rate (%)**	
Clark Fork River Site #	**Site Name**	**Long-term Benthic Algal Biomass (mg Chl *a*/m^2, growing season) Mean [median]**	**TN**	**TP**	**TN**	**TP**
15.5	Clark Fork above Missoula	96 [80]	90th	95th	10.2%	5.4%
22	Clark Fork at Huson	72 [52]	76th	96th	24.0%	3.8%
25	Clark Fork above Flathead	35 [20]	100th	99th	0.1%	1.5%
					Grand Mean:	**7.5%**
					Grand Median:	**4.6%**
					Maximum:	**24.0%**
					Minimum:	**0.1%**

Table A4-2. Sites on the Clark Fork River (CFR) Consistently Exceeding the Maximum Benthic Algae Standard (Growing Season, 1998-2006).

			Percentile in Site's Nutrient Frequency Distribution Matching CFR Standards		Criteria Exceedance Rate (%)	
Clark Fork River Site #	**Site Name**	**Long-term Benthic Algal Biomass (mg Chl *a*/m^2, growing season) Mean [median]**	**TN**	**TP**	**TN**	**TP**
9	Clark Fork at Deer Lodge	180 [147]	23rd	50th	77.0%	50.0%
10	Clark Fork above Little Blackfoot River	163 [117]	48th	12th	52.0%	88.0%
18	Cark Fork at Shuffields	197 [181]	50th	72nd	50.4%	27.7%
					Grand Mean:	**57.5%**
					Grand Median:	**51.2%**
					Maximum:	**88.0%**
					Minimum:	**27.7%**

Discussion: The main assumption of this analysis was that the magnitudes of the Clark Fork River nutrient criteria, which were established as standards for the river, are correct. That is, if the nutrient standards are achieved, then summertime algae levels should be kept below the established nuisance thresholds. It was assumed that, as has previously been shown, both N and P co-limit in the Clark Fork River (Lohman and Priscu, 1992); (Dodds, et al., 1997). It was further assumed that the algae standard (150 mg Chl *a*/m^2, site mean per sampling event) will protect beneficial uses. Regarding the later, research completed since the Clark Fork River standards were adopted in 2002 show that 150 mg Chl *a*/m^2 (site mean) is identified as a nuisance threshold by the Montana public majority (Suplee, et al., 2009). If all these assumptions hold true, then reasonable exceedance rates for the 9 year dataset can be derived and used as a case study. It would have been ideal to have a true population of data (rather than a subset of data for a single river over a specific time period) with which to carry out this analysis. But such data are not readily available, and the long-term dataset examined here will have to serve as a proxy.

Comparison of Clark Fork River sites 15.5, 22, and 25 (don't exceed algae standard; **Table A4-1**) *vs.* 9, 10, and 18 (do exceed algae standard; **Table A4-2**) show a clear separation in the consistency of compliance with the river's numeric nutrient standards. It is clear from **Table A4-2** that if the exceedance rate is about 50% then nuisance algae growth will almost certainly occur. But when the exceedance rate is ca. 5-10%, nuisance algae is unlikely to occur (**Table A4-1.**) For purposes of estimating a protective nutrient criteria exceedance rate, the range of exceedance rates from these site groups needs to be considered as well. Note that an exceedance rate *of as much as* 24% does not result in excess benthic algae in some cases (site 22; **Table A4-1**). On the other hand, notice that an exceedance rate *of as little as* 27.7% can result in non-compliance with the algae standard (site 18; **Table A4-2**). Thus, an exceedance rate around 25% probably represent a threshold; if about 25% of the dataset exceeds the nutrient criteria, then there are roughly equal odds that the site could have nuisance algae (or not). This is partially supported

by the fact that the single site with borderline algae conditions (site 12, Clark Fork River at Bonita; 144 mg Chl *a*/m^2) had a TN exceedance rate of 30.8%.

Conclusion: These analyses show that over a 9 year period (1998-2006) sites on the Clark Fork River that have consistently exceeded the nuisance algae standard (150 mg Chl *a*/m^2, summertime max) have TN and TP exceedance rates with a central tendency around 54%. On the other hand, sites that did not exceed the benthic algae standards had TP and TN exceedance rates with a central tendency around 6%. Within each group (sites that do not exceed algae standards, those that do; **Tables A4-1** and **A4-2**), individual sites had exceedance rates as high as or as low as about 25%. This suggests that 25% may be an exceedance rate threshold where the ability to assure compliance with the algae standard becomes tenuous. Given that about 50% is certainly too high of an exceedance rate and will not protect beneficial uses, approximately 10% is probably too restrictive, and 25% is borderline, it is recommended that a nutrient exceedance rate be set to 20%.

(2.2) 2011 Analysis.

The 12-year (1998-2009) nutrient and algae dataset for the Clark Fork River was very large, and was first reduced prior to statistical analyses. Data reduction followed the following general pattern: At any given site (e.g., CFRPO-12), for any given year (e.g., 2005), and for any given parameter (e.g., TP concentration), the data were reduced to a monthly mean for each summer month (June, July, August, or September). First, quality control duplicates collected on the same day were reduced to a mean (TN data was not analyzed directly until 2009 and so, for 1998-2008 data, TN is the sum of TKN and NO_{2+3} samples collected simultaneously during a sampling event). Next, the mean of all individual days where sampling occurred within a month was calculated, resulting in a monthly mean. Nutrient sampling effort varied considerably from site-to-site and from year-to-year, and we did not want heavily sampled months or years to be over-represented in the dataset in the final analysis. In the manner we reduced the data, therefore, each monthly value carries equivalent weight, with some summer months being better characterized (i.e., sampled more days) than others.

For benthic algae samples, up to 20 spatially-dispersed replicates were collected at a site during any given sampling event. Algae sampling events occurred only once a month. Thus, for a given site/year/month, the benthic algae mean calculated was the value used.

We next determined if each mean nutrient concentration, computed on a month-by-month basis, was above or below the Clark Fork River's applicable standards (TP or TN). This was only carried out for sites and times which had corresponding benthic algae samples. Then, we determined the proportion of months during a summer, at a site, that exceeded the river's nutrient criteria. For example, if a site in 2008 was sampled in June, July, August, and September, and June and August exceeded the TN standard, the TN exceedance rate for summer '08 would be 0.5 (50%). Each exceedance rate was then associated with its corresponding "Max Summer Chl *a*" value (exceedance rate as X, Max Summer Chl *a* as Y). Max Summer Chl *a* is the highest mean monthly Chl *a* value encountered during the summer at a site, per ARM 17.30.631. TN or TP data that were collected *after* the Max Summer Chl *a* event occurred were not included (e.g., if the Max Summer Chl *a* occurred in August, we did not include in the analysis the September TN or TP data for that site/year). Finally, least squares regressions (with 95% confidence intervals) were run for TN exceedance rate vs. Max Summer Chl *a* and TP exceedance rate vs. Max Summer Chl *a,* combining all sites and years together. The results are shown on the next page in **Figure A4-1.**

Regression statistics for both regressions were significant ($p << 0.01$). Using the line equations shown in **Figure A4-1**, 150 mg Chl *a*/m^2 (i.e., the maximum allowable benthic Chl *a* level for a summer; ARM 17.30.631) equates to a 26% exceedance rate of the TN standard and a 31% exceedance of the TP standard. The equivalent exceedance rates corresponding to the upper 95% confidence intervals (which are more conservative) are about 11% and about 5% for TN and TP, respectively.

These Clark Fork River data demonstrate that, across 10 sites with 12 year's worth of monitoring, there is a significant, definable relationship between benthic algal growth and the frequency of exceedance of the river's nutrient standards. That is, sites which frequently exceed the nutrient standards have higher levels of benthic algae. Sites that experience greater than about 25-30% exceedance of the nutrient standards will develop nuisance benthic algal growth, i.e., growth equal to or greater than 150 mg Chl *a*/m^2.

The analytical approach taken in 2008 (**2.1** above) was more coarse than what we have done here, in that it lumped all data by site and then looked to see how often that site—over the long haul—exceeded the nutrient standards. This analysis, in contrast, looks at each site and each summer as an individual event, and then collectively evaluates all the data together, regardless of location along the river (**Figure A4-1**). Interestingly, the overall results between the earlier analysis and the current one are largely the same, in spite of the different analytical approaches. If we continue to assume that the nutrient standards on the Clark Fork River are largely correct in magnitude, then this latest analysis indicates we would want to keep exceedance rates of the applicable nutrient standards between 5-31%, if we want to keep benthic algae below nuisance levels. Since these results correspond nicely to the earlier analysis, we continue to recommend that nutrient criteria exceedance rates be set at 20%.

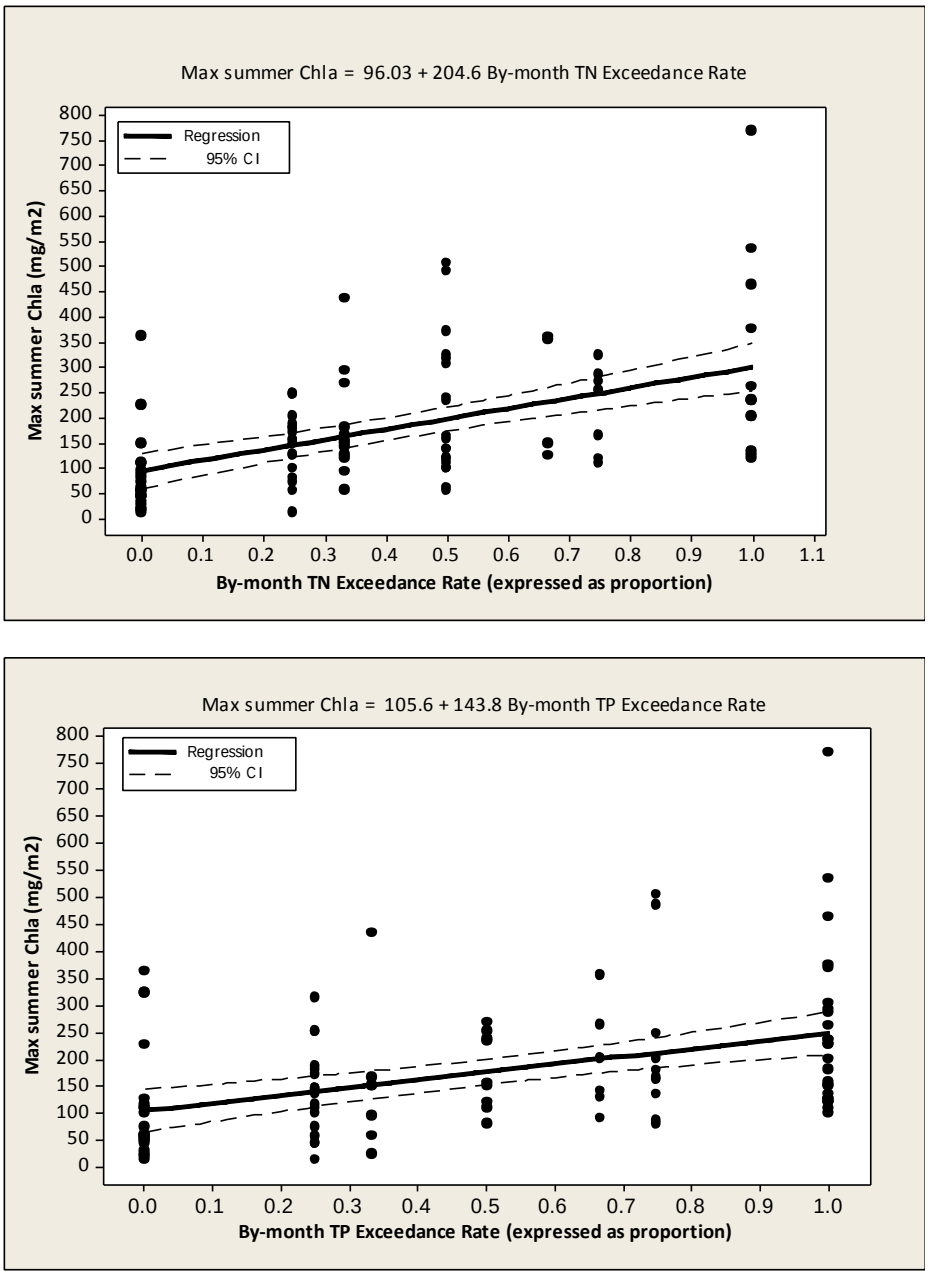


Figure A4-1. Least squares regression for TN exceedance rate vs. Max Summer Chl *a* (upper panel) and TP exceedance rate vs. Max Summer Chl *a* (lower panel), for ten Clark Fork River monitoring sites (1998-2009). Dotted lines are the 95% confidence intervals. Both regressions are significant ($p << 0.01$).

A.5.0 Minimum Number of Nutrient Samples

The final consideration is minimum nutrient sample size. A "nutrient" sample refers to a nutrient type, such as TP or TN Sample sizes apply to each nutrient type, and not to the total number of nutrient samples collected from a stream segment. So, if 7 TN and 7 TP samples were collected from a segment they would not represent 14 samples, but rather 7 of TN and 7 of TP. There is extensive discussion of determining appropriate sample size on a study-by-study basis in USEPA (2002). However, the

recommendations made here for determining compliance with the numeric nutrient standards are meant to apply generally to all Montana wadeable streams, mainly for purposes of 303(d) listing/delisting. *Please note that these sample size minimums do not apply to biological samples (e.g. benthic algae or diatoms) that may be collected concurrently with the nutrient samples.*

For unlisted streams, those for which the form of the null hypothesis is "complies with standard" (**Section A.4.2.1**), the implication for making a type II (beta) error is that a truly non-compliant stream segment would be incorrectly declared compliant. This is a scenario DEQ wants to minimize, and so the probability of such an outcome should be reduced well below 50%, i.e., well below that of a coin flip. The Exact Binomial Test in the accompanying spreadsheet tool can be used to estimate minimal sample sizes. In the test it can be seen that if alpha (type I) error is set to 0.25, exceedance rate (p) to 0.2, and effect size/gray zone (p2) to 0.15 (entered value = 0.35), then a Beta (type II) error of 0.35 is achieved with 12 samples. (Note in the spreadsheet that introducing lower and lower alpha values causes beta error to increase and, therefore, many more samples are needed to try to balance alpha and beta errors.) Twelve samples is about as low an **n** that can be used and still have roughly balanced (0.25 *vs*. 0.35) alpha and beta errors that are each well below 50%.

For listed streams, a similar approach is used. Listed streams are those for which the form of the null hypothesis is "does not comply with standard". In this scenario, the implication of making a type I (alpha) error is that a truly non-compliance stream segment is incorrectly declared compliant; again, this is a scenario. DEQ wants to minimize Setting the alpha to 0.25, exceedance rate (p) to 0.2, and effect size/gray zone (p2) to 0.15 (entered value = 0.05), a beta error of 0.14 (14%) can be achieved with 13 samples. This is a reasonable balance of type I and II errors, and provides a total sampling effort about the same as that for unlisted streams. Given these considerations, it is recommended that:

- For new, unlisted stream segments, a minimum of 12 independent samples for any given nutrient be collected for compliance determination.

- For 303(d)-listed stream segments that already have one 1 nutrient criteria exceedance for a given nutrient, a minimum of 13 independent samples (this total can include newly collected as well as previously collected samples) should be used for compliance determination.

- For listed streams with 13-18 total samples that already have 2 or more exceedances for a given nutrient, the default conclusion is that the stream segment has failed the Exact Binomial Test (no further sampling required at this time). Run the t-test as well and incorporate results in decision matrix.

- For listed streams that have > 18 samples for a given nutrient, set alpha to 0.25, exceedance rate to 0.2 and effect size to 0.15 in the Exact Binomial Test, and determine if the reach is (or is not) in compliance with the Exact Binomial Test. Carry out the same for the T-test.

- If a very large dataset (> 300 samples) is available for a particular stream, then lower type I (alpha) and type II (beta) error can be achieved with higher confidence in the results. Use the special feature of the Exact Binomial Test to help define these confidence levels. Confer with Standards Section if needed.

APPENDIX B. DETAILS ON ASSESSMENT METHODOLOGIES FOR WADEABLE MOUNTAIN AND TRANSITIONAL STREAMS

The following provides the rationales used for the selection of the assessment tools. It also provides the rationales for selected impact thresholds. This information is summarized in **Section 4.0** of the main document.

B.1.0 ASSESSMENT OF BENTHIC ALGAL GROWTH

An evaluation of statistical uncertainty in the site averages calculated using DEQ's standard procedure for collecting and analyzing benthic Chl *a* is detailed in **Appendix A** of the Chl *a* SOP (Montana Department of Environmental Quality, 2011c). To summarize: if a benthic Chl *a* sampling event has followed the SOP, DEQ is confident that—for a typical wadeable stream— at least 80% of the time the measured Chl *a* average calculated will be within ±30% of the true average. Given this known variability, decision points pertaining to benthic algae growth and harm-to-uses have been developed, and are further detailed in **Sections B.1.1** and **B.1.2** below

B.1.1 BENTHIC ALGAL CHL *A* LEVELS AND THE RECREATION USE

It is reasonable that, once a site's true average algal level exceeds about 150 mg Chl *a*/m^2, impairment to the recreational use has occurred. This is shown in **Figure B1-1**. But we also have to account for the uncertainty around the Chl *a* measurement. Shown are the three photographs that bracket the acceptable-unacceptable threshold, per Suplee *et al.* (2009) each with their interval widths (based on the 20 Chl *a* replicates associated with each photo) calculated at the 90% confidence level. Once algae levels have reached the lower bound of photo E (photo E's lower confidence bound = 169 mg Chl *a*/m^2), the acceptability threshold has already been exceeded. This is because the public majority finds the algae level shown in the photo to be highly undesirable. The gray zone in **Figure B1-1** represents the zone where public acceptability rapidly transitions from "OK" to "Not OK". Going forward, any measured average Chl *a* value DEQ believes could plausibly be as high as 165 mg Chl *a*/m^2 (in the gray zone, and at upper confidence bound of photo F, but still below the lower confidence bound of photo E) should be considered an exceedance.

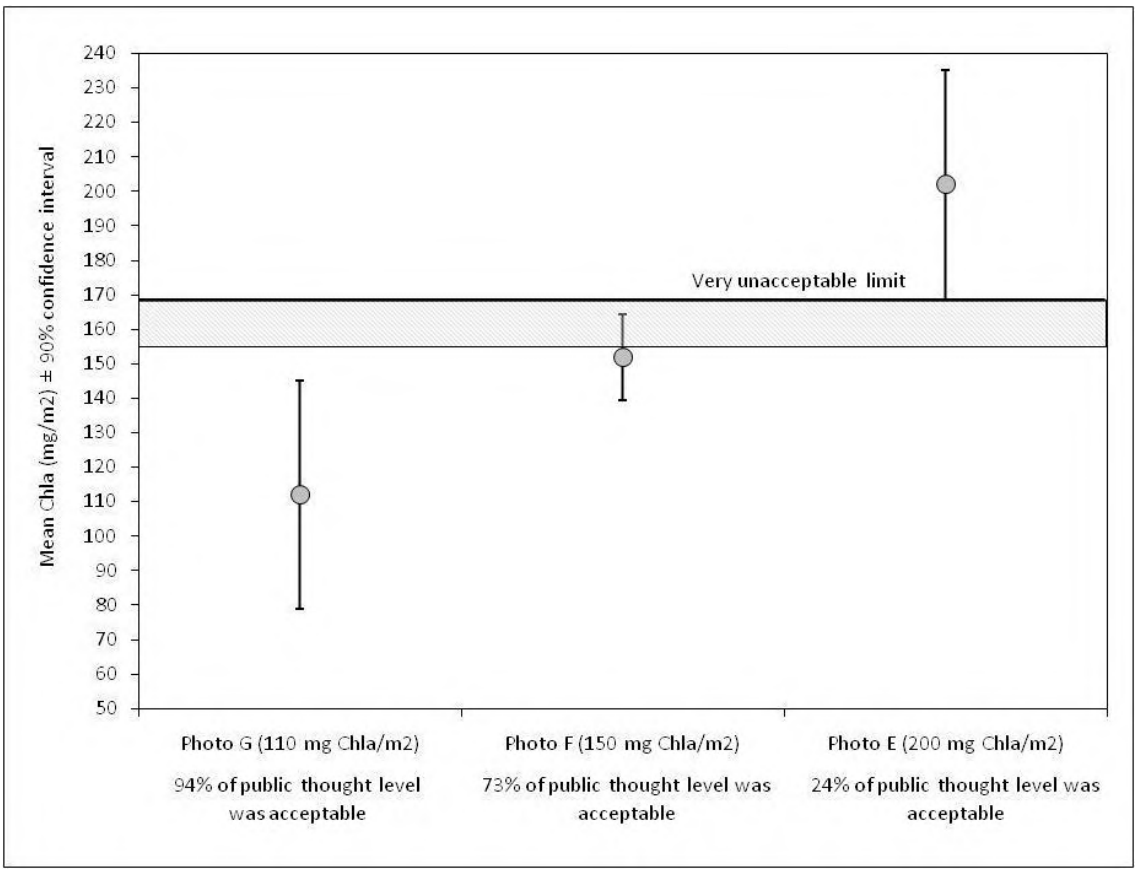


Figure B1-1 Averages (Gray Dots) and 90% Confidence Bands for the Three Photos Bracketing the Acceptable-unacceptable Threshold, per Suplee et al. (2009) The gray band shows the algae level range across which public opinion rapidly shifts from acceptable to unacceptable.

Returning to DEQ's algae-sampling protocol, the average that is calculated for any given sampling event has a definable interval width and, when the upper bound of that interval reaches about 165 mg Chl *a*/m^2, an impairment at that site should be considered to have occurred. Using the approach outlined in Appendix A of DEQ (Montana Department of Environmental Quality, 2011c) and given that ***n*** = 11, Coefficient of Variation (CV) = 73%, DEQ can be 80% certain that the true benthic algae average may be as high as 165 mg Chl *a*/m^2 when the measured average is ≥ 129 mg Chl *a*/m^2. Therefore, any sampling event for which the measured benthic algal Chl *a* average is **≥ 129 mg Chl *a*/m^2** should be considered an exceedance, and in violation of ARM 17.30.637(1)(e).

B.1.2 Benthic Algal Chl *a* Levels Relative to Late-season Dissolved Oxygen Problems, and Potential Impacts to Fish and Associated Aquatic Life

In 2009, DEQ commenced a BACIP (Before After Control Impact Paired) design, whole-stream nutrient addition study to better understand the exact way stream changes are manifested due to nitrogen and phosphorus pollution. We wanted to understand the relationship between these changes and stream

beneficial uses (Montana Department of Environmental Quality, 2010a)[16]. In summer 2010 soluble nitrogen and phosphorus were added to a reach (High Dose reach) of the stream, and major changes occurred as a result. One of the most interesting findings was the temporal manner in which stream dissolved oxygen (DO) problems occurred, and the relationship of those DO levels to measured benthic algae levels.

After nutrient additions began in early August 2010 and then continued throughout summer 2010, DO standards that protect fish (1 Day minima in DEQ-7;(Montana Department of Environmental Quality, 2010b)) were never exceeded — nor even approached —in the High Dose reach. This was true in spite of large daily DO swings (**Figure B1-2**)[17]. Relative to the upstream Control reach, DO increased dramatically during the day, but did not at night fall much below the Control reach values (**Figure B1-2**). Benthic algal production (growth) exceeded respiration throughout most of the summer and this, in conjunction with adequate re-aeration due to the stream's flow, likely prevented nighttime DO levels from dropping below the DO standards. However, in fall, the growing season's accumulated algal growth began to senesce en masse and we observed large amounts of decaying algae on the stream bottom in early October. The decaying algae induced a high oxygen demand which was concentrated near the bottom, in affect acting like a sediment oxygen demand (SOD), and which in turn led to exceedances of the DO standards. (The YSI in **Figure B1-2** was monitoring DO about 20 cm off the stream bottom, in a run.) The DO standards exceedances all occurred late in the season, after robust benthic algae growth had ended.

It should be noted that two other YSIs (one deployed upstream and another further downstream of the one in **Figure B1-2**, thus bracketing the High Dose reach) simultaneously recorded DO concentrations, none of which violated DO standards, even in October (data not shown). This was apparently due to longitudinal changes in stream morphology (e.g., width/depth relationships) and their affect on stream re-aeration, and dead algae accumulation on the bottom. We calculated that for DO to decline from what was measured by the YSI just upstream of the High Dose reach to that recorded in early October in **Figure B1-2** would require a SOD higher than any we could locate in the literature[18]. This suggests that DO concentrations were not uniform from stream surface to bottom, but rather, a bottom-to-surface DO gradient likely existed. Our analyses further indicated that DO was probably near zero near the bottom, and then near saturation at the surface. These findings suggest that DO problems of this nature can be both longitudinally and vertically patchy along the stream channel.

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[16] Although this study was carried out in a C-3 warm water fishery stream, the stream's key characteristics relative to algal growth make it a reasonable comparison to western Montana gravel-bottom streams, as we have done here. The stream has a gravel dominated substrate, perennial flow of about 4-6 CFS in summer, a water surface slope of 0.4%, and riffles are common throughout, as are pools. The dominant filamentous algae that grew during the study was *Cladophora*, which is also commonly found in western MT streams.

[17] Routine QC checks of the instrument, including mid-project calibration, routine instrument cleaning, etc. was undertaken The low DO values measured are considered valid measurements.

[18] Stream water biochemical oxygen demand (BOD) samples were also collected in early October at the site. They were non-detect, thus the DO consumption had to be coming from the decaying algal material on the bottom.

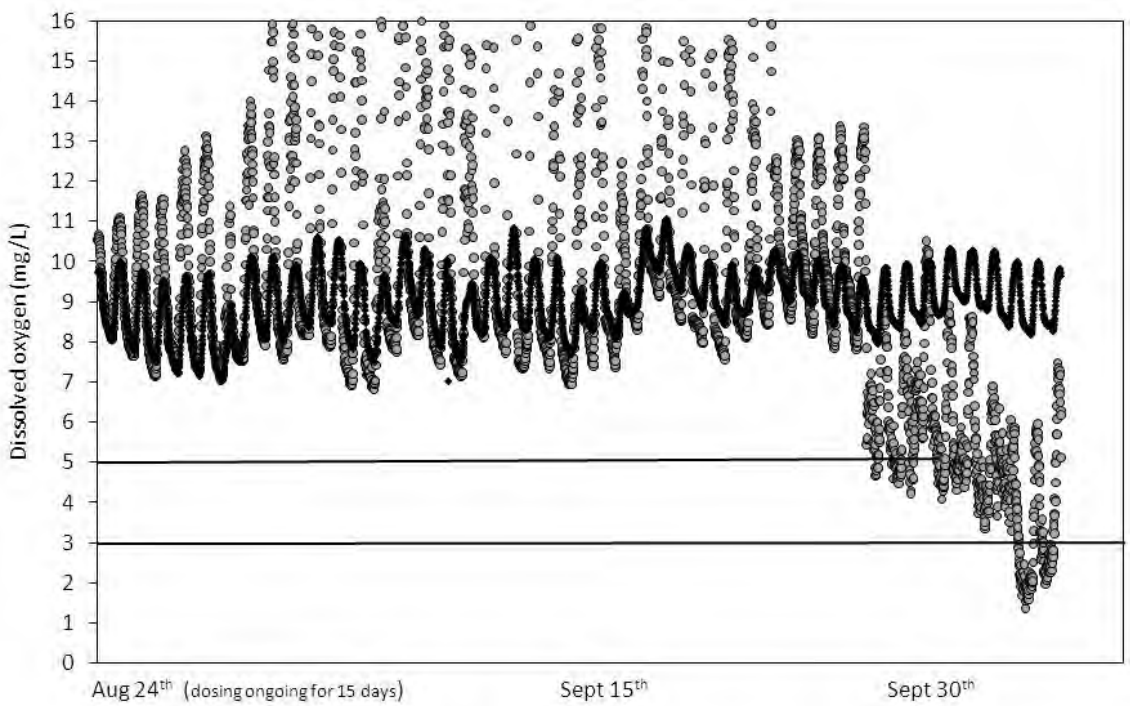


Figure B1-2. Temporal Changes in Dissolved Oxygen (DO) Levels in the High Dose Study Reach as Measured by Deployed YSI Instrument, 2010. Gray dots are DO observations at the High Dose Reach, collected at 15 min intervals. For comparison, the black line oscillating around 9 mg/L is the DO measured by another YSI in the upstream Control reach, which did not receive nutrient additions. The horizontal black lines are (upper) the adult fish and (lower) juvenile fish DO standards for this stream.

Returning to the High Dose reach's benthic-algal growth, benthic algae Chl *a* levels at the end of the growing season reached 127 mg Chl *a*/m^2 (**Table B1-1**). It is apparent from **Figure B1-2** that this level of benthic algae was sufficient to induce DO violations along the channel when the algae died and decomposed en masse. The implication of this finding is that the late-season average benthic algae we measured (127 mg Chl *a*/m^2) has the potential, in wadeable streams, to cause DO standards exceedances in the post-summer period, probably in late September or October. Although this study was carried out in a C-3 warm water fishery stream, the stream's late season water temperatures (which ranged from about 12-16 ^{0}C) are comparable to what is observed in typical western Montana gravel bottom streams at that time of the year. While it is true that water temperature strongly affects DO concentration, we would not expect western Montana streams manifesting similar algal densities to be able to compensate (i.e., maintain DO above standards) due to their having cooler water temperatures, as their temperatures are often about the same at that time of the year.

Table B1-1. Benthic Algae Density Measured at the High Dose Study Site, 2010

Sampling Date	Reach average benthic-algae density (mg Chl *a*/m^2)	Chl *a* replicates' CV(%)*	Reach average AFDW density (g/m^2)	AFDW replicates' CV(%)*
August 26, 2010	**111**	123	**26**	93
September 8, 2010	**116**	97	**34**	45
September 22, 2010	**87**	82	**37**	61
October 6, 2010	**127**	90	**33**	73

* 11 replicates were collected for each sampling event. Less than 11 were used to calculate reach average AFDW because core samples, if collected, are not included in the calculation of average reachwide AFDW density.

As mention at the beginning of **Section B.1**, there is a definable level of uncertainty around any given benthic Chl *a* average, but there is no way to know if the values in **Table B1-1** are at the low end, high end, or in the middle of that range. We'll assume here that the 127 mg Chl *a*/m^2 measured is, in fact, accurate. Thus, to be protective and assure that DO problems that could harm fish and associated aquatic life are prevented from occurring, we recommend that when a site's average benthic Chl *a* exceeds **120 mg Chl *a*/m^2** it is too high, and should therefore be considered an impact to fish and associated aquatic life.

B.1.3 Benthic Algal AFDW Levels and Harm-to-use Thresholds

Ash Free Dry Weight (AFDW) collected from natural stream-sediment surfaces is a useful measurement for estimating algal biomass. The laboratory method basically oxidizes and reports back the mass of all organic material in the sample (American Public Health Association, 1998). It is useful in that it provides an additional means of assessing accumulated algal biomass independent of Chl *a.* Chl *a* levels tend to be highest during peak growth, and then decline later as the Chl *a* molecules degrade as the algae senesce (Stevenson, et al., 1996). If an assessor samples a stream late in the season, they may find fairly low Chl *a* values in spite of the presence of a large biovolume of algal material. Thus, a site that may truly have an excess algae problem could potentially be assessed as unimpaired simply due to the fact that the samples were collected late in the season.

For this reason, we recommend that AFDW be determined for all samples when Chl *a* is collected. AFDW can be determined from the same sample in a subsequent analysis that follows the Chl *a* analysis Site average. AFDW can be determined from individual replicates, or as a weighted average.

Note: AFDW results from core samples should <u>never</u> be included in determining a site's average AFDW. The method measures organic material from the entire core sample, not just the surface where the algae are growing, and will therefore over-report AFDW.

DEQ has not collected AFDW using the 11-transect method long enough to be able to carry out the type of statistical uncertainty calculations used for Chl *a* (Appendix A of (Montana Department of Environmental Quality, 2011c). However, there are good estimates of what comprises too much algal AFDW. In Suplee et al. (2009), the threshold Chl *a* level of 150 mg/m^2 corresponds to 36 g AFDW/m^2 In New Zealand, extensive analysis of algal AFDW resulted in a recommendation of 35 g AFDW/m^2 as the maximum level for gravel/cobble streams, to protect recreation use (Biggs, 2000). Note in **Table B1-1** above that the late season AFDW corresponding to 127 mg Chl *a*/m^2 (the Chl *a* level linked to the late-season DO problems) is 33 g/m^2 Long -term monitoring in the Clark Fork River (1998-2009) shows that the average summer AFDW at sites that do not develop nuisance algae (i.e., they are consistently <150 mg Chl *a*/m^2) ranged from 17 to 48 g AFDW/m^2 (mean: 27 g AFDW/m^2). Given the values presented, we recommend that site average AFDW (i.e., mean of the 11 replicates collected at a site, replicates being only templates or hoops) should be no greater than **35 g AFDW/m^2.** This value should be protective of both fish and aquatic life and recreation uses.

B.1.4 Some Additional Considerations Regarding Benthic Algae Sampling

Recently, DEQ has instituted an economization practice that consolidates all hoop, core, or template samples from a sampling event together, so that only three (at most) Chl *a* samples need to be analyzed,

instead of eleven. While unquestionably thrifty, the ability to determine the replicates' variance has been lost. All of the Chl *a* confidence calculations discussed in **Section B.1.1** assume that a sampling event will manifest a typical replicate CV of 73%, but this is an assumption In cases where it is very important to truly know the replicates' CV, the replicates should each be analyzed separately..

Cases may also arise where an entity is not satisfied with the level of confidence or interval widths DEQ has presented here. Collecting 11 samples in a stream reach is already a time consuming and expensive procedure, and we consider the confidence level (80%) and interval width (± 30% of the mean) to be satisfactory for algae sampling. If an entity (regulated or otherwise) desires higher levels of precision, than it is our recommendation that the financial cost to achieve those levels fall to the entity.

If more precision is wanted, how many more algae samples should be collected? Long term sampling of benthic Chl *a* by Dr. Vicki Watson on the Clark Fork River shows that with about 20 replicates, one can be 90% confident that the measured average Chl *a* is within ± 20% of the true average. For benthic algae sampling, which is inherently noisy, this is a fairly high degree of confidence. For DEQ's wadeable stream method, this would involve placing 20 transects instead of 11 along a site, with algae collection occurring at each of the 20 transects using the systematic approach (R, L, C, repeat) described in the SOP.

B.2 Assessment Using Biometrics

DEQ has used diatom-algae assemblages and macroinvertebrate assemblages for many years to make assessment of stream water quality and condition. Some of these metrics are being incorporated into the process for assessing excess nitrogen and phosphorus pollution. Details of each are given in the **Sections B.2.1** and **B.2.2** below.

B.2.1 Biometrics Based on Diatom Algae

DEQ has been using benthic diatoms to assess water quality since the 1970s. Earlier approaches used diagnostic and descriptive biometrics based on quasi-universal ecological attributes of diatom species and observed structural characteristics of benthic diatom associations (Bahls, et al., 2008). The current approach (initiated in 2004) uses regional classification, stream reference sites, a priori knowledge of stressors in streams, and discriminant function analysis to identify "increaser" taxa that respond to specific stressors and in a predictable way (Teply and Bahls, 2006; Bahls, et al., 2008; Tepley and Bahls, 2005). The metrics were specifically developed to indicate the likelihood of nitrogen and phosphorus impairment, have been developed for many regions of the state, and can function properly in the presence of other major pollutants (Teply, 2010a; Teply, 2010b). Please see the periphyton SOP (Montana Department of Environmental Quality, 2011b) for details. Each sample will provide the probability of a nutrient problem, such as in this example:

> *This indicates that the sample represents a stream that has about a 65% percent probability of being impaired due to nutrients (nitrogen or phosphorus) under 303(d) guidelines. This probability is based on past evidence of taxa associated with nutrient-impaired streams in the Northern/Canadian Rockies Stream Group. Nutrient Increaser Taxa do not discriminate other causes of impairment and this result does not*

indicate whether the stream may or may not be impaired due to other causes.

Diatom nutrient-increaser metrics are available for the Northern and Canadian Rockies ecoregions, the Idaho Batholith ecoregion, and a series of level-IV ecoregions that predominate along the Rocky Mountain Front (i.e., mountain-to-plains transitional zones). **Note: There is currently no validated nutrient-increaser model for use in the Middle Rockies ecoregion.** As of this writing, a sample that indicates **>51%** probability of impairment by nutrients should be considered to indicate the sample is from a site with excess nutrient problems. Findings based on diatom samples are not, however, stand alone, and need to be incorporated with other data per the decision framework described in **Section 3.0** of the main document.

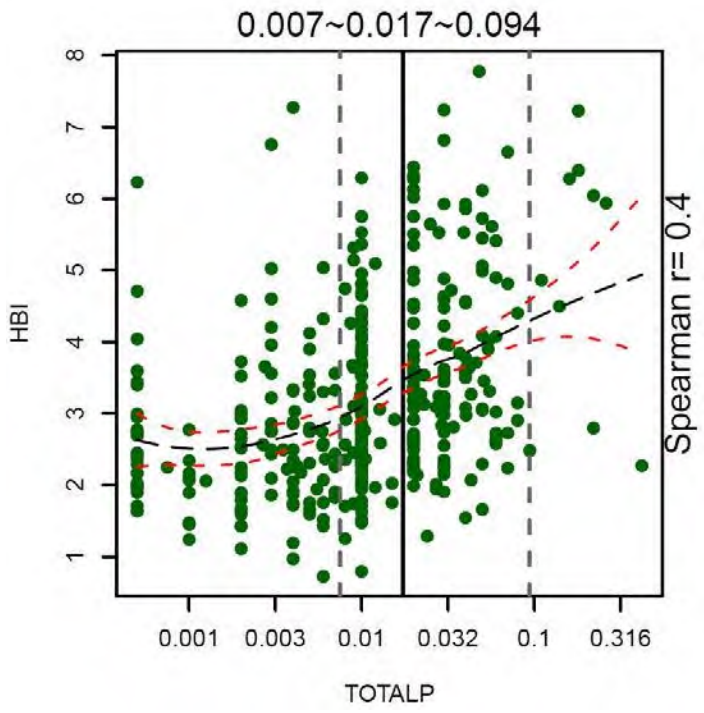
B.2.2 Biometrics Based on Macroinvertebrates

Benthic macroinvertebrates have been used for a long time as indicators of stream water quality (e.g., (Hilsenhoff, 1987; Barbour, et al., 1999). Recently, DEQ and EPA carried out a correlation analysis using Montana data to examine the relationship between stream nutrient concentrations and benthic macroinvertebrate metrics (Tetra Tech Inc., 2010). Among the metrics, one (Hilsenhoff Biotic Index, or HBI) is sufficiently well understood and showed a sufficiently patterned response to stream nutrient gradients in Montana's mountainous regions that we believe it can be used as a secondary response variable to help assess nutrient impacts. How the metric will be incorporated with other effect variables was discussed in **Section 3.0.** We here define a biological threshold for the HBI metric, giving consideration to the fact that almost all mountainous streams in Montana are to be maintained suitable for "growth and propagation of salmonid fishes and associated aquatic life" (A-Closed, A-1, B-1, C-1 classes), or "growth and marginal propagation of salmonid fishes and associated aquatic life" (B-2, C-2 classes).

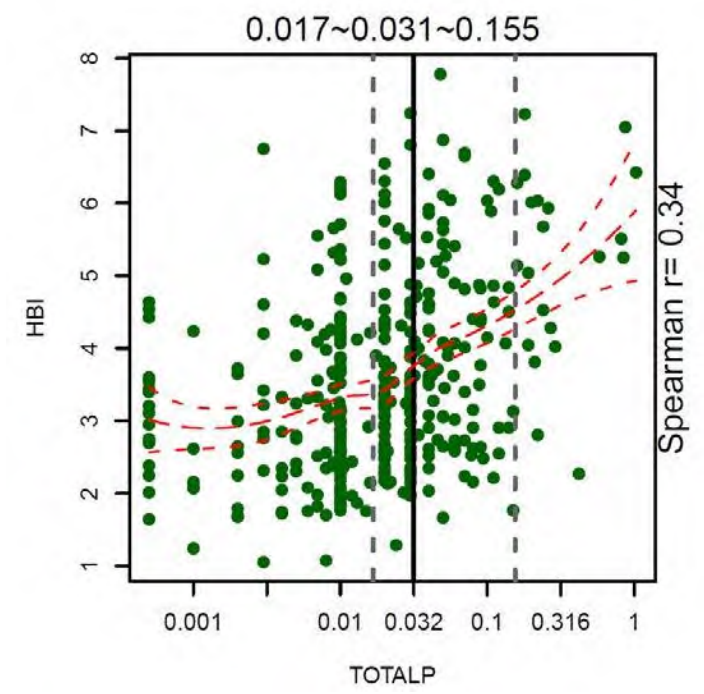
B.2.2.1 Hilsenhoff Biotic Index

HBI is based on tolerance values. A large number of macroinvertebrate taxa have been assigned a numeric value which represents the organism's tolerance to organic pollution (Barbour et al., 1999). HBI is then calculated as a weighted average tolerance value of all individuals in a sample. Higher index values indicate increasing tolerance to pollution.

Figure B2-1(A) shows the HBI *vs.* TP correlation in mountainous-region streams (Tetra Tech Inc., 2010). The data are from the "Mountains" site class (a.k.a. Mountains bioregion)(Montana Department of Environmental Quality, 2006). The Mountains Bioregion comprises stream sites whose catchments are mainly in the Middle Rockies, Canadian Rockies, Northern Rockies, and Idaho Batholith ecoregions and where elevation is greater than 1700 m, precipitation is greater than 700 mm/year, and annual mean daily maximum temperature is < 11°C. Also shown is the same data, but this time aggregated simply by level III ecoregion rather than bioregion (**Figure B2-1(B)**); note the very similar patterns. This indicates that ecoregions and bioregions work about equally well as geospatial frameworks to segregate macroinvertebrate data for the purpose of correlation to stream nutrient concentrations.



A. Mountains bioregion.



B Middle Rockies ecoregion.

Figure B2-1. HBI metric vs. TP.

The relationship between HBI and TN is shown below (**Figure B2-2**), aggregated by ecoregions (only Middle Rockies ecoregion shown). As for TP *vs*. HBI, there is a noisy but discernable (and significant) relationship between nutrients and the HBI score.

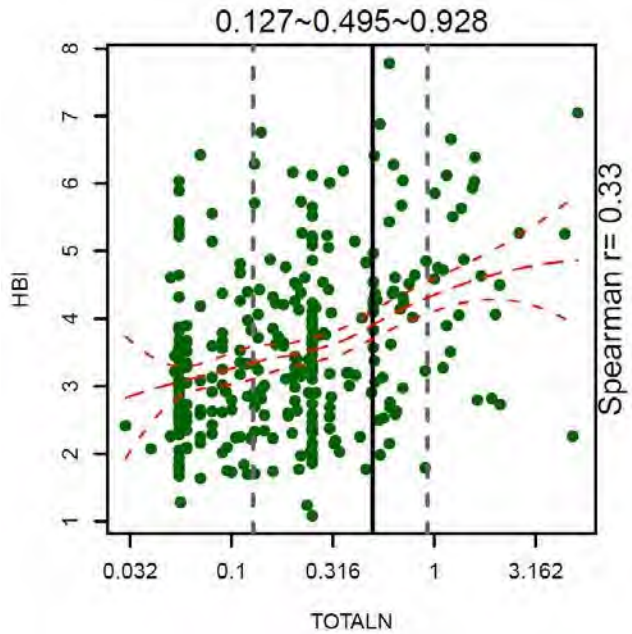


Figure B2-2. HBI Macroinvertebrate Metric vs. Stream TN Concentration Data are aggregated by ecoregions (Middle Rockies ecoregion shown).

Several components of **Figures B2-1 (A) and (B)** and **Figure B2-2** require explanation. Change-point analysis (Qian, et al., 2003) shows the statistically-derived point in the dataset where a shift, or threshold, in Y has occurred relative to X. In the figures, it is the vertical black line, bracketed to the right and left by its 90% confidence interval as dashed grey lines. This change-point, ± its 90% confidence interval, is shown as a numeric value at the top of each figure. The curving dashed lines running left to right are the locally-weighted regression line (LOWESS) and associated 90% confidence limit as dashed red lines. The Spearman's *rho* correlation value (Conover, 1999) between nutrient and metric is shown on the right side of each figure.

B.2.2.2 Interpreting the Macroinvertebrate Metric Correlations to Nutrients

Although **Figure B2-1** and **Figure B2-2** demonstrate significant correlations (parametric least-squares regression), they show large amounts of scatter. Numerous factors contribute to this scatter. For example, macroinvertebrates are separated from the direct effects of nutrient increases by one trophic level (i.e., nutrients directly influence aquatic plants and algae, and changes in plant species and biomass in turn influences macroinvertebrates). This is why they are considered secondary data, per the **Section 3.0** decision framework. In addition, environmental factors (natural and human-caused) other than nutrients influence macroinvertebrate populations, adding to the scatter in the relationships between the metrics and the nutrients. This is especially true for these data, which have been compiled over relatively large spatial areas and incorporate many different streams sampled over a long period of time (> 15 years). In spite of the scatter, there are patterns that can be discerned. Note, for example, that when TP is greater than 0.15 mg/L in **Figure B2-1(B)**, the likelihood of a stream having an HBI score <4 is very low.

The following section provides more detail on how **Figure B2-1** and **Figure B2-2** (and related data) were used to interpret the macroinvetebrate metrics relative to nutrients.

B.2.2.3 Patterns Observed Between HBI and Change-points, and Previously Used DEQ Thresholds

DEQ has in the past used thresholds for stream impairment using the HBI metric (Bukantis, 1998). Mountainous and intermountain valley regions used HBI values of 3.0 and 4.0, respectively, as the threshold values between full support and impairment of aquatic life (Bukantis, 1998). Hilsenhoff (1987) notes that the transition between Very Good water quality (slight organic pollution) and Good water quality (some organic pollution) is an HBI score of 4.5. How do the thresholds relate to the data seen in **Figures B2-1** and **Figure B2-2**? Results are shown below in **Table B2-1,** and include data from the Middle Rockies, the Mountains Bioregion, and the Low Valleys Bioregion.

Table B2-1. Nutrient Concentrations on the LOWESS Regression Line Corresponding to Specified Thresholds.
(Statistical Changepoint or Macroinvertebrate Metric Value) for **Figures B2-1, B2-2**, and Similar Graphs. Data are grouped by bioregions and ecoregions. Only Middle Rockies ecoregion shown due to insufficient data on other ecoregions.

			Corresponding Nutrient Concentration	
MT Bioregion	**Parameter**	**Metric Threshold Value**	**TP (mg/L)**	**TN (mg/l)**
Mountains bioregion	**Changepoint**		**0.02**	**0.46**
Mountains bioregion	HBI	4.0	0.07	1.15
Mountains bioregion	HBI	4.5	0.19	beyond graph
Low Valleys bioregion	**Changepoint**		**0.04**	**0.32**
Low Valleys bioregion	HBI	4.0	0.04	0.32
Low Valleys bioregion	HBI	4.5	0.19	1.35
Middle Rockies Ecoregion	**Changepoint**		**0.03**	**0.50**
Middle Rockies Ecoregion	HBI	4.0	0.04	0.55
Middle Rockies Ecoregion	HBI	4.5	0.16	1.7

One observation that can be made about **Table B2-1** is that, in any given region, HBI scores of 4.5 can correspond to nutrient concentrations much higher than the corresponding changepoint concentrations, whereas nutrient concentrations matching an HBI of 4.0 usually match fairly closely to the changepoint concentrations. These results suggest that an HBI score of 4.0 is a meaningful threshold relative to stream nutrient concentrations in western Montana streams. This conclusion stems from the fact that an HBI of 4.0 has previously been recommended as a threshold by DEQ for this region (Bukantis 1998), and that the data show a statistically-significant threshold (i.e., a change in biological structure relative to nutrients) at an HBI of 4.0. An HBI score of 4.0 is also meaningful from the perspective of water quality protection, as scores of 4.5 indicates transition into conditions where some organic pollution is already noted (Hilsenhoff, 1987). Thus, if elevated nutrients are suspected and an HBI score of >4 is encountered, there is a good chance that excess nutrients are causing the problem.

APPENDIX C. DETAILS ON ASSESSMENT METHODOLOGIES FOR WADEABLE PLAINS STREAMS

Benthic algae levels discussed in **Appendix B**, **Section B.1.1** are appropriate for the mountainous and transitional (mountain-to-plains) region of the western part of the state. Many eastern Montana plains streams are ecologically different from western Montana streams, and therefore the results from the public perception algae survey (Suplee et al., 2009) should probably not be universally applied to them. Montana plains streams often become intermittent, are generally low gradient, commonly have mud bottoms and are often turbid, and frequently have substantial macrophyte populations. It is not uncommon in these streams to see macrophytes intermixed with filamentous algae and floating masses of green algae; these conditions are even occasionally observed in plains streams minimally impacted by people (i.e., plains reference streams). These situations make measurement of benthic algal biomass in plains streams difficult and complicated. Further, our analysis shows that 4% of the sampling-event averages from plains reference streams have benthic algae >150 mg Chl *a*/m^2, whereas none of the sampling-event averages for benthic algae in western Montana reference streams even approach this value (e.g., the highest sampling-event average for a western Montana reference site was 76 mg Chl *a*/m^2). These findings, taken together, suggest that benthic algae measurement is probably not the best assessment tool for determining impairment for plains region streams. As such, we recommend that benthic algal biomass not be used to assess plains streams.

The following sections discuss the assessment tools we believe are more appropriate for assessing wadeable streams of the plains region.

C.1.0 ASSESSMENT USING DIATOM ALGAE BIOMETRICS

Nutrient-increaser diatom metrics have been developed for plains wadeable streams using the same methods as the diatom metrics presented in **Appendix B.** The metrics will indicate the likelihood of nitrogen and phosphorus impairment, and can function properly in the presence of other common pollutants such as sediment and metals (Teply, 2010a; Teply, 2010b). Please see DEQ's periphyton SOP (Montana Department of Environmental Quality, 2011b) for details. Each periphyton sample will provide the probability of a nutrient problem, such as in this example:

> *This indicates that the sample represents a stream that has about a 25% percent probability of being impaired due to nutrients (nitrogen or phosphorus) under 303(d) guidelines. This probability is based on past evidence of taxa associated with nutrient-impaired streams in the Warm-water Stream Group. Nutrient Increaser Taxa do not discriminate other causes of impairment and this result does not indicate whether the stream may or may not be impaired due to other causes.*

As of this writing, a sample that indicates **>51%** probability of impairment by nutrients should be considered to indicate the sample is from a site with excess nutrient problems. Findings based on diatom samples are not, however, stand alone, and need to be incorporated with other data per the decision framework described in **Section 3.0** of the main document.

C.2.0 Assessment Using the Difference Between the Daily Maximum Dissolved Oxygen Concentration and the Daily Minimum Dissolved Oxygen Concentration (Delta)

We initially considered using DEQ's DO standards for routine assessment of plains streams. But examination of long-term DO datasets, including those from the plains-stream dosing study (**Appendix B, Section B.1.2**), showed that DO standards are a fairly insensitive way to assess nutrient impacts in plains streams. We found only a low number of instances where streams that we know have excess nutrient impacts consistently violated the DO standards, and some nutrient-impacted stream sites never violated the DO standards at all (at least during summer and early fall).

We found that streams that have high daily DO **delta** (i.e., the daily maximum DO minus the daily minimum DO) may *eventually* manifest DO standards violations late in the year, when the algae die and decompose en masse (see **Appendix B**, **Section B.1.2**). To address this, the DO monitoring-period could be extended (e.g., to the end of October or early November), but this is not always practical given the unpredictable onset of winter and its affect on road access, retrieval of deployed instruments, etc. In lieu of extending the monitoring season, measurement of summer and early fall DO deltas has great potential as an assessment tool. Others have found that DO delta is related to harm to aquatic life. In Minnesota, strong positive correlations are found between the percent tolerant fish and the magnitude of the DO deltas. At DO deltas <4.5 mg/L, tolerant fish are usually <10% of the total fish population, but when DO deltas are > 4.5 mg/L tolerant fish become a substantial proportion of the population. Conversely, sensitive fish exhibit a wide range of values at DO deltas <4 mg/L, but above 4.5 mg/L they decline to 10% or less of the fish population(Minnesota Pollution Control Agency, 2010). The state of Minnesota is recommending that, for the northern plains regions at its southern end, measured DO deltas should not exceed 5.0 mg/L.

DO delta is also shown to be lower in reference streams. In Tennessee, the maximum DO delta value reported in a wadeable reference stream is 4.0 mg/L, whereas about 45% of impacted streams assessed have measured DO deltas greater than 4.0 mg/L (Arnwine and Sparks, 2003).

We calculated DO deltas for Montana plains <u>reference</u> streams. There were a total of 177 day's worth of delta values from the Box Elder Creek Control reach (Montana Department of Environmental Quality, 2010a) and the Little Beaver Creek Reference Site, collected in 2009 and 2010. 90% of the daily DO deltas from these reference sites were less than 5.3 mg/L. The single highest DO delta measured was 6.6 mg/L, from Little Beaver Cr, which is influenced by the presence of macrophytes. (Also, on about 10% of occasions, DO at the Little Beaver Cr reference site dropped just below the juvenile fish standard of 5.0 mg DO/L; however, it never got close to the adult fish DO standard of 3.0 mg/L.)

No fish data were collected contemporaneously with the reference site DO data, however fish populations have been evaluated in both of these streams (Bramblett, et al., 2005) at alternative reference sites (BoxElder_382_W and LittleBe_410_W) not far downstream. These alternative reference sites had among their fish populations substantial proportions of sensitive/intolerant species, especially Box Elder Creek in the Little Beaver Creek site 17% of the fish captured were considered sensitive/intolerant. Sensitive/intolerant species are typically the first species to disappear due to chemical and physical perturbations (e.g., low DO)(Barbour, et al., 1999). Assuming DO patterns at the alternative reference sites are roughly comparable to those which we monitored, the fish data suggest

that a healthy fishery is being maintained in spite of occasionally high DO deltas and occasional exceedances of the juvenile fish criterion[19].

We employed change-point analysis (Qian, et al., 2003) to help identify any DO delta thresholds. We had 11 plains-stream locations, in both reference (Suplee, et al., 2005) and non-reference condition, which had continuous instrument-measured DO data (**Table C2-1**). These sites comprised both perennial and intermittent streams. Delta values were calculated, resulting in over 550 days of DO delta values. Each location was assigned a rating (1 through 4) representing our BPJ assessment of how strongly it was impacted by nutrients, and these ratings were associated with the corresponding DO delta values. The ratings used were: 1 = no known nutrient impact; 2 = low nutrient impact; 3 = medium nutrient impact; 4 = high nutrient impact. In quite a few cases the ratings could be very accurately assigned, as some sites were reference sites and some were part of the nutrient dosing study (e.g., all DO deltas associated with the High-dose reach were assigned a rating of 4). Change-point analysis was then run on the dataset with delta values on the X axis and their corresponding rating scores on the Y A highly significant ($p << 0.001$) change-point was identified at 6.0 mg/L (the 90% confidence interval for the change-point was 5.5 mg/L to 6.6 mg/L). Essentially, analysis showed that in moving from sites rated 3 to sites rated 4, the magnitude of the DO deltas ramped up dramatically, with the threshold of this change occurring at 6.0 mg/L.

Table C2-1. Long-term DO Monitoring Sites in Plains Streams

Station ID	Continuous Data Time Range	DO observations time-step (min)
Y26BOXEC08-upstream	Aug 25 to Sept 30, 2010	15
Y26BOXEC08-downstream	Aug 24 to Sept 30, 2010	15
Y26BOXEC04	July 26 to Sept 26, 2009 AND July 19 to Oct 7, 2010	15
Y26BOXEC09-upstream	Aug 25 to Sept 30, 2010	15
Y26BOXEC09-downstream	Aug 11 to Sept 30, 2010	15
Y27LBVRC02	Aug 30 to Sept 25, 2008 AND Aug 29 to Oct 8, 2010	15
Y27LBVRC04	Aug 30 to Sept 24, 2008 AND July 29 to Oct 8, 2010	15
M22CTWDC03	July 22 to July 24, 2003	30
M22BSPRC10	Aug 17 to Aug 20, 2003	30
Y27LBVRC12	Aug 30 to Sept 25, 2008	15
Y27LBVRC01	July 28 to Sept 24, 2009	15

We then estimated false-positive and false-negative rates, and made comparisons to the reference data, using the datasets above. Data were aggregated to create two basic groups (ratings 1, 2 = nutrient un-impacted; ratings 3, 4 = nutrient impacted), and 65 observations were then randomly drawn for false positive/negative analysis (35 from the un-impacted group, 30 from impacted group). Results are shown in **C2-2** below.

[19] Dr. Robert Bramblett (MSU fishery biologist; personal communication, March 11, 2010) provided species counts and IBI scores for the two sites Box Elder Creek's score was quite good (77) Dr. Bramblett noted that the Little Beaver Creek site's IBI was being reduced (score 55) by the presence of northern pike; this non-native predatory fish plays a large role in reducing metric scores in the Bramblett IBI He noted that the site's habitat was simple and northern pike were crowded in with their prey, but otherwise, the Little Beaver Creek site seemed healthy.

Table C2-2. Statistics Associated with DO Delta Threshold

DO Delta Threshold	% of all reference-site deltas > threshold	Estimated false positive rate*	Estimated false negative rate†
≥ 6.0	3%	9%	77%
≥ 5.3	**10%**	**23%**	**63%**
≥ 5.0	15%	26%	60%
≥ 4.0	26%	54%	53%

* The probability that a truly un-impacted site is found to have a DO delta value great than the threshold.
† The probability that a truly impacted site is found to have a DO delta value less than the threshold

The change-point threshold (DO delta of 6.0 mg/L) is probably too high for assessment, as it has a particularly high false-negative rate (77%; **Table C2-2**). Other DO thresholds between 6.0 and 4.0 mg/L were also evaluated. A DO delta threshold of 4.0 mg/L provides good balance between alpha and beta error, but its ability to determine impact is no better than a coin flip. It also allows far too many of the deltas from the reference sites to be exceedances (in fact, almost all observations from one reference site are >4.0 mg/L). The reality is, sites with excess nutrient problems do not manifest high DO deltas every single day throughout the summer, due to the vagaries of clouds, weather, and wind, which means that as an assessment tool DO delta will inherently have high false-negative rates. We selected 5.3 mg/L as the threshold for these reasons:

- It has false positive and negative rates comparable to what was found for the diatom-based nutrient increaser metrics applicable to this region(Teply, 2010b)
- It is very close to the lower bound of the 90% confidence interval (i.e., DO delta of 5.5 mg/L) of the change-point, as determined from the change-point analysis
- It keeps the proportion of reference-site data exceeding the threshold to no more than 10%
- It is in fairly good agreement with the threshold recommended by Minnesota for their plains region (i.e., DO delta of 5.0 mg/L)to protect fish and aquatic life

C.2.1 Instantaneous DO Monitoring in Wadeable Plains Streams

DEQ assessment can continue to rely on instantaneous measurements of DO. The following guidelines are recommended for instantaneous DO data collection.

When to Measure, Minimum: Without question the best time to measure the lowest daily DO is at dawn. DO in streams and standing waters is usually at its daily low just before sunrise (e.g., (Odum, 1956; Teply, 2010b; Boyd, et al., 1978; Madenjian, et al., 1987; Quinn and Gilliland, 1989). DO measurements in streams at other times of the day usually cannot give a reliable estimation of the nighttime low, especially in plains streams, because numerous other factors (e.g., wind speed and direction, air temperature, the stream's sediment oxygen demand, presence/absence of aquatic macrophytes) play a role in the rate of DO decline per unit time. Simple models incorporating various environmental factors have been used to estimate dawn DO in aquaculture ponds (e.g., (Boyd, et al., 1978; Madenjian, et al., 1987), and more sophisticated models can simulate diel DO cycles in streams and rivers (e.g., QUAL2K; (Chapra, et al., 2008). However, numerous input variables are required to run these models, making these impractical approaches for routine stream DO assessment. Therefore, we recommend dawn DO measurements be taken. We examined a number of plains stream diel DO plots, including both reference and non-reference sites, and found that **the most appropriate time window for capturing the DO daily minima is between dawn (or pre-dawn) and 8:00 am.** This time frame should be adhered to when sampling during the summer growing season (June 16th to September 30th).

The assessor should make notes of weather conditions at the time (approximate wind speed and direction, cloud cover).

When to Measure, Maximum: The DO concentration maximum in flowing streams usually occur after solar noon, commonly around 4:00 pm. The combined effects of plant/algae respiration, plant/algae primary production (which peaks around solar noon), and flow and re-aeration influences on DO saturation result in the typical sinusoidal DO patterns observed each day (Odum, 1956; Chapra and Di Toro, 1991). We examined the continuous recordings of DO for plains stream (both flowing and intermittent), and found that most daily DO peaks occurred between 2:30 pm and 5:00 pm. Time of year appeared to have no discernable effect, and the exact timing of the DO peak seemed more influenced by local factors (probably clouds, and wind velocity and direction). **We recommend that monitoring for the DO maximum occur between 2:30 pm and 5:00 pm.** Measurements need not be taken continuously during that period; checking stream DO every 15-30 minutes should be sufficient to catch the peak. You may need to stay somewhat beyond 5:00 pm if the values are still climbing. DEQ's main hand-held instrument for DO measurement is the YSI 85. This instrument has a 50 reading, manual-entry memory which can be used for collecting daily DO maximums. Set the instrument up in situ and then leave it on between 2:30 pm and 5:00 pm; record readings every 15-30 minutes by depressing the ENTER button for two seconds. Data may be downloaded later.

For the purpose of calculating DO delta, at least 3 DO sampling events should be taken in each assessment reach. Temporal independence of DO measurements is not a concern, since DO delta can be quite variable on a day-to-day basis. Each sampling event DO delta can considered on its own merits. Therefore, there is no reason to wait for 30 days to collect a subsequent DO measurement at a site. The assessor may collect DO data each day while they are in the area. This will also help increase the number of sampling events collected from an assessment reach.

C.3.0 Biochemical Oxygen Demand

Biochemical oxygen demand is one of the oldest water quality assessment tools, first recommended for use by the English Royal Commission on Sewage Disposal in the early 1900s (Hynes, 1966). It is a standardized test carried out over 5 days that measures the amount of putrescible material in water, which consumes oxygen as it decomposes. It is also one of the required measurements for wastewater nationally under the National Secondary Treatment Regulations (40 CFR part 133).

Montana has no standard for ambient BOD_5 in streams (although wastewater facility effluent and mixing zones are held to BOD_5 requirements). Nevertheless, the following guidelines for BOD_5 are commonly followed in many parts of the world:

- 1-2 mg BOD_5/L: Very clean water, little biodegradable waste
- 3-5 mg BOD_5/L: Moderately clean water, some biodegradable waste
- 6-9 mg BOD_5/L: Many bacteria, much biodegradable matter
- ≥10 mg BOD_5/L: Very bad, large amounts of biodegradable wastes in the water

The method used at the DPHHS Environmental Laboratory currently has a detection limit of 4 mg BOD_5/L (which coincides with the Royal Commission's recommendation that 4 mg BOD_5/L not be exceeded; Hynes, 1966). In plains streams we have found that otherwise healthy streams (i.e., reference sites) can have values in the 6-9 range fairly often. We recommend a value of 8.0 mg/L as a threshold for concern in plains streams.

APPENDIX D - REFERENCES

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ATTACHMENT E

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION III
1650 Arch Street
Philadelphia, Pennsylvania 19103-2029

SEP 23 2014

Ms. Melanie Davenport, Director
Division of Water Quality Programs
Virginia Department of Environmental Quality
629 E. Main Street
P.O. Box 1105
Richmond, Virginia 23218

Dear Ms. Davenport:

On December 12, 2013, the U.S. Environmental Protection Agency (EPA) partially approved the Virginia Department of Environmental Quality's (VADEQ) 2012 Section 303(d) list but took no action on the assessment status related to algal growth impacts to recreation uses in the North Fork Shenandoah River, South Fork Shenandoah River and mainstem Shenandoah River. EPA has completed its review of this issue. For the reasons set forth in the enclosed Rationale, EPA approves Virginia's 2012 Section 303(d) list including the previously deferred segments North Fork Shenandoah River, South Fork Shenandoah River and mainstem Shenandoah River.

EPA's decision to defer action on algal impacts to recreation uses in the Shenandoah River was, in part, to allow EPA more time to evaluate information submitted by the Shenandoah Riverkeeper and other available information. The Riverkeeper's information, in the form of visual observations and statements of users of the River, warrants serious consideration and further investigation and EPA has been working closely with the Commonwealth to ensure this occurs. EPA has requested that VADEQ work towards developing an assessment methodology and impairment threshold to evaluate algal impacts to recreation uses of Virginia's rivers and streams that can be used for future Integrated Reports.

EPA is aware that VADEQ is preparing the 2014 Section 303(d) list for public review. As we have for other cycles, EPA will be providing comments to Virginia on the draft 2014 list. EPA expects VADEQ to evaluate all readily available and applicable water quality information including information related to excess algal growth on North Fork Shenandoah River, South Fork Shenandoah River and mainstem Shenandoah River.

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We appreciate the continued cooperation from VADEQ staff in evaluating the water quality issues in the Shenandoah Basin. If you have any questions, please feel free to contact me, or have your staff call Ms. Evelyn MacKnight at (215) 814-5717 or Mr. Bill Richardson at (215) 814-5675.

Sincerely,

Jon M. Capacasa, Director
Water Protection Division

Enclosure

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RATIONALE FOR APPROVAL OF VIRGINA DEPARTMENT OF ENVIRONMENTAL QUALITY 2012 SECTION 303(d) LIST

I. Purpose

On December 12, 2013, the U.S. Environmental Protection Agency (EPA) partially approved the Virginia Department of Environmental Quality's (VADEQ) 2012 Section 303(d) list. EPA conducted a review of Virginia's 2012 Section 303(d) list and supporting documentation and information. Based on that review, with the exception of the assessment status related to algal growth impacts to recreation uses in the North Fork Shenandoah River, South Fork Shenandoah River and mainstem Shenandoah River (collectively referred to as the Shenandoah River), EPA determined that the Commonwealth's list of water quality limited segments (WQLSs) still requiring Total Daily Maximum Loads (TMDLs) met the requirements of Section 303(d) of the Clean Water Act (CWA or the Act) and EPA's implementing regulations. At that time, EPA deferred its final decision on the Section 303(d) listing status of the Shenandoah River with respect to algal growth impacts to recreation uses.

This constitutes EPA's rationale for approving the remainder of VADEQ's 2012 Section 303(d) list, specifically, the listing status of the Shenandoah River with respect to algal growth impacts to recreation uses. Parts I-III of EPA's December 12, 2013 Rationale for Approval of Virginia Department of Environmental Quality 2012 Section 303(d) List is incorporated herein by reference.

IV. Assessment Status of the Shenandoah River Related to Recreation Use as Impacted by Algal Growth

The Shenandoah River is located in northern Virginia and drains 1,955,982 acres of land. Land use is dominated by forest (56.0%) and agriculture (33.4%) with a portion of developed land (9.6%). The Shenandoah River basin is composed of three subbasins (8-digit United States Geologic Survey Hydrologic Unit Codes (HUC)). The three subbasins are the South Fork of the Shenandoah River (HUC 02070005), North Fork of the Shenandoah River (HUC 02070006), and the Shenandoah River (HUC 02070007). The North Fork Shenandoah River and South Fork Shenandoah River join in Front Royal, Virginia to form the Shenandoah River. The Shenandoah River flows into the Potomac River at Harpers Ferry, West Virginia. The Potomac River eventually drains to the Chesapeake Bay.

As part of the public comment process on VADEQ's draft 2012 Section 303(d) List, on April 23, 2012 the Shenandoah Riverkeeper provided VADEQ with information purporting to demonstrate impairment of Virginia's recreational (primary and secondary contact) use and a portion of Virginia's narrative water quality criteria due to excessive algal growth in the Shenandoah River and/or its North and South Forks (collectively "the River"). Subsequently, on October 16, 2012, the Shenandoah Riverkeeper and Earthjustice Legal Defense Fund (Earthjustice) provided the same information to EPA. The Shenandoah Riverkeeper and

Earthjustice then provided to EPA additional information that had not been provided to VADEQ on October 31, 2012, September 23, 2013 and November 7, 2013.

The additional information provided to EPA not provided to VADEQ consisted of a large number of photographs depicting what appear to be substantial algal growth at various locations on the River. The Riverkeeper's photographs include photographs taken along or in the river and aerial photographs. The Riverkeeper also provided statements by approximately 80 stakeholders asserting that their use and enjoyment of the River have been diminished by algal growth. The stakeholder statements were also supplied to VADEQ during the public comment period. The concerns expressed by the stakeholders include decreased fish population,[1] algal clumps on lures, fishing lines and hooks, slippery river bottom making wading difficult, difficulty kayaking, unpleasant aesthetic appearance, and unpleasant odor.

The Shenandoah Riverkeeper asserts that the photographs and testimonials establish that the Shenandoah River is impaired for primary and secondary recreation use as defined below, and does not achieve the following portions of Virginia's water quality standards:

9VAC25-260-5

"Primary contact recreation" means any water-based form of recreation, the practice of which has a high probability for total body immersion or ingestion of water (examples include but are not limited to swimming, water skiing, canoeing and kayaking).

Secondary contact recreation" means a water-based form of recreation, the practice of which has a low probability for total body immersion or ingestion of waters (examples include but are not limited to wading, boating and fishing).

9VAC25-260-20. General criteria.

A. State waters, including wetlands, shall be free from substances attributable to sewage, industrial waste, or other waste in concentrations, amounts, or combinations which contravene established standards or interfere directly or indirectly with designated uses of such water or which are inimical or harmful to human, animal, plant, or aquatic life.

Specific substances to be controlled include, but are not limited to: floating debris, oil, scum, and other floating materials; toxic substances (including those which bioaccumulate); substances that produce color, tastes, turbidity, odors, or settle to form sludge deposits; and substances which nourish undesirable or nuisance aquatic plant life. Effluents which tend to raise the temperature of the receiving water will also be

[1] While a number of the testimonials attached to the Riverkeeper's letter refer to fish kills, the Riverkeeper does not appear to be asserting that the algal blooms are causing an aquatic life impairment or are responsible for fish kills. TMDLs for PCBs and mercury to address fish consumption have been established for portions of the mainstem Shenandoah River and the North and South Forks, and Virginia's Section 303(d) list identifies other portions of the South Fork as impaired for fish consumption due to PCBs and mercury and in need of a TMDL.

controlled. Conditions within mixing zones established according to 9VAC25-260-20 B do not violate the provisions of this subsection.

V. Status of Algal Growth Impacts on Recreation in Shenandoah River on VADEQ's 2012 Integrated Report

For purposes of its 2012 Integrated Report, VADEQ identified the impact of algal growth on recreation use of the Shenandoah River under Part 2(B), which identifies "waters [that] are of concern to the state but no water quality standard exists for a specific pollutant, or the water exceeds a state screening value or toxicity test." Waters in this category remain a priority for monitoring and assessment.

In response to the information provided by the Shenandoah Riverkeeper as part of the public process, VADEQ stated that its approach for purposes of assessing the recreational use is based upon human health concerns and therefore is focused on bacteria levels.[2] Indeed, TMDLs for bacteria have been prepared for portions of the North and South Forks and mainstem Shenandoah River, while other portions remain listed as impaired for the recreation use due to excessive levels of bacteria. DEQ noted that the Riverkeeper was not asserting a human health concern, but rather advocating listing based upon an aesthetic component of the recreational use. DEQ asserted that it does not have a systematic method for collecting and evaluating the presence of algal growth to determine whether there is a subjective nuisance.

DEQ further noted that low levels of dissolved oxygen often are associated with excessive algal growth and that the low levels of dissolved oxygen that would be expected to accompany excessive algal growth were not documented in the River. According to DEQ, trends analysis indicated that levels of nutrients in the River (nitrogen and/or phosphorus, which are often a causative factor of excessive algal growth) were either stable or trending downward. DEQ stated that it was in the process of developing riverine freshwater nutrient standards that, once established, would facilitate identification and restoration of waters subject to excessive algal growth.

DEQ identified 17 TMDLs for tributaries to the Shenandoah River addressing either nutrients or sediment which, when fully implemented, would lower causative factors and therefore decrease algal growth in the River. In addition, DEQ pointed out that that the Chesapeake Bay TMDL includes allocations for individual and aggregate sources of nutrients to the Shenandoah River, and that Virginia's Watershed Implementation Plan (WIP) for the Chesapeake Bay TMDL calls for controls of point and nonpoint sources of nitrogen, phosphorus and sediment to the Shenandoah River watershed to achieve those TMDL allocations. While intended to address the impairments to the downstream Chesapeake Bay, the Chesapeake Bay TMDL and WIP controls should reduce sources of sediment and nutrients to the River and thus decrease causative factors for algal growth. DEQ noted that in 2011, the first year for compliance with Virginia's Chesapeake Bay Nutrient Discharge Watershed General Permit, "the Shenandoah basin's significant dischargers achieved substantial nutrient reductions. The total

[2] EPA guidance in its Water Quality Standards Handbook (2d ed.) (Section 2.1.3) focuses on bacteria in connection with protecting the recreational use.

nutrient loads discharged were well below their aggregate annual WLA -- Total Nitrogen (TN) was only 48% of the annual WLA and Total Phosphorus (TP) was just 67% of the annual WLA. Comparing 2011 to 2009 discharge figures, the TN load was reduced by 561,000 pounds per year; the TP load was 169,000 pounds per year lower." DEQ also described controls expected to be implemented by non-point sources that also should have the effect of lowering nutrient availability in the watershed.

Finally, due to lack of methodology and rigorous quality assurance/quality controls, DEQ categorized the Riverkeeper's information as Level II citizen monitoring data. As stated in VADEQ's *2012 Water Quality Assessment Guidance Manual*, only Level III data is used in Virginia for listing determinations. Level II data may be used to establish an observable effect. In this case, DEQ stated: "We believe it is appropriate to recognize these sections of the river as having an observed effect of aquatic algae. Therefore, we propose to modify the 2012 Draft Water Quality 305(b)/303(d) Integrated Report accordingly and list these waters under VA Category 2B ("*waters are of concern to the state but no water quality standard exists for a specific pollutant, or the water exceeds a state screening value or toxicity test*") for the recreational use. This designation means that these areas will remain a priority for monitoring and assessment in the future and will be evaluated when water quality standards related to nutrients and supporting indicators are available for free flowing rivers and streams."

VI. EPA's Actions Taken in Response to Shenandoah Riverkeeper's Concerns

To improve data and information on water quality and address the need for an assessment methodology to evaluate algal impacts to recreation uses, VADEQ and EPA have agreed to cooperate in a pilot study to develop a means to evaluate spatial and temporal extent of algal growth in Virginia's non-tidal flowing waters in a quantitative and repeatable way. EPA has provided $80,000 in funding for the study, which is ongoing and being performed by the Interstate Commission for the Potomac River Basin (ICPRB) and be conducted on the Shenandoah River. ICPRB is utilizing citizen scientists to collect algal cover data at pilot sites on the Shenandoah River in an effort to develop a repeatable assessment methodology that will provide data that can be used to systematically assess algal impacts to the recreation use. To date, ICPRB has trained members of the Friends of the Shenandoah River and Friends of the North Fork (Shenandoah River) on algal cover measurement techniques and the volunteers have been collecting algal cover data throughout the summer of 2014. ICPRB staff are in regular contact with citizen scientists to track progress and answer questions. A final report that includes results of the pilot study and an assessment methodology that can utilized by VADEQ is expected by early 2015. The assessment technique being developed by ICPRB is using a similar methodology that was developed for West Virginia as a model. West Virginia has successfully employed the approach for assessing algal impacts to recreation uses on four West Virginia waterbodies. EPA recommends and has offered to fund a Virginia specific user survey to better understand the amount and duration of algal growth that impacts recreation of the river user population as a whole.

VII. Analysis of Algal Growth Impact on the Shenandoah River for VADEQ's 2012 Section 303(d) List

In compiling their Section 303(d) lists, States must assemble and evaluate all existing and readily available data (40 C.F.R. 130.7(b)(5)); identify all waterbodies that fail to meet currently applicable water quality standards (33 U.S.C. 1313(d)(1)(A)); and submit a biennial list of such waters to EPA for approval (40 C.F.R. 130.7(d)(1)). *Cf. Sierra Club, Inc. v. Leavitt,* 488 F.3d 904, 913-14 (11th Cir. 2007) (state cannot avoid obligation to assemble and evaluate all existing and readily available data through state law limiting age of data that can be considered). In this instance, it is unclear from DEQ's response whether it has evaluated the information provided by the Riverkeeper. On the one hand, it appears that VADEQ identified an observable effect and placed the River in Category 2(B) of its Integrated Report based upon the Riverkeeper's information. On the other hand, VADEQ also appears to have stated that it declined to evaluate the information due to lack of a systematic way to identify and evaluate data on algal blooms and due to a policy decision to limit assessment of the recreation use to human health, rather than aesthetic, concerns.

While a State has discretion on how to consider the value (weight) of certain data based upon issues such as a lack of quality assurance/quality control, the State may not decline to evaluate data at all. Since it is unclear whether DEQ has evaluated the Riverkeeper's information as required by 40 CFR 130.7(b)(5), EPA has an obligation to take action to ensure that the federal requirement that all data be evaluated is satisfied.

Accordingly, EPA has evaluated the information provided by the Riverkeeper. Certain information provided to EPA was <u>not</u> provided to VADEQ as part of the public notice process. VADEQ gives members of the public at least two opportunities to submit data in connection with every Section 303(d) list cycle: an initial data call prior to development of the draft list and public notice and comment on the draft list. EPA strongly encourages members of the public to avail themselves of the processes provided by VADEQ. Circumstances where information is given to EPA for consideration outside the processes provided by VADEQ should be limited so as to preserve the integrity of the established public process.[3]

For the reasons set forth herein, EPA does not believe that the information provided in connection with the 2012 Section 303(d) List by the Riverkeeper provides an adequate record to support a determination by EPA to disapprove Virginia's 2012 Section 303(d) List. As further described below, however, EPA expects that DEQ make material progress toward identifying a means for evaluating information on algal growth in the Shenandoah River against the applicable water quality standards. If additional necessary information is provided in future listing cycles, and DEQ has not made progress toward identifying a means to evaluate that information, EPA will consider those factors in future Section 303(d) list reviews.

[3] EPA also notes that, for purposes of its 2012 Section 303(d) List, Virginia sought data reflecting water quality conditions for the time period through December 31, 2010. Some of the information provided by the Riverkeeper purports to represent river conditions outside the time period covered by Virginia's 2012 Section 303(d) List.

As a general matter, EPA agrees that visual observation and statements by water users can support a determination that one or more narrative criteria or designated uses are not being achieved, provided there is sufficient information to allow the State or EPA to identify the spatial and temporal extent of the impairment and provided such evidence is generally consistent with other evidence in the record. In this instance, however, the existing and readily available information when viewed as a whole presents a mixed picture. The materials supplied by the Riverkeeper provide information that there are at certain times substantial algal growth at certain locations in the River and that certain users have experienced diminished use and enjoyment of certain areas within the River. Other information, however, indicates that at least some portions of the River do not appear to suffer from excessive algal growth. Specifically, conditions consistent with what would be expected to occur in association with the types of large, long-lasting algal growth, described by the Riverkeeper's submission do not appear present in portions of the River. In addition, aerial photographs provided by the Shenandoah Riverkeeper, while providing supporting evidence, do not by themselves establish algal presence as it is difficult to distinguish in aerial photographs potentially impairing algal growth from beneficial submerged aquatic vegetation.

EPA recognizes that ambient water quality sampling locations may not fully coincide with the locations of algal growth depicted in the information supplied by the Riverkeeper. Nevertheless, the existing and readily available information presents a mixed picture. On the one hand, the Riverkeeper asserts the entire 244 miles that comprise the Shenandoah mainstem and its North and South Forks are impaired by substantial algal growth. On the other hand, other data, such as information related to dissolved oxygen levels in portions of the River, appear inconsistent with the effects of long-term and substantial algal growth at certain locations. Written and verbal statements provided by the Riverkeeper also present a mixed picture. On the one hand, the Riverkeeper has provided statements by stakeholders that the algal growth lasts for long periods of time and that their use and enjoyment of the River have been diminished. On the other hand, the Riverkeeper acknowledges that portions of the River continue to be used for fishing, boating and swimming. The existing and readily available information does not provide EPA with a basis for determining spatial and temporal extent of impairment, including which portions of the River suffer from the effects of long-term substantial algal growth and which do not.

VADQ has established 43 assessment units along the 244 miles of the mainstem Shenandoah River, North Fork Shenandoah River and South Fork Shenandoah River. There are eight assessment units on the mainstem Shenandoah River comprising a total of approximately 38 river miles. The South Fork Shenandoah River contains 13 assessment units along approximately 101 river miles. The North Fork Shenandoah River contains 22 assessment units encompassing approximately 105 river miles.

The Riverkeeper has not asserted that the algal growth presents a threat to human health, nor is EPA aware of any potential human health threat. Rather, the Riverkeeper asserts that the impairment of the aesthetic component of the narrative water quality criteria has diminished recreational use and enjoyment of the River ("interfere ... with designated uses"; "undesirable or nuisance aquatic plant life"). EPA generally agrees that the views of users of a waterbody may be considered in determining whether the aesthetic enjoyment of the waterbody has been so

impaired as to interfere with a designated recreational use. Different individuals, however, may have different tolerance levels for the presence of excess algal growth when engaged in recreational activities. Accordingly, different individuals may consider different amounts of algae as interfering with their use and enjoyment of the River or as presenting a nuisance. Here, the Riverkeeper has presented communications from approximately 80 individuals who responded to outreach from the Riverkeeper. The approximate population living in counties surrounding the River is 370,000 people, and that number likely is increased with users who live outside these counties and travel to utilize the River. It is unclear whether the method used by the Shenandoah Riverkeeper to collect these 80 user statements yields a representative sample of the views of the general population of users of the River. This is one reason why EPA proposes that a user survey be conducted. In addition, while the Riverkeeper has provided a map showing the locations of complaints regarding river conditions received and the seasons in which those complaints occurred, there is little information to determine the temporal duration of the observations, as it is acknowledged that rainfall generally scours algal growth.[4]

Because the concept of nuisance is one generally determined by state common law, EPA generally prefers that the State make determinations in the first instance related to whether subjective and aesthetic water quality conditions are being achieved or whether levels of algae present a "nuisance" or "interfere .... with" the recreational use of their waters by their citizens.[5] Nevertheless,, states may not indefinitely defer making such a determination by postponing methodology development, especially when presented with evidence that there may be adverse water quality conditions.

Accordingly, EPA has evaluated the information supplied by the Riverkeeper and determined that it provides evidence of substantial algal growth at certain locations for an unclear duration. Other information would seem to indicate that certain portions of the River do not appear to reflect conditions indicative of excessive algal growth. It remains unclear whether the views of the individuals submitted by the Riverkeeper are representative of those of the general population of users of the River.

The Riverkeeper's information warrants serious consideration and further investigation. Moreover, this is the second list cycle in which the Riverkeeper has presented information. Virginia may not indefinitely postpone evaluation of use impairment when it is presented with information in the form of visual observations and statements by users of the River. EPA anticipates that the Riverkeeper may provide additional information as part of the 2014 and 2016 listing cycles that will address some of the record issues identified here by EPA. It is EPA's expectation that Virginia will take concrete steps toward developing a method to evaluate the visual and testimonial information related to excess algal growth on the Shenandoah mainstem and its North and South Forks that may be provided for the 2014 and future list cycles. If information sufficient to determine the spatial and temporal extent of substantial algal growth

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[4] EPA does agree that some evidence of duration can be inferred where the size of the algal bloom can be ascertained from the photographs.

[5] In this way, measurement of the recreational use differs from the aquatic life use. As to aquatic life, there are measures, such as multi-metric indices, which allow impacts to the naturally occurring aquatic community to be objectively measured.

and substantial interference with primary and secondary contact recreation by the general population of users is provided and Virginia has not made substantial progress toward developing a method for evaluating such information, EPA will consider those factors in connection with future Section 303(d) lists.

EPA anticipates that ICPRB will outline an assessment methodology that will improve EPA and DEQ's ability to evaluate spatial and temporal extent of algal growth and its impact on River enjoyment and can be applicable to other non-tidal flowing waters in Virginia.
In any case, it is EPA's expectation that DEQ will commit to taking concrete steps and develop a timeline for evaluating algal growth impacts on the recreation use in flowing non-tidal waters. Such steps could include incorporating the assessment methodology provided by ICPRB into DEQ's assessment methodology that is used for Section 303(d) Listing. For an assessment methodology that evaluates algal impacts to recreation uses to be complete, EPA encourages VADEQ to develop an impairment threshold that can be used as part of the assessment methodology for 303(d) listing decisions.

ATTACHMENT F

Shenandoah Riverkeeper
P.O. Box 405
Boyce, VA 22620
540.837.1479
jeff@shenandoahriverkeeper.org
www.shenandoahriverkeeper.org

September 23, 2010

Darryl M. Glover
Water Quality Monitoring and Assessment Manager
Virginia Department of Environmental Quality
P.O. Box 1105
Richmond, VA 23218-1105

Delivered via Email Attachment: Darryl.Glover@deq.virginia.gov; dmglover@deq.virginia.gov

RE: Comments on 2010 305(b)/303(d) Integrated Report

Dear Mr. Glover,

Please accept these comments from Shenandoah Riverkeeper and Potomac Riverkeeper on Virginia's Draft 2010 305(b)/303(d) Water Quality Assessment Integrated Report.

Both Shenandoah and Potomac Riverkeeper's mission are to protect and restore water quality in the Shenandoah and Potomac Rivers respectively. We share over 3000 members who use and enjoy these rivers and whose use we also try to protect through community action and enforcement of environmental laws.

I am writing to express my concerns pertaining to the methods currently used to evaluate the Shenandoah River for the 303(d) listing process. The Draft Report lists a total of nine discrete segments of the North and South Forks of the Shenandoah River that are impaired due to three reasons: mercury in fish tissue as a violation of the "fish consumption" use; E. coli levels as a violation of the "recreation" use; and benthic macro-invertebrate assessments as a violation of the "aquatic life" use. In addition to those issues, there are several other important indicators not cited by the Department of Environmental Quality (DEQ) in the Draft Report that should be seriously considered under (1) the aquatic life use and (2) the recreation use.

Aquatic Life Use Impairment

Virginia's Water Quality Standards (WQS) state that "all state waters…are designated for the following uses: … the propagation and growth of a balanced, indigenous population of aquatic life, including game fish, which might reasonably be expected to inhabit them…." 9 VAC 25-260-10(A). The "General Criteria" of the WQS demand that "State waters, including wetlands, shall be free from

substances attributable to sewage, industrial waste, or other waste in concentrations, amounts, or combinations which contravene established standards *or interfere directly or indirectly with designated uses of such water* or which are inimical or harmful to human, animal, plant, or aquatic life" (emphasis added). 9 VAC 25-260-20(A). In the 2010 Draft Integrated Report, the benthic macro-invertebrate assessment is the only parameter noted as showing non-attainment of the aquatic life use in three sections of the Shenandoah River. However, the river is suffering from several problems that appear to be causing a violation of the aquatic life use: (1) fish kills, (2) intersex traits, (3) and lack of native vegetation. DEQ must evaluate these phenomena as factors in listing the river as violating the aquatic life use.

Fish Kills

DEQ and other state agencies have extensively studied the fish kills in the Shenandoah basin. The fish kills demonstrate that the river is not supporting aquatic life like a properly functioning ecosystem should, and in fact indicate that the river's ecosystem is significantly out of balance.

The fish kills occur annually with varying levels of mortality. The worst years were 2004, 2005, and 2007. During 2006, 2008, 2009, and 2010, the river experienced lower-level fish kills and numerous fish in the river developed abnormal sores and appeared visibly ill in the spring of each year. The fish kills have been consistently worst during periods when pre-spawn and spawning coincide with late March or Early April runoff events that trigger turbidity and high water temperatures. The chronic low-level fish kills seem to be spurred by several species of the bacteria *Aeromonas*, but also Columnaris and possibly contributing Largemouth Bass Virus. But the reason for the major, widespread fish kills in certain years is currently unknown though two diverging theories exist. One group of scientists as USGS have reported findings of compromised immune function and theorize there may be a relationship between the high prevalence of intersex and the severity of fish kills. Several species of important game fish are affected, including non-native smallmouth bass, but also native redbreast sunfish, rock bass, and suckers. The fish kills have triggered an unnatural shift in species composition in which species with less exacting habitat needs displace the Shenandoah's normal species. For example, largemouth bass can be found where historically only smallmouth bass were found, carp turn up where previously only suckers were present, and green sunfish and bluegill move into areas previously dominated by redbreast sunfish. These shifts have lasted for several years over wide areas of the river but remain in others.

I believe that, based on studies conducted and commissioned by DEQ, the River is not supporting a balanced population of fish, including game fish, and that DEQ should include the kills as a reason for listing the river as violating the aquatic life standard. Several reports documenting the fish kills and studies of various possible causes can be found at http://www.deq.virginia.gov/info/srfishkillhistory.html (History of Fish Kills in the Shenandoah, by DEQ); http://www.dgif.virginia.gov/fishing/fish-kill/ (Shenandoah and James River Fish Disease and Mortality Investigation, by DGIF); http://www.deq.virginia.gov/export/sites/default/info/documents/FishkillReport-

final.pdf (The Economic Impact of the 2005 Shenandoah River Fish Kill: A Preliminary Assessment, by the Center for Energy and Environmental Sustainability);
http://www.deq.virginia.gov/export/sites/default/info/documents/2007.02.23ScienceTeamReport.pdf (Fish Kills in the Shenandoah River Basin: Preliminary Report of the Shenandoah Basin Science Team, by faculty from Virginia Commonwealth University and Virginia Tech).
New information corroborating evidence that multiple bacteria, immuno-suppression, environmental and contaminant factors are likely contributors to the fish health decline will be provided very soon in USGS paper "**MORTALITY OF CENTRARCHID FISHES IN THE POTOMAC DRAINAGE:** 2 **SURVEY RESULTS AND OVERVIEW OF POTENTIAL CONTRIBUTING FACTORS"**

Intersex (Testicular Oocytes)

Intersex traits in smallmouth bass have been found with alarming regularity in the Shenandoah system. Between 80% and 100% of the male smallmouth bass are producing eggs in their testicles. The trait is not evolutionary, nor is it an appropriate genetic adaptation which increases species fitness because additional reproductive capability is not a result. Most rivers contain a very low level of fish with intersex traits, and the lowest levels are associated with rivers where human impacts are lowest. Therefore, the intersex phenomenon is most likely attributable to human impacts. Studies have shown that intersex male smallmouth are less fit to reproduce and do not protect nests as well as healthy males. They also show a decreased ability to rear young to survival age. The eggs produced by male smallmouth are not fertile, viable eggs, and therefore do not serve any reproductive purpose.

The exceedingly high occurrence of the intersex trait in male smallmouth bass hampers the "propagation and growth of a balanced, indigenous population of aquatic life, including game fish, which might be reasonably expected to inhabit them," and therefore is a basis for 303(d) listing. This is a difficult topic because environmental conditions for Smallmouth Bass spawning have been excellent during several years post fish kill. This could be used as an argument that intersex is not effecting reproduction but the Riverkeeper contends that anecdotal evidence of good spawning of one species is NOT a basis for ignoring the intersex phenomena for many reasons. The first reason is that no information about native redbreast sunfish and rock bass populations and reproduction exists. Yet years of fish populations surveying have shown that redbreast but especially Rock Bass populations have NOT rebounded and populations remain depressed over large areas of the river system. Additionally, egg production by male bass may not represent the problem, but may only be a readily discoverable histo-pathological indicator of contaminant exposure and evidence that other problems exist. Surely, additional study should be commissioned during a TMDL process and should be considered as an argument for listing this impairment.

Basic facts about the intersex traits can be found at http://www.deq.state.va.us/info/fishqa.html (DEQ; Questions and Answers about

the discovery of intersex smallmouth bass in the Shenandoah and Calfpasture Rivers). A USGS article on the subject published in the Journal of Aquatic Animal Health in 2007 can be accessed at http://www.antietamflyanglers.org/docs/jaahintersex.pdf ("Intersex (Testicular Oocytes) in Smallmouth Bass from the Potomac River and Selected Nearby Drainages").

Submerged Aquatic Vegetation

The entire Main Stem of the Shenandoah has experienced near complete loss of healthy stands of submerged aquatic vegetation (SAV). Both types of native grasses, *Vallisneria* and Stargrass (*Heteranthera*), began disappearing in 2002 and currently show no signs of recovery. High turbidity, illustrated by chronic planktonic algae blooms, suspended sediments and an overabundance of carp during the majority of the year, most likely led to the decline of the SAV. Stands of stargrass that have developed in pool areas are habitually unhealthy, exhibiting delayed growth initiation, very sparse leafing or brown leafs. Additionally most stands are overwhelmed by algal communities. The resulting lack of SAV creates an environment in which the algae can bloom in the presence of nutrients in the river without competition, which leads to prodigious and problematic algal blooms. Several organisms that provide key roles in healthy river ecosystem processes need SAV. Juvenile insects and fish rely on the grasses as key habitat. SAV also acts as a filtering mechanism that removes sediment suspended in the water column. Until SAV is restored and algae levels are limited, there cannot be a balanced population of aquatic life.

Ironically, in other portions of the river, the rooted grasses get so thick that they impair travel by recreational river users. It is common for sections like that between Hazard Mill and Bentonville Bridge to become so choked with stargrass that the outfitters cease putting innertubers on the river.

I assert that a river system with little native vegetation and algae over-abundance in one area, , yet overabundance of grasses sometimes and and algae blooms at others is experiencing severe nutrification problems which leads to boom or bust cycles and provides an additional reason to be listed as impaired.

Recreational Use Impairment

Virginia's Water Quality Standards state that "all state waters…are designated for the following uses: recreational uses, e.g., swimming and boating…." 9 VAC 25-26-10(A). Currently, the E.coli bacteria level is the only standard used by the Department of Environmental Quality to assess whether the recreational use is being met. 9 VAC 25-260-170(A). E.coli levels are used to protect "primary contact recreational uses in surface waters," which is defined to mean "any water-based form of recreation, the practice of which has a high probability for total body immersion or ingestion of water (examples include but are not limited to swimming, water skiing, canoeing, and kayaking)." Additionally, "secondary contact recreation" is defined to include "wading, boating, and fishing." 9 VAC 25-260-5.

There are two key factors other than E.coli that must be addressed when making the determination as to whether or not the Shenandoah River is attaining its recreational use: (1) turbidity and (2) algae growth. High levels of turbidity and algae have significant adverse effects on swimming, fishing, and paddling, all of which are quintessential recreational uses the Shenandoah River is known for.

Turbidity

The turbidity problems create prolonged periods of muddy water that result in swimmers, paddlers, and fishermen completely avoiding the River. While some turbidity is naturally occurring in any water body, the turbidity levels found in the Shenandoah are often unacceptable. Average turbidity in the Shenandoah watershed has increased during the last decade from an average of about 2.0 NTU in 1997 to approximately 10.0 NTU in 2007. Turbidity levels above 7.0 NTU symbolize severely impaired, cloudy water. Clear, unimpaired waters usually exhibit turbidity between 0 and 4.0 NTU. The consistently increasing levels of turbidity cause substantially prolonged periods in which the river is not suitable for recreational use. A report by the Friends of the Shenandoah River discussing turbidity data can be found at http://www.fosr.org/reports/WQ-Shenandoah2007.pdf ("The Status of Water Quality in the Rivers and Tributaries of the Shenandoah River Watershed," August, 2007).

Fishermen rely on clear water because game fish use clear water to identify and target lures and bait. As a professional fishing guide, there have always been long periods of time following rain events which would render the river unfishable. In those instances I would travel to other waterways. My records estimate that over the course of the winter and spring, 25%-50% of the time the river is unusable, depending on precipitation, due to turbidity. Angler surveys also reveal that they do not use the river to fish when the river is turbid. Swimmers turn away from river when they can't see down into the water for fear of health problems, and fear of unknown underwater hazards.

Algae

Algae growth has become so prolific that it renders the Shenandoah virtually unusable for recreation in many places consistently during many periods of the year. The algae grows in unnaturally large quantities that can clog portions of the river, making swimming and fishing impossible or undesirable on large stretches of the river. Recently, several species of blue-green algae have shown up repeatedly and the stench is often equated to raw sewage or rotting broccoli. In late October of 2009, I witnessed a filamentous periphytic algae bloom on the South Fork of the Shenandoah that covered the bottom of the River from bank to bank for approximately fifty miles from Front Royal to Luray. It can be seen growing on top of native grasses in the attached Photo #1. Even after the river cleared in the spring, there was still algae covering the rocks on a seventy-five mile stretch of river on the South Fork from Island Ford to Front Royal (Photo #2 attached). During the summer of 2010, the major algae bloom began in the first part of July.

I received complaints from many landowners, fishermen, fishing guides and outfitters whose livelihood depends on the river. One landowner on the South

Fork, near Burners Bottom, complained that the algae was "so thick and smelled so bad" that they ceased using the river. The landowner commented that it was the worst algae bloom he had ever seen. On July 8, 2010, I received a message from a reporter for the Winchester Star who stated that her child's swim trunks smelled like sewage when he came home after swimming in the river. The swim trunks were not salvageable. An article in the Winchester Star highlighted the concern, available here: http://www.winchesterstar.com/articles/view/algae_bloom ("Algae Bloom," July 10, 2010, The Winchester Star). Upon inquiry John Gibson, owner of Downriver Canoe Company, noted complaints from canoeists describing the river as reeking of raw sewage and possibly diminished the enjoyment of canoers and tubers. Don Roberts from Front Royal Canoe Company also said his customers complained of odor problems on the river during a period of low flow this July. He believes this is the worst algae bloom he has ever seen. Shenandoah River Outfitters also confirmed the river contained the algae near Luray and that the excessive algae. Several fishermen were also affected by the algae on both the Shenandoah and the section of the Potomac immediately below the confluence with the Shenandoah. Fishermen complained of both the smell and the fact that it was impossible to fish large sections of the river because the algae immediately fouled hooks.

This summer's low flow conditions were by no means unique to this year, and do not provide a sufficient explanation as to why the river experienced such a heavy bloom. By comparison, I surveyed the Rappahannock River from Remington to Kelly's Ford during the period July to September, 2010 when both systems were experiencing extremely low flow conditions. Only the Shenandoah experiences heavy algae blooms and cyanobacteria (blue green algae Oscillotoria). The Rappahannock exhibited comparatively non-existent algal blooms. Where periphytic algae existed, the species assemblage was quite different from the Shenandoah and consisted of species more readily identified with moving rivers. The Shenandoah's assemblages were wide and varied and prolific literature suggests nutrification is the limiter (also the key) to these types of algal blooms.

I surveyed our summer algae bloom during the days of July 7th and 8th and found that the blooms began well above Cootes's Store on the upper most section of the North Fork and continued downstream almost 100 miles to the confluence with the South Fork. I was surprised in fact, to observe that the planktonic algae blooms started literally in the very location that the river originated this summer where it came out of the cobblestone in the secluded agricultural tributaries of the North Fork in Fulk's Run. I also personally witnessed the same bloom extending throughout the Main Stem of the Shenandoah from the confluence of the North and South Forks all the way to the West Virginia border. At the same time, the North Fork was darkened by green plankton, had floating chunks of foul smelling algae, and filaments which covered the native grasses and bottom of the river (Photos #3 and #4 attached). We are also submitting a host of photos and videos named accurately in the title demonstrating the algae problems.

Harold Marshall, Professor in the Department of Biological Sciences at Old Dominion University, identified many types of algae from samples I collected from the Main Stem of the Shenandoah on August 27, 2010. The types identified included several cyanobacteria filamentous taxa: *Oscillatoria limosa*, *Oscillatoria*

princeps, and *Lyngbya sp*. There were also numerous diatoms including representatives of the following genera: *Pinnularia*, *Achnanthes*, *Tabellaria*, *Navicula*, *Gomphonema*, and *Gyrosigma*. The cyanobacteria, commonly known as blue-green algae, is of particular concern. Some forms of cyanobacteria naturally produce toxins called microcystins. Microcystins have been found in the livers of fish in the South Branch of the Potomac River, but there have not been any studies conducted to assess whether or not fish in the Shenandoah contain the microcystins. If present in the Shenandoah, humans could potentially be exposed to the toxins through swimming or by consuming fish harboring microcystins. For studies of other water bodies that reference the blue-green algae toxicity issue, see http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2761.2001.00273.x/abstract ("Confirmation of catfish, *Ictalurus punctatus* (Rafinesque) mortality from *Microcystis* toxins," Journal of Fish Diseases, 2001).

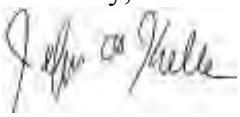
The explosive algae growth is so prolific that it directly affects the normal recreational uses of the River. The unnatural levels impede swimming and fishing so severely that the Shenandoah River is not attaining the designated use of recreation, as required by the Clean Water Act and the Virginia Water Quality Standards.

Conclusion

In my capacity as the Shenandoah Riverkeeper, I am committed to protecting and restoring the Shenandoah River and its tributaries. Until the nutrient and sediment inputs are properly controlled and managed on this river system, the River will not be able to provide the recreational opportunities or harbor the aquatic life it has historically supported and which are protected by the Clean Water Act. I request that Virginia list the entire stretches of both the North and South Forks, as well as the Main Stem of the Shenandoah River, as impaired waters that should be classified under Category 5 due to the aquatic life and recreational use impairments explained above. A proper TMDL is the only way to control the excessive pollution in the River since there are no applicable freshwater nutrient criteria in Virginia's Water Quality Standards.

Please contact me at (540) 837-1479 or email me at Jeff@Shenandoah riverkeeper.org if you have any questions about these comments. I appreciate the opportunity to comment on this subject.

Sincerely,



Jeff Kelble
Shenandoah Riverkeeper
www.shenandoahriverkeeper.org

ATTACHMENT G

GUIDANCE FOR CITIZEN NOMINATION OF STATE SURFACE WATERS FOR INCLUSION IN VIRGINIA DEPARTMENT OF ENVIRONMENTAL QUALITY'S ANNUAL WATER QUALITY MONITORING PLAN. IN ACCORDANCE WITH §62.1-44.19:5.F OF THE CODE OF VIRGINIA, WATER QUALITY MONITORING INFORMATION AND RESTORATION ACT.

PURPOSE OF GUIDANCE DOCUMENT

This document provides detailed guidance on implementation of §62.1-44.19:5.F, the section of the Water Quality Monitoring, Information and Restoration Act that provides for requests from the public regarding specific segments that should be included in the Virginia Department of Environmental Quality's (DEQ) annual Water Quality Monitoring Plan.

VIRGINIA DEPARTMENT OF ENVIRONMENTAL QUALITY CONTACT

Questions concerning this document should be addressed to Mr. Stuart Torbeck, Virginia Department of Environmental Quality, P.O. Box 1105, Richmond, Virginia 23218 at (804) 698-4461 or the DEQ in-state toll free number (1-800-592-5482). The street address is 629 East Main Street, Richmond, Virginia 23219.

BACKGROUND ON THE GUIDANCE DOCUMENT

During the 1997 legislative session, the General Assembly enacted the Water Quality Monitoring, Information and Restoration Act (the Act). The Act directs DEQ to provide a procedure for citizens of the Commonwealth to nominate portions of lakes, streams, and rivers of Virginia for water quality monitoring by DEQ. Citizens can send their nominations to DEQ via the procedures described below. Nominations received by April 30, 2010 will be considered for inclusion in DEQ's annual monitoring plan for the 2011 calendar year. The monitoring plan will be finalized after considering the citizen's nominations for inclusion. DEQ will respond to each request in writing, stating the reasons for accepting or denying each nomination. DEQ's response is due by August 31 for nominations received between January 1, and April 30.

PROCESS TO REQUEST ADDITIONAL MONITORING

Any person may request that a specific body of water be included in DEQ's annual water quality monitoring plan. Each request received between January 1 and April 30, shall be reviewed when DEQ develops or updates the annual water quality monitoring plan. Such requests shall include, at a minimum (i) a geographical description of the water body recommended for monitoring; (ii) the reason the monitoring is requested, and (iii) any water quality data that the petitioner may have collected or compiled. Please see the nominating form Attachment 1.

Please note that the monitoring program covered by this process is directed at the surface waters of the state. Private ponds, privately owned lakes and any other body of water not deemed to be "State Waters" are ineligible.

Nominations can be submitted by mail, fax, email, or hand delivered to the receptionist's desk at our Central Office at 629 East Main Street, Richmond, Virginia.

Mailing Address:
Mr. Stuart Torbeck
VA Dept. of Environmental Quality
P.O. Box 1105
Richmond, VA. 23218

Street Address (FedEx):
Mr. Stuart Torbeck
VA Dept. of Environmental Quality
629 East Main Street
Richmond, VA 23219

FAX:
Attention Stuart Torbeck
VA Dept of Environmental Quality
804-698-4032

E- MAIL
charles.torbeck@deq.virginia.gov

Use of the nomination form shown in Attachment 1 is preferred. All nominations with the minimum of information as outlined above will be accepted for review.

TIMELINE

Nominations received between **January 1 and April 30, 2012** will be considered for inclusion in DEQ's Water Quality Monitoring plan for the following calendar year (2013). DEQ will respond in writing on its approval or denial of each nomination by August 31, 2012. The DEQ 2012 monitoring plan will be made available for public inspection. A notice of availability of the annual monitoring plan will be placed in the Virginia Register and on DEQ's web site at *http://www.deq.virginia.gov/water*

ATTACHMENT 1

REQUEST TO INCLUDE A WATER SEGMENT IN DEQ'S ANNUAL MONITORING PLAN

Name: Jeff Kelble **Date:** April 27, 2012

Mailing Address: Shenandoah Riverkeeper, P.O. Box 405
Street

City: Boyce **State:** VA **Zip:** 22620

E-mail address: Jeff@shenandoahriverkeeper.org

Telephone: Home: cell 540-533-6465 **Business:** 540-837-1479 **Fax:**

Geographic description of the water body:

(1) Name of the water body or segment proposed for monitoring:

South Fork Shenandoah River, North Fork Shenandoah River, and Main Stem of Shenandoah River all to be monitored for nuisance aquatic vegetation and nutrient impairment

(2) Description of the upstream and downstream boundaries of the water body proposed for monitoring. Attach a map (preferably a photocopy of a 7.5 minute quad USGS topographic map) which delineates the boundaries:

Please see attached map, but generally we are requesting that the entire named portions of the North Fork, South Fork, and Main Stem of the Shenandoah River be monitored for algae, other nuisance aquatic vegetation and nutrient impairment. The North Fork begins at approximately Brock's Gap at the confluence of the German River and Capon Run. The South Fork Shenandoah Begins in Port Republic at the Confluence of South River and the North River. The Main Stem Shenandoah originates in Front Royal at the Confluence of the North and South Fork of the Shenandoah and flows to Harper's Ferry West Virginia where it terminates at the confluence with the Potomac River.

(3) Reason for requesting that this water body be monitored:

The Shenandoah river system currently suffers from serious algae blooms through much of the year. By directly interfering with a designated use (recreation), these blooms, by themselves, violate Virginia's General Criteria within the Water Quality Standards for nuisance aquatic plant life (aka the "narrative standard) . We are requesting that DEQ monitor the algae blooms and consider listing the North Fork, South Fork, and Main Stem of the Shenandoah River on Virginia's 305(b)/303(d) Water Quality Assessment Integrated Report based on the extent of the algal growth and its effect on recreational use.

(4) Attach any water quality data that you have collected or compiled. Include the name of the organization/entity that generated the data.

Mail to:
Stuart Torbeck
VA DEQ
PO Box 1105
Richmond, VA. 23218

FedEx To:
Stuart Torbeck
VA DEQ
629 East Main Street
Richmond, VA 23219

Fax to: **Stuart Torbeck, VA DEQ**
804-698-4032

E-MAIL: charles.torbeck@deq.virginia.gov

ATTACHMENT H

COMMONWEALTH of VIRGINIA

DEPARTMENT OF ENVIRONMENTAL QUALITY
Street address: 629 East Main Street, Richmond, Virginia 23219
Mailing address: P.O. Box 1105, Richmond, Virginia 23218
Fax (804) 698-4500 TDD (804) 698-4021
www.deq.virginia.gov

L. Preston Bryant, Jr.
Secretary of Natural Resources

David K. Paylor
Director

(804) 698-4000
1-800-592-5482

July 27, 2012

Mr. Jeff Kelble
Shenandoah River Keeper
P. O. Box 405
Boyce, Va. 22620

Dear Mr. Kelble.

Thank you for your submitted request for monitoring on the North Fork, South Fork and main stem of the Shenandoah River. Don Kain at the DEQ Valley Regional Office in Harrisonburg has reviewed your request.

Shenandoah-North Fork-South Fork and Main Stem: We regret to inform you that you request cannot be honored for the following reasons, Virginia does not currently have a water quality standard for nuisance aquatic vegatation.or nutrients in riverarine systems, and also quantitative criteria for assessment of algae do not currently exist.

Evaluation of nuisance algae and other aquatic plant growth would be best conducted through a special, dedicated study that allows a quantitative, systematic assessment of conditions at multiple sites over multiple seasons in conjunction with related water quality constituents (pH, alkalinity, hardness, flow, water clarity, etc.). DEQ's regional monitoring program is neither staffed nor funded for such a study. This type of study may be best conducted by a researcher with specific expertise in this area. We appreciate the nominations submitted. The citizen nomination process is an important resource to help DEQ identify water bodies for sampling. DEQ makes every effort to have monitoring data available to the public. If you would like to learn more about current DEQ sampling efforts, please visit the website :
http://www.deq.virginia.gov/Programs/Water/WaterQualityInformationTMDLs/WaterQualityMonitoring/CitizenMonitoring/FollowupMonitoring.aspx

If you have any questions about DEQ monitoring in your area, please contact Don Kain at (540)-574-7815 or by e-mail at Donald.Kain@deq.virginia.gov

C. Stuart Torbeck Jr.
Water Quality Data Liaison
cc: Don Kain DEQ Valley Regional Office

Shenandoah Riverkeeper
P.O. Box 405
Boyce, VA 22620
540.837.1479
keeper@shenandoahriverkeeper.org
www.shenandoahriverkeeper.org

April 23, 2012

Via e-mail (John.Kennedy@deq.virginia.gov)
John M. Kennedy
Office of Water Monitoring and Assessment
Virginia Department of Environmental Quality
P.O. Box 1105
Richmond, VA 23218-1105

RE: Comments on 2012 305(b)/303(d) Water Quality Assessment Integrated Report

Dear Mr. Kennedy,

Please accept the following comments from the Shenandoah Riverkeeper regarding Virginia's draft 2012 305(b)/303(d) Water Quality Assessment Integrated Report. These comments were prepared with advice and assistance from the Environmental Law and Conservation Clinic at the University of Virginia School of Law.

Shenandoah Riverkeeper is a program of the Potomac Riverkeeper Inc., a 501(c)(3) non-profit corporation. Shenandoah Riverkeeper's mission is to use citizen action and enforcement to protect and restore water quality in the Shenandoah River Watershed for people, fish, and aquatic life. Shenandoah Riverkeeper and Potomac Riverkeeper are grass-roots organizations with over 2,000 combined members. These members use the Shenandoah and Potomac Rivers to swim, fish, boat, and recreate. Some are landowners along the river, and many use the river for business uses and as drinking water.

We request that the North Fork, South Fork, and Main Stem of the Shenandoah River be added to the list of Category 5 impaired waters due to significant algae blooms, which violate Virginia's nuisance aquatic plant life standard and interfere with the public's recreational use of these waters. Although portions of these waters are already listed for a variety of impairments, the state should add the algae blooms as an additional cause of impairment.

Most of the Shenandoah system currently suffers from significant algae blooms through much of the year. These blooms, by themselves, violate Virginia's water quality standards. The General Criteria explicitly provide:

> State waters, including wetlands shall be free from substances attributable to sewage, industrial waste, or other waste in concentrations, amounts, or combinations which contravene established standards or *interfere directly or indirectly with designated uses* of such water or which are inimical or harmful to human, animal, plant, or aquatic life.
>
> Specific substances to be controlled include, but are not limited to . . . substances which *nourish undesirable or nuisance aquatic plant life*.[1] (emphasis added.)

As will be described more fully below, algae blooms that occur in the Shenandoah system clearly amount to "undesirable or nuisance aquatic plant life," which alone violates the general criteria. In addition, these blooms seriously impair recreational uses of these rivers. The Commonwealth's water quality standards make such recreational use a designated use of all state waters,[2] and the impairment of that use constitutes violations of water quality standards.

[1] 9 VAC 25-260-20(A).
[2] 9 VAC 25-260-10 (A).

I. The Nature and Extent of the Algae Blooms Constitute Nuisance Aquatic Vegetation and Impair Recreational Use of the Rivers.

In my role at Shenandoah Riverkeeper, but also as a professional fishing guide for nearly a decade and recreational user for two decades, I have witnessed the severe algae blooms that hinder recreational use of the rivers. In my observations, the algae can and do bloom at nearly any time of year and excessive nutrients are always available in the system to fuel algae growth. It is quite rare in fact, to float a stretch of river and not see portions of the water column or the river bottom that have very significant growth of one form or another nearly year round. With the availability of nutrients at all times, it seems that a handful of different algae species responds when other water conditions are conducive to the growth of that particular algae. The variables seem to include: a) sunlight angle b) water clarity c) presence of turbidity d) temperature e) length of day f) pH g) alkalinity and h) hardness. These parameters are in constant fluctuation and contribute to the annual variability in the presence of algae.[3]

During late October of 2009, I took it upon myself to make more formal and comprehensive observations of the algae. During that month, I observed a filamentous periphytic algae in the South Fork of the Shenandoah River that covered the floor of the River from bank to bank. The particular algae bloom extended approximately fifty miles from Front Royal to Luray. In the spring of that same year, even though the river had cleared some, algae could be seen on rocks along a seventy-five mile stretch of the river from Island Ford to Front Royal. I have previously provided DEQ with photos that confirm these observations during my comments on the previous 2010 305(b)/303(d) Water Quality Assessment Integrated Report Draft, and which were part of the record during the time when EPA approved the previous draft 2010 303(D)/305(B) Water Quality Assessment Integrated Report. I would like to incorporate those comments, photos, and observations by reference in these comments.[4]

In July of 2010, I began receiving complaints from landowners and businesses regarding a major algae bloom on the South Fork. In particular, one landowner referred to the bloom as the worst algae bloom he had ever seen, and he further stated that he was no longer using the river because the algae was "so thick and smelled so bad." In that same month, I had a concerned mother contact me, because after her child swam in the river his swim trunks smelled like sewage. The woman happened also to be a reporter for the Winchester Star, and shortly thereafter an article in the paper highlighted algae problem. That article can be found at: http://www.winchesterstar.com/articles/printable/358092.

The algae bloom in July of 2010 also impacted local businesses. Multiple outfitters, including Downriver Canoe Company, Front Royal Canoe Company, and Shenandoah River Outfitters reported to me negative impacts from the algae blooms. In some cases, they reported that customers had complained about diminished enjoyment of the river due to the severe algal growth. Fishermen also complained that the algae in the Shenandoah River ruined their recreational experience. In particular they were frustrated, because the algae would foul their hooks making it impossible to fish large segments of the river. They were also disgusted by the stench of the algae. These complaints, and others, were also detailed in our comments regarding the 2010 305(b)/303(d) Water Quality Assessment Integrated Report.

Since the algae blooms of 2009 and 2010, I have continued to monitor the algae blooms on the River, and it is clear that they still impair recreational use and are a nuisance to those who use the River. The annual pattern has not changed and we continue to see periodic riverwide planktonic blooms after river rises, and then large periods of various periphytic and floating algae during the rest of the year.

During the spring and summer of 2011 for example, the Main Stem Shenandoah, through its entire reach exhibited the worst planktonic bloom I have seen to date. The water literally was the color of thick green paint for over three months. Fish were sluggish and wildlife activity around the river was down to nearly zero. This did not clear up until the sun angle and temperatures lowered in September. The river was completely unsuitable for recreational use. Also generally, we continue to see a handful of other algae during large parts of the summer and fall months that covered the bottom of the river and/or the surfaces over large swaths of river. In repeated attempts to use the river last summer, I was turned off by either a heavy carpeting of filamentous algae covering the bottom of the river, or chunks and mats of algae floating around on the surface.

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[3] Summers, James, *Assessment of Filamentous Algae in the Greenbrier River And Other West Virginia Streams* (West Virginia DEP DWWM, December 17, 2008).

[4] Letter from Jeff Kelble, Shenandoah Riverkeeper (Sep. 23, 2010) (on record with Shenandoah Riverkeeper).

Most recently, in order to collect information for these comments, I solicited comments from stakeholders in order to better understand how algae in the Shenandoah River has continued to impacted recreational use of the river. From the over 80 responses we received, it is clear that the algae continues to be a nuisance and violate the recreational designated use set forth in the Virginia Water Quality Standards. Those letters, which are appended to this comment and that we incorporate in our official comments, speak for themselves and detail the location and approximate dates of the observed nuisance algae by recreational river users and landowners.

DEQ should note that these letters include observations of nuisance algae blooms at points along nearly the entire lengths of the North Fork, South Fork, and Main Stem Shenandoah. DEQ should also note that the areas most often mentioned are the areas most heavily used by the public. This does not necessarily mean algae problems are either limited to or focused in these areas. Quite the contrary. During my tenure as Riverkeeper I have documented repeatedly that when certain algae bloom, they are present from the top of the North Fork and South Fork and run all the way to the confluence with the Main Stem and on down to Harper's Ferry and the terminus of the river.

This is the case with the late spring/early summer planktonic blooms. This river wide bloom is most often associated with 1) the early May bloom of a wooly green bottom covering algae, 2) the mid-summer blooms of cyanobacteria (blue-green toxic oscillotoria algae as identified by Harold Marshal of ODU) and other dark green filamentous bottom covering and star-grass coating algae, and 3) the bright green filamentous algae that blooms almost overnight in late September/early October when the native grasses die back naturally.[5] On August 27, 2010, Shenandoah Riverkeeper drew two samples of water, one from the Main Stem Shenandoah at Route 7, the other from the Rappahannock River 300 yards downstream of the Remington Route 29 Business Bridge. The water sample and algae were preserved in a red fixative that Dr. Marshal had provided to Shenandoah Riverkeeper several days prior. Among other things this is what we derived from this single simple test:

1) The algae community on the Shenandoah wase different than the Rappahannock
2) The algeas found on the Shenandoahn are indicative of high nutrient levels and warm weather (summer sample)
3) There were several species of blue green algae, aka Cyanobacteria present which are known nuisance algaes and which account for bad odors, floating mats and the production of mycrocystin which is a liver toxin

In general, the complaints we are attaching highlight the excessive algae growth that occurs during the winter and mid-summer months in the North Fork, South Fork, and Main Stem of the Shenandoah River. Virtually all of the letters complain of a planktonic algae bloom following rain events in the winter, as well as filamentous algae ("rock snot") and "bubbly" algae that covers the surface of the river in the summer months. That said, many complaints reiterate the point that although it is especially bad in the winter and summer, algae growth remains a nuisance throughout the year.

It is worth pointing out a few specific examples of how the excessive algae has made swimming, fishing, and boating both unpleasant and difficult. Colby Trow, the owner of a local fly shop and guide service, states that he has photos of the river for each month of the past ten years, and it is difficult to find a single photo that doesn't illustrate the excessive algae growth. He also describes being embarrassed when clients ask him why the river is so dirty. William Amshey, states in his letter that he avoids wet wading in the river for "fear of infection." Another citizen, Andrew Riccobono, states that he travels as far as Pennsylvania or New York to fish, because at certain times of the year his flies become "covered in green muck after every cast" while fishing in the Shenandoah. Yet another citizen, Beau Morgan, describes the algae as sometimes being so thick that "one could hardly get a canoe to glide across it." After describing his frustrations associated with trying to fish in the Shenandoah River and the foul smell that emanates from the algae, Bill Millhouser says plainly, "I just cannot use the River due to the odors and annoyance."

We are attaching a map that shows the location of the algae blooms either observed directly by Jeff Kelble or reported in the letters that we are submitting to DEQ. The map clearly demonstrates that the entire reach of North Fork, South Fork, and mainstem Shenandoah are plagued by undesirable nuisance algae blooms every year, although reports are concentrated around the most heavily used areas.

These citizen letters demonstrate the frustration felt by landowners and river uses but represent only a few examples of the many Virginia citizens whose recreational use of the Shenandoah River has been diminished due to the excessive algal

[5] E-mail from Harold G. Marshall, Professor, Old Dominion University, (Sep. 22, 2010, 2:29 PM) (Attachment I to these comments).

growth. The unnatural levels of algae in these parts of the river clearly violate the requirement in the General Criteria of the water quality standards that waters be free from substances that cause the growth of undesirable or nuisance aquatic plant life. In addition, all the Commonwealth's waters have recreation as a designated use, and the algae blooms frustrate and impair that use.

II. The Algae Blooms Violate Water Quality Standards, Even in the Absence of Numeric Criteria or Thresholds for Nutrients.

In response to our similar comments on the 2010 Integrated Report, DEQ stated that it could not list the Shenandoah River due to algal growth, because "no listing threshold exists for nutrients, algae, freshwater submerged aquatic vegetation, and undetermined potential immune suppressors in these waters." This response implies that in order to list those portions of the river, a numeric criterion would have to be set and exceeded. This position is contrary to the Clean Water Act. The narrative standards that DEQ has established are just as enforceable as a numeric standard would be, and the facts set out above demonstrate a violation of those standards. The information we are submitting today about the algae blooms in the Shenandoah system show quite plainly that pollution is fueling the growth of undesirable and nuisance aquatic plant life, and that the algae blooms are impairing recreational uses of the river, both in violation of state water quality standards.

In *PUD No. 1 of Jefferson County v. Washington Dept. of Ecology*,[6] the Supreme Court of the United States stated that "EPA has not interpreted § 303 to require States to protect designated uses exclusively through enforcement of numerical criteria."[7] The Court in that case went on to say that:

> Petitioners also appear to argue that use requirements are too open ended, and that the Act only contemplates enforcement of the more specific and objective 'criteria.' But this argument is belied by the open ended nature of the criteria themselves. . . . We think petitioners' attempt to distinguish between uses and criteria loses much of its force in light of the fact that *the Act permits enforcement of broad narrative criteria based on, for example, 'aesthetics.'*[8] (emphasis added.)

The Court further explained that the idea of *only* enforcing specific numeric criteria is ridiculous, because that would imply that States would have to "study to a level of great specificity each individual surface water to ensure the criteria applicable to that water are sufficiently detailed and individualized to fully protect that water's designated uses."[9]

The point is simple: "listing thresholds" are not necessary in order to designate the South Fork, North Fork, and Main Stem of the Shenandoah River impaired due to excess algae. The fact that the algae blooms rise to the level of "undesirable or nuisance" aquatic plant life and hinder recreational use of these waters is enough to list the waters as impaired. In our research, we have been unable to find any regulations or guidance from either DEQ or EPA that would suggest otherwise.

The various citizen letters submitted with these comments show that the algae blooms in the Shenandoah system are excessive, are aesthetically unpleasing, cause foul odors, make it frustratingly difficult and sometimes impossible to fish in the river, make it unpleasant to wade and swim in the river, and even make it difficult to navigate the river with a canoe. The general criteria, applicable to all waters of the state, require that waters "shall be free from substances attributable to sewage, industrial waste, or other waste" in amounts which interfere with designated uses. The criteria specifically provide that "substances to be controlled include . . . substances which nourish undesirable or nuisance aquatic plant life."[10] The algae blooms we have documented plainly exceed any threshold for "undesirable and nuisance aquatic plant life," and DEQ is responsible for adding that impairment to the impaired waters list.

In addition, all state waters are designated for "recreational uses, e.g., swimming and boating."[11] The significant, unpleasant, and pervasive algae blooms in the Shenandoah River straightforwardly interfere with those uses. This is an impairment of water quality standards under the Clean Water Act just as much as if a numeric criteria were violated. DEQ

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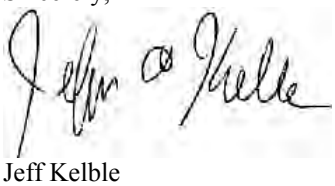
[6] 511 U.S. 700 (1994).
[7] *Id.* at 715.
[8] *Id.* at 715-16.
[9] *Id.* at 717-18.
[10] 9 VAC 25-260-20(A).
[11] 9 VAC 25-260-10 (A).

does not need a numeric threshold to determine that these portions of the Shenandoah River violate the General Criteria set forth in the Virginia Water Quality Standards.

We ask that DEQ to add violations of the nuisance aquatic plant life and recreational use standards to the listed causes of impairment for the North Fork, South Fork and Main Stem Shenandoah River in the state's 2012 305(b)/303(d) Water Quality Assessment Integrated Report. If DEQ fails to take this step, it will be a great disservice to the citizens of Virginia who value the recreational opportunities that the Shenandoah River has historically provided. We look forward to working with both DEQ and EPA to provide any additional information you might need in connection with this issue. If you have any questions regarding this request, please contact Jeff Kelble at: jeff@shenandoahriverkeeper.org or (540) 837-1479. We appreciate your time and consideration and are grateful for the opportunity to provide comments.

Sincerely,



Jeff Kelble

CC: Melanie Davenport

Thank you for your comments on the 2012 Draft Water Quality 305(b)/303(d) Integrated Report regarding the presence of algae in the Shenandoah Basin. The Department appreciates your concern for the Shenandoah River and its tributaries and your organization's efforts to protect and preserve the uses of this important natural resource. However, DEQ believes that listing the entire stretches of the North Fork, South Fork and mainstem Shenandoah Rivers as impaired for recreational use due to the presence of "nuisance" algae is inappropriate.

Virginia's EPA-approved 2012 Water Quality Assessment Guidance states:

> **Impaired waters needing a TMDL are those waters that do not meet Water Quality Standards due to a pollutant(s).** A pollutant, as defined in 40 CFR 122.2, means any dredged spoil, solid waste, incinerator residue, filter backwash, sewage, garbage, sewage sludge, munitions, chemical wastes, biological materials, radioactive materials (except those regulated under the Atomic Energy Act of 1954, as amended (42 U.S.C. 2011 et seq.)), heat, wrecked or discarded equipment, rock, sand, cellar dirt and industrial, municipal, and agricultural waste discharged into water. (*emphasis added*)

As you noted in your comments, the 9 VAC 25-260-20(A) General Criteria provides as follows:

> State waters, including wetlands shall be free from **substances** attributable to sewage, industrial waste, or other waste in concentrations, amounts, or combinations which contravene established standards, or interfere directly or indirectly with designated uses of such water, or which are inimical or harmful to human, animal, plant, or aquatic life.
>
> Specific **substances** to be controlled include, but are not limited to . . . substances which nourish undesirable or nuisance aquatic plant life. (*emphasis added*).

Algae itself is not a pollutant to be controlled within the meaning of the General Criteria, whereas the presence of nutrients, depending on their source and impacts, may be. However, at this time DEQ has no reliable methodology for assessing what may or may not be a "nuisance" nor has it reliable data demonstrating in what amounts and under what conditions nutrients might cause such a "nuisance" and therefore prompt a listing threshold. As no listing threshold exists, there also exist no attainment goals for these uses and subsequent delisting.

As outlined in the 2012 Virginia Water Quality Assessment Guidance, recreational use assessment includes swimming and other primary and secondary contact recreation uses such as water skiing and pleasure boating. Determinations of "fully supporting" versus "impaired" are based on violations of the e-coli bacteria in freshwater and enterococci bacteria in saltwater and transition zones. Refer to the excerpt from Table 1: Designated Use Matrix from Virginia's Water Quality Assessment Guidance below.

5.	Recreation/Swimming Use	Conventional Pollutant (E. coli and enterococci bacteria) and/or VDH beach closures/advisories or other available bacteria data. Previously listed fecal coliform impairments with no additional conventional bacteria data.

Virginia's bacteria water quality standard is based on the EPA-approved human health risk criteria of an illness rate of 8 in 1,000 individuals at the instantaneous Water Quality Standard of 235 cfu/100 ml. The risk levels are based on swimming, where water frequently gets in the mouth, nose, and eyes. DEQ believes that the use of a human health risk based standard is the most appropriate to determine recreational use support. Impaired waters are defined as those with chronic, recurring or ***human health*** (*emphasis added*) related WQ Standard violations using Quality Assurance/Quality Control (QA/QC) approved ambient monitoring data, special study data and/or other programmatic in-stream data collections.

Listing the entire stretches of the North Fork, South Fork and mainstem Shenandoah Rivers based on anecdotal and subjective observations would be an abandonment of the agency's long standing use of the human health risk-based numerical criteria for recreational use. To date, no scientific cause and effect documentation has produced a water quality criterion to evaluate human health risks associated with the presence of algae. The proposal to list the entire stretches of these rivers would be based on an "aesthetic" narrative standard alone. Virginia has not listed waters solely based on narrative criteria in previous assessment cycles. While the aquatic life use can be impaired due to the general narrative criteria for benthic macroinvertebrates, it is supported through the surrogate data from the Virginia Stream Condition Index. Validated monitored data is and has been the basis for DEQ's listing of impaired waters in Virginia. This approach allows for consistency from assessment cycle to assessment cycle.

DEQ evaluated data from many and varied sources for the 2012 Integrated Report. These data are broken into two general categories for assessment purposes. The first type of data is QA/QC approved "monitored" data. These data come from the collection and analysis of chemical, biological, and/or physical samples taken by DEQ and/or other DEQ-approved data submitted during the reporting period. These data are referred to as Level III and are considered the highest quality data. The 303(d) Impaired Waters list is comprised of only QA/QC approved monitored data due to the assessment confidence associated with the QA/QC monitoring requirements. Monitored data are obtained using EPA accepted methods and DEQ approved protocols.

All non-DEQ monitoring submittals, except U.S. Geological Survey chemical data submittals, must provide a sampling and analysis protocol and all field data for review. The second type of data used in assessment is considered "evaluated" data. These physical, chemical and/or biological data are primarily obtained from sources where there is not an EPA accepted sampling protocol and/or the use of non-DEQ approved sampling and analysis protocols. These data are considered Level II and of lower quality with less confidence in their results; they are not used for listing waters as fully supporting or impaired. If these types of data indicate water quality problems, the water is characterized as having an observed effect. To date, there are no studies reliably and numerically correlating nutrient levels with

the presence of "nuisance" algae to the substantial exclusion of other causal conditions. Further, algae have no systematic method of data collection and evaluation to allow a determination of what might be categorized as a "nuisance". Observations of algae growth can only be evaluated based on a subjective, anecdotal criteria and would be classified as Level II data.

DEQ monitors and assesses as many miles of Virginia's streams and rivers as resources allow. The North Fork, South Fork and mainstem Shenandoah Rivers are no exception. These rivers are systematically monitored and assessed against all available uses that monitoring data can provide.

Algae growth has not been linked to impairments that have been identified on stretches of the Shenandoah Basin rivers and streams. Typically, excessive algae can cause low dissolved oxygen levels in affected waters. These conditions have not been documented in these waters. As nutrients and algae are natural parts of a riverine system, it is very difficult to determine the "natural" amount for a riverine eco-system and when those levels exceed what is appropriate for a "fully supported" aquatic system. Algae blooms vary according to time and season and are unpredictable. Other available data do not correlate nutrient levels to an increased algae presence. Trend data evaluated in the 2012 Integrated Report indicate either no change or declining trends for nitrogen and phosphorus levels on the North Fork and South Fork Shenandoah Rivers. See table below:

Shenandoah Basin Trend Stations: Status 1991-2010

Station	Total Nitrogen	Total Phosphorus
1BNFS000.57	Declining	No change
1BNFS010.34	Declining	No change
1BNFS070.67	No change	No change
1BNFS093.53	No change	No change
1BSSF003.56	Declining	Declining
1BSSF054.20	Declining	Declining
1BSSF100.10	Declining	Declining

Stream Codes:

NFS North Fork Shenandoah River
SSF South Fork Shenandoah River

Virginia DEQ recognizes that the presence of excess nutrients can be among the factors leading to algal growth and thus, it is sensible that waters with notable algae growth be evaluated for the presence of excess nutrients, as well as other potentially-contributing factors. DEQ is in the process of developing riverine freshwater nutrient standards which will allow the Department the ability to list streams/rivers as impaired for nutrients and by default address algal growth and bloom issues in the future. While nutrient criteria have not yet been adopted, the development of these standards has been ongoing. The Academic Advisory Committee comprised of representatives from Virginia Tech, Virginia Commonwealth University, Virginia Institute of Marine Science and the University of Virginia has completed its report to the agency and we are now reviewing their recommendations. Once these criteria have been established, they will be considered for inclusion in Virginia's Water Quality Standards through the Triennial Review Process which updates, adds and deletes standards to better reflect the most recent science, trends and knowledge of pollutant effects.

As mentioned above, the development of these standards is a very complex undertaking as it is not a simple function of setting a fixed, maximum allowable amount of nutrients, as all waters are different and varied criteria based on other factors are necessary to determine impairment. As you mentioned in your comments, different algae species respond when conditions are conducive to the growth of that particular algae. Those variables include sunlight angle, water clarity, presence of turbidity, temperature, length of day, pH, alkalinity and hardness. These parameters are in constant fluctuation and contribute to the annual variability in the presence of algae.

As with all river systems, conditions in the headwater streams and tributaries have a significant impact on conditions in the mainstem. The exchange of nutrients and sediment loads to the system occur primarily in these smaller, extensive watersheds and are transported to the mainstem. In the South Fork Shenandoah River, stressor analysis determined the cause of the benthic impairment was phosphorus entering the system upstream of the impaired reach. Through the development of the TMDL, it was determined that the needed phosphorus load reduction could not be achieved in the impaired reach as the reductions required were more than the inputs located in that reach. Reductions upstream would be necessary to restore the benthic community. Implementation of TMDL's developed for phosphorus in the upstream tributaries was determined to provide the needed phosphorus load reductions. This situation led EPA to re-classify the benthic aquatic life use impairment on the South Fork Shenandoah River from Category 5A (Impaired, requiring a TMDL) to Category 4A (Impaired, no TMDL required), based on the approved TMDL's upstream that outlined the necessary phosphorus reductions to return the aquatic life use to a fully supporting status. This study confirms that the listing of the mainstem waters in the Shenandoah Basin for the presence of algae based upon nutrients and the subsequent development of a TMDL would be ineffective in addressing the presence of algae because the nutrients present are being transported from upstream sources.

Local TMDLs, and to a lesser extent implementation plans, have been developed on 17 tributaries to the Shenandoah basin rivers which have identified sediment and nutrients as causes for benthic impairments. These TMDL studies and implementation plans identify reductions and practices required to meet these loads and are outlined below.

North Fork Shenandoah River	**South Fork Shenandoah River**
Turley Creek - Sediment	Muddy Creek - Sediment & Phosphorus
Long Meadow Run – Sediment & Nitrogen	Mossy Creek – Sediment
Smith Creek - Sediment	Mill Creek (Rockingham) - Sediment & Phosphorus
Holmans Creek - Sediment	Pleasant Run - Sediment & Phosphorus
Mill Creek - Sediment	Moffett Creek – Sediment
Toms Brook Sediment	Upper Middle River – Sediment
	Lewis Creek - Sediment, Lead & PAH's
Mainstem Shenandoah	Christians Creek – Sediment
Spout Run - Sediment	South River - Sediment & Phosphorus
	Quail Run - Ammonia & Total Residual Chlorine
	South Fork Shenandoah River - Sediment & Phosphorus

Although the designated uses to be restored and protected by these TMDLs may differ from the purpose of the impaired listing which is being sought, those that deal with nutrients and sediments are expected

to help improve the algal conditions as well. One example of how these existing and developing TMDLs can contribute to the cleanup of Shenandoah waters from the standpoint of nutrients is the South River TMDL (approved by EPA 12/9/10), which was developed to address bacteria and benthic impairments. This TMDL calls for phosphorus and sediment load reductions of 51% and 39%, respectively, in order to meet the established TMDL endpoint. Phosphorus must be reduced by 70% from cropland, pasture, transitional and residential uses; along with a 70% reduction of the phosphorus load from septic systems. An Implementation Plan (IP) was developed in October 2010 by the Dept. of Conservation and Recreation along with multiple stakeholders in the region. The IP, which covers both the South River and Christians Creek, was approved by the State Water Control Board in June 2012, and is available at this DEQ webpage:
http://www.deq.virginia.gov/Portals/0/DEQ/Water/TMDL/ImplementationPlans/southchristiansip.pdf.
The IP calls for significant pollution reduction actions with staged implementation over 20 years, employing multiple Best Management Practices across all developed land use types, such as:

- Livestock exclusion with riparian buffers
- Constructed wetlands
- Continuous no-till , conservation tillage and small grain cover crops
- Pet waste education programs
- Streambank stabilization
- Bioretention filters, sedimentation basins and other urban stormwater controls

In addition to the TMDLs and IPs focused on local waters, it is very important to recognize the development and approval of the far-reaching and comprehensive Chesapeake Bay TMDL and the Virginia Watershed Implementation Plan (WIP). The entire Shenandoah basin is included in the watershed that is subject to the Chesapeake Bay TMDL and extensive controls on the point and nonpoint source inputs of nitrogen, phosphorus and sediment are called for under the WIP, many of which are already in operation. Implementation of the WIP for Chesapeake Bay restoration will also have the coincidental benefit of improving local water quality conditions in the headwater areas. Significant municipal and industrial point sources are required to either upgrade to near state-of-the-art nutrient reduction technology or make other arrangements (e.g., nutrient credit exchange) to reduce their nutrient discharges and meet assigned nutrient waste load allocations (WLA); other smaller discharges are required to maintain "capped" loadings at their current design capacity. In 2011, the first year for compliance with Virginia's Chesapeake Bay Nutrient Discharge Watershed General Permit, the Shenandoah basin's significant dischargers achieved substantial nutrient reductions. The total nutrient loads discharged were well below their aggregate annual WLA -- Total Nitrogen (TN) was only 48% of the annual WLA and Total Phosphorus (TP) was just 67% of the annual WLA. Comparing 2011 to 2009 discharge figures, the TN load was reduced by 561,000 pounds per year; the TP load was 169,000 pounds per year lower.

For nonpoint sources, particularly agriculture, the WIP contemplates a significant expansion of the coverage of best management practices to control polluted runoff. Of the agricultural practices contained in Virginia's WIP, the most widely used practices (nutrient management, buffers, soil conservation and livestock exclusion) are expected to be at 90% or greater in 2025 in terms of coverage on all land that is suitable for receiving such practices. In addition, the WIP prescribes 75,000 tons of manure to be exported from Rockingham and Page counties to outside the Chesapeake Bay watershed annually. Beyond reductions achieved to date, within Virginia's entire Chesapeake Bay watershed, the

WIP calls for an additional 10 million pounds of reductions in nitrogen delivered to the Bay and approximately 1.8 million pounds of phosphorus reduction by 2025.

The comments you provided are constructive and we share your concerns. The Virginia DEQ recognizes that the reports included with these comments indicate that excessive algae may exist in various locations in the Shenandoah Basin.

Virginia DEQ does not have specific criteria or standards to directly address the algae issues you have identified, and while the nutrient study process underway discussed above can seem to be frustratingly slow, it is imperative that the agency develop accurate, scientifically valid and defensible tools to determine the level of nutrients that reach the point of impairment. A premature listing prior to development of these standards strains the agency's credibility and objectivity in the eyes of both the regulated community and the public at large.

We believe it is appropriate to recognize these sections of the river as having an observed effect of aquatic algae. Therefore, we propose to modify the 2012 Draft Water Quality 305(b)/303(d) Integrated Report accordingly and list these waters under VA Category 2B ("*waters are of concern to the state but no water quality standard exists for a specific pollutant, or the water exceeds a state screening value or toxicity test*") for the recreational use. This designation means that these areas will remain a priority for monitoring and assessment in the future and will be evaluated when water quality standards related to nutrients and supporting indicators are available for free flowing rivers and streams. DEQ just received (July 2012) the aforementioned report from the agency's Academic Advisory Committee entitled, A "Screening Approach" for Nutrient Criteria in Virginia. That report is now under review to see if the findings have relevance to the development of a methodology for assessing whether or not a "nuisance" aquatic plant condition exists in a waterbody.

APPENDIX I

Shenandoah River Keeper
PTOX Cyanobacteria Screening Report

Prepared: May 22, 2014
Prepared By: GreenWater Laboratories

Samples	Collected
NF2b Farmers Mill	4/18/14
SF2b Island Ford	4/18/14

Methods

One mL from each of the samples (NF2b Farmers Mill and SF2b Island Ford) collected on April, 18 2014 was preserved with Lugol's iodine solution and allowed to settle. The samples were scanned at 100X for the presence of potentially toxigenic (PTOX) cyanobacteria using a Nikon Eclipse TE100 Inverted Microscope equipped with phase contrast optics. Higher magnification was used as necessary for identification.

Results

NF2b Farmers Mill 140418
Microscopic observation of the NF2b Farmers Mill sample collected on 4/18/2014 revealed the dominance of the filamentous cyanobacteria *Phormidium* cf. *favosum* (Fig. 1). *Phormidium autumnale* and *P. favosum* share many morphological traits and are mainly separated based on habitat, slight differences in average trichome width and frequency of sheath formation. *P. autumnale* is described from mesotrophic to eutrophic streams and rivers, and *P. favosum* mostly from cold, flowing waters on limestone substrates. The trichomes observed in this sample fit the description for *P. favosum*. *Phormidium autumnale* and *Phormidium favosum* are both potential anatoxin producers.

Recommendations
Toxin analysis for anatoxin is recommended at this time.

SF2b Island Ford 140418
Microscopic observation of the SF2b Island Ford sample collected on 4/18/2014 revealed the presence of a few filaments of the potentially toxic (PTOX) cyanobacteria *Oscillatoria limosa* (Fig. 2). Some strains of *Oscillatoria limosa* have been found to produce the hepatotoxin microcystin.

Recommendations
Toxin analysis is not recommended at this time.

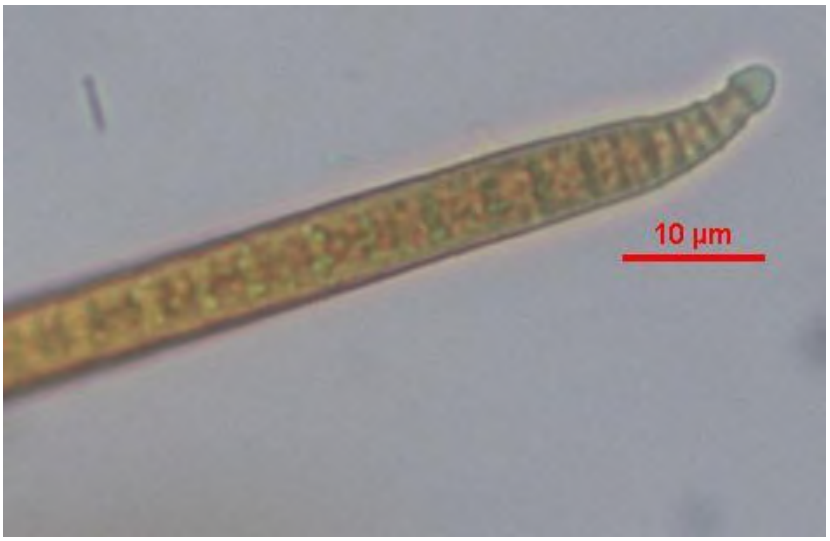


Fig. 1 *Phormidium* cf. *favosum* NF2b Farmers Mill 400X (scale bar = 10µm)

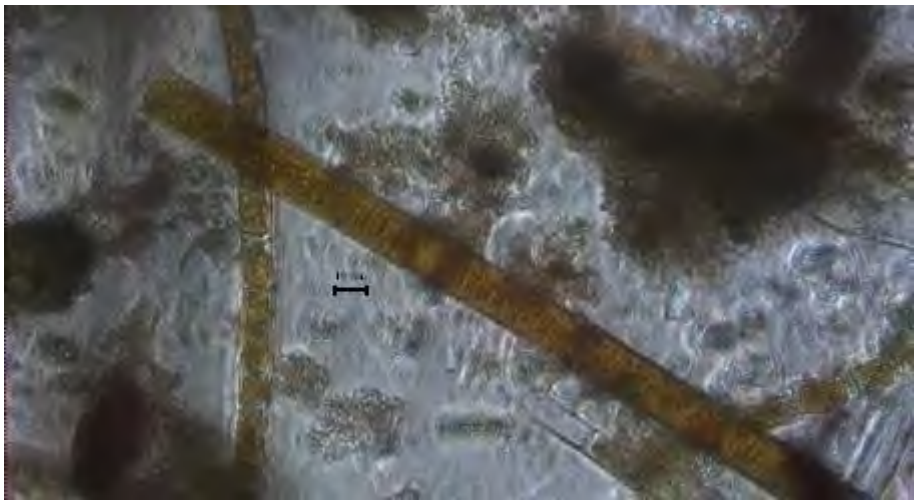


Fig. 2 *Oscillatoria limosa* SF2b Island Ford 400X (scale bar = 10µm)

Submitted by: Nara Souza, M.S.

Date: 5/22/2014

Microcystin Data Report

Project: Shenandoah River Keeper

Sample ID	Site	Sample Date
NF1a	Buckton	4/13/14
SF2a	Shenandoah State Park	4/18/14

Toxins – Microcystin (MC)

Sample Prep – Samples were ultrasonicated to lyse cells and release toxins. A duplicate sample of NF1a was spiked (lab fortified matrix, LFM) with 1.0 µg/L MCLR and a recovery level calculated.

Analytical Methodology – A microcystins (MC) enzyme linked immunosorbent assay (ELISA) from Abraxis LLC was utilized for the quantitative and sensitive congener-independent detection of MCs. The ELISA kit is sensitive down to a limit of detection/quantification (LOD/LOQ) of 0.15 µg/L. The average Lab Fortified Blank (LFB) recovery was 110%; the LFM recovery for NF1a was 94%.

Summary of Results

Sample#	MC levels (µg/L)
NF1a	ND
SF2a	ND

LOD/LOQ = 0.15 µg/L MC
ND = not detected above the LOD/LOQ

Submitted by: _____
Mark T. Aubel, Ph.D.
Date: 5/23/14

Anatoxin-a Report
(Project: Shenandoah Riverkeeper)

Sample Identification	Collection Date
Farmer's Mill (NF2b)	4/18/2014

Toxins – Anatoxin-a (ANTX-A)

Sample Prep – The sample was ultra-sonicated to lyse all cells and release toxins. Strata X solid phase extraction (SPE) was utilized to achieve a 10x pre-concentration for ANTX-A analysis, with a duplicate lab fortified matrix (LFM) prepared at 0.1 µg/L (pre-extraction).

Analytical Methodology – Liquid chromatography/ mass spectrometry/ mass spectrometry (LC-MS/MS) was utilized for the determination of ANTX-A. The $[M+H]^+$ ion for ANTX-A (166 *m/z*) was fragmented and the product ion (91 *m/z*) was monitored. The current LOD is 0.05 µg/L for ANTX-A

Summary of Results
(µg/L)

Sample	ANTX-A (ELISA)
Farmer's Mill (NF2b)	ND
Detection Limits	*0.05*

ND = Not detected above detection limit

Submitted by: Mark T. Aubel, Ph.D.
Date: 6/12/14

Shenandoah River Keeper
PTOX Cyanobacteria Screening Report

Prepared: June 11, 2014
Prepared By: GreenWater Laboratories

Samples	Collected
NF-4 Farmers Mill	5/7/14
NF-5 Farmers Mill	5/14/14
NF-6 Farmers Mill	5/21/14

Methods

One mL from each sample (NF-4 Farmers Mill, NF-5 Farmers Mill and NF-6 Farmers Mill) collected was preserved with Lugol's iodine solution and allowed to settle. Entire samples were scanned at 100X for the presence of potentially toxigenic (PTOX) cyanobacteria using a Nikon Eclipse TE100 Inverted Microscope equipped with phase contrast optics. Higher magnification was used as necessary for identification.

Results

NF-4 Farmers Mill 140507
No potentially toxic (PTOX) cyanobacteria were observed in the NF-4 Farmers Mill sample collected on 5/7/14.

Recommendations
Toxin analysis is not recommended at this time.

NF-5 Farmers Mill 140514
No potentially toxic (PTOX) cyanobacteria were observed in the NF-5 Farmers Mill sample collected on 5/14/14.

Recommendations
Toxin analysis is not recommended at this time.

NF-6 Farmers Mill 140521
Microscopic observation of the NF-6 Farmers Mill sample collected on 5/21/14 revealed the presence of the cyanobacteria *Phormidium* sp. (Figs. 1-2). Most of the sample was composed of short and/or decomposing trichomes. Note that some species in these genera have been known to produce toxins.

Recommendations
Toxin analysis is not recommended at this time.

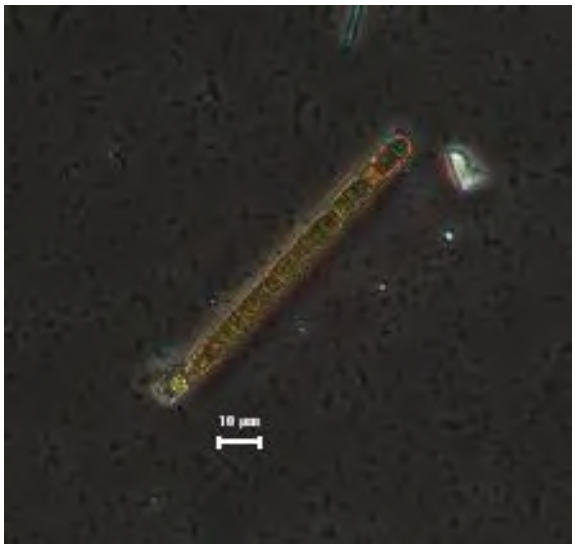


Fig. 1 *Phormidium* sp. 400X (scale bar = 10µm)

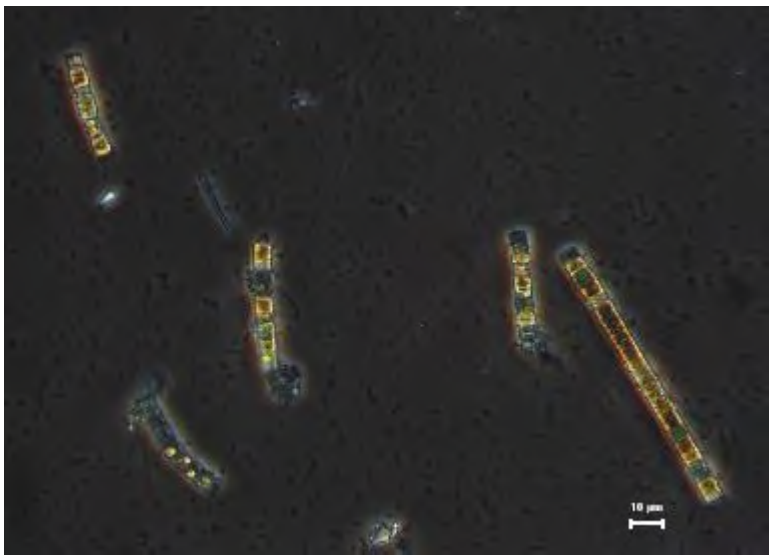


Fig. 2 *Phormidium* sp. 400X (scale bar = 10µm)

Submitted by: Nara Souza, M.S.

Date: 6/11/2014

GreenWater Laboratories
205 Zeagler Drive
Suite 302
Palatka FL 32177
Ph (386) 328-0882
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Contact:
markaubel@greenwaterlab.com
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National Testing Laboratories

MICROCYSTIN RESULTS

Tested on: **5/19/2014**
Method: **Enzyme-Linked ImmunoSorbent Assay (ELISA)**
Analyte: **Microcystins**
Analyzed by: **Amanda Foss**

Sample ID Collection Date	Initial Conc. Factor	Dilution Ratio	Assay Value, ug/L	Final Dilution Factor	Avg. LFB Recovery(%)	Avg. LFM Recovery(%)	Final Concentration (ug/L)	Average (ug/L)
NF1a (Buckton)	1x	none	0.11	1	110	94	ND	**ND**
4/13/2014			0.08	1			ND	
SF2a (Shenandoah St Park)	1x	none	0.12	1	110	–	ND	**ND**
4/18/2014			0.13	1			ND	

ND = Not detected above LOD/LOQ
LOD/LOQ = 0.15 µg/L
LFB = 1.0 µg/L MCLR
LFM = 1.0 µg/L MCLR

Submitted by: Amanda Foss
Amanda Foss, M.S.
Date: 5/23/2014

Submitted to: Brendan Young
Shenandoah Riverkeeper
157 Peyton St
Winchester VA 22601
(703) 867-7878
Brendan@potomacriverkeeper.org

Fwd: Algae Stuff

People

Thank you Ryan!
---------- Forwarded message ----------
From: **Braham, Ryan** <rbraham@usgs.gov>
Date: Fri, Jan 23, 2015 at 8:03 AM
Subject: Re: Algae Stuff
To: Jeff Kelble <jeff@shenandoahriverkeeper.org>
Cc: "Blazer, Vicki" <vblazer@usgs.gov>

Hi Jeff,

I am so sorry for not including that. These samples were all collected on July 18, 2013 between 10am and 1pm.

Ryan

On Thu, Jan 22, 2015 at 4:50 PM, Jeff Kelble <jeff@shenandoahriverkeeper.org> wrote:

> Ryan,
> one last thing, cause this is awesome, what dates were these samples taken?
> Jeff
>
> On Thu, Jan 22, 2015 at 4:12 PM, Braham, Ryan <rbraham@usgs.gov> wrote:
>
> > Hi Jeff,
> >
> > I just ran our in-house ELISA for total microcystin on filtered water samples from our 3 Shenandoah sites (North Fork Shenandoah @ Strasburg, South Fork Shenandoah @ Front Royal, and Mainstem Shenandoah @ Millville Dam). These are grab water samples collected in July 2013. These data are presented as ng/mL (ppb). The North Fork site was below the detection limit of 0.0015 and recorded at 1/2 that value at 0.0008. The South Fork site was 0.0050 (SD=0.0010) and the mainstem site was 0.0088 (SD=0.0000). These data were analyzed today and have yet to undergo complete scrutiny. Thus, they should be considered preliminary data which is subject to revision. I have not had the time to run any other samples from these sites but hope to have a more complete dataset in the near future. Enjoy!
> >
> > Ryan